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Evolution of a Project Linking Plasmalogens to Cognitive Deficits in Schizophrenia: A Feasibility Study

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Evolution of a Project Linking Plasmalogens to Cognitive Deficits in Schizophrenia: A Feasibility Study

by

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Abstract

Schizophrenia (SCZ) is one of the most severe and debilitating psychiatric disorders, affecting approximately 1% of the population. SCZ has historically been defined by the presence of psychotic symptoms; however, focus has now turned to cognitive deficits as a significant challenge for individuals with this disorder. Cognitive deficits can include a lack of attention, slower processing speeds, impaired social cognition, and impaired working memory. Current antipsychotics do not improve cognitive functioning. It is known that free phospholipids within the blood, including plasmalogens, are frequently altered in SCZ. Using monophasic lipid extraction and liquid-chromatography-mass-spectrometry (LC-MS), this project attempted to identify an assay of lipids within a control participant's blood sample. This project also obtained Measurement and Treatment Research to Improve Cognition in SCZ (MATRICS™) Consensus Cognitive Battery (MCCB™) data from 2 control participants. This study also discusses the feasibility of continuing the project in a sample population, as the multiple roadblocks (i.e., multiple changes in laboratories, illnesses, and the onset of coronavirus disease (COVID-19) restrictions, presented to be a significant challenge in recruitment of both participants with SCZ and healthy controls.

Key Words: Schizophrenia, cognition, cognitive deficits, plasmalogens, lipids, LC-MS.

Preface

This thesis is an original, unpublished, independent work, by the author J. Bist. The experiments reported in Chapters 3-5 were covered by Ethics Certificate #16-1004 (REB#16-1004), issued by the University of Calgary Conjoint Health Research Ethics Board (CHREB) for the project "Lipid analysis and assessment of cognitive function in schizophrenia" on November 16th, 2017. Ethics requirements were renewed annually.

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List of Symbols, Abbreviations, & Nomenclature

A	Alpha
ß	Beta
Δ	Delta
Γ	Gamma
M	Micro
Ω	Omega
°C	degrees Celsius
µL	Microliters
2-AG	2-arachinodylglycerol
5-HT	Serotonin
5-HT_{2A}	serotonin receptor 2A
5-HT_{2C}	serotonin receptor 2C
$^{13}C_1$-DMBNHS	$^{13}C_1$-*S*,*S*′-dimethylthiobutanoylhydroxysuccinimide ester
AA	arachidonic acid
AADHAP-R	acyl/alkyl-dihydroxyacetone phosphate reductase
AADHAP-S	acyl/alkyl-dihydroxyacetone phosphate synthase
AAGAT	alkyl/acyl-glycerol-3-phosphate acyltransferase
AB	Alberta
AC	adenylyl cyclase
Ach	Acetylcholine
AD	Alzheimer’s disease
AEA	*N*-arachidonoylethanolamine or anandamide
AHS	Alberta Health Services

Amu	atomic mass units
ANOVA	Analysis of Variance
BACS	*Brief Assessment of Cognition in Schizophrenia:* Symbol Coding
BCL	buffy coat layer
BMI	body mass index
BMP	bis(monoacyl)glycerol phosphate
BVMT-R™	*Brief Visuospatial Memory Test– Revised*
cAMP	cyclic adenosine monophosphate
CBR_1	cannabinoid receptor 1
CBR_2	cannabinoid receptor 2
CBD	Cannabidiol
$CHCl_3$	Chloroform
CH_3OH	Methanol
CHR	clinically high-risk
CHREB	Conjoint Health Research Ethics Board
Cm	Centimeters
CNS	central nervous system
COVID-19	coronavirus disease 2019
cPA	cyclic phosphatidic acid
C-PT	choline-phosphotransferase
CPT-IP	*Continuous Performance Test– Identical Pairs*
CSF	cerebrospinal fluid
D_2Rs	dopamine type-2-*like* receptors
DA	Dopamine

DAG	Diacylglycerol
DCA	dicarboxylic acid
DHA	docohexaenoic acid
DHAP	dihydroxyacetone phosphate
DHAP-AT	dihydroxyacetone phosphate acyltransferase
DHAP-S	dihydroxyacetone phosphate synthase
DMF	Dimethylformamide
DSM-5™	Diagnostic and Statistical Manual, Fifth Edition™
eCBs	Endocannabinoids
EDTA	ethylenediaminetetraacetic acid
E-PT	ethanolamine-phosphotransferase
ER	endoplasmic reticulum
ESI	electrospray ionization
$FAAH_{1/2}$	fatty acid amide hydrolase 1 or 2
FAs	fatty acids
FEP	first-episode psychosis
Fluency	*Category Fluency:* Animal Naming
FMC	Foothills Medical Centre
G	centrifugal force
GABA	γ-amino-butyric acid
GAD67	glutamic acid decarboxylase 67
GHR	genetic high-risk
GLU	Glutamate
GPA	1-0-alkyl-2-hydroxy-*sn*-glycerophosphate

GPC	choline glycerophospholipid
GPCRs	G-protein coupled receptors
GPE	ethanolamine glycerophospholipid
GWAS	genome-wide association studies
$[^{X}H_{Y}]$	hydrogen isotope
HMRB	Heritage Medical Research Building
HMRC	Heritage Medical Research Clinic
HVLT-R™	*Hopkins Verbal Learning Test™–Revised*
I_2	Iodine
IPA	isopropyl alcohol
K^+	potassium ion
Kg	weight in kilograms
L	volume in litres
LC-MS	Liquid chromatography-mass spectrometry
LMU	Lincoln Memorial University
LNS	*Letter-Number Span*
LPA	lysophosphatidic acid
LPAe	ether lysophosphatidic acid or ether-LPA
LPC	Lysophosphatidylcholine
LPCe	ether-lysophosphatidylcholine or ether-LPC
LPE	Lysophosphoethanolamine
LPEe	ether-lysophosphoethanolamine or ether-LPE
LPG	Lysophosphatidylglycerol
LPI	Lysophosphatidylinositol

M	mols per litre (mol/L) or molar concentration
MAG	Monoacylglycerol
MAGL	monoacylglycerol ligase
MATRICS™	Measurement and Treatment Research to Improve Cognition in Schizophrenia™
MCCB™	MATRICS Consensus Cognitive Battery™
MetS	metabolic syndrome
Min	time in minutes
M.I.N.I.™	Mini International Neuropsychiatric Interview™
Ml	volume in millilitres
mM	1/1000 mol/L or millimolar concentration
MS	mass spectrometry
MSCEIT™	*Mayer-Salovey-Caruso Emotional Intelligence Test:* Managing Emotions
MS/MS	tandem mass spectrometry
m/z	mass-to-charge ratio
N_2	Nitrogen
Na^+	sodium ion
NAB®	*Neuropsychological Assessment Battery®:* Mazes
NAPE	*N*-acyl-phosphatidylethanolamine
NAPS	*N*-acyl-phosphatidylserine
$NH_4CH_3CO_2$	ammonium acetate
NIMH	National Institute of Mental Health
NMDAR	*N*-methyl-D-aspartate receptor
NW	Northwest
PA	phosphatidic acid

PAP	phosphatidic acid phosphatase
PC Pls	phosphatidylcholine plasmalogens
PD	Parkinson’s disease
PE Pls	phosphatidylethanolamine plasmalogens
PFC	prefrontal cortex
PG	Phosphatidylglycerol
PH	Phosphohydrolase
Pin	Phosphatidylinositol
P.I.	Principle Investigator
PKA	protein kinase A
PLA_2	phospholipase A_2
PlsC	choline plasmalogens
PlsE	ethanolamine plasmalogens
PNS	peripheral nervous system
Ppm	parts per million
PRS	polygenic risk score
PS	Phosphatidylserine
PtdCh	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdSer	Phosphatidylserine
PUFAs	poly-unsaturated fatty acids
Q-ToF	quadrupole time-of-flight
RBCs	red blood cells
RT	retention time

SCZ	Schizophrenia
Sec	time in seconds
SK	Saskatchewan
SM	Sphingomyelin
Sn	stereospecific numbering
SSA	Schizophrenia Society of Alberta
TAGs	triacylglycerides
TEA	triethylamine
THC	Δ^9-tetrahydrocannbidiol
TMT	*Trail Making Test:* Part A
TN	Tennessee
TRW	Teaching, Research, and Wellness
U of C	University of Calgary
USA	United States of America
USB	universal serial bus
V	electrical potential in volts
v:v or v:v:v	volume per volume concentration
WMS®-III	*Wechsler Memory Scale*®– *Third Edition:* Spatial Span

CHAPTER 1: INTRODUCTION

Goals of the Research Project

The goals for this research project were to: a) Obtain a lipid assay from both individuals with schizophrenia (SCZ) and healthy controls, b) using the obtained lipid assays, determine the lipid species present and a semi-quantification of the detected lipids that are present in both individuals with SCZ and healthy controls, c) measure cognitive performance using the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS™) Consensus Cognitive Battery (MCCB™) in both individuals with SCZ and healthy controls, and d) use multivariate statistical analyses to compare differences between both the measure of cognitive performance, as determined by the MCCB™, and the lipid species and semi-quantifications present that were obtained through the lipid assays, both in individuals with SCZ and healthy controls. We hypothesized that altered blood lipid levels may be associated with a lowered standardized score on the MCCB™. For more information on the aims for this study, see Chapter 2*: Aims, Hypothesis, and Rationale*.

The original goals of this study could not be obtained due to multiple changes of laboratories, illnesses, and recruitment complications due to environmental restrictions to prevent coronavirus disease 2019 (COVID-19). We conclude that a future study using these methods would be considered feasible. The implementation of this protocol may provide useful information regarding lipids, including plasmalogens, as a possible biomarker for diagnosis of SCZ.

Background

Current State of Knowledge in Schizophrenia

Schizophrenia (SCZ) is a severely debilitating mental disorder with a worldwide prevalence of approximately 1%, and is characterized by positive, negative, and cognitive symptoms (Marder & Cannon, 2019). Typically, symptoms of the disorder first appear in late teens to early twenties, and before onset of the disorder, there is often a time frame from a few months to a couple of years during which a person experiences subtle decline in behaviour and functioning, often referred to as the prodromal stage (Goff, 2021; Kane et. al, 2019; Marder & Cannon, 2019). Dysfunction in this stage can occur in areas such as occupational functioning, interpersonal relationships, and academics (Marder & Cannon, 2019). As well, there is a slightly higher incidence rate in males in comparison to females (Chestnukh et al., 2021).

The etiology of SCZ remains unclear; however, there is evidence for both genetic and environmental risk factors (Hsu et al., 2020; Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020). Prenatal and childhood environmental risk factors for developing SCZ include maternal malnutrition, infection, fetal hypoxia, parental separation, and sexual or physical abuse (Keefe & Kahn, 2017; Marder & Cannon, 2019). Other environmental risk factors include smoking, diabetes, lack of exercise, an association to increased susceptibility with cannabis use, socioeconomic status, and brain injury (Marder & Cannon, 2019; Gerlach et al., 2019; Goldman & Mangurian, 2020). In addition to these risk factors, there are numerous genes and transcription factors that contribute to an increased risk for developing SCZ (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020). Scientists believe that SCZ could be caused by small

gene variations, such as insertions, deletions, or inversions that affect brain pathways (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020).

Epidemiology

Susceptibility to SCZ is both genetic and environmental, and heritability factors explain approximately 80% of the risk to develop SCZ (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020). The risk to develop SCZ is increased for a child with a first-degree relative with SCZ (Chou et al., 2016; McCleery & Nuechterlein, 2019). In monozygotic twin studies, there is approximately an 81% chance for the second twin to inherit SCZ if one already has the disorder (Chou et al., 2016). New technological advances have now made genome-wide association studies (GWAS) possible, and it was found that over 100 loci are associated with SCZ itself (McCutcheon, Marques, & Howes, 2020). Since SCZ is considered a polygenic disorder, GWAS have enabled scientists to later discover polygenic risk scores (PRS) for individuals (McCutcheon, Marques, & Howes, 2020). PRS allow for scientists to determine the risk of inheriting SCZ and there is evidence that scientists may be able to predict successful treatments depending on the number and type of alleles associated with SCZ that an individual will carry (Goff, 2021; McCutcheon, Marques, & Howes, 2020). For example, it has been discovered that deletion of allele 22q11.2 is associated with a 30 to 40% increase in the lifetime risk of developing SCZ (McCutcheon, Marques, & Howes, 2020). Despite these technological advances, it is difficult for scientists to determine specific alleles for SCZ itself, as many other psychiatric disorders overlap in both risk factors and alleles (McCutcheon, Marques, & Howes,

2020). In addition to this, no pharmacologic treatments have been successful for all symptoms to date (Goff, 2021).

Many individuals suffering from SCZ struggle with daily functioning, including enduring the burden of discrimination, social stigma, and reduced socioeconomic status, and these disadvantages persist throughout adulthood (Kaddurah-Daouk et al., 2012; Marder & Cannon, 2019). For many individuals, it is difficult to obtain work due to persistence of SCZ symptoms, and individuals diagnosed with SCZ have an increased mortality rate at approximately 12 to 15 years earlier when compared to healthy controls (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020). In addition to this, cognitive deficits are now considered a predictor of occupational functioning (Tripathi, Kar, & Shukla, 2018). Cognitive remediation measures are now being implemented and studied by researchers to improve cognitive deficits, and thereby aiding the individual in occupational functioning.

Diagnostic Criteria

SCZ is diagnosed by the presence of positive and negative psychotic symptoms that have been present for a minimum period of 6 months (McCutcheon, Marques, & Howes, 2020). Positive psychotic symptoms include hallucinations, delusions, disorganized speech, and disorganized or catatonic behavior (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020). Negative psychotic symptoms include avolition, alogia, and affective flattening (Marder & Cannon, 2019; McCutcheon, Marques, & Howes, 2020).

According to the *Diagnostic and Statistical Manual of Mental Disorders- Fifth Edition*™ (DSM-5™), SCZ is formally diagnosed by the presence of at least two of the following items,

with presence for a minimum of one month, and at least one of the items must be 1, 2, or 3: 1) delusions, 2) hallucinations, 3) disorganized speech, 4) grossly disordered or catatonic behaviour, and 5) negative symptoms (American Psychiatric Association, 2013). Other diagnostic criteria include social and/or occupational dysfunction with the exclusion of other factors such as substance use, general medical conditions, and autism or developmental delay (American Psychiatric Association, 2013).

Ætiology and Pathophysiology

Dopamine Hypothesis of Schizophrenia

The most accepted theory for the pathophysiological dysfunction in SCZ is the *dopamine (DA) hypothesis.* The DA hypothesis emerged in the 1950s with the discovery of chlorpromazine, the first anti-psychotic drug available to treat positive symptoms of SCZ (Goff, 2021; Madras, 2013; Moghaddam & Javitt, 2012; Nestler et al., 2015). This scientific development allowed for the discovery of the therapeutic target: DA type-2-*like* receptors (D_2Rs) (Nestler et al., 2015). In addition to DA's role in motor function, DA plays multiple roles in attention, cognition, executive functioning, motivation, and behaviour (Nestler et al., 2015). The DA hypothesis stipulates that symptoms of SCZ are due to dysfunction in DA regulation throughout the brain, particularly due to overactivity of D_2Rs (Madras, 2013; Moghaddam & Javitt, 2012). D_2Rs are inhibitory G-protein-coupled receptors (GPCRs) that decrease the activity of cyclic adenosine monophosphate (cAMP) by inhibiting adenylyl cyclase (AC) and thereby producing cellular responses such as reducing protein kinase A (PKA) (Nestler et al., 2015). D_2Rs, which are both pre- and post-synaptic, are expressed in various areas of the brain,

including the striatum, frontal cortex, amygdala, and hippocampus, many of which have been found to be affected in SCZ (McCutcheon, Abi-Dargham, & Howes, 2019; Nestler et al., 2015). Additionally, D_2R expression correlates positively with the efficacy of antipsychotics (Nestler et al., 2015; McCutcheon, Abi-Dargham, & Howes, 2019).

Chlorpromazine, initially developed in 1951, was the first anti-psychotic drug created to help individuals with SCZ (Nestler et al., 2015). Chlorpromazine is a potent antagonist at D_2Rs (Nestler et al., 2015). Eventually, other antipsychotics were developed that all function as D_2R antagonists. Examples include haloperidol, clozapine, quetiapine, risperidone, and olanzapine. More recently, partial DA agonists, including aripiprazole, brexpiprazole and cariprazine, have also shown efficacy in the treatment of SCZ. Non-pharmacological treatments, frequently used in addition to pharmacotherapy, include cognitive behavioural therapy (CBT) and cognitive remediation therapy (Nestler et al., 2015; Jauhar, Laws & McKenna, 2019; Tripathi, Kar, & Shukla, 2018; Vita et al., 2021). Haloperidol, for example, has a high affinity for D_2Rs at both the synaptic terminal and presynaptic terminal; however, scientists do not fully understand the mechanism behind the production of antipsychotic effects due to this affinity (Nestler et al., 2015). D_2Rs can be modulated through other neurotransmitters (e.g., GABAergic, serotonergic, noradrenergic, cholinergic, and peptidergic); therefore, it is not fully understood how the dysfunction in dopaminergic system alone can cause psychotic effects (Nestler, 2015).

Other Hypotheses of Schizophrenia

Other hypotheses focus on neurotransmitters and receptors beyond DA, to gain a better understanding of the ætiology of SCZ. Different neurotransmitters including glutamate (GLU),

serotonin (5-HT), acetylcholine (ACh), γ-amino-butyric acid (GABA), and endocannabinoids (eCBs) have been implicated in the ætiology of SCZ over the last 25 years (Brisch et al., 2014; Goff, 2021). These hypotheses were put forth because the available pharmaceutical treatments, such as D_2R antagonists and partial D_2R agonists, do not work for every individual, and these treatments are not effective for all symptoms experienced by individuals with SCZ (i.e., negative symptoms and cognitive deficits) (Madras, 2013; Zhou et al., 2020). Additionally, serious side effects of antipsychotic drugs (e.g., tardive dyskinesia), has prompted more research into mechanisms involved in the ætiology of psychosis to develop alternative medications (Nestler et al., 2015).

Glutamate

GLU is the main excitatory neurotransmitter in the central nervous system (CNS) (Moghaddam & Javitt, 2012). The *Glutamate Hypothesis of Schizophrenia* postulates a hypofunction of the *N*-methyl-D-aspartate receptors (NMDARs) (Moghaddam & Javitt, 2012). NMDAR hypofunction results in excess GLU in the synapse which can cause excitotoxicity to surrounding neurons (Moghaddam & Javitt, 2012). Excitotoxicity can result in neurodegeneration and subsequently, can contribute to the development of symptoms of SCZ (Moghaddam & Javitt, 2012).

Serotonin

An early model of serotonin receptor 2A (5-HT_{2A}) antagonism helped to produce second generation antipsychotics, including risperidone and olanzapine (Chestnykh et al., 2021; Goff,

2021). It is also known that 5-HT antagonists help to ameliorate extrapyramidal side effects of other antipsychotic drugs (Yang & Tsai, 2017). The interaction between DA and 5-HT is difficult for researchers to study due to the wide range and amount of both receptor types within many areas of the brain (Brisch et al., 2014; Yang & Tsai, 2017; Chestnykh et al., 2021).

γ-Aminobutyric Acid

GABA is the primary inhibitory neurotransmitter in the CNS and is receiving increased interest from scientists studying cognitive deficits in SCZ (Chang et al., 2016). The *GABA Deficiency Hypothesis of Schizophrenia* states that there is decreased glutamic acid decarboxylase 67 (GAD67), as well as decreased number of GABAergic neurons in individuals with SCZ due to an unknown mechanism (Chang et al, 2016). GAD67 is a precursor of GABA within cortical neurons, and this enzyme is critical for GABA regulation in the CNS. NMDARs are also found on GABAergic neurons. GABAergic neurons are mostly inhibitory to the postsynaptic neuron; however, this inhibition is important to regulate messages being sent from one neuron to another (Chang et al., 2016). If GABAergic neurons are not being stimulated due to NMDAR hypofunction, overactivation of the postsynaptic neuron can occur, resulting in dysfunction (Chang et al., 2016).

Acetylcholine

Another theory for the development of SCZ is the *Muscarinic Hypothesis of Schizophrenia*, which proposes that a decrease in muscarinic cholinergic neurotransmission underlies symptoms of SCZ, including cognitive symptoms (Raedler et al., 2007). ACh is a

widely distributed neurotransmitter and plays a crucial role in areas including sensory perception, memory, motor functioning, arousal, sleep, and psychosis; however, it has been difficult to create a drug targeting ACh due to ACh playing multiple roles outside the CNS (e.g., modulation of blood flow and heart rate) (Raedler et al., 2007). An extensive review from Raedler et al., 2007, shows strong support that the ACh system is affected in SCZ through post-mortem, neuroimaging, and neuropharmacological studies.

Endocannabinoids

There was increased interest and research into the mechanisms behind marijuana plant, *Cannabis sativa,* after evidence was shown for medicinal uses (e.g., nociception, anti-inflammation) (Zou & Kumar, 2018). Additionally, new laws in North America now permit recreational use; however, long-term effects are unknown (Volk & Lewis, 2019; Zou & Kumar, 2018).

Shortly after the discovery of both the psychoactive component, Δ^9-tetrahydrocannabidiol (THC) and cannabidiol (CBD), cannabinoid receptors and eCBs were also discovered to interact in many areas of the body (Katona & Freund, 2012; Marzo & Petrocellis, 2012). The discovery of the interaction between eCBs and cannabinoid receptors prompted new research into neuromodulation within SCZ (Navarrete, Diez, & Araque, 2014; Minichino et al., 2019). Cannabinoid 1 receptor (CBR_1) and cannabinoid 2 receptor (CBR_2) are widely distributed throughout the brain, and there is evidence that both the ligands and receptors of the eCB system can modulate the activity of other neurotransmitters including DA, ACh, and GLU (Fakhoury, 2017; Zou & Kumar, 2018; Minichino et al., 2019). *N*-arachidonoylethanolamine (AEA) and 2-

arachidonylglycerol (2-AG) are lipids that are synthesized in the post-synaptic cell membrane and bind as agonists to CBR_1 and CBR_2 on the presynaptic cell membrane using retrograde neuromodulation (Fakhoury, 2017; Zou & Kumar, 2018; Minichino et al., 2019).

Research into eCBs and their interactions has aided in the discovery of some differences that have been found between the endogenous ligands AEA and 2-AG. AEA has a high affinity to CBR_1 but acts as a partial agonist to this receptor (Zou & Kumar, 2018). 2-AG has a medium-to-low affinity to both CBR_1 and CBR_2 but acts as a full agonist to both receptors (Zou & Kumar, 2018). Both endogenous ligands are synthesized and regulated within neuronal cells separately. Increased concentration in the synapse will allow transport proteins to take up AEA and 2-AG (Fakhoury, 2017). Inside the presynaptic cell, AEA will be degraded by fatty acid amide hydrolase ($FAAH_{1/2}$) and 2-AG will be degraded by monoacylglycerol ligase (MAGL) (Fakhoury, 2017).

It is currently unknown how eCBs and the associated ligands regulate observed symptoms in SCZ. Post-mortem and animal studies have reported that there is evidence of a reduced number of CBR_1 and CBR_2 receptors for both individuals with a diagnosis of SCZ and for animals treated with phencyclidine (PCP) (Fakhoury, 2017). AEA and 2-AG are increased in cerebrospinal fluid (CSF), plasma, and whole blood samples from individuals with SCZ (Schneider et al., 2017; Volk & Lewis, 2019). Increased 2-AG and AEA acting on CBR_1 and CBR_2 are known to reduce neuronal GLU release in SCZ, which can contribute to GLU dysfunction (Schneider et al., 2017).

Phospholipid Hypothesis of Schizophrenia

Lipid Structure and Synthesis Overview

Lipids are amphipathic, fundamental, organic molecules that are utilized by the human body for several processes (Tracey et al., 2018). Lipids are diverse, and there are over 2000 different known lipid species in the mammalian system (Tracey et al., 2018). Lipids can be classified into 5 categories: fatty acids (FAs), triacylglycerides (TAGs), phospholipids, sterol lipids, and sphingolipids (Tracey et al., 2018). FAs are an essential component of all lipid types, most often generated as-needed by the cell in the cytosol, and can be further categorized into long-, medium-, or short-chain FAs, which thereby produces variations in functions (Tracey et al., 2018). TAGs, which only play a small role in neuronal lipid metabolism, are commonly found within adipose tissue, and are composed of a glycerol backbone along with 3 FA chains (Tracey et al., 2018). Sterol lipids, often found in the body as cholesterol, and in a tetracyclic ring structure with a hydroxyl group attached to one end of the ring (Tracey et al., 2018). TAGs are usually created and stored within the ER, whereas sterol lipids and sphingolipids are more concentrated within lipid rafts at the plasma membrane (Tracey et al., 2018).

Phospholipids Overview

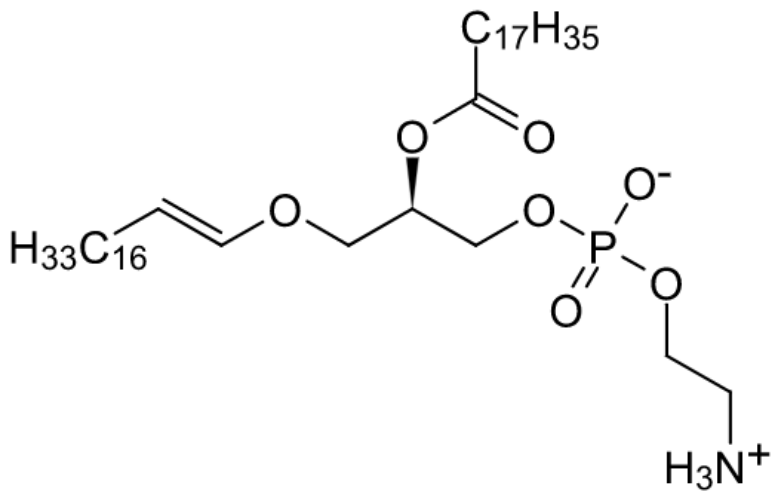
Phospholipids are fundamental components of the plasma membrane animal cells and are critical components of neuronal tissue (Tracey et al., 2018). Phospholipids are composed of a glycerol backbone, 2 FAs, a hydrophilic head group, and a phosphate group (Tracey et al., 2018). Phospholipids are categorized into two groups: glycerophospholipids and phosphosphingolipids (Tracey et al., 2018). Glycerophospholipids can be further categorized into plasmalogens, more

specifically phosphatidylcholine plasmalogens (PC Pls), phosphatidylethanolamine plasmalogens (PE Pls), phosphatidylserine (PS), phosphatidylinositol (PIn), and phosphatidylglycerol (PG) (Tracey et al., 2018).

Plasmalogens

Plasmalogens are important components of plasma membranes, and they are found in high concentrations in brain tissue, specifically in neuronal cell membranes and myelin (Wallner & Schmitz, 2011). In healthy individuals, plasmalogens constitute approximately 20% of the total phospholipids in the cell membranes throughout the entire body (Braverman & Moser, 2012). It is known that there are lower levels of blood plasmalogens in individuals with SCZ (Kaddurah-Daouk et al., 2012).

Figure 1. *Structure of a Plasmalogen, Including sn-1, sn-2, and sn-3 Positions*



Note. This figure shows the *sn*-1, *sn*-2, and *sn*-3 positions within the plasmalogen structure. The *sn*-1 position contains a vinyl-ether double bond, the *sn*-2 position contains poly-unsaturated

fatty acids (PUFAs), and the *sn*-3 position carries a distinguishing head group. From '*Plasmalogen*' by Ronald Mattern, 2009, Wikimedia Commons (https://commons.wikimedia.org/wiki/File:Plasmalogen.png). In the public domain.

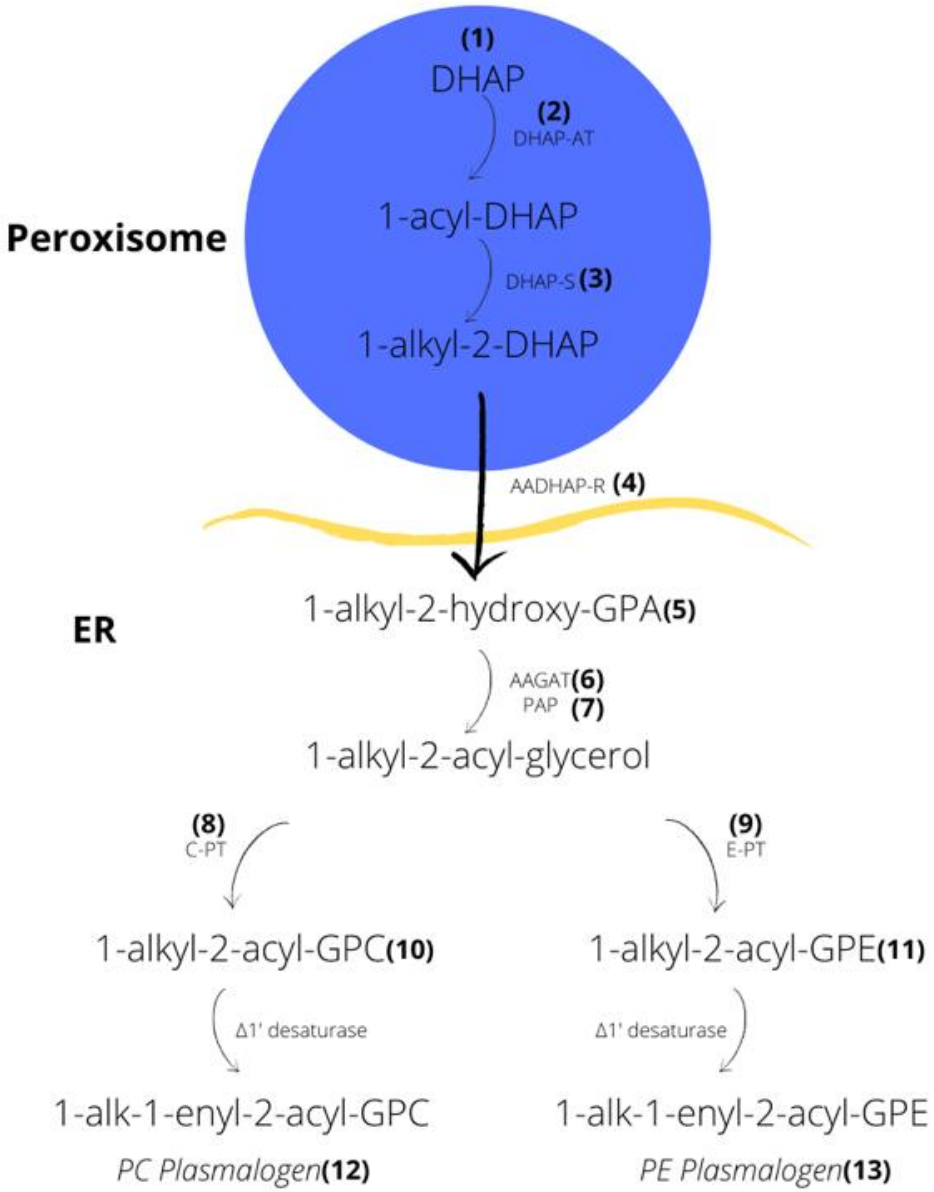
Plasmalogens have a unique, specialized structure and synthesis. Figure 1 shows the locations of the *sn*-1, *sn*-2, and *sn*-3 positions. Attached to their glycerol backbone, phospholipids have a vinyl-ether double bond at the *sn*-1 position, PUFAs on the *sn*-2 position, and a distinguishing polar head group at the *sn*-3 position (Braverman & Moser, 2012; Wallner & Schmitz, 2011). At the *sn*-2 position, the vinyl-ether double bond is important for removing free radicals, and without this double bond, a cell would be at risk for damage (Kaddurah-Daouk et al., 2012; Wallner & Schmitz, 2011). At the *sn*-2 position, the PUFAs that are most common include: arachidonic acid (AA) and docosahexaenoic acid (DHA). At the *sn*-3 position, plasmalogens mainly contain ethanolamine or choline, but could also rarely contain inositol, serine, or threonine (Braverman & Moser, 2012).

The biosynthesis of plasmalogens begins in the extracellular membrane and intercellular matrix of the peroxisome, and plasmalogens complete their synthesis in the extracellular membrane and extracellular matrix of the endoplasmic reticulum (ER) (Brites, Waterham, & Wanders, 2003; Braverman & Moser, 2012). The first step of biosynthesis involves replacement of the *sn*-1 fatty acid with a fatty alcohol and this process begins through esterification of dihydroxyacetone phosphate (DHAP) with acyl-CoA ester using dihydroxyacetone phosphate acyltransferase (DHAP-AT) (Brites, Waterham, & Wanders, 2003; Braverman & Moser, 2012). This reaction is further catalyzed by alkyl/acyl-dihydroxyacetone phosphate synthase (AADHAP-S), with the product being 1-alkyl-DHAP (Brites, Waterham, & Wanders, 2003; Braverman & Moser, 2012). Next, the ketone group in 1-alkyl-DHAP is further reduced and has a phosphate group removed through use of acyl/alkyl-dihydroxyacetone phosphate reductase

(AADHAP-R) and phosphohydrolase (PH), which results in a substrate called 1-alkyl-2-acyl-glycerol (Brites, Waterham, & Wanders, 2003). Subsequently, through further reduction, the plasmalogens are further modified through various enzymes (e.g., phosphotransferases and desaturases) and the distinction between PE Pls and PC Pls emerge (Brites, Waterham, & Wanders, 2003; Braverman & Moser, 2012).

Plasmalogens function correctly when there is a balance between biosynthesis and catabolism (Wallner & Schmitz, 2011; Leppik et al., 2019). Catabolism of plasmalogens first occur through the activation of plasmalogen-specific phospholipase A_2 (PLA_2) (Wallner & Schmitz, 2011). This type of PLA_2 catabolizes plasmalogens at a normal rate in healthy individuals, aids in lipid membrane maintenance, and act as second messengers throughout the body (Wallner & Schmitz, 2011). Plasmalogens are involved in multiple processes throughout the body, including ion transport, membrane fusion, and regulation of cholesterol (Wallner & Schmitz, 2011). When PLA_2 catabolizes a plasmalogen, AA and DHA are released and can act as second messengers within the body (Berger et al., 2006; Evans et al., 2003; Farooqui, Farooqui, & Horrocks, 2008). AA, DHA, and their products (i.e., eicosanoids) are essential products for synaptic plasticity, including pruning, and neuronal migration (Berger et al., 2006). SCZ patients have a higher amount of PLA_2 activity (Farooqui, Farooqui, & Horrocks, 2008).

Figure 2. *Plasmalogen Synthesis Within a Cell*



Note. This figure summarizes the steps of plasmalogen synthesis within a cell. (1) dihydroxyacetone phosphate (DHAP), (2) dihydroxyacetone phosphate acyltransferase (DHAP-AT), (3) dihydroxyacetone phosphate synthase (DHAP-S), (4) acyl/alkyl-dihydroxyacetone

phosphate synthase (AADHAP-S), (5) 1-0-alkyl-2-hydroxy-*sn*-glycerophosphate (GPA), (6) alkyl/acyl-glycerol-3-phosphate acyltransferase (AAGAT), (7) phosphatidic acid phosphatase (PAP), (8) choline-phosphotransferase (C-PT), (9) ethanolamine-phosphotransferase (E-PT), (10) choline glycerophospholipid (GPC), (11) ethanolamine glycerophospholipid (GPE), (12) phosphatidylcholine plasmalogen (PC Plasmalogen), (13) phosphatidylethanolamine plasmalogen (PE Plasmalogen). Adapted from 'Functions of plasmalogen lipids in health and disease,' by Braverman & Moser (2012), and 'Functions and biosynthesis of plasmalogens in health and disease,' by Brites et al. (2004). Copyright by Braverman & Moser (2012). Copyright 2004 by Brites et al. Reprinted with permission. See Figure 8 in Appendix K for permissions.

Explanation of the Phospholipid Hypothesis of Schizophrenia

Accumulation of evidence over the past 25 years contribute to the viewpoint that phospholipids, particularly plasmalogens, play a role in the pathophysiology of SCZ (Horrobin, Glen, & Vaddadi, 1994; Peet et al., 1994; Glen et al., 1994; Horrobin, 1998; Bois, Deng, & Huang, 2005; Tessier et al., 2016; McCleary & Nuechterlein, 2019; Frajerman et al., 2021). *Plasmalogens,* a subclass of phospholipids, are found throughout serum lipoproteins and tissue membranes within animal cells (Wallner & Schmitz, 2011). Plasmalogens are found throughout the nervous system, which contribute to maintaining cell fluidity and removing free radicals. Several neurological disorders, including Alzheimer's Disease (AD) and Parkinson's Disease (PD) show a decreased level of plasmalogens within various tissues (Wallner & Schmitz, 2011; Braverman & Moser, 2012; Kaddurah-Daouk et al., 2012; Schneider et al., 2017).

Horrobin (1998) stated that due to observations of individuals with SCZ (e.g., alterations in ventricular size, neurodevelopment differences, perinatal risk factors, onset in adolescence and early adulthood, sex differences, and seasonal variations in onset), there must be a biochemical basis or substrate associated with this disorder (Horrobin, 1998). Horrobin (1998) argues that the

phospholipids are a potential candidate for guiding future research studies. Today, SCZ is viewed as a neurodevelopmental disorder, which suggests that a focus on early detection and intervention can improve functional and occupational outcomes for individuals with SCZ substantially (Horrobin et al., 1991; Horrobin, Glen, & Vaddadi, 1994; Kaddurah-Daouk et al., 2012; McCleery & Nuechterlein, 2019; Frajerman et al., 2021). While the other hypotheses of SCZ described above may give a partial understanding of SCZ symptomology, this hypothesis states that dysfunctional phospholipid metabolism contributes to all positive, negative, and cognitive symptoms observed (Tessier et al., 2016).

Tessier and al. (2016) describe that with aid from new advances in technology (e.g., liquid chromatography-mass spectrometry), it is now possible to measure the changes in lipids under different conditions to be able to test the hypothesis further. With neurolipidomics becoming a rapidly expanding area of research, brain lipids may be indirectly assessed via membrane lipids in cells such as red blood cells (RBCs) (Tessier et al., 2016). By assessing human blood serum, which contains thousands of different metabolites that can reflect an individual's state of health, differences in metabolites could help to identify incongruence between patients with a dysfunctional state vs. controls (Prata, Mechelli, & Kapur, 2014; Levine et al., 2015). Identifying these differences in lipid metabolites will help to clarify the largely unknown pathophysiology of SCZ.

Other Research in Plasmalogens and Schizophrenia

In recent years, several studies have examined blood-based biomarkers, including plasmalogens, and metabolic changes in SCZ (Kaddurah-Daouk et al., 2012; McEvoy et al., 2013; Wu et al., 2013; Wood et al., 2015; Chan et al., 2015; Tessier et al., 2016; Solberg et al.,

2016; Petrikis et al., 2017; Yan et al., 2018). Lipid abnormalities, particularly plasmalogens, have also been observed in several other disorders, including AD and gastrointestinal cancer (Hartmann, Kuchenbecker, & Grimm, 2007; Igarashi et al., 2011; Messias et al., 2018).

A study by Kaddurah-Daouk et al. (2012) looked at 20 drug-naïve patients experiencing a first psychotic episode and 20 unmedicated patients experiencing relapses in comparison to healthy controls. These researchers reported lower levels of both PC Pls and PE Pls in both drug-naïve patients and unmedicated patients experiencing relapses (Kaddurah-Daouk et al., 2012). These lower levels of plasmalogens appeared to be a trait of SCZ in general, and the level of plasmalogens did not specify specifically whether the patient was experiencing a relapse or first-episode psychosis (FEP) (Kaddurah-Daouk et al., 2012).

Another study by Tessier et al. (2016) examined 74 antipsychotic-medicated and clinically stable SCZ outpatients in comparison to 40 healthy controls. Cognitive function was assessed, using the Continuous Performance Test, Salience Attribution Test, and Wisconsin Card Sorting Test (Tessier et al., 2016). Results from this study showed that sphingomyelin (SM) was most associated with a SCZ diagnosis; however, they found that plasmalogens specifically were also significantly altered (Tessier et al., 2016). Results also showed that cognitively, performance on these 3 tests were poorer in the SCZ group compared to healthy controls (Tessier et al., 2016). The researchers suspected that due to the lower scores in the cognitive tests, this may be related to abnormal DA signalling; therefore, more research will be needed in this area (Tessier et al., 2016).

In another study by Wood et al., (2015), researchers examined plasmalogens in both plasma and platelets 23 patients with SCZ and 27 age-matched controls. Results showed that both PC Pls and PE Pls were decreased in patients with SCZ by 23% and 45% respectively

(Wood et al., 2015). Additionally, DHA was decreased by approximately 30% in both plasma and platelets for patients with SCZ (Wood et al., 2015).

A recent study has examined external white matter post-mortem tissue from individuals with SCZ (Ghosh, Dyer, & Beasley, 2017). It was found that PC Pls and PE Pls were quantified in prefrontal cortex (PFC) tissue, and that these plasmalogens were lower in the SCZ group when compared to the control group (Ghosh, Dyer, & Beasley, 2017). The researchers concluded that white matter circuits may be prone to dysfunction in SCZ, and that more research needs to be done in this area (Ghosh, Dyer, & Beasley, 2017). Despite this, there has been mixed results. For example, researchers Hamazaki, Choi, & Kim (2010) found that there was no change in DHA within the hippocampal region of post-mortem tissue. Hamazaki et al. (2016) then did a follow-up study on PUFAs in the corpus callosum but were unable to find any significant lipid differences in this area as well. The researchers concluded that both DHA decreases and altered lipid structure or metabolism may be specific to other areas of the brain for individuals with SCZ (Hamazaki, Choi, & Kim, 2010; Hamazaki et al., 2016).

Other Lipid Disturbances in Schizophrenia

Sphingomyelin

Another type of lipid associated with both SCZ and cortical stress are sphingolipids. SM is the most prominent of the sphingolipid class found in animal cell membranes (particularly within myelin sheath and axonal membranes), and SM is composed of ceramide and various hydrophilic head groups (Schneider et al., 2017; Tessier et al., 2016; Wood, 2019). The hydrophilic head groups interact with each other to create strong domains in a cell membrane called lipid rafts (Wood, 2019). SM can be hydrolyzed to ceramide and free fatty acids, which

can then alter the cell membrane environment (Schneider et al., 2017; Tessier et al., 2016; Wood, 2019). Low ceramide levels have been linked to both clinical and cognitive symptoms in SCZ; however, further testing is required (Schneider et al., 2017; Tessier et al., 2016).

Antipsychotic Use

Researchers have examined first generation (i.e., haloperidol, chlorpromazine & fluphenazine) and second-generation antipsychotic medications (i.e., risperidone, olanzapine & quetiapine), and have found these medications are effective for managing positive symptoms; however, these antipsychotics do not improve negative and cognitive symptoms (Zhou et al., 2020; Marder & Cannon, 2019). Antipsychotics are widely used to treat positive symptoms of SCZ; however, there are multiple side effects such as development of metabolic syndrome (MetS), dyslipidemia, and obesity (Almeida et al., 2020).

In a previous study by researchers Kaddurah-Daouk et al. (2012), plasmalogen levels did not change in response to antipsychotic treatment in participants with FEP; therefore, the researchers concluded that plasmalogens are not directly affected by antipsychotics, and the lowered levels observed in SCZ were a result of the illness itself. A study by Almeida et al. (2020) examined blood plasma lipid assays of patients 6 weeks after treatment with either risperidone, olanzapine, or quetiapine. Results from the study showed that depending on the antipsychotic treatment that was given, different classes of lipids, including plasmalogens, were affected differently (Almeida et al., 2020). Since the study had only examined a short period of time of 6 weeks, it is unknown how these lipid differences would affect an individual for a longer time period.

Smoking

In terms of general smoking, a study by An et al. (2016) examined serum lipid profiles in 104 individuals with SCZ that smoked and 26 individuals with SCZ that did not smoke. With smoking being normally linked to abnormal blood lipid profiling, it was expected to change the serum lipid profiles; however, no significant differences between blood lipids were found when both groups were compared (An et al., 2016). In terms of cannabis, further research is required for determining if there is a link between blood lipid levels and intake of cannabis (Gerlach et al., 2019).

Cognitive Deficits in Schizophrenia

History of Cognitive Deficits in Schizophrenia

Historically, although Kræpelin first described SCZ as "dementia praecox," with descriptions of the illness based on cognitive deficits, pharmacological treatment has been focused on reducing positive symptoms (McCleery & Nuechterlein, 2019). Cognitive impairment is still not recognized as an official symptom of SCZ per the DSM-5™; however, there is debate around including it, as individuals with SCZ experiencing cognitive deficits describe these symptoms as prominent and persistent (American Psychiatric Association, 2013; McCleery & Nuechterlein, 2019). Additionally, cognitive deficits are not improved with current antipsychotic medications (American Psychiatric Association, 2013; McCleery & Nuechterlein, 2019). It is thought that SCZ is a disorder of higher order cognition and dysfunctional sensory processes, and to determine additional factors surrounding cognitive deficits in SCZ, more research is needed (Tang & Niznikiewicz, 2020).

Prevalence and Risk for Cognitive Deficits

It is estimated that up to 80% of individuals with psychotic disorders experience significant cognitive deficits (McCleery & Nuechterlein, 2019). There are some individuals diagnosed with SCZ that do not experience cognitive deficits; however, it is argued that all individuals diagnosed with SCZ experience some degree of cognitive impairment, and these individuals would likely have performed better on cognitive tests if they had not developed SCZ (McCleery & Nuechterlein, 2019). For the approximately 80% of individuals with SCZ that do experience cognitive deficits, cognitive impairment is observed in at least one standard deviation below the population mean (McCleery & Nuechterlein, 2019).

Individuals classified as *clinically high-risk* (CHR) to developing psychosis can show significant cognitive impairment during controlled tests (McCleery & Nuechterlein, 2019). CHR individuals are defined as being within the prodrome or individuals at increased risk for developing psychosis (McCleery & Nuechterlein, 2019). These individuals can rapidly decline academically, socially, and vocationally before the development of SCZ (McCleery & Nuechterlein, 2019). Additionally, there is evidence of attenuated cognitive deficits appearing in *genetically high-risk* (GHR) groups (such as individuals with a first degree relative of an individual with SCZ) (McCleery & Nuechterlein, 2019). A meta-analysis by Snitz, MacDonald, and Carter (2006), showed that first-degree relatives of an individual with SCZ show an attenuated deficit in attention, processing speed, working memory, reasoning, and problem solving. This evidence suggests individuals with a genetic susceptibility can also experience cognitive deficits that are prominent and difficult to treat.

McCleery & Nuechterlein (2019) argue that cognitive impairment should be forefront to a SCZ diagnosis. This study works to examine the relationship between cognitive deficits in SCZ

and observed blood lipids, particularly plasmalogens (McCleery & Nuechterlein, 2019). *Cognition*, for the purposes of this research study, will be defined as a measurement of cognitive performance in relation to memory, attention, executive function, problem-solving, processing speed, and the ability to understand social cues and emotions.

Cognitive Testing and Using the MCCB™

With cognitive deficits being a core feature of SCZ, the National Institute of Mental Health (NIMH) put forth an initiative to bring standardization, reliability, and validity to cognitive testing in SCZ (Nuechterlein & Green, 2016). This NIMH-based initiative resulted in development of the MCCB™ (Nuechterlein & Green, 2016).

Description and Features of the MATRICS™ Consensus Cognitive Battery (MCCB™)

The MCCB™ consists of 10 tests and measures cognitive performance within 7 different cognitive domains (Nuechterlein & Green, 2016). It is a standardized battery that is administered and scored by individuals with proper training, and it is supervised by a psychologist or psychiatrist (Nuechterlein & Green, 2016). The 10 tests within the MCCB™ include: 1) Brief Assessment of Cognition in Schizophrenia (BACS): Symbol Coding, 2) Category Fluency: Animal Naming (Fluency), 3) Trail Making Test (TMT): Part A, 4) Continuous Performance Test– Identical Pairs (CPT-IP), 5) Wechsler Memory Scale®–Third Edition (WMS®-III): Spatial Span, 6) Letter-Number Span (LNS), 7) Hopkins Verbal Learning Test™– Revised (HVLT-R™), 8) Brief Visuospatial Memory Test– Revised (BVMT-R™), 9) Neuropsychological Assessment Battery® (NAB®): Mazes, and 10) Mayer-Salovey-Caruso

Emotional Intelligence Test (MSCEIT™): Managing Emotions (Nuechterlein & Green, 2016). The 7 cognitive domains include: speed of processing, attention/vigilance, working memory (both verbal and non-verbal), verbal learning, visual learning, reasoning and problem solving, and social cognition (Nuechterlein & Green, 2016). For a detailed list of tests and domains covered in each test, including dependent variables for each test section, see Table 3 in Appendix D.

Each cognitive domain can be compared to normative data in T-scores and percentiles found in the MCCB™ Manual (Nuechterlein & Green, 2016). Features of the MCCB™ include high test-retest reliability, high utility as a repeated measure, demonstrated relationship to functional outcome, highly rated tolerability by respondents, and convenient administration and scoring (Nuechterlein & Green, 2016).

CHAPTER 2: AIMS, HYPOTHESES, AND RATIONALE

Aims

The aims of this study could not be obtained, due to change of laboratories, illnesses, and COVID-19 restrictions (also see Chapter 1, *Goals of the Research Project*, for a brief introduction to the goals for this study). Roadblocks to continuation of this study can be found in Chapter 5, *Discussion*. Aim 1 was to obtain lipid assays using Liquid Chromatography-Mass Spectrometry (LC-MS), from both individuals with schizophrenia (SCZ) and healthy controls. Aim 2 was to be able to detect lipid species within the obtained lipid assays and obtain a semi-quantification of these detected lipids in both individuals with SCZ and healthy controls. Aim 3 was to measure cognitive performance using the MCCB™ in both individuals with SCZ and healthy controls. Aim 4 was to then use multivariate statistical analyses to compare differences between both the cognitive performance, as determined by the MCCB™, and the detected lipid species and semi-quantifications that were obtained through the lipid assays, both in individuals with SCZ and healthy controls.

Hypothesis

Regarding Aim 1 and 2, we hypothesized that a blood lipid assay would be able to be obtained through using LC-MS, and from this assay, we would be able to determine the lipid species and semi-quantifications present, for both individuals with SCZ and healthy controls. We further hypothesized that individuals with SCZ would have differing semi-quantifications of

specific lipid species, when compared with healthy controls. For plasmalogens, we hypothesized that semi-quantifications for PC Pls and PE Pls would be lower than healthy controls. Regarding Aim 3, we hypothesized that measures of cognitive performance could be obtained using MCCB™, and this test would be able to be obtained from both individuals with SCZ and healthy controls. We further hypothesized that the MCCB™ results will show average T-scores for both completion of individual MCCB™ sections and overall cognitive domains, for both individuals with SCZ and healthy controls; however, we also hypothesized that individuals with SCZ would have lower average T-scores in comparison to healthy controls. Regarding Aim 4, we hypothesized that we can then use multivariate statistical analyses to compare differences between both the measure of cognitive performance, as determined by the MCCB™, and the lipid species and semi-quantifications present that were obtained through the lipid assays, both in individuals with SCZ and healthy controls. We further hypothesized that alterations in blood lipid species and semi-quantifications for individuals with SCZ, specifically regarding plasmalogens, will be associated with increased cognitive difficulties in SCZ, when in comparison to healthy controls.

Rationale

There is a lack of research in relation to both plasmalogens and cognitive deficits in SCZ; therefore, it would be beneficial to observe if there are relationships between these using multivariate statistical analyses, as lower levels of plasmalogens (including alterations of other significant lipids) have been previously reported (Kaddurah-Daouk et al., 2012; Tessier et al., 2016; Wood, 2019). Researchers suspect that low plasmalogen levels could be a potential biomarker for diagnosis of SCZ; therefore, plasmalogens are considered a viable candidate

(Kaddurah-Daouk et al., 2012; Tessier et al., 2016). Currently, there are no reliable or validated biomarkers for SCZ; however, researchers are exploring PUFAs, which include plasmalogens, as potential biomarkers for this disorder (Frajerman et al., 2021).

Historically, FAs have been considered pivotal indicators for disease states in humans (Zhou et al., 2020). Phospholipids play a major role in the maintenance of neuronal cell membranes within the CNS and Peripheral Nervous System (PNS), including maintenance of the myelin sheath (Zhou et al., 2020). With the multiple roles that lipids play throughout the body, there can be significant implications of a change in lipid quantifications for the observed pathology of SCZ (Farooqui, Farooqui, & Horrocks, 2008; Zhou et al., 2020). Increased PLA_2 activity can result in disruption of neuronal cell membrane fluidity, which in turn disrupts functioning for all types of membrane-dependent proteins, including Na^+-K^+-ATPase, adrenergic receptors, and the uptake of norepinephrine and serotonin; therefore, changes to plasmalogens is predicted to precede the onset of clinical symptoms for SCZ (Farooqui, Farooqui, & Horrocks, 2008). It has also been reported that there are higher levels of PLA_2 activity in individuals with SCZ at all stages of the disorder and both with and without antipsychotic use (Kaddurah-Daouk et al., 2012; Zhou et al., 2020). Higher PLA_2 activity can lead to further breakdown of the plasmalogens needed to maintain neuronal cell membranes within the body (Farooqui, Farooqui, & Horrocks, 2008; Zhou et al., 2020). Zhou et al. (2020) also report that higher PLA_2 activity in individuals with SCZ is positively correlated with positive symptoms, an increase in illness duration, and number of episodes that an individual with SCZ experiences. Additionally, higher PLA_2 activity results in altered levels of AA and DHA within individuals in SCZ (Farooqui, Farooqui, & Horrocks, 2008; Zhou et al., 2020). It is known that supplementation with cod fish oil, which is a source of beneficial omega-3 FAs, can aid in incorporating additional DHA, an

anti-inflammatory lipid, into cell membranes within the body (Zhou et al., 2020). Considering that there is evidence for disturbances in lipid pathways in SCZ, particularly with plasmalogens, this study aimed to investigate the relationship between both cognitive deficits and levels of plasmalogens in patients with SCZ. We hypothesized that individuals with SCZ would have lower average T-scores in comparison to healthy controls, and this is due to current research describing cognitive impairment in SCZ (Nuechterlein & Green, 2016; McCleery & Nuechterlein, 2019). There is no current research comparing cognitive performance and observed blood lipids in SCZ.

CHAPTER 3: MATERIALS AND METHODS

Experimental Design

Overall Study Information

This study was an experimental study, and planned to involve two cohorts of 25 participants, with one cohort consisting of individuals with schizophrenia (SCZ), and the other cohort consisting of healthy control participants. The independent variable for this study was the cognitive test results using the MCCB™. The dependent variable was the amounts of plasmalogens found within the blood samples.

Inclusion and Exclusion Criteria

Inclusion criteria for all participants were to include being age 18 to 50, and ability to consent to study procedures (including taking a blood sample). For healthy matched controls, an additional inclusion criterion is completion of the Mini International Neuropsychiatric Interview™ (M.I.N.I.™). For SCZ participants, they must also have a confirmed diagnosis of SCZ for a minimum of 1 year. Exclusion criteria for all participants include alcohol abuse or dependence, illicit drug abuse or dependence, evidence of another neuropsychiatric disorder or evidence of a secondary neurodegenerative disease, evidence of a condition that affects blood coagulants, pregnancy, body mass index (BMI) over 35, any other medical condition that results in being unable to provide a blood sample, and any other unknown reasons that result in being unable to take a blood sample. In control participants, a personal family history or immediate

family history of a neuropsychiatric disorder or neuropsychiatric disease were exclusion criteria. For inclusion and exclusion criteria that would be used for this study, see Table 1 below, *Inclusion and Exclusion Criteria*.

Table 1. *Inclusion and Exclusion Criteria*

Schizophrenia Participants		Control Participants	
Inclusion Criteria	**Exclusion Criteria**	**Inclusion Criteria**	**Exclusion Criteria**
BMI less than 35	Alcohol or illicit drug abuse and/or dependence	BMI less than 35	Alcohol or illicit drug abuse and/or dependence
Age 18-50	Evidence of a secondary neuropsychiatric and/or neurodegenerative disease	Age 18-50	Evidence of and/or immediate family history of a secondary neuropsychiatric and/or neurodegenerative disease
Confirmed diagnosis of SCZ	Condition and/or use of medication that affects blood coagulants or lipids (ex. Statins)	Completion of M.I.N.I.™	Condition and/or use of medication that affects blood coagulants or lipids (ex. Statins)
Minimum duration of illness of 1 year starting from first symptoms present	Pregnancy		Pregnancy

Participants

Number of Participants

In accordance with other research studies in the area of plasmalogens, the sample amount was set at attempting to recruit a minimum of 50 participants: 25 with a confirmed diagnosis of SCZ, and 25 control participants. In total, it was expected to recruit approximately 56

participants, after accounting for a 10% dropout rate in each group of participants, to the nearest whole number.

Obtaining M.I.N.I.™ Data

The M.I.N.I.™ assesses participants for psychiatric disorders, and this test has a high validity while also available in a wide variety of languages. Healthy matched controls were to be determined using the M.I.N.I.™ English Version 7.0.0, which assesses DSM-5™ criteria. This brief test was to act as a formal platform for ensuring inclusion and exclusion criteria for control participants within the study.

Obtaining MCCB™ Assessment Data

All participants were to take the MCCB™, in which T-scores in the following cognitive domains were to be obtained: speed of processing, attention/vigilance, working memory (both verbal and non-verbal), verbal learning, visual learning, reasoning and problem solving, and social cognition. In preparation for this study, Jessica Bist obtained MCCB™ training through NeuroCog Trials, and became certified as a rater on June 28th, 2016.

Preparing Participants for a Blood Sample

Blood samples were not to be fasted. In the past, fasted blood samples were thought to reduce variability in lipid assays after food intake; however, studies have shown that blood lipids

in fasted versus non-fasted states are not significantly different (Lai & James, 2014). There is an exception to this rule for lipid assays, but this only includes lipid assays that observe the levels of cholesterol and triglycerides in the blood (Lai & James, 2014). The exception is that there may be increased variability in a sample depending on if the participant was in upright or supine position; therefore, to mitigate this risk, the procedure for blood drawing was to be standardized, and all samples were taken in the supine position (Lai & James, 2014).

For serum samples to be properly processed, 2.5ml of whole blood were to be collected into a purple top ethylenediaminetetraacetic acid (EDTA) Vacutainer© tube by a professional phlebotomist at the Heritage Medical Research Clinic (HMRC). With consent, an extra 2.5ml of whole blood was to be collected and remain de-identified for the United States-based study team led by Dr. Paul Wood at Lincoln Memorial University (LMU), in Harrogate, Tennessee (TN), United States of America (USA). Processed plasma and red blood cell (RBC) samples were to be shipped to this study team at LMU to help create the lipid assays and aid in the statistical analysis for this study.

The blood was to then be placed immediately on ice until processed. Within 30min, the samples were to then be centrifuged at 3000g for 15min at room temperature. Upper supernatant was to be transferred to a clean 1.5ml microfuge tube without disturbing the buffy coat layer (BCL). The BCL was then to be aspirated and then subsequently discarded. 1ml of RBCs were then transferred to a clean 1.5ml microfuge tube. Samples were to then be stored at -80°C for up to 2 months or until ready to ship. For the full extraction method, see Appendix J, *Lipid Extraction Method for Blood Serum Sample*.

The analysis of de-identified and processed blood samples was to be used to monitor alterations in membrane lipids in the same participants, in accordance with their local ethics board.

Data Analysis Using LC-MS

Our project was to use an Accurate Mass Quadruple Time-of-Flight LC-MS (6230 Q-ToF) with Mass Hunter (Agilent) software. To extract lipids, we used a monophasic lipid extraction method with derivatization (See Appendix J).

Monophasic lipid extraction began with diluting the serum sample with a mixture of methanol/chloroform/water (2:1:0.74). This allowed for separation of polar and nonpolar lipids in the sample. Additives of $^{13}C_1$-*S*,*S*′-dimethylthiobutanoylhydroxysuccinimide ester ($^{13}C_1$-DMBNHS), iodine (I_2), and methanol (CH_3OH) then allows for derivatization into aminophospholipids and plasmalogens. After applying standards, these samples were normalized and quantified using the LC-MS with Agilent software. Candidate identifications of lipids were to be identified using Metlin (a tandem mass spectrometer [MS/MS] metabolomics) database.

About LC-MS

LC-MS is a technique that allows for separation of physical entities from a sample, such as the various phospholipids found within a blood sample (Yan et al., 2018). For this study, free FAs within the blood plasma, which includes plasmalogens were quantified using LC-MS, in

accordance with the procedures outlined by Ryan & Reid (2016). For the monophasic lipid extraction method used for this study, see Appendix J.

LC-MS Parameters

The parameters for the LC-MS were adapted from both Anand et al. (2016) and Ryan & Reid (2016). A processed blood sample was injected into a 6230 ToF LC-MS (Agilent Technologies) through an ESI mode source operated in positive ion mode. A syringe injected samples at the flow rate of 2 microlitres per minute (2 µl/min). The microspray needle had a diameter of 50 micrometers (50µm). Capillary voltage was set to 3500 volts (3500V). Mass spectrometry (MS) data was collected from 100-3000 mass-to-charge ratio (100-3000m/z) with an acquisition rate of 1 spectrum/sec. Dry and nebulizer gas was optimized for the most stable flow, with dry gas being set to 5L/min at 325°C and nebulizer gas being set to a pressure of 1.03bar.

Specific Lipid Identification Using LC-MS

Specific lipid identification for the control blood sample is unobtainable for this study due to missing raw data files. In future studies, raw LC-MS data from control and SCZ blood samples would be processed using a chosen software such as *MassLynx 4.2* or *MS-DIAL*, similarly to previous studies (Knittelfelder et al., 2014; Yan et al., 2018). Once processed, MS spectra can be compared against a chosen lipid database, such as Metlin (a general, online database) or MSDIAL-LipidDBs-VS23-FiehnO (if using MS-DIAL software) (Yan et al., 2018).

Ethics

Recruitment and Consent

This study was approved through the University of Calgary Conjoint Health Research Ethics Board (CHREB). Each participant was to be required to review and sign a consent form. Participants were made aware that their participation was voluntary, and withdrawal from the study could be requested at any time. Confidentiality of documentation were to be ensured by keeping all documentation on an encrypted universal serial bus (USB) and in a locked drawer, and in a locked room at Foothills Medical Centre (FMC). Reimbursement was to be given for transportation, parking, and meals.

Participants with a well-established and confirmed diagnosis of SCZ were to be recruited through case managers from Unit 24 at FMC and similar programs in Calgary, Alberta (AB), Canada. Case managers from Unit 24 FMC will inform patients about the study, and case managers were to ask the possible participant for their permission for the primary researcher to contact them over the phone. Other programs that participants were attempted to be recruited from included Carnat Centre, South Health Campus, Sheldon Chumir Mental Health Clinic, Northwest (NW) Mental Health Clinic, The Schizophrenia Society of Alberta (SSA), and other private offices. We were also attempting to recruit through poster and social media advertisements. Attempts to recruit were stalled due to multiple change in laboratories, illnesses, and complications due to COVID-19 restrictions.

Each participant was to undergo a brief phone screening, and those who met study criteria would then be invited in-person to sign a consent form. With consent, SCZ group

participants would need to provide their Alberta Health Number to confirm a primary diagnosis of SCZ within the last year. Control participants would not need to provide their Alberta Health Number; however, control participants would need to complete the M.I.N.I.™

Additionally, participants were to continue their personalized treatment program as directed by their psychiatrist. No participant would need to start or stop taking antipsychotics or any other medication as a prerequisite for this study.

Risks to Participants

Participants were fully informed of all risks associated with the study. This study required access to medical records for participants within the SCZ group, and this was to be used for obtaining a confirmed diagnosis of SCZ, duration of illness, current treatments, and current medications. Control group participants would not need to give access to their medical records. To assess participants for psychiatric disorders and ensure that these participants were not currently experiencing a mental health diagnosis, the M.I.N.I.™ was implemented for all control group participants. With consent, the following data was also to be obtained from all participants: age, sex, weight (kg), height (cm), education (in years), and use of supplements. Immediately after a participant would take the MCCB™, identifying information would be coded in an Excel file and stored safely in a locked cabinet on a USB in the Teaching, Research, and Wellness (TRW) building. From each participant, a blood plasma sample would then be obtained and stored at -80°C at the Heritage Medical Research Building (HMRB), and from this, we were then to ship these samples to LMU, in order to generate a lipid assay using LC-MS for each participant.

In terms of the blood draw, this study also carried a small amount of risk for possible discomfort, bruising, swelling, and/or redness. All blood draws also carry the possibility of infection occurring afterwards. To mitigate risks, the blood sample was to be taken by a professional phlebotomist at the HMRC within the TRW building at FMC. This would have helped to ensure that there is less risk for discomfort, bruising, swelling and/or redness. The principal investigator (P.I.) was to be available if complications were to occur from the blood draw.

Possible complications from the MCCB™, include that the participant may become fatigued, distressed, angry, and/or frustrated. Frequent breaks were permitted to mitigate risk of fatigue. If a participant was to require immediate medical assistance, the P.I. was to be available.

Statistical Analysis

For estimating sample size in future studies, it would be beneficial to complete a power calculation, to accurately determine the best sample population size. From the power calculation, we would then be able to determine if there is clinical significance through finding a p-value. Two-way Analysis of Variance (ANOVA) with Tukey-Kramer post-hoc analysis was to be used to further analyze data sets and compare the differences between plasmalogens and other pertinent lipids from both individuals with SCZ and control participants.

CHAPTER 4: RESULTS

Demographic Information for Control Participants

This study was able to collect MCCB™ data from a total of 2 control participants. Both participants were Caucasian and female. Other information collected for this study included age (years), sex (male or female), height (cm), weight (kg), education (years), and use of supplements. For a detailed description of demographic information collected for this study, see Table 2 below.

Table 2. *Demographic Data for Control Participants*

	001	002	Average
Age (years)	23	22	22.5
Sex (M or F)	F	F	N/A
Height (cm)	167	170	168.5
Weight (kg)	56.88	80.74	68.81
Education (years)	14	15	14.5
Handedness	Right	Right	N/A
Ethnicity	Caucasian	Caucasian	N/A
Supplements-Vitamins	C, B_{12}	None	N/A
Supplements- Other	Iron, Magnesium	None	N/A
Time to Complete MCCB™ (min)	75	80	77.5
Time to Complete M.I.N.I.™ (min)	22	19	20.5

M.I.N.I.™ Data Collected

Results from the M.I.N.I.™ indicated that the control participants within this study had no evidence of a mental health diagnosis.

Average T-Scores Per MCCB™ Test for Control Participants

For average T-Scores per MCCB™ cognitive test, see Table 4 in Appendix E. For a graph of average T-Scores per MCCB™ cognitive test, see Figure 6 in Appendix G. In terms of individual scores, the Trail Making Test (TMT), Hopkins Verbal Learning Test- Revised (HVLT-R), and Continuous Performance Test (CPT-IP) scores for participant 001 and the CPT-IP score for participant 002 were below average when compared against age- and gender- corrected normative scores for the MCCB™. All other test scores were average when compared against age- and gender-corrected normative scores for the MCCB™. Participant 001 had lower individual test scores in TMT, HVLT-R, and CPT-IP and participant 002 had a lower test score in CPT-IP; however, the overall test scores result in an average overall composite score.

Expected MCCB™ Test Results for Additional Controls and Individuals with Schizophrenia

For additional controls, we would expect to see individual test scores that compare to the age- and gender- corrected normative scores for the MCCB™. For individuals with SCZ, we would expect to see lower individual test scores when compared to controls.

Average T-Scores Per MCCB™ Cognitive Domain for Control Participants

For average T-Scores per MCCB™ cognitive domain, see Table 5 in Appendix F. For a graph of average T-Scores per MCCB™ cognitive domain, see Figure 7 in Appendix H. Overall composite scores are average against age- and gender- corrected normative scores for the MCCB™.

Expected MCCB™ Cognitive Domain Results for Additional Controls and Individuals with Schizophrenia

For additional controls, we would expect to see similar overall composite scores when compared with age- and gender- corrected normative scores for the MCCB™. For individuals with SCZ, we would expect to see a lower overall composite score, and lower scores in all 7 cognitive domains when compared with controls.

Extraction of Blood Lipids Using Q-ToF LC-MS (6500 Series)

Blood lipids were successfully extracted from one participant using an Agilent 6230 LC/Q-ToF using the lipid extraction method from Appendix J. See Figures 3, 4, and 5 (within Appendix A, B, and C respectfully) for chromatogram and LC-MS data. See Figure 9 in Appendix L for copyright permissions.

Expected LC-MS Results for Controls and Individuals with Schizophrenia

After identifying lipid entities, we would expect to see lower levels of PE Pls and PC Pls in individuals with SCZ when compared to healthy controls.

CHAPTER 5: DISCUSSION

Our study demonstrated that we were able to extract entities from blood serum using the first lipid extraction method that was created by Dr. Brechenmacher and Dr. Khan (See Appendix J for the lipid extraction method and see Figure 9 in Appendix L for copyright permissions). Our study also demonstrated that M.I.N.I.™ and MCCB™ data could be obtained from all control participants. This study was to initially begin through a collaboration with a private company in Saskatoon, Saskatchewan (SK) called *Phenomenome*; however, the company underwent receivership while the legal department of the University of Calgary was negotiating an intellectual property agreement, and the project was unable to continue through collaborating with this company. Soon after, the principal and primary investigators located Dr. Schriemer's laboratory at the University of Calgary (U of C), that could help process lipid samples, and the study was re-established. At this point in the study, Dr. Khan and Dr. Brechenmacher helped with development of a methodology for lipid analysis, and we were able to analyze one sample from a control individual, in order to test if plasma lipids were able to be found using the developed extraction method (See Appendix J for *Lipid Extraction Method for Blood Serum Sample* and Figure 9 in Appendix L for copyright permissions). After obtaining additional ethics approval, we were notified by this laboratory that they were no longer able to support this study due to a change in directors and a novel focus of this laboratory on protein analysis rather than lipid analysis. Since there was an inability to continue at the U of C laboratory, research was to continue at U of C in coordination with Dr. Paul Wood's laboratory at Lincoln Memorial University (LMU) by using a different plasma extraction method created by Dr. Paul Wood (See Appendix I for lipid extraction method [*Plasma Sample Analysis*] and Figure 10 in Appendix M for copyright permissions). Samples were to be shipped on a monthly basis on dry ice from U of

C to LMU. Our rationale for the initial set-up and continuing this study is described in Chapter 2: *Aims, Hypothesis, and Rationale*. More information regarding challenges to completion of the study is discussed further in this chapter, in the section titled *Roadblocks*.

In terms of LC-MS results, the chromatogram in Figure 3 (Appendix A) shows the total abundance of all negatively charged ions in the blank control (i.e., the solvent) as it was infused into the LC-MS over a period of 2 minutes, without using an LC-MS column to separate compounds. This figure shows that entities were identified. Figure 4 (Appendix B) shows the mass spectrum taken at 0.711min, and this figure highlights the abundance of background ions, as measured as m/z on the x-axis, that were present in the blank solvent; therefore, this further indicates that the contribution to the sample from solvents that were used is minimal. The spectrum from the blood of a control participant is shown in Figure 5 (Appendix C). This figure indicates that there are a variety of different ions present in the blood plasma sample. For example, the ions 367.2464 and 393.2624 represent individual compounds found in blood plasma, which may or may not be indicative of blood lipids. Due to lack of retention of raw data, identities of individual compounds were unable to be fully identified. For example, retention time (RT) for a compound to be passed through the column is another parameter that is required for proper assignment of compound identity; however, this information was not available in the data retained. In future studies, it is recommended that all experimental raw data be stored securely (with consent and permission), as with the laboratory changes, it was unknown that this information would be beneficial to retrieval of compound identities and for simplifying the search within a chosen lipid database (e.g., *Metlin*).

It is expected that individuals with SCZ will have reduced levels of plasmalogens, more specifically PC Pls and PE Pls when compared with controls, due to evidence of higher PLA_2

activity in individuals with SCZ (Farooqui, Farooqui, & Horrocks, 2008; Zhou et al., 2020). In addition, alongside variations in gene expression, lipids within the blood are often dysregulated in the pathogenesis of SCZ (Schneider et al., 2017). Completing a full assay of all blood lipids can aid in identifying differences in other lipids, aside from plasmalogens. Some other candidate lipids, in which changes may be observed, include SM, 2-AG, and/or AEA. For example, a recent study by Wood (2019), showed that there were increased *N*-acyl-phosphatidylserines (NAPS), *N*-acyl-phosphatidylethanolamines (NAPEs), and sulfatides in individuals with SCZ, which are known neuronal stress markers. Increased sulfatide levels are also known to interfere with neuronal myelin sheaths, which could also contribute to cognitive symptoms in SCZ (Wood, 2019).

There are currently no known studies that show a definitive link between free phospholipids within blood plasma to the levels of phospholipids found within neuronal membranes; however, plasma free FAs have historically been used to represent the levels of phospholipids in membrane fatty acids in SCZ, and researchers describe that there is some evidence to support this link (Solberg et al., 2015; Wood et al., 2015; Zhou et al., 2020). For example, it is known that supplementation with ω-3 FAs (i.e., PUFAs) causes AA and DHA to be incorporated into the total phospholipids within the plasma, platelets, and membranes throughout the body; however, more research is needed in this area (Solberg et al., 2015; Wood et al., 2015; Zhou et al., 2020).

It is important to note as well, that since the release of the original phospholipid hypothesis published by Horrobin, Glen, & Vaddadi (1994), some modifications have been made to this hypothesis, and these changes may influence future studies and/or area of study focus. For example, Eggers (2012) had extended the hypothesis to include that overactivity of PLA_2 may be

due to a stress response of 5-HT_{2A} and 5-HT_{2C} receptors. It is thought that 5-HT_{2C} receptors may play a role as a future drug target, as these receptors can influence DA levels (Pogorelov et al., 2017).

It is known that cultural and socioeconomic factors, including physical and mental states (e.g., fatigue, hunger, or motivation) can interfere with MCCB™ scores. Results for the control participants in this study show that the T-Scores for each test and T-Scores per cognitive domain that were obtained for all participants are considered average when compared against age- and gender-corrected normative scores for the MCCB™.

Roadblocks, Feasibility, & Limitations

There were multiple roadblocks to completing the initial study. The original thesis project began in September 2015 and was set to work with through the company, *Phenomenome*, located in Saskatoon, SK, Canada. This bio-tech company focused on the analysis of lipids in biological samples and was to help with lipid analysis of blood samples. The agreement to help with lipid analysis eventually fell through due to the company entering into receivership. Additionally, it was difficult to sign an agreement with a private company outside of the U of C due to intellectual property issues. Due to both of these issues, the decision was made to continue the project with a laboratory at the U of C. A local research lab at the U of C was able to analyze our samples; however, a method needed to be created for sample analysis using LC-MS. This was further developed with the help of Dr. Khan and Dr. Brechenmacher and tested using a control blood sample; however, approximately a year and a half into the project, the lab was unable to continue with the project due to both a change in management and a new focus on protein

analysis. Through multiple leads, a new lab was found that would be able to complete the lipid analysis at Lincoln Memorial University (LMU) in Harrogate, TN, USA. Blood samples were to be prepared in Calgary for analysis and then shipped from Calgary, AB, Canada to Harrogate, TN. Ethics requirements were increased throughout the time working with this lab, due to the nature of working with another university in a different country. During the duration of this study, several members of the supervisory committee had developed illnesses and new health challenges, which had also affected the timeline of this project. Additionally, during the progression of this project, Dr. Glenda MacQueen had passed away on March 27th, 2020. She was a valued member of this project's Supervisory Committee, and her mentorship and guidance will be greatly missed.

Overall recruitment for the study was low due to multiple factors: a) change in laboratories and change in ethics requirements for each laboratory; b) stricter inclusion and exclusion criteria, and c) recruitment for most individuals to be sought through use of mental health case managers. Each change in laboratory brought along a change in ethics requirements that affected recruitment of possible participants. For example, with the change from the bio-tech laboratory from Saskatoon, SK, to the laboratory at the U of C, time was needed to find this laboratory, develop a methodology for analysis, and then re-submit ethical requirements before recruitment could be started. Two control participants were recruited in 2019 through the use of a website called *Kijiji*, which was approved through Institutional Review Board at U of C. Individuals with SCZ did not contact the researcher with interest about the study during the study period. Even though this study had received ethics approval, we also encountered difficulties involving other agencies and/or associations within the community to help with recruitment. Concerns included additional clinical and ethical guidelines as well as additional guidelines surrounding personal health information and

maintenance of confidentiality. In early 2020 we were starting to attempt recruitment of individuals with SCZ through case managers at Alberta Health Services (AHS). The COVID-19 pandemic resulted in a sudden cessation of all research activities at the U of C, and the study was unable to continue.

Due to roadblocks identified earlier in this discussion (i.e., change of laboratories, lack of recruitment, illnesses, the passing of a Supervisory Committee member, and the disruption of research at U of C due to the COVID-19 pandemic), this project was unable to continue. We have provided preliminary data on the feasibility of carrying out a similar project in the near future. This study is feasible once COVID-19 restrictions lift, and the study can be carried out with persistence in collaboration and communication to case managers throughout mental health services, agencies, and/or associations. To our knowledge, there are no current studies linking plasmalogens in individuals with SCZ to cognitive deficits; therefore, by carrying out a similar study in the future, individuals with SCZ can benefit with increased knowledge in this area, and researchers can determine if more investigations are warranted. It would also be a benefit to understand if lipids can affect cognitive performance results during the MCCB™.

There were some limitations to this study. Our study was able to determine that there were entities within the serum sample; however, we are unable to determine if these entities were in fact lipids, due to a lack of raw data. In future studies, raw data would be obtained and stored, and these lipids would then be able to be identified within a database such as *Metlin*. Based on the small sample size, there is an increased margin of error and higher variability in the data set. A larger sample size and an increase to the number of participants within the experimental group will overcome these limitations.

Lessons Learned and Recommendations for Future Studies

For future study recruitment, it will be beneficial to develop working relationships with case managers through AHS or other agencies. Future researchers may also consider developing working relationships with private agencies and/or associations that directly support individuals with SCZ, as this may aid in study recruitment. It is recommended that future researchers contact directors and/or agency case managers prior to the recruitment phase of the study, in order to a) present the importance of research in this area; b) be aware of additional guidelines and program specific requirements that may interfere with recruitment; c) obtain support from the agency and d) provide assurance that an individual's personal health information will be protected. Future studies can increase number of posts to Kijiji and/or develop a system for when to refresh a post, as potential participants will only be able to see an advertisement if it falls under specific categories. Verbal feedback from presentations of this research (prior to the COVID-19 pandemic) suggested that difficulties with recruitment may also have been due to the design of the recruitment poster and/or strictness of inclusion and exclusion criteria. Based on this feedback, poster design was altered in early 2019. The newly designed poster aided in gathering more interest in the study from potential participants; however, many individuals did not meet the strict inclusion and exclusion requirements to participate either as a subject with schizophrenia or as a control subject. For example, this study required control individuals to have no recent marijuana or alcohol use in addition to having no personal history of mental illness. These inclusion and exclusion criteria created a hindrance for potential participants signing up. Future studies can focus on reduction of criteria that may cause hinderance in participation in this study. For example, future studies may consider increasing the required BMI for the study to include more individuals. Overall, future

research in this area will require focus on recruitment of SCZ participants, and with this, researchers will then be able to complete comparisons of plasmalogen levels between individuals with SCZ and healthy controls.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions and Future Directions

There were multiple roadblocks that affected completion of the initial study (i.e., multiple changes in laboratories, strict inclusion and exclusion criteria, physical illness of supervisors, and the onset of coronavirus disease (COVID-19) restrictions), and these were significant challenges in recruitment of both participants with SCZ and healthy controls. Despite these factors, this study provided current background information within the field of SCZ research, and this project creates an opening for future researchers to continue this study in a larger sample population. This project is considered feasible with an ease of COVID-19 restrictions and continued collaboration and communication (for recruitment purposes) between case managers within AHS, external agencies, and/or associations that support individuals with SCZ. Cognitive deficits continue to be a significant challenge for individuals with SCZ, and with plasmalogens being a critical component of brain and neuronal tissue that is altered in individuals with SCZ, further investigation on this topic is justified (American Psychiatric Association, 2013; McCleery & Nuechterlein, 2019; Schneider et al., 2017).

CHAPTER 7: REFERENCES

Almeida, V. de, Alexandrino, G. L., Aquino, A., Gomes, A. F., Murgu, M., Dobrowolny, H., … Martins-de-Souza, D. (2020). Changes in the blood plasma lipidome associated with effective or poor response to atypical antipsychotic treatments in schizophrenia patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 101, 109945. Retrieved from https://doi.org/10.1016/j.pnpbp.2020.109945

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC: Author.

An, H.-M., Tan, Y.-L., Tan, S.-P., Shi, J., Wang, Z.-R., Yang, F.-D., … Zhang, X.-Y. (2016). Smoking and Serum Lipid Profiles in Schizophrenia. *Neuroscience Bulletin*, 32(4), 383–8. Retrieved from https://doi.org/10.1007/s12264-016-0022-0

Anand, S., Young, Sydney, A., Esplin, M. S., Peaden, B., Tolley, H. D., Porter, T. F., … Graves, S. W. (2016). Detection and confirmation of serum lipid biomarkers for preeclampsia using direct infusion mass spectrometry. Journal of Lipid Research, 57(4), 687–696. Retrieved from https://doi.org/10.1194/jlr.p064451

Berger, G. E., Smesny, S., & Amminger, G. P. (2006). Bioactive lipids in schizophrenia. *International Review of Psychiatry*, 18(2), 85–98. Retrieved from https://doi.org/10.1080/09540260600583072

Bois, T. M. du, Deng, C., & Huang, X.-F. (2005). Membrane phospholipid composition, alterations in neurotransmitter systems and schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29(6), 878–888. Retrieved from https://doi.org/10.1016/j.pnpbp.2005.04.034

Braverman, N. E., & Moser, A. B. (2012). Functions of plasmalogen lipids in health and disease. *Biochimica et Biophysica Acta*, 1822(9), 1442–52. Retrieved from https://doi.org/10.1016/j.bbadis.2012.05.008

Brisch, R., Saniotis, A., Wolf, R., Bielau, H., Bernstein, H.-G., Steiner, J., … Gos, T. (2014). The Role of Dopamine in Schizophrenia from a Neurobiological and Evolutionary Perspective: Old

Fashioned, but Still in Vogue. *Frontiers in Psychiatry*, 5, 47. Retrieved from https://doi.org/10.3389/fpsyt.2014.00047

Brites, P., Waterham, H. R., & Wanders, R. J. A. (2004). Functions and biosynthesis of plasmalogens in health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1636(2–3), 219–231. Retrieved from https://doi.org/10.1016/j.bbalip.2003.12.010

Chan, M. K., Krebs, M.-O., Cox, D., Guest, P. C., Yolken, R. H., Rahmoune, H., … Bahn, S. (2015). Development of a blood-based molecular biomarker test for identification of schizophrenia before disease onset. *Translational Psychiatry*, 5(7), e601. Retrieved from https://doi.org/10.1038/tp.2015.91

Chang, C.-Y., Chen, Y.-W., Wang, T.-W., & Lai, W.-S. (2016). Akting up in the GABA hypothesis of schizophrenia: Akt1 deficiency modulates GABAergic functions and hippocampus-dependent functions. Scientific Reports, 6(1), 33095. Retrieved from https://doi.org/10.1038/srep33095

Chestnykh, D. A., Amato, D., Kornhuber, J., & Müller, C. P. (2021). Pharmacotherapy of schizophrenia: Mechanisms of antipsychotic accumulation, therapeutic action and failure. *Behavioural Brain Research*, 403, 113144. Retrieved from https://doi.org/10.1016/j.bbr.2021.113144

Chou, I.-J., Kuo, C.-F., Huang, Y.-S., Grainge, M. J., Valdes, A. M., See, L.-C., … Doherty, M. (2016). Familial Aggregation and Heritability of Schizophrenia and Co-aggregation of Psychiatric Illnesses in Affected Families. *Schizophrenia Bulletin*, 43(5), sbw159. Retrieved from https://doi.org/10.1093/schbul/sbw159

Eggers, A. E. (2012). Extending David Horrobin's membrane phospholipid theory of schizophrenia: overactivity of cytosolic phospholipase A(2) in the brain is caused by overdrive of coupled serotonergic 5HT(2A/2C) receptors in response to stress. *Medical Hypotheses*, 79(6), 740–3. Retrieved from https://doi.org/10.1016/j.mehy.2012.08.016

Evans, D. R., Parikh, V. V., Khan, M. M., Coussons, C., Buckley, P. F., & Mahadik, S. P. (2003). Red blood cell membrane essential fatty acid metabolism in early psychotic patients following antipsychotic drug treatment. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 69(6), 393–399. Retrieved from https://doi.org/10.1016/j.plefa.2003.08.010

Fakhoury, M. (2017). Role of the Endocannabinoid System in the Pathophysiology of Schizophrenia. *Molecular Neurobiology*, 54(1), 768–778. Retrieved from https://doi.org/10.1007/s12035-016-9697-5

Farooqui, A. A., Farooqui, T., & Horrocks, L. A. (2008). Metabolism and Functions of Bioactive Ether Lipids in the Brain, 107–127. Retrieved from https://doi.org/10.1007/978-0-387-77401-5_6

Frajerman, A., Chaumette, B., Lamazière, A., Tessier, C., Nuss, P., Krebs, M. O., & Kebir, O. (2021). P.115 Membrane lipids in early phases of psychosis: potential biomarkers for a personalised medicine? *European Neuropsychopharmacology*, 44, S11–S12. Retrieved from https://doi.org/10.1016/j.euroneuro.2021.01.022

Gerlach, J., Koret, B., Gereš, N., Matić, K., Prskalo-Čule, D., Vrbanc, T. Z., … Filipčić, I. (2019). Clinical Challenges in Patients with First Episode Psychosis and Cannabis Use: Mini-Review and a Case Study. *Psychiatria Danubina*, 31(Suppl 2), 162–170.

Ghosh, S., Dyer, R. A., & Beasley, C. L. (2017). Evidence for altered cell membrane lipid composition in postmortem prefrontal white matter in bipolar disorder and schizophrenia. *Journal of Psychiatric Research*, 95, 135–142. Retrieved from https://doi.org/10.1016/j.jpsychires.2017.08.009

Glen, A. I. M., Glen, E. M. T., Horrobin, D. F., Vaddadi, K. S., Spellman, M., Morse-Fisher, N., … Skinner, F. S. (1994). A red cell membrane abnormality in a subgroup of schizophrenic patients: evidence for two diseases. *Schizophrenia Research*, 12(1), 53–61. Retrieved from https://doi.org/10.1016/0920-9964(94)90084-1

Goldman, M. L., Pincus, H. A., & Mangurian, C. (2020). Schizophrenia. *The New England Journal of Medicine*, 382(6), 583–584. Retrieved from https://doi.org/10.1056/nejmc1915943

Hamazaki, K, Maekawa, M., Toyota, T., Dean, B., Hamazaki, T., & Yoshikawa, T. (2016). Fatty acid composition of the postmortem corpus callosum of patients with schizophrenia, bipolar disorder, or major depressive disorder. *European Psychiatry : The Journal of the Association of European Psychiatrists*, 39, 51–56. Retrieved from https://doi.org/10.1016/j.eurpsy.2016.05.007

Hamazaki, Kei, Choi, K. H., & Kim, H.-Y. (2010). Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species.

Journal of Psychiatric Research, 44(11), 688–93. Retrieved from https://doi.org/10.1016/j.jpsychires.2009.11.017

Hartmann, T., Kuchenbecker, J., & Grimm, M. O. W. (2007). Alzheimer's disease: the lipid connection. *Journal of Neurochemistry*, 103(s1), 159–170. Retrieved from https://doi.org/10.1111/j.1471-4159.2007.04715.x

Horrobin, D. F. (1998). The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. Schizophrenia Research, 30(3), 193–208. Retrieved from https://doi.org/10.1016/s0920-9964(97)00151-5

Horrobin, D. F., Glen, A. I. M., & Vaddadi, K. (1994). The membrane hypothesis of schizophrenia. Schizophrenia Research, 13(3), 195–207. Retrieved from https://doi.org/10.1016/0920-9964(94)90043-4

Horrobin, D. F., Manku, M. S., Hillman, H., Iain, A., & Glen, M. (1991). Fatty acid levels in the brains of schizophrenics and normal controls. Biological Psychiatry, 30(8), 795–805. Retrieved from https://doi.org/10.1016/0006-3223(91)90235-e

Hsu, M.-C., Huang, Y.-S., & Ouyang, W.-C. (2020). Beneficial effects of omega-3 fatty acid supplementation in schizophrenia: possible mechanisms. *Lipids in Health and Disease*, 19(1), 159. Retrieved from https://doi.org/10.1186/s12944-020-01337-0

Huang, J. H., Park, H., Iaconelli, J., Berkovitch, S. S., Watmuff, B., McPhie, D., … Karmacharya, R. (2016). Unbiased Metabolite Profiling of Schizophrenia Fibroblasts under Stressful Perturbations Reveals Dysregulation of Plasmalogens and Phosphatidylcholines. *Journal of Proteome Research*, 16(2), 481–493. Retrieved from https://doi.org/10.1021/acs.jproteome.6b00628

Igarashi, M., Ma, K., Gao, F., Kim, H.-W., Rapoport, S. I., & Rao, J. S. (2011). Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer's disease prefrontal cortex. *Journal of Alzheimer's Disease : JAD*, 24(3), 507–17. Retrieved from https://doi.org/10.3233/jad-2011-101608

Jauhar, S., Laws, K. R., & McKenna, P. J. (2019). CBT for schizophrenia: a critical viewpoint. *Psychological Medicine*, 49(08), 1233–1236. Retrieved from https://doi.org/10.1017/s0033291718004166

Kaddurah-Daouk, R., McEvoy, J., Baillie, R., Zhu, H., Yao, J. K., Nimgaonkar, V. L., … Nasrallah, H. A. (2012). Impaired plasmalogens in patients with schizophrenia. *Psychiatry Research*, 198(3), 347–352. Retrieved from https://doi.org/10.1016/j.psychres.2012.02.019

Kane, J. M., Agid, O., Baldwin, M. L., Howes, O., Lindenmayer, J.-P., Marder, S., … Correll, C. U. (2019). Clinical Guidance on the Identification and Management of Treatment-Resistant Schizophrenia. *The Journal of Clinical Psychiatry*, 80(2). Retrieved from https://doi.org/10.4088/jcp.18com12123

Katona, I., & Freund, T. F. (2012). Multiple Functions of Endocannabinoid Signaling in the Brain. *Annual Review of Neuroscience*, 35(1), 529–558. Retrieved from https://doi.org/10.1146/annurev-neuro-062111-150420

Keefe, R. S. E., & Kahn, R. S. (2017). Cognitive Decline and Disrupted Cognitive Trajectory in Schizophrenia. *JAMA Psychiatry*, 74(5), 535. Retrieved from https://doi.org/10.1001/jamapsychiatry.2017.0312
Laboratory Guide to the Methods in Biochemical Genetics. (2008). Retrieved from https://doi.org/10.1007/978-3-540-76698-8

Kern, R. S., Gold, J. M., Dickinson, D., Green, M. F., Nuechterlein, K. H., Baade, L. E., … Marder, S. R. (2011). The MCCB impairment profile for schizophrenia outpatients: Results from the MATRICS psychometric and standardization study. *Schizophrenia Research*, 126(1–3), 124–131. Retrieved from https://doi.org/10.1016/j.schres.2010.11.008

Knittelfelder, O. L., Weberhofer, B. P., Eichmann, T. O., Kohlwein, S. D., & Rechberger, G. N. (2014). A versatile ultra-high performance LC-MS method for lipid profiling. *Journal of Chromatography B*, 951, 119–128. Retrieved from https://doi.org/10.1016/j.jchromb.2014.01.011

Lai, R., & James, W. (2014, March 26). Lipids - Fasting versus Non-Fasting. Retrieved November 19, 2020, from https://www.albertahealthservices.ca/assets/wf/lab/wf-lab-bulletin-lipids-fasting-vs-non-fasting-collection.pdf

Leppik, L., Parksepp, M., Janno, S., Koido, K., Haring, L., Vasa, E., & Zilmer, M. (2019). Profiling of Lipidomics Before and After Antipsychotic Treatment in First-Episode Psychosis. *SSRN Electronic Journal*. Retrieved from https://doi.org/10.2139/ssrn.3220097

Levine, S. Z., Rabinowitz, J., Uher, R., & Kapur, S. (2015). Biomarkers of treatment outcome in schizophrenia: Defining a benchmark for clinical significance. *European Neuropsychopharmacology*, 25(10), 1578–1585. Retrieved from https://doi.org/10.1016/j.euroneuro.2015.06.008

Madras, B. K. (2013). History of the Discovery of the Antipsychotic Dopamine D2 Receptor: A Basis for the Dopamine Hypothesis of Schizophrenia. *Journal of the History of the Neurosciences*, 22(1), 62–78. Retrieved from https://doi.org/10.1080/0964704x.2012.678199

Marder, S. R., & Cannon, T. D. (2019). Schizophrenia. The New England Journal of Medicine, 381(18), 1753–1761. Retrieved from https://doi.org/10.1056/nejmra1808803

Marzo, V. D., & Petrocellis, L. D. (2012). Why do cannabinoid receptors have more than one endogenous ligand? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1607), 3216–3228. Retrieved from https://doi.org/10.1098/rstb.2011.0382

Mattern, R. (2009). Plasmalogen [Image]. Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Plasmalogen.png

McCleery, A., & Nuechterlein, K. H. (2019). Cognitive impairment in psychotic illness: prevalence, profile of impairment, developmental course, and treatment considerations . *Dialogues in Clinical Neuroscience*, 21(3), 239–248. Retrieved from https://doi.org/10.31887/dcns.2019.21.3/amccleery

McCutcheon, R. A., Marques, T. R., & Howes, O. D. (2020). Schizophrenia—An Overview. *JAMA Psychiatry*, 77(2), 201–210. Retrieved from https://doi.org/10.1001/jamapsychiatry.2019.3360

McCutcheon, R. A., Abi-Dargham, A., & Howes, O. D. (2019). Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms. *Trends in Neurosciences*, 42(3), 205–220. Retrieved from https://doi.org/10.1016/j.tins.2018.12.004

McEvoy, J., Baillie, R. A., Zhu, H., Buckley, P., Keshavan, M. S., Nasrallah, H. A., … Kaddurah-Daouk, R. (2013). Lipidomics Reveals Early Metabolic Changes in Subjects with Schizophrenia:

Effects of Atypical Antipsychotics. *PLoS ONE*, 8(7), e68717. Retrieved from https://doi.org/10.1371/journal.pone.0068717

Messias, M. C. F., Mecatti, G. C., Priolli, D. G., & Carvalho, P. de O. (2018). Plasmalogen lipids: functional mechanism and their involvement in gastrointestinal cancer. Lipids in Health and Disease, 17(1), 41. Retrieved from https://doi.org/10.1186/s12944-018-0685-9

Minichino, A., Senior, M., Brondino, N., Zhang, S. H., Godwlewska, B. R., Burnet, P. W. J., … Lennox, B. R. (2019). Measuring Disturbance of the Endocannabinoid System in Psychosis. *JAMA Psychiatry*, 76(9), 914–923. Retrieved from https://doi.org/10.1001/jamapsychiatry.2019.0970

Moghaddam, B., & Javitt, D. (2012). From Revolution to Evolution: The Glutamate Hypothesis of Schizophrenia and its Implication for Treatment. Neuropsychopharmacology, 37(1), 4–15. Retrieved from https://doi.org/10.1038/npp.2011.181

Navarrete, M., Díez, A., & Araque, A. (2014). Astrocytes in endocannabinoid signalling. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1654), 20130599. Retrieved from https://doi.org/10.1098/rstb.2013.0599

Nestler, E. J., Hyman, S. E., Holtzman, D. M., & Malenka, R. C. (2015). *Molecular neuropharmacology: A foundation for clinical neuroscience*. New York: Mc Graw Hill education.

Nuechterlein, K. H., & Green, M. F. (2016). MATRICS Consensus Cognitive Battery: Manual, Third Edition. Los Angeles, California: MATRICS Assessment, Inc.

Peet, M., Laugharne, J. D. E., Horrobin, D. F., & Reynolds, G. P. (1994). Arachidonic Acid: A Common Link in the Biology of Schizophrenia? *Archives of General Psychiatry*, 51(8), 665–666. Retrieved from https://doi.org/10.1001/archpsyc.1994.03950080077012

Petrikis, P., Voulgari, P., Boumba, V., Archimandriti, D., Skapinakis, P., & Mavreas, V. (2017). Changes in the cytokine profile in first episode, drug-naïve patients with psychosis after short-term antipsychotic treatment. European Psychiatry, 41(S1), S371. Retrieved from https://doi.org/10.1016/j.eurpsy.2017.02.382

Pogorelov, V. M., Rodriguiz, R. M., Cheng, J., Huang, M., Schmerberg, C. M., Meltzer, H. Y., … Wetsel, W. C. (2017). 5-HT2C Agonists Modulate Schizophrenia-Like Behaviors in Mice.

Neuropsychopharmacology, 42(11), 2163–2177. Retrieved from https://doi.org/10.1038/npp.2017.52

Prata, D., Mechelli, A., & Kapur, S. (2014). Clinically meaningful biomarkers for psychosis: A systematic and quantitative review. *Neuroscience & Biobehavioral Reviews*, 45, 134–141. Retrieved from https://doi.org/10.1016/j.neubiorev.2014.05.010

Raedler, T. J., Bymaster, F. P., Tandon, R., Copolov, D., & Dean, B. (2007). Towards a muscarinic hypothesis of schizophrenia. *Molecular Psychiatry*, 12(3), 232–246. Retrieved from https://doi.org/10.1038/sj.mp.4001924

Ryan, E., & Reid, G. E. (2016). Chemical Derivatization and Ultrahigh Resolution and Accurate Mass Spectrometry Strategies for "Shotgun" Lipidome Analysis. *Accounts of Chemical Research*, 49(9), 1596–1604. Retrieved from https://doi.org/10.1021/acs.accounts.6b00030

Schneider, M., Levant, B., Reichel, M., Gulbins, E., Kornhuber, J., & Müller, C. P. (2017). Lipids in psychiatric disorders and preventive medicine. Neuroscience & Biobehavioral Reviews, 76, 336–362. Retrieved from https://doi.org/10.1016/j.neubiorev.2016.06.002

Snitz, B. E., MacDonald, A. W., & Carter, C. S. (2006). Cognitive Deficits in Unaffected First-Degree Relatives of Schizophrenia Patients: A Meta-analytic Review of Putative Endophenotypes. Schizophrenia Bulletin, 32(1), 179–194. Retrieved from https://doi.org/10.1093/schbul/sbi048

Solberg, D K, Bentsen, H., Refsum, H., & Andreassen, O. A. (2015). Association between serum lipids and membrane fatty acids and clinical characteristics in patients with schizophrenia. *Acta Psychiatrica Scandinavica*, 132(4), 293–300. Retrieved from https://doi.org/10.1111/acps.12388

Solberg, Dag K, Bentsen, H., Refsum, H., & Andreassen, O. A. (2016). Lipid profiles in schizophrenia associated with clinical traits: a five-year follow-up study. *BMC Psychiatry*, 16(1), 299. Retrieved from https://doi.org/10.1186/s12888-016-1006-3

Tang, Y., & Niznikiewicz, M. A. (2020). Neuroimaging in Schizophrenia, 241–300. Retrieved from https://doi.org/10.1007/978-3-030-35206-6_14

Tessier, C., Sweers, K., Frajerman, A., Bergaoui, H., Ferreri, F., Delva, C., … Nuss, P. (2016). Membrane lipidomics in schizophrenia patients: a correlational study with clinical and cognitive

manifestations. Translational Psychiatry, 6(10), e906–e906. Retrieved from https://doi.org/10.1038/tp.2016.142

Tracey, T. J., Steyn, F. J., Wolvetang, E. J., & Ngo, S. T. (2018). Neuronal Lipid Metabolism: Multiple Pathways Driving Functional Outcomes in Health and Disease. *Frontiers in Molecular Neuroscience*, 11, 10. Retrieved from https://doi.org/10.3389/fnmol.2018.00010

Tripathi, A., Kar, S. K., & Shukla, R. (2018). Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies. *Clinical Psychopharmacology and Neuroscience*, 16(1), 7–17. Retrieved from https://doi.org/10.9758/cpn.2018.16.1.7

Vita, A., Barlati, S., Ceraso, A., Nibbio, G., Ariu, C., Deste, G., & Wykes, T. (2021). Effectiveness, Core Elements, and Moderators of Response of Cognitive Remediation for Schizophrenia. JAMA Psychiatry, 78(8). Retrieved from https://doi.org/10.1001/jamapsychiatry.2021.0620

Volk, D. W., & Lewis, D. A. (2019). Insights Into the Pathophysiology of Endocannabinoid Signaling in Schizophrenia. JAMA Psychiatry, 76(9), 887–888. Retrieved from https://doi.org/10.1001/jamapsychiatry.2019.0844

Wallner, S., & Schmitz, G. (2011). Plasmalogens the neglected regulatory and scavenging lipid species. *Chemistry and Physics of Lipids*, 164(6), 573–89. Retrieved from https://doi.org/10.1016/j.chemphyslip.2011.06.008

Wood, P. L. (2019). Targeted lipidomics and metabolomics evaluations of cortical neuronal stress in schizophrenia. Schizophrenia Research, 212, 107–112. Retrieved from https://doi.org/10.1016/j.schres.2019.08.003

Wood, P. L., Unfried, G., Whitehead, W., Phillipps, A., & Wood, J. A. (2015). Dysfunctional plasmalogen dynamics in the plasma and platelets of patients with schizophrenia. Schizophrenia Research, 161(2–3), 506–510. Retrieved from https://doi.org/10.1016/j.schres.2014.11.032

Wu, X., Huang, Z., Wu, R., Zhong, Z., Wei, Q., Wang, H., … Zhang, J. (2013). The comparison of glycometabolism parameters and lipid profiles between drug-naïve, first-episode schizophrenia patients and healthy controls. Schizophrenia Research, 150(1), 157–162. Retrieved from https://doi.org/10.1016/j.schres.2013.07.051

Yan, L., Zhou, J., Wang, D., Si, D., Liu, Y., Zhong, L., & Yin, Y. (2018). Unbiased lipidomic profiling reveals metabolomic changes during the onset and antipsychotics treatment of schizophrenia disease. *Metabolomics: Official Journal of the Metabolomic Society*, 14(6), 80. Retrieved from https://doi.org/10.1007/s11306-018-1375-3

Yang, A. C., & Tsai, S.-J. (2017). New Targets for Schizophrenia Treatment beyond the Dopamine Hypothesis. *International Journal of Molecular Sciences*, 18(8), 1689. Retrieved from https://doi.org/10.3390/ijms18081689

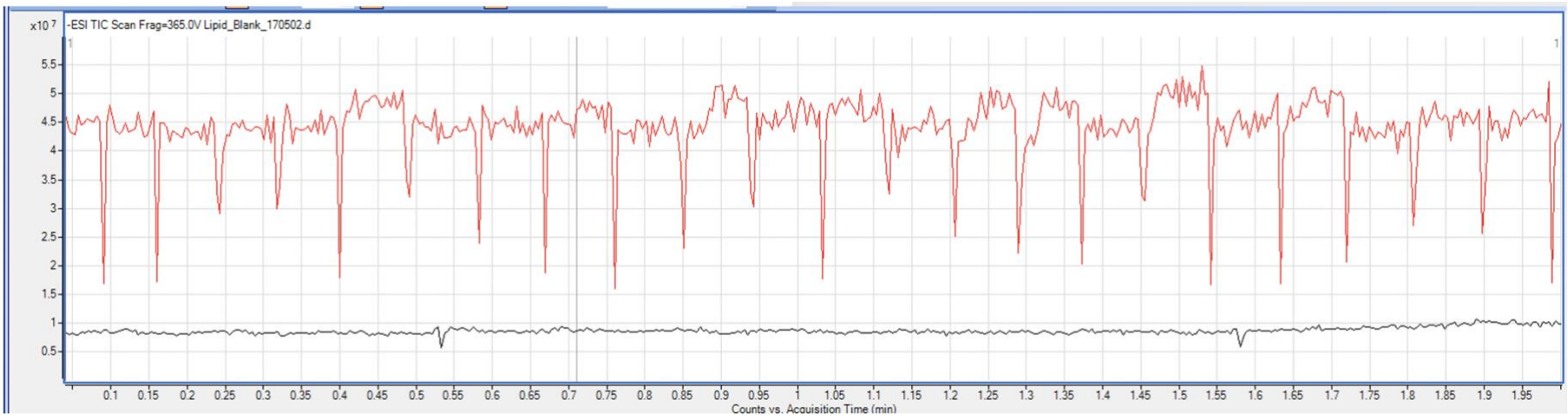
Zhou, X., Long, T., Haas, G. L., Cai, H., & Yao, J. K. (2020). Reduced Levels and Disrupted Biosynthesis Pathways of Plasma Free Fatty Acids in First-Episode Antipsychotic-Naïve Schizophrenia Patients. *Frontiers in Neuroscience*, 14, 784. Retrieved from https://doi.org/10.3389/fnins.2020.00784

Zou, S., & Kumar, U. (2018). Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *International Journal of Molecular Sciences*, 19(3), 833. Retrieved from https://doi.org/10.3390/ijms19030833

CHAPTER 8: APPENDICES

Appendix A

Figure 3. *Chromatogram of Blood Lipid Sample Using Electrospray Ionization (ESI) in a Control Participant*



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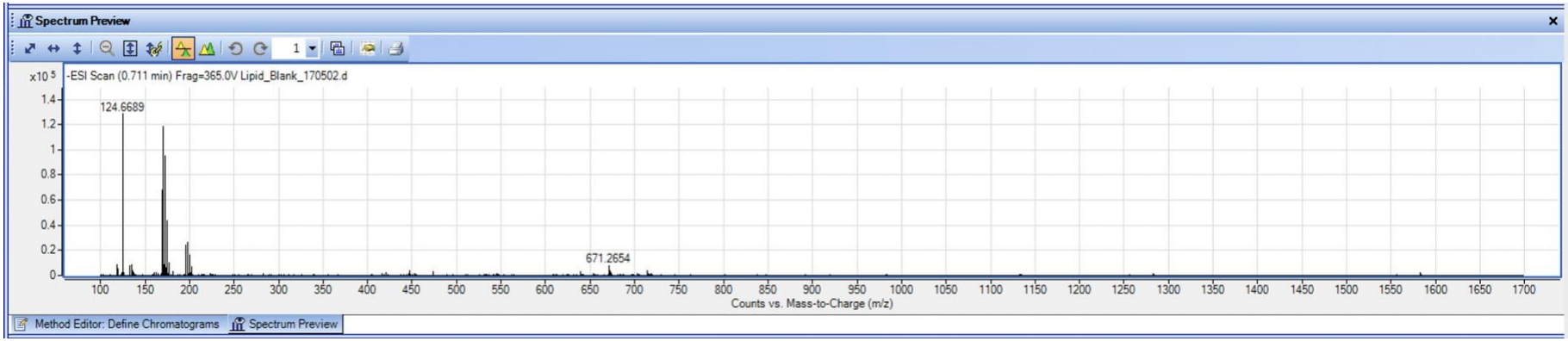
Appendix B

Figure 4. *Mass Spectrum of a Sample Containing 2:1:0.74 Ratio of Methanol, Chloroform, and Water at 0.711min*



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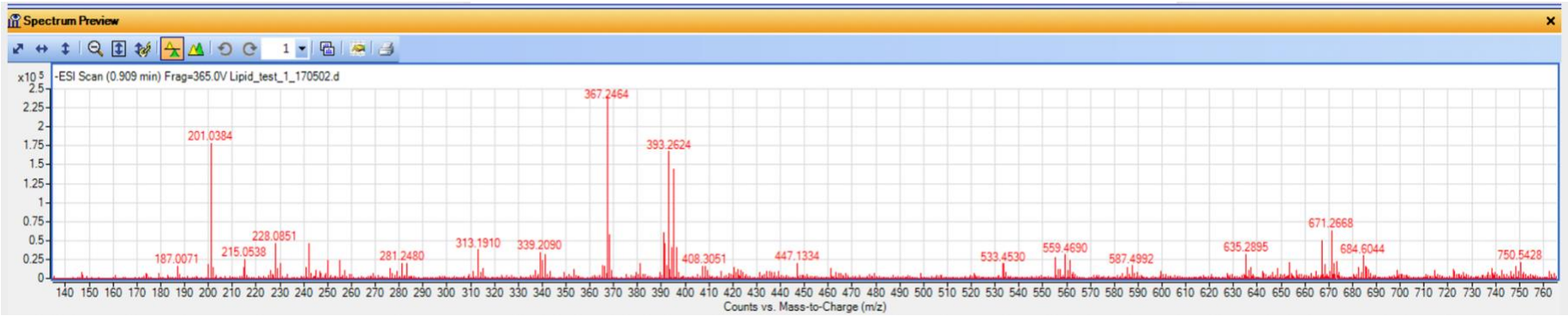
Appendix C

Figure 5. *Mass Spectrum of Blood Lipid Sample from a Control Participant*



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Appendix D

Table 3. *Descriptions of Each Section of the MATRICS™ Consensus Cognitive Battery (MCCB™) and Subsequent Dependent Variables*

Cognitive Domains	**MCCB Test**	**Dependent Variables**
Speed of Processing	*Trail Making Test* (TMT): Part A	Correctly connect circled numbers in ascending order within 300 seconds.
	Brief Assessment of Cognition in Schizophrenia (BACS): Symbol Coding	Number of correctly matched symbols to numbers within 90 seconds.
	Category Fluency: Animal Naming (Fluency)	Number of animals correctly named within 60 seconds.
Attention/Vigilance	*Continuous Performance Test—Identical Pairs* (CPT-IP)	Average d-prime (using false alarms) across 3 trials of 2-, 3-, and 4-digits number recognitions.
Working Memory	*Letter Number Span (LNS)*	Number of correctly ordered randomized letter-number chains.
	Wechsler Memory Scale®—Third Edition (WMS®-III): Spatial Span	Number of correct sequences tapped by participant after viewing the administrator complete the sequence, in both forward and backward conditions.
Verbal Learning	*Hopkins Verbal Learning Test—Revised*™ (HVLT-R™)	Average number of words correctly identified from a 12-item list over 3 trials.
Visual Learning	*Brief Visuospatial Memory Test—Revised*™ (BVMT-R™)	Average score for image reproduction over 3 trials.

Reasoning and Problem Solving	*Neuropsychological Assessment Battery*® (NAB®): Mazes	Raw score given depending on time to complete mazes.
Social Cognition	*Mayer-Salovey-Caruso Emotional Intelligence Test* (MSCEIT™): Managing Emotions	Responses are scored against a consensus for self-regulation of emotions.

Note. Adapted from 'MATRICS™ Consensus Cognitive Battery,' by Nuechterlein & Green (2016), and 'The MCCB™ Impairment Profile for Schizophrenia Outpatients: Results from the MATRICS™ Psychometric and Standardization Study,' by Kern et al. (2011). Copyright Nuechterlein & Green (2016). Copyright Kern et al. (2011). Reprinted with permission. See Figure 11 in Appendix N for copyright permissions.

Appendix E

Table 4. *Individual T-Scores Per Section of MATRICS™ Consensus Cognitive Battery (MCCB™) For Each Participant, Including Average T-Score Per Section*

Test	**001**	**002**	**Average**
TMT	43	51	47
BACS SC	57	49	53
HVLT-R	44	51	47.5
WMS-III SS	53	59	56
LNS	58	58	58
NAB Mazes	62	39	50.5
BVMT-R	56	45	50.5
Fluency	53	44	48.5
MSCEIT ME	51	56	53.5
CPT-IP	41	41	41

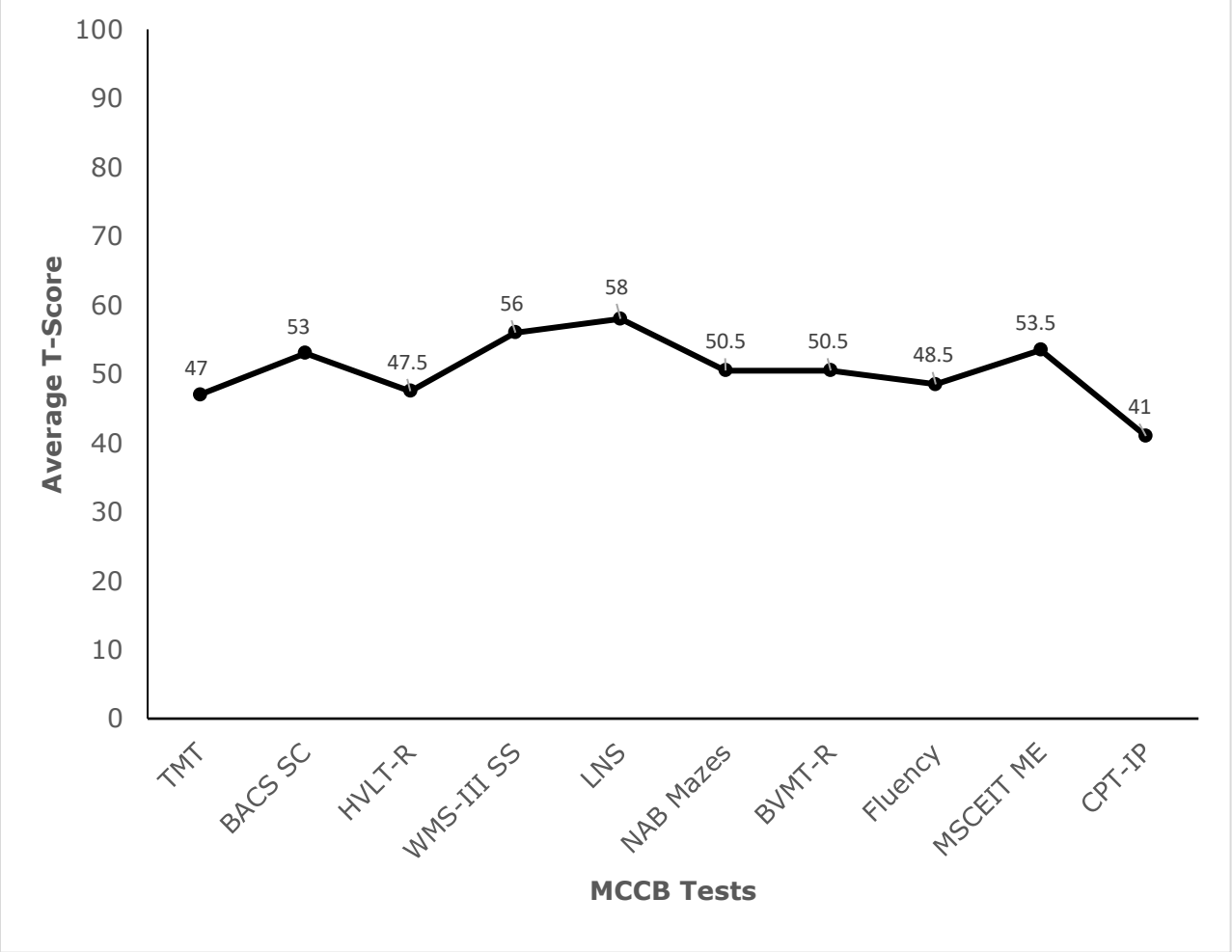
Appendix F

Table 5. *Individual T-Scores Per Cognitive Domain of MATRICS™ Consensus Cognitive Battery (MCCB™) For Each Participant, Including Average T-Score Per Section*

Cognitive Domain	**001**	**002**	**Average**
Speed of Processing	51	47	49
Attention/Vigilance	41	41	41
Working Memory	57	61	59
Verbal Learning	44	51	47.5
Visual Learning	56	45	50.5
Reasoning and Problem Solving	62	39	50.5
Social Cognition	51	56	53.5
Overall Composite	53	48	50.5

Appendix G

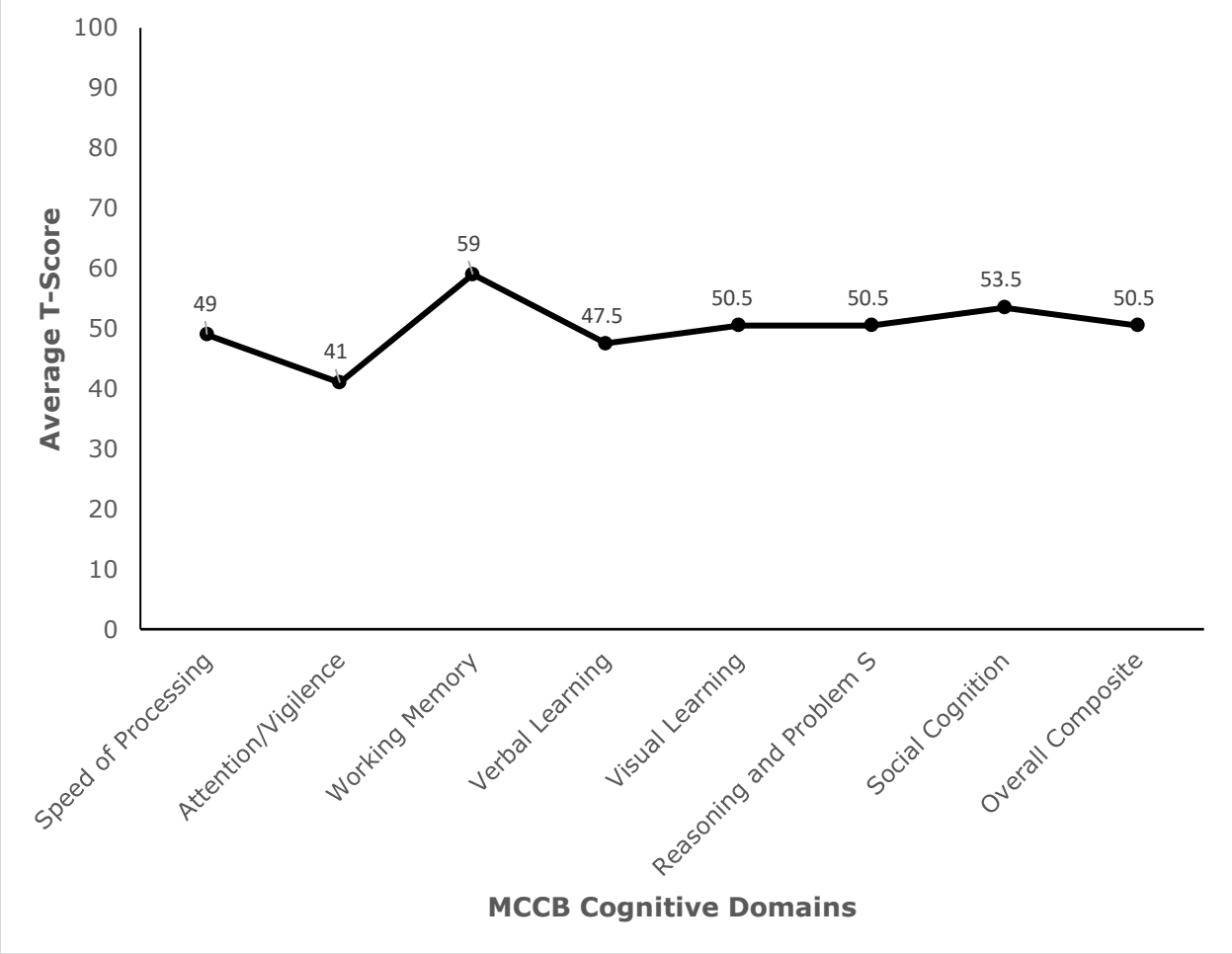
Figure 6. *Average T-Scores Per MATRICS™ Consensus Cognitive Battery (MCCB™) Test for Control Participants*



Note. Average MATRICS™ Consensus Cognitive Battery (MCCB™) T-scores per test for control participants (age- and gender- corrected).

Appendix H

Figure 7. *Average T-Scores Per MATRICS™ Consensus Cognitive Battery (MCCB™) Cognitive Domain for Control Participants*



Note. Average MATRICS™ Consensus Cognitive Battery (MCCB™) T-scores per cognitive domain for control participants (age- and gender- corrected).

Appendix I

Plasma Sample Analysis

The following method was to be used to analyze plasma samples. Created and reprinted with permission from Dr. Paul Wood. See Figure 10 in Appendix M for permissions.

Plasma or RBC samples will be processed utilizing tert-butyl methylether and CH_3OH for extraction of lipids. The extraction solution will contain [$^2H_8$]arachidonic acid, [$^2H_3$]phytanic acid, [$^2H_4$]hexacosanoic acid, [$^{13}C_{16}$]palmitic acid, [$^2H_7$]cholesterol sulfate, [$^2H_5$]MAG 18:1, [$^{13}C_3$]DAG 36:2, [$^2H_{31}$]PtdEtn 34:1, [$^2H_{54}$]PtdEtn 28:0, [$^2H_{31}$]PtdCh 34:1, [$^2H_{54}$]PtdCh 28:0, [$^2H_{62}$]PtdCh 32:0, [$^2H_{31}$]SM 16:0, [$^2H_{31}$]PtdSer 36:1, and [$^2H_{31}$]PA 34:1 as internal standards. Lipid extracts will be dried by centrifugal vacuum evaporation prior to dissolution in IPA:CH_3OH:$CHCl_3$ (4:2:1) containing 7.5mM ammonium acetate ($NH_4CH_3CO_2$). Shotgun lipidomics will be performed utilizing high-resolution (140,000 at 200 atomic mass units (amu) data acquisition, with sub-ppm mass accuracy on an orbitrap mass spectrometer (Thermo Q Exactive) with successive switching between polarity modes. Washes between samples with hexane/ethyl acetate (3:2) are used to minimize ghost effects. This lipidomics analytical platform assays approximately 3000 potential lipids across 56 lipid classes.

In positive ion ESI, the cations of choline plasmalogens (PlsC), phosphatidylcholines (PtdCh), lysophosphatidylcholines (LPC), formyl-LPC, lysophosphoethanolamies (LPE), acrolein-LPE adducts, ether-LPE (LPEe), acrolein-LPEe adducts, ether-lysophosphocholines (LPCe), sphingomyelins, hydroperoxy-PtdCh, hydroperoxy-PlsC, monoacylglycerols (MAG), acylcarnitines, ceramides, galactosyl-ceramides, lactosyl-ceramides, N-acyl-amino acids, N-acyl-phosphatidylethanolamines (NAPEs), and N-acylphosphatidyserines (NAPS) and the

ammonium adducts of diacylglycerols (DAG), monogalactosyl-DAGs, digalactosyl-DAGs, cholesterol esters, hydroperoxy-cholesterol esters, triacylglycerols, and alkenyl-acylglycerols will be quantitated and lipid identities validated by MS/MS.

In negative ion ESI, the anions of ethanolamine plasmalogens (PlsE), phosphatidylglycerols (PG), phosphatidylethanolamines (PtdEtn), phosphatidic acids (PA), cyclic phosphatidic acids (cPA), phosphatidylinositols (PIn), phosphatidylserines (PS), sterol sulfates, lysophosphatidylethanolamines (LPE), lysophosphatidylinositols (LPI), lysophosphatidic acids (LPA), ether-LPA (LPAe), dicarboxylic acids (DCA), hydroxyl-DCA, dihydroxy-DCA, lysophosphatidylglycerols (LPG), ceramides, sulfatides, NAPEs, formyl-LPE, bis(monoacyl)glycerol phosphates (BMP), hydroperoxy-PS, N-acyl-amino acids, NAPS, acrolein-PlsE adducts, acrolein-PtdEtn adducts, acrolein-PS adducts, ceramide-PtdEtn, and FAs will be quantitated and lipid identities validated by MS/MS.

Appendix J

Lipid Extraction Method for Blood Serum Sample

The following method was to be used to extract lipids using liquid chromatography-mass spectrometry (LC-MS). Created and reprinted with permission from Dr. Morgan Khan and Dr. Laurent Brechenmacher. See Figure 9 in Appendix L for copyright permissions.

Monophasic Lipid Extraction:

Serum samples are to be diluted to a volume of 250µl of 40% methanol. To this, methanol (1.25ml), chloroform (0.675ml), and water (0.35ml) were added to create a monophasic extraction mixture with a final ratio of 2:1:0.74 (1.35ml:0.675ml:0.5ml) methanol/chloroform/water, including water and methanol present in the initial samples.

1. Dilute serum to a final volume of 250µl at 40% methanol.
2. Vortex samples for 1min, and then incubated at room temperature for 30min with shaking.
3. Extracts were then centrifuged at 2,000g for 30min at room temperature to precipitate proteins, and the supernatants were collected.
4. Protein pellets were then resuspended in water (0.25ml) by vortexing, and reextracted with chloroform/methanol (1:2, v:v, 1.0ml) by incubation and centrifugation as described above.
5. The supernatants from the repetitive extractions were collected and pooled (5.05ml), and the pellets were discarded.

6. The monophasic lipid extracts containing both polar and nonpolar lipids were immediately dried under nitrogen and resuspended in 250µl isopropanol/methanol/chloroform (4:2:1, v:v:v) (at a concentration of 20 mg/ml retina tissue extracted).
7. Reconstituted lipid extracts were centrifuged at 2,000 g to remove any residual particulates, then transferred to 2.0ml glass vials and stored at -80°C until further use.

Functional Group Derivatization:

Prior to MS analysis in positive ionization mode, half of each retina lipid extract from each of the above extraction methods was subjected to sequential functional group selective modification of i) amine-containing PE and PS lipids using $^{13}C_1$-DMBNHS; and ii) the O -alkenyl ether double bond of plasmalogen lipids using iodine and methanol.

1. Derivatization of aminophospholipids with $^{13}C_1$-DMBNHS:

a) 5µL of the stock lipid extract was dried under a stream of nitrogen, then redissolved in 40µL of 39:1.1 $CHCl_3$/0.00625M TEA and vortexed for 30sec.
b) 1µL of 0.00625M $^{13}C_1$-DMBNHS in dimethylformamide (DMF) was then added to the lipid mixture and vortexed for 30min.
c) The reactions were quenched by drying under a stream of nitrogen (N_2) and were subjected to I_2/CH_3OH derivatization of vinyl ether bonds prior to MS analysis.

2. Derivatization of plasmenyl vinyl ether bonds with I_2 and CH_3OH:

a) For cell extract reactions, 5µL of the stock SW480 lipid was dried under a stream of N_2, then redissolved in 60µL ice cold 2:1 $CHCl_3$/CH_3OH with 2mM ammonium bicarbonate

and 1.33mM I_2 (estimated to be a 10-fold molar excess with respect to the total plasmenyl lipid concentration).

b) The solutions were reacted in an ice bath for 5min and dried under a stream of N_2.

c) The resulting products were then re-dissolved in 200μL 4:2:1 IPA/CH_3OH/$CHCl_3$ containing 20mM ammonium formate for immediate analysis by MS.

MS Analysis:

Forty microlitres (40μL) of reconstituted, derivatized (for positive ionization mode analysis), or nonderivatized (for negative ionization mode analysis) lipid samples were then transferred into individual wells of a Whatman Multi-Chem 96-well plate (Sigma Aldrich) and dried under N_2. Immediately before analysis, lipid extracts were resuspended in IPA:CH_3OH:$CHCl_3$ (4:2:1, v:v:v) containing 20mM ammonium formate.

Appendix K

Figure 8. *Copyright Permissions for Adapted Figure (Figure 2)*

From: Wanders, R.J.A. [redacted]
Subject: Re: Permission to Use Adapted Figure in Thesis- Jessica Bist
Date: April 16, 2021 at 1:20 AM
To: Jessica Bist [redacted]

[△EXTERNAL]

Dear Jessica,
You have my full permission to use the adapted figure!
Best regards.
Prof.dr. Ronald JA Wanders
PS If possible, I would love to receive an electronic copy if your Thesis just for my own interest and curiosity for your work.

Verstuurd vanaf mijn iPad

Op 15 apr. 2021 om 16:18 heeft Jessica Bist [redacted] het volgende geschreven:

Hi Dr. Wanders,

I am a Master's student at the University of Calgary, studying plasmalogens in schizophrenia. I am currently putting together the final version of my thesis.

I've created an adapted figure for my thesis on plasmalogen synthesis based on your 2004 review paper titled, "Functions and biosynthesis of plasmalogens in health and disease."

Is it okay to use the figure in my final version of my thesis? I will need written permission to use it in my thesis.

I have attached the adapted figure I created to this email.

Let me know.

Thank you!
Jessica L. Bist

[redacted]

<Adapted Figure- Plasmalogen Synthesis- Jessica Bist.docx>

VUmc disclaimer : www.vumc.nl/disclaimer
AMC disclaimer : www.amc.nl/disclaimer

From: Nancy Elise Braverman, Dr [redacted]
Subject: Re: Permission to Use Adapted Figure in Thesis- Jessica Bist
Date: April 19, 2021 at 9:41 AM
To: Jessica Bist [redacted]

[△EXTERNAL]

Hi Jessica,
You are welcome to use this.
I would also add the peroxisome membrane enzyme FAR1, as it controls cellular plasmalogen levels by feedback inhibition.
My best wishes, Nancy

Nancy Braverman, MS, MD, FACMG
[redacted]
phone: [redacted]
FAX: [redacted]

Recognize and embrace human individuality- including our copy number variants, mutant genes, differences in transcription, regulation and methylation/ Reconnaître et adopter l'individualité humaine, ce qui comprend les variations du nombre de copies, les gènes mutants, ainsi que les différences de transcription, de régulation et de méthylation.

From: Jessica Bist
Sent: Thursday, April 15, 2021 10:09 AM
To: Nancy Elise Braverman, Dr
Subject: Permission to Use Adapted Figure in Thesis- Jessica Bist

Hi Dr. Braverman,

I am a Master's student at the University of Calgary, studying plasmalogens in schizophrenia. I am currently putting together the final version of my thesis.

I've created an adapted figure for my thesis on plasmalogen synthesis based on your 2012 review paper titled "Functions of plasmalogen lipids in health and disease."

Is it okay to use the figure in my final version of my thesis? I will need written permission to use it in my thesis.

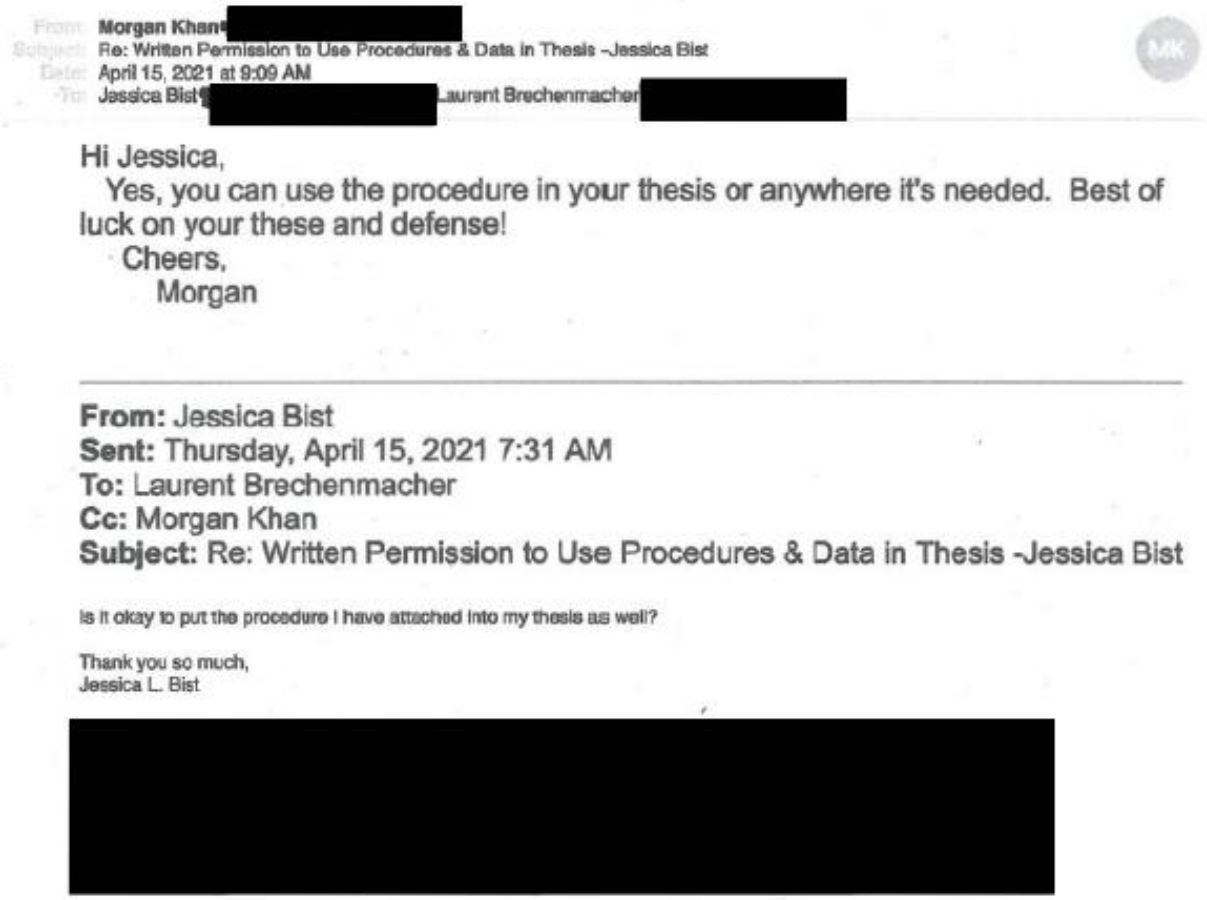
I have attached the figure I created to this email.

Let me know.

Note. Copyright permissions were obtained through e-mail communications from Dr. Braverman and Dr. Wanders for the creation and insertion of the adapted figure (Figure 2).

Appendix L

Figure 9. *Copyright Permissions for Lipid Extraction Method for Blood Serum Sample and Use of Collected Data (Appendix A-C, Appendix J)*



From: Morgan Khan[redacted]
Subject: Re: Written Permission to Use Procedures & Data in Thesis -Jessica Bist
Date: April 15, 2021 at 9:09 AM
To: Jessica Bist[redacted] Laurent Brechenmacher[redacted]

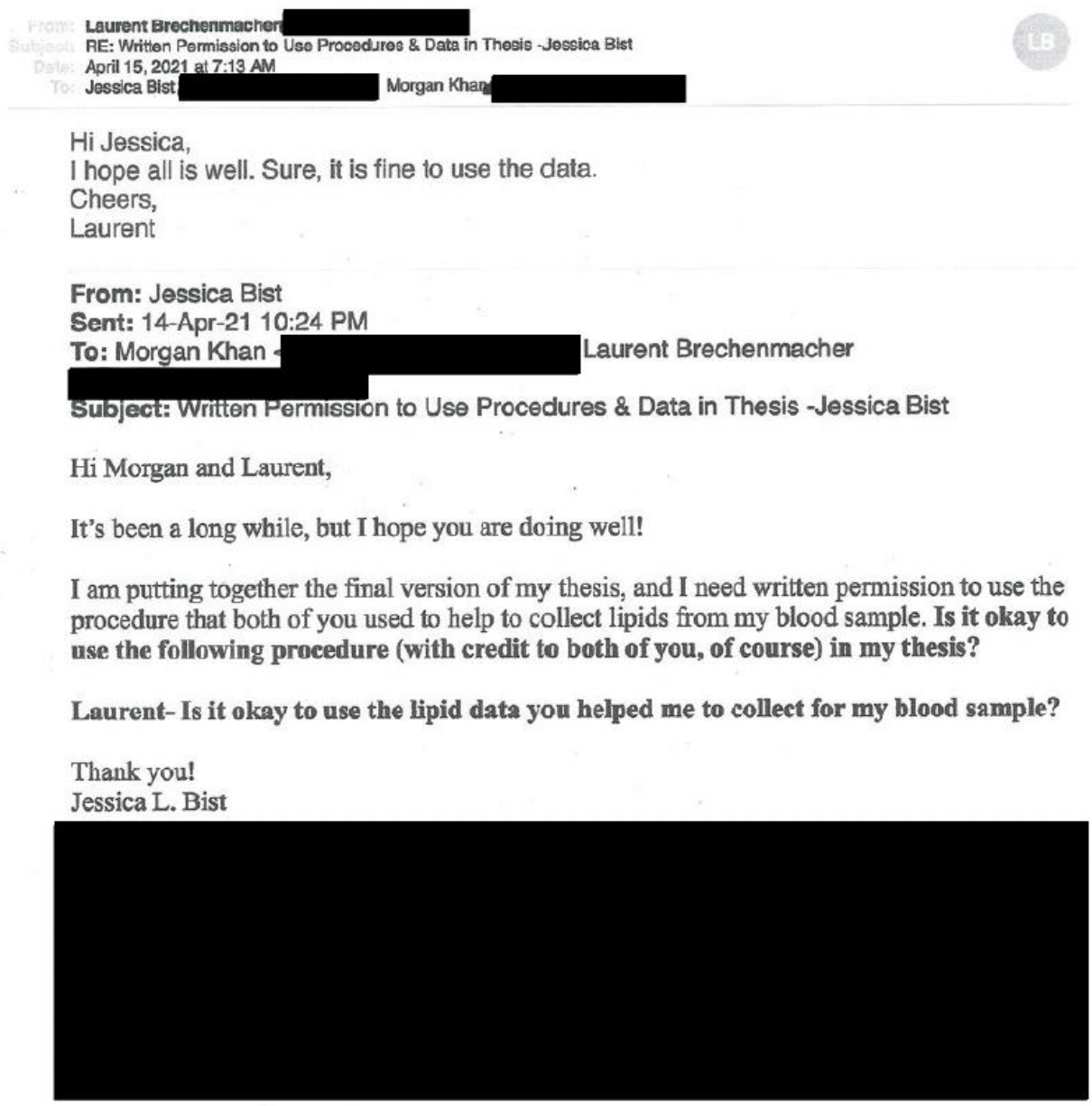
Hi Jessica,
Yes, you can use the procedure in your thesis or anywhere it's needed. Best of luck on your these and defense!
Cheers,
Morgan

From: Jessica Bist
Sent: Thursday, April 15, 2021 7:31 AM
To: Laurent Brechenmacher
Cc: Morgan Khan
Subject: Re: Written Permission to Use Procedures & Data in Thesis -Jessica Bist

Is it okay to put the procedure I have attached into my thesis as well?

Thank you so much,
Jessica L. Bist

[redacted]



From: Laurent Brechenmacher [redacted]
Subject: RE: Written Permission to Use Procedures & Data in Thesis -Jessica Bist
Date: April 15, 2021 at 7:13 AM
To: Jessica Bist [redacted] Morgan Khan [redacted]

Hi Jessica,
I hope all is well. Sure, it is fine to use the data.
Cheers,
Laurent

From: Jessica Bist
Sent: 14-Apr-21 10:24 PM
To: Morgan Khan [redacted] Laurent Brechenmacher [redacted]
Subject: Written Permission to Use Procedures & Data in Thesis -Jessica Bist

Hi Morgan and Laurent,

It's been a long while, but I hope you are doing well!

I am putting together the final version of my thesis, and I need written permission to use the procedure that both of you used to help to collect lipids from my blood sample. **Is it okay to use the following procedure (with credit to both of you, of course) in my thesis?**

Laurent- Is it okay to use the lipid data you helped me to collect for my blood sample?

Thank you!
Jessica L. Bist

[redacted]

Note. Copyright permissions were obtained from Dr. Morgan Khan and Dr. Laurent Brechenmacher for the Lipid Extraction Method for Blood Serum Sample (Appendix J). Additionally, copyright permission was obtained from Dr. Laurent Brechenmacher for use of the collected data through liquid chromatography-mass spectrometry (LC-MS) (See Figures 3-5 in Appendix A-C).

Appendix M

Figure 10. *Copyright Permissions for Plasma Sample Analysis (Appendix I)*

From: **Wood, Paul** [redacted]
Subject: Re: Our Study
Date: November 24, 2020 at 7:25 AM
To: Jessica Bist [redacted]

PW

[△EXTERNAL]

Jessica:

That is unfortunate. The answers are yes to both.

Good luck
Paul

From: Jessica Bist
Sent: Monday, November 23, 2020 10:08 AM
To: Wood, Paul
Cc: Thomas Raedler (AHS)
Subject: Our Study

Dear Dr. Wood,

I hope you are well.

We have been unable to continue recruitment due to COVID-19 restrictions in Calgary, Alberta. In order to still be able to graduate from my program, I am currently trying to write up a thesis with the data that I do have, into a feasibility study.

I was wondering, is it possible to put the following process for processing plasma samples into my thesis (with credit to you, of course)? I have attached what you sent me previously.

I was also wondering about the statistics that would have been used for my study. In your previous publications, you used student t-test to analyze the data sets. I was thinking, with the addition of the MCCB (Matrics scores) (which is a categorical dependent variable), we may have needed to use two-way ANOVA or factorial logistic regression. Were you planning to use a similar method in this study as well?

Thank you!

Jessica L. Bist

[redacted]

Phone (Cell) [redacted]
Phone (Work) [redacted]

Note. Copyright permissions were obtained from Dr. Paul Wood for the Plasma Sample Analysis (Appendix I).

Appendix N

Figure 11. *Copyright Permissions for Adapted Table 3, 'Descriptions of Each Section of the MATRICS™ Consensus Cognitive Battery (MCCB™) (Appendix D).*

From: Keith Nuechterlein [redacted]
Subject: Re: Permission for Use of Adapted Table-- Jessica Bist
Date: May 3, 2021 at 10:56 PM
To: Jessica Bist [redacted]

KN

[△EXTERNAL]

Dear Jessica,

You have my permission to use the Table in your thesis. Using the table in a published article later may require further adaptation.

Keith Nuechterlein, PhD

On Mon, May 3, 2021, 9:36 PM Jessica Bist <[redacted] wrote:

Hi Dr. Nuechterlein,

I am a Master's student at the University of Calgary, studying plasmalogens in schizophrenia. I am currently putting together the final version of my thesis.

I created an adapted table, based on the MCCB Manual—Third Edition.

Is it okay to use the table in my final version of my thesis? I will need written permission to use it in my thesis.

I have attached the table I created to this email, in both word and PDF just incase.

Let me know.

Thank you!

Jessica L. Bist

[redacted]

From: Robert Kern [redacted]
Subject: RE: Perm ss on for Use of Adapted Tab e- Jess ca B st
Date: May 4, 2021 at 10:28 AM
To: Jess ca B st [redacted]

RK

[redacted]

Jessica,
You have my permission to use the table in your thesis.
Good luck with your thesis!
Best regards,
-- Bob K.

Robert S. Kern, PhD
[redacted]

From: Jessica Bist [redacted]
Sent: Monday, May 3, 2021 9:29 PM
To: [redacted]
Subject: Permission for Use of Adapted Table- Jessica Bist

Hi Dr. Kern,

I am a Master's student at the University of Calgary, studying plasmalogens in schizophrenia. I am currently putting together the final version of my thesis.

I've created an adapted table, based on your 2011 paper titled "The MCCB Impairment Profile for Schizophrenia: Results from the MATRICS Psychometric and Standardization Study."

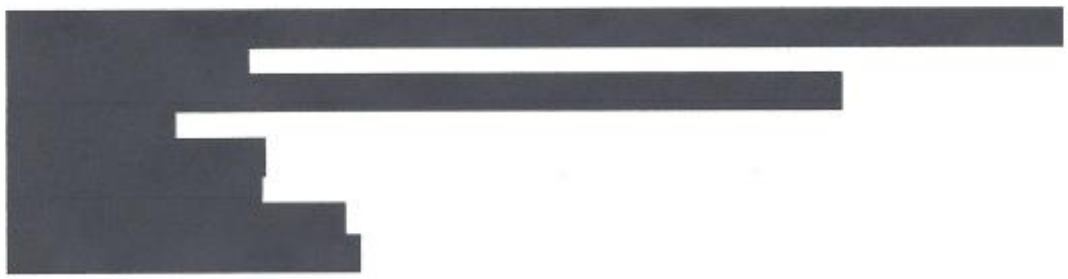
Is it okay to use the table in my final version of my thesis? I will need written permission to use it in my thesis.

I have attached the table I created to this email, in both word and PDF just incase.

Let me know.

Thank you!

Jessica L. Bist



Note. Copyright permissions were obtained from Dr. Nuechterlein and Dr. Kern for the adapted table (Table 3, Appendix D).