COMT GENOTYPE, SCHIZOPHRENIA, AND DOPAMINE TRANSMISSION

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DEDICATION

It would be impossible to list everyone who has provided me with support and assistance in reaching this point, but I would be remiss if I did not specifically mention the formative contributions of my parents Tom and Susan Birchfield, Mary Jane Conner, and Pamela Stuntz.

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Catechol-o-methyltransferase (COMT) catabolism is the primary mechanism for dopamine signal deactivation in the dorsolateral prefrontal cortex, an area of the brain associated with working memory. Working memory deficits are found in persons with schizophrenia and their unaffected siblings. A single nucleotide polymorphism of the COMT gene results in a MET→VAL shift at codon 158, increased enzyme thermostability, and increased enzymatic activity. The hypothesized result of this shift is decreased dopamine transmission in the brain area associated with working memory due to increased dopamine catabolism. The current study analyzed the effect of COMT genotype and schizophrenia on the mRNA expression of genes known to be influenced

by dopamine signal transmission with qPCR of RNA extracted from high-quality, fresh-frozen postmortem dorsolateral prefrontal cortex tissue. While no significant difference was observed between genotypes, a significant effect of diagnosis was found for the D_1 dopamine receptor, COMT, and tyrosine hydroxylase, the rate-limiting step of dopamine synthesis. The current findings support a model decreased dopamine synthesis and increased catabolism leading to deficient dopamine signal transmission in persons with schizophrenia.

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LIST OF ABBREVIATIONS

3-MT – 3-methoxytyramine

AAADC – aromatic amino acid decarboxylase

AC – adenylate cyclase

cAMP – cyclic adenosyl monophosphate

cDNA – complimentary deoxyribonucleic acid

COGS – Consortium on the Genetics of Schizophrenia

COMT – catechol-o-methyltransferase

DA – dopamine

DARPP-32 – dopamine and cAMP regulated phosphoprotein of 32 kDa

DAT – dopamine active transporter

DLPFC – dorsolateral prefrontal cortex

DOPA – 3,4-dihydroxyphenylalanine

DOPAC – 3,4-dihydroxyphenylacetic acid

DSM-IV-TR – Diagnostic & Statistical Manual of Mental Disorders, 4th ed., text revision

FDA – US Food and Drug Administration

fMRI – functional magnetic resonance imaging

GABA – gamma aminobutyric acid

GAPDH – glyceraldehyde 3-phosphate dehydrogenase

HPRT – hypoxanthine-guanine phosphoribosyltransferase

HVA – homovanillic acid

ICD – International Classification of Diseases

MAO – monoamine oxidase

MATRICS – Measurement & Treatment Research to Improve Cognition in Schizophrenia

MB-COMT – membrane-bound COMT

Met - methionine

MPTP – 1-methyl-4-phenyl-1,2,4,6,-tetrahydropyridine

mRNA – messenger ribonucleic acid

NIMH – National Institute on Mental Health

PET – positron emission tomography

PFC – prefrontal cortex

S-COMT – soluble COMT

SNP – single nucleotide polymorphism

TH – tyrosine hydroxylase

Val - valine

VMAT – vesicular monoamine transporter

WHO – World Health Organization

CHAPTER ONE

CONCISE OVERVIEW OF THE LITERATURE

The Dopamine Hypothesis of Schizophrenia

Schizophrenia is a multifaceted disorder involving a complex interaction of genetic and environmental factors (Tandon, Keshavan, & Nasrallah, 2008b). One of the oldest and most widely scrutinized theories of the pathogenesis of schizophrenia is the dopamine (DA) hypothesis (Davis, Kahn, Ko, & Davidson, 1991). In its most basic form, increased activity and hyperstimulation of receptors in DA pathways innervating the striatum are responsible for positive symptoms including hallucinations and delusions. Conversely, decreased activity and hypostimulation of receptors in DA pathways innervating the frontal lobes are responsible for negative and cognitive symptoms including avolition, social withdrawal, and executive function deficits (Crow, 1981; Toda & Abi-Dargham, 2007).

Dopamine and COMT Catabolism

During dopaminergic transmission, the neurotransmitter is released pre-synaptically and diffuses across the synaptic cleft to stimulate post-synaptic D_1-type or D_2-type receptors. D_1-type receptors (D_1 and D_5) activate adenylate cyclase (AC) in the post-synaptic neuron, and D2-type receptors (D_2, D_3, and D_4) inhibit post-synaptic AC as well as act as pre-synaptic autoreceptors (Missale et al., 1998). DA deactivation occurs primarily through two mechanism, active transport and catabolism. The dopamine active

transporter (DAT) predominates in striatal tissue and has been estimated to have a 1000 fold greater affinity for dopamine than the catabolizing enzyme catechol-o-methyltransferase (COMT; Chen et al., 2004). DAT, however, is not widely found in frontal lobe tissue where catabolism is thought to play a pivotal role in dopamine signal deactivation (Lewis et al., 2001; Matsumoto et al., 2003). COMT is found throughout the brain, kidneys, and liver (Matsumoto et al., 2003; Tenhunen et al., 1994) and deactivates dopaminergic signaling by catabolizing the neurotransmitter into 3-methoxytyramine (3-MT) which is subsequently metabolized into homovanillic acid (HVA) by monoamine oxidase (MAO; Axelrod & Tomchick, 1958; Karoum, Chrapusta, & Egan, 1994). COMT is found primarily in the brain in its longer, membrane-bound form and throughout the rest of the body in its shorter, soluble form (Tenhunen, Salminen, Jalanko, Ukkonen, & Ulmanen, 1993).

Several single nucleotide polymorphisms (SNP's) of the COMT gene have been studied including one located in exon 4 at codon 158 (Bertocci et al., 1991; Lundstrom, Salminen, Jalanko, Savolainen, & Ulmanen, 1991). At this location a SNP shift of methionine (Met) to valine (Val) has been shown to significantly increase enzymatic activity of the enzyme even when it is expressed at similar levels (Chen et al., 2004; Lotta et al., 1995). This is believed to be the result of changes in COMT protein integrity due to increased thermostability (Scanlon, Raymond, & Weinshilboum, 1979). Presumably, an increased genetic load of the higher-activity COMT-Val allele will correlate with increased DA catabolism and decreased DA signaling in the frontal lobes. The associated decrease in signaling would not be evident in striatal tissue where DA signal deactivation occurs primarily through DAT transport (Sesack et al, 1998).

Dopamine and Working Memory

Working memory (WM) and executive functioning deficits are demonstrated both in patients with schizophrenia and their unaffected siblings when compared to matched controls (P. S. Goldman-Rakic, 1994). These deficits have been associated with abnormal prefrontal cortex (PFC) functioning and decreased PFC DA signaling (Abi-Dargham et al., 2002; D. R. Weinberger, Berman, Suddath, & Torrey, 1992). Radiotracer labeling and positron emission tomography (PET) scanning demonstrate an upregulation in D_1 receptors in the frontal lobes, but not striatum, of patients with schizophrenia compared to controls, an effect presumed to be an ineffective compensatory mechanism in response to decreased PFC DA signaling (Abi-Dargham et al., 2002). With regards to COMT genotype, neuropsychological tests demonstrate a decrease in WM and executive function with COMT-Val allele load (Egan et al., 2001). These decreases are seen independent of a schizophrenia diagnosis but severely exacerbated by it (T. E. Goldberg et al., 2003). These findings are echoed in radiotracer labeling of D_1 receptors in controls compared by COMT genotype, as studies have demonstrated an increase in radiotracer binding in the PFC with COMT-Val allele load, indicative of decreased dopamine signaling and associated frontal lobe functioning (Slifstein et al., 2008). Although associated with WM deficits similar to those seen in schizophrenia, few studies have demonstrated a significant correlation between COMT genotype and schizophrenia diagnosis (Karayiorgou et al., 1998).

CHAPTER TWO

REVIEW OF THE LITERATURE

Introduction

Schizophrenia is a multifaceted disease involving a complex interaction of biological and environmental factors (Tandon, Keshavan, & Nasrallah, 2008b), and lifetime prevalence is commonly estimated to be between 0.5% and 1.5% of the population (Matthysse & Kidd, 1976). In the United States alone in 2002, schizophrenia related expenditures were estimated to be $22.7 billion in direct healthcare costs, $9.3 billion in direct non-healthcare costs such as homeless shelters and clinical research, and $32.3 billion in indirect costs such as unemployment and caregiver expenses (McEvoy, 2007). These figures do not account for emotional toll of the disorder paid by patients and their loved ones. While much progress has been made, further understanding of the disease process is needed in order to more effectively treat and manage this illness.

Symptomatology of Schizophrenia

While the causes of schizophrenia are obscure, it is clinically well characterized. Similar symptoms were rated in seven different countries in the World Health Organization (WHO) International Pilot Study of Schizophrenia (Sartorius et al., 1974). The most frequently occurring symptoms (summarized on Table 1) are still descriptive of the symptoms seen today. Because of the similarities in symptom presentation across

countries and cultures, clear diagnostic guidelines for schizophrenia have been established in the DSM-IV-TR (American Psychiatric Association, 2000) and tenth revision of the International Classification of Diseases and Related Health Problems (ICD-10) (World Health Organization, 1992).

Because presentation of characteristic schizophrenia symptoms is heterogeneous, efforts have been made to cluster symptoms into subgroups. Such analyses have sought to determine if schizophrenia is a single illness with a single pathophysiology or group of correlated syndromes as some have suggested (Carpenter & Buchanan, 1994). Three distinct symptom domains have been consistently identified and include the positive symptom domain (hallucinations, delusions), negative symptom domain (avolution, anhedonia, social withdrawal), and cognitive symptom domain (thought disorder, cognitive deficits) (Andreasen, Arndt, Alliger, Miller, & Flaum, 1995; Arndt, Alliger, & Andreasen, 1991; Grube, Bilder, & Goldman, 1998; Kay & Sevy, 1990; Lenzenweger, Dworkin, & Wethington, 1991; Liddle, 1987).

Cognitive Deficits as a Symptom Domain

Cognitive deficits are a hallmark of schizophrenia, and it has been suggested that impairment in cognition represents the core of schizophrenia (J. M. Gold, 2004; P. S. Goldman-Rakic, 1994). These deficits are stable throughout the course of the illness and are not secondary phenomena resulting from other symptoms (T. E. Goldberg et al., 1990; Park, Püschel, Sauter, Rentsch, & Hell, 1999). Literature reviews, meta-analyses, and structural equation modeling of a range of neuropsychological variables reveal the presence of a "general factor" of cognitive impairment in schizophrenia that in one study

accounted for 63.6% of the variance of test scores (Dickinson, Ramsey, & Gold, 2007; Dickinson, Ragland, Gold, & Gur, 2008; J. Gold, Hahn, Strauss, & Waltz, 2009; Heinrichs & Zakzanis, 1998). The cognitive deficits are broad, stable, and affect most cognitive domains by an average of one standard deviation compared to matched controls (as review by Palmer, Dawes, & Heaton, 2009). Cognitive dysfunction associated with schizophrenia is evident long before the first psychotic episode, possibly as early as six years old according to one meta-analysis (Woodberry, Giuliano, & Seidman, 2008), and affected persons experience further deterioration with the onset of the illness (Seidman, Buka, Goldstein, & Tsuang, 2006). A cross-sectional study conducted at four locations in the United States demonstrated similar levels of cognitive impairment both in chronic and recent-onset schizophrenia (Sponheim et al., 2010), and persons with schizophrenia are likely further impaired by their lack of insight into their dysfunction (Medalia & Thysen, 2008). The deficits result in a substantial impairment social functioning (M. F. Green, 1996), including such aspects as job tenure (J. M. Gold, Goldberg, McNary, Dixon, & Lehman, 2002). Cognitive dysfunction is also found in unaffected first-degree relatives of persons with schizophrenia and are likely the result of genetic factors that represent a predisposition to the illness (Faraone et al., 1995; Faraone et al., 2000).

One of the most coordinated and concerted efforts to understand schizophrenia-related cognitive deficits comes from the National Institute of Mental Health (NIMH) Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative (M. F. Green & Nuechterlein, 2004). The purpose of the MATRICS initiative is to evaluate and support the development of pharmacotherapies for the treatment of cognitive dysfunction in schizophrenia (Marder & Fenton, 2004). Based

on their review of the literature, seven domains were selected for assessment of cognitive functioning and included working memory, attention/vigilance, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing, and social cognition (M. F. Green et al., 2004). Gold (2004) identified several reasons for the importance of cognitive deficits as a target for therapeutic intervention, including the high frequency of characteristic cognitive deficits in schizophrenia, the stability of those deficits, and the independence of cognitive deficits from other symptom manifestations. The initiative has identified and evaluated functionally meaningful measures of cognitive improvement to aid in the development, testing, and approval of new pharmacotherapies by the U.S. Food and Drug Administration (FDA) (M. F. Green et al., 2008).

Schizophrenia and Working Memory

One domain of cognition extensively researched in schizophrenia is working memory (WM), which "refers to a brain system that provides temporary storage and manipulation of the information necessary for such complex cognitive tasks as language comprehension, learning, and reasoning," (Baddeley, 1992). WM deficits are associated with schizophrenia (P. S. Goldman-Rakic, 1994; Keefe, Lees-Roitman, & Dupre, 1997; Park & Holzman, 1992; Park, Holzman, & Goldman-Rakic, 1995), and there is some evidence that this domain of cognition is impaired beyond the general pattern of cognitive deficits of schizophrenia (Braff et al., 1991). WM dysfunction is evident whether the "online" content is externally or internally generated (Petrides, Alivisatos,

Meyer, & Evans, 1993), and it does not seem to be the result of amotivation or lack of attention, as patients who previously could not perform a WM task achieved similar levels of functioning as controls when given continuous instruction (T. E. Goldberg, Weinberger, Berman, Pliskin, & Podd, 1987). After instructions were removed, however, patients were forced to rely on their WM abilities and performed the task at their baseline levels (T. E. Goldberg et al., 1987).

WM deficits in schizophrenia appear to be heritable with one study by the Consortium on the Genetics of Schizophrenia (COGS), a seven-site collaboration assessing the heritability of vulnerabilities to schizophrenia, estimating the heritability coefficient to be as high as 0.39 (Greenwood et al., 2007). As part of the Dunedin Multidisciplinary Health and Developmental Study, a cohort of 1037 persons were administered a number of neuropsychological measures at various points of development over 30 years (Reichenberg et al., 2010). Study authors demonstrated WM deficits to be present before the manifestation of schizophrenia, and WM ability continued to lag behind both healthy individuals and those who later developed other psychiatric illnesses (Reichenberg et al., 2010). WM deficits are co-segregated within the illness and families with patients demonstrating a high degree of deficits compared to first degree relatives who in turn demonstrated poorer WM performance than controls (Conklin, Curtis, Katsanis, & Iacono, 2000; Park et al., 1995). Additionally, these WM deficits, found both in treated and untreated persons with schizophrenia, are resistant to medication even when other symptoms improve (Park et al., 1999). The evidence in support of WM dysfunction in schizophrenia make it an important target for research in understanding the disease process.

Working Memory and the Dorsolateral Prefrontal Cortex

The prefrontal cortex (PFC) is a brain area long considered to be crucial for WM processes . While some researchers have sought to subdivide the PFC into multiple regions according to specific WM functions, the only one that is consistently implicated in WM is the dorsolateral prefrontal cortex (DLPFC) (Levy & Goldman-Rakic, 2000). In the human brain, the DLPFC is comprised of Brodmann's areas 9 and 46 occupying the middle part of the superior and middle frontal gyri with a considerable proportion lying deep within the middle frontal sulcus (A. M. Owen, 1997). The DLPFC sends projections to the striatum and in turn receives projections back from the mesencephalon through the thalamus (Alexander, DeLong, & Strick, 1986). This circuit of which the DLPFC is a critical component is thought to contribute to WM processes (P. Goldman-Rakic, 1999).

The involvement of the DLPFC in WM has been well established based on evidence from single unit recordings in nonhuman primates and from neuroimaging studies of humans (P. S. Goldman-Rakic, 1999; Petrides et al., 1993) In experiments with rhesus monkeys trained to perform working memory tasks of different levels of difficulty in different sensory modalities Levy (2000) demonstrated that only bilateral DLPFC, rather than dorsomedial prefrontal cortex, lesions resulted in impairment in spatial WM tasks regardless of the level of task difficulty. Further evidence comes from the studies of persons with excisions of the frontal cortex. Significant deficits were observed on a computerized test of spatial working memory even at the least challenging level of task difficulty (Owen et al., 1996). Only at the most difficult level of the task difficulty were these deficits seen in patients with other brain injuries such as unilateral

temporal lobe lesions or unilateral amygdalo-hippocampectomy lesions. More recently, functional brain imaging studies have documented the engagement of the DLPFC in WM tasks in healthy individuals (Egan et al., 2001; Karlsgodt et al., 2007; D. S. Manoach et al., 1997). Such experiments often require the participant to perform a working memory task such as the N-Back Test, Wisconsin Card Sorting Test, or Sternberg Item Recognition Task (Sternberg, 1966) while in a functional MRI (fMRI) or during positron emission tomography (PET) scanning.

In his review of neuroimaging studies of WM in multiple neurologic populations Hillary (2006) conceptualized conditions that increase the requirements of the basal neural network to meet demands of the environment as a "cerebral challenge." Such conditions may be temporary (i.e. sleep deprivation), task related (i.e. increasing task demands), or permanent (i.e. aging or other condition resulting in neurological impairment). Neuroimaging studies of patients with medical conditions involving a major neurological component such as multiple sclerosis, HIV, and traumatic brain injury demonstrated increased activation of the DLPFC compared to healthy controls on WM tasks. Schizophrenia may also represent a "cerebral challenge" as a number of functional brain imaging studies of WM in patients with schizophrenia have documented abnormal activity in the DLPFC (Karlsgodt et al., 2007; Kim et al., 2010; D. S. Manoach et al., 1999; D. S. Manoach et al., 2000; D. S. Manoach et al., 1997) which is often related to poorer performance on working memory tasks (Barch, Shelineb, Csernansky, & Snyderc, 2003; Karlsgodt et al., 2007; D. S. Manoach et al., 1999).

Pattern of DLPFC Activation

In addition to the involvement of the DLPFC in WM processes of patients with schizophrenia and healthy controls, the pattern of DLPFC activation in relation to WM load is a central issue in the literature. In healthy individuals, it is generally observed that with increasing task difficulty, the DLPFC is increasingly activated (Karlsgodt et al., 2007; D. S. Manoach et al., 1997); with some studies showing a drop off as cognitive demands become excessive (T. E. Goldberg et al., 1998). However, there are conflicting findings when analyzing WM load and DLPFC activation in patients with schizophrenia. While some have demonstrated hypoactivation of the DLPFC during WM tasks in patients compared with control subjects (Barch et al., 2003; Cannon et al., 2005; Driesen et al., 2008; D. R. Weinberger, Berman, & Zec, 1986) others have instead demonstrated hyperactivation (Callicott et al., 2000; D. S. Manoach et al., 1999; D. S. Manoach et al., 2000).

In order to synthesize these apparent discrepant findings, authors have suggested a number of different models of DLPFC activation and WM load. For example, Manoach (2003) has suggested that the relationship takes the shape of an inverted U-curve, whereby low WM load results in low DLPFC activation, moderate WM load results in high DLPFC activation, and high WM load results in low DLPFC as cognitive resources reach their capacity (Figure 1). In this model, patients with schizophrenia have a leftward shift in their DLPFC activation curve. WM load considered low for a healthy control actually would represent moderate WM load for the proband who would have relatively higher DLPFC activation. WM load considered moderate for a healthy control would represent high WM load for the proband who would have relatively low DLPFC

activation as cognitive resources reached their capacity. Others have argued for a cross-over between-subjects model whereby healthy controls demonstrate a linear increase in DLPFC activation with increasing WM load, whereas probands demonstrate a linear decrease in DLPFC activation with increasing WM load (Karlsgodt et al., 2009). More recent studies have argued not for task-related hypo-frontality or hyper-frontality (i.e. increased vs. decreased PFC activation compared to control subjects on a given task), but for inefficient DLPFC functioning that may be manifested in either direction depending on task demands (Kim et al., 2010; Potkin et al., 2009). Just as unaffected relatives of persons with schizophrenia demonstrate an intermediate level of WM impairment compared to their affected relative and healthy controls (Conklin et al., 2000), so too do they demonstrate an intermediate level of DLPFC inefficiency (Karlsgodt et al., 2007).

A meta-analysis by Glahn and colleagues (2005) documented hypofrontality of schizophrenic subjects during N-back tasks but also noted increased activation of other brain areas including the anterior cingulate. The authors caution against restricting the focus of research to DLPFC activation and suggest examining the wider network that supports the task. Indeed, recruitment of other brain areas during WM tasks has also been noted in patients with schizophrenia, including the basal ganglia, thalamus, parietal lobe and middle temporal gyrus during similar WM tasks (Kim et al., 2010; D. S. Manoach et al., 2000). Meyer-Lindenberg (2001) demonstrated abnormal patterns of connectivity in persons with schizophrenia, whereby probands tended to activate the inferior temporal lobe, hippocampus, and cerebellum during WM tasks. In contrast control subjects activated the DLPFC and cingulate gyrus. Schizophrenia has long been considered an illness characterized by frontal lobe impairment and dysfunction of the

corticolimbic neural network involved in WM processes (D. R. Weinberger et al., 1992; D. R. Weinberger, Aloia, Goldberg, & Berman, 1994).

Working Memory and Dopamine

One of the oldest and most widely scrutinized theories of the pathogenesis of schizophrenia is the dopamine hypothesis (Davis et al., 1991) In its most basic form, increased activity and hyperstimulation of receptors in DA pathways innervating the striatum are responsible for positive symptoms including hallucinations and delusions. Conversely, decreased activity and hypostimulation of receptors in DA pathways innervating the frontal lobes are responsible for negative and cognitive symptoms including avolition, social withdrawal, and executive function deficits (Crow, 1981; Toda & Abi-Dargham, 2007).

Typical DA Synapse

At a typical DA synapse in the DLPFC, DA synthesis occurs in the pre-synaptic neuron in a linear fashion after the catabolism of the amino acid tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (TH), the rate limiting step in DA synthesis (Figure 2; Kumer & Vrana, 1996). DOPA is subsequently decarboxylized by aromatic amino acid decarboxylase (AAADC) to form DA. DA is stored pre-synaptically after uptake by vesicular monamine transporter 2 ($VAMT_2$) and is later released into the synaptic cleft where it interacts with postsynaptic receptors including D_1-type (D_1 and D_5) and D_2-type (D_2, D_3, and D_4) DA receptors or pre-synaptically with

D_2-type DA autoreceptors (Figure 3; Missale, Nash, Robinson, Jaber, & Caron, 1998; Qi, Miller, & Voit, 2008).

The D_1 DA receptor is the most widely expressed DA receptor in the primate neocortex (Hurd, Suzuki, & Sedvall, 2001; Lidow, Goldman-Rakic, Gallager, & Rakic, 1991) and is found primarily on post-synaptic dendritic spines (Smiley, Levey, Ciliax, & Goldman-Rakic, 1994). D_1 is a G protein-coupled receptor that stimulates the cyclic adenosine monophosphate (cAMP) pathway through adenylate cyclase (AC) leading to expression of immediate early genes (Missale et al., 1998) D_1 receptors will downregulate or upregulate (decrease or increase in quantity in response to an external variable) in response to respective increased or decreased extracellular DA. In DAT-knockout mice (DAT-ko, mice bred to express no DAT), which have significantly elevated extracellular DA, Giros and colleagues measured decreased D_1 mRNA compared to DAT-wild type mice (DAT-wt) (Giros, Jaber, Jones, & Wightman, 1996). Conversely, mice engineered to over-express DAT have decreased extracellular DA and upregulated D_1 receptors (Ghisi et al., 2009). Chronic administration of NMDA antagonists decreases extracellular DA and increases D_1 receptor binding sites in the primate PFC (Tsukada et al., 2005).

The D_2 DA receptor is significantly less abundant than D_1 in the primate neocortex, perhaps as low as 5% - 10% that of D_1 (Hurd et al., 2001; Lidow et al., 1991). D_2 is a G protein-coupled receptor that inhibits the cAMP pathway through inhibition of AC. It modulates DA release(Missale et al., 1998), and is the primary target for traditional antipsychotic medications (Seeman, 2010). D_2 regulation in response to extracellular DA appears similar to the D_1 receptor. Upregulation of D_2 receptors was

observed in mice over-expressing DAT that have low levels of DA (Ghisi et al., 2009), and decreased D_2 mRNA was observed in DAT-ko mice with high levels of extracellular DA (Giros et al., 1996). Furthermore, low levels of D_2 receptors were found in abusers of methamphetamine which increases extracellular DA by blocking DAT (Volkow et al., 2001).

Dopamine Transmission and Working Memory

DLPFC neurons are part of a complex circuitry with a number of excitatory, inhibitor, and modulatory connections linking the PFC, midbrain, and striatum (Figure 4; for reviews see D. A. Lewis & Gonzalez-Burgos, 2006; D. A. Lewis & Gonzalez-Burgos, 2007). The DLPFC sends projections to the midbrain and in turn receives projections back from the mesencephalon through the thalamus (Alexander et al., 1986). Along with the neurotransmitters gamma-aminobutyric acid (GABA) and glutamate, dopamine (DA) transmission seems to be an important factor for optimal functioning of this circuitry by regulating its excitability through the formation of a synaptic complex with excitatory inputs on 1.) dendritic spines of PFC pyramidal neurons and 2.) GABA interneurons which in turn synapse in a linear array on PFC pyramidal axon initial segments (P. Goldman-Rakic, Leranth, Williams, Mons, & Geffard, 1989; P. Goldman-Rakic, 1996; Seamans, Gorelova, Durstewitz, & Yang, 2001). Increased DA transmission in the PFC is results in decreased DA transmission in striatal subcortical structures (Grace, 1993; Kolachana, Saunders, & Weinberger, 1995) and increased DA transmission back to the frontal cortex (A. Meyer-Lindenberg et al., 2002). The dopaminergic "tuning" of GABA interneurons and pyramidal neurons in the DLPFC is believed to give rise to

synchronized, oscillating firing at 30–80 Hz (Whittington & Traub, 2003). Oscillations at this range of frequencies in the human DLPFC increase in proportion to working memory load (Howard et al., 2003). This functional circuit may be compromised in persons with schizophrenia at least in part by disturbance of dopaminergic tuning of the temporal integration mechanism of inhibitory interneurons (P. S. Goldman-Rakic, Muly, & Williams, 2000; S. Williams & Boksa, 2010).

The administration of antagonists of the D_1 dopamine receptor, the most widely expressed dopamine receptor in the prefrontal cortex (Hurd et al., 2001; Lidow et al., 1991), results in impairment in WM tasks in nonhuman primates (Sawaguchi & Goldman-Rakic, 1991). Conversely, administration of full D_1 receptor agonists has been shown in some studies to improve WM task performance (Arnsten, Cai, Murphy, & Goldman-Rakic, 1994) and reduce age-related and antipsychotic-induced WM deficits in these subjects (Castner, Williams, & Goldman-Rakic, 2000; Castner & Goldman-Rakic, 2004). Along with acetylcholine and glutamate, DA transmission has been identified by the MATRICS initiative as a target for pharmacological agents for the treatment of cognitive impairments in schizophrenia (Buchanan, Freedman, Javitt, Abi-Dargham, & Lieberman, 2007), specifically the D_1 receptor (P. Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004). DA transmission specifically in the DLPFC seem particularly important for WM processes. The mesocortical DA tract provides widespread innervation to neocortical areas including the DLPFC (Levitt, Rakic, & Goldman-Rakic, 1984; D. A. Lewis, Campbell, Foote, Goldstein, & Morrison, 1987), and administration of D_1 antagonists directly in the DLPFC of nonhuman primates, particularly around the

principal sulcus, results in WM deficits in a dose-dependent manner (Sawaguchi & Goldman-Rakic, 1994).

Radiotracer labeling of D_1 receptors by [^{11}C]NNC 112 and positron emission tomography (PET) scanning have demonstrated an upregulation in D_1 receptors in the DLPFC of patients with schizophrenia compared to controls (Abi-Dargham et al., 2002). The increased radiotracer binding was strongly associated with poorer performance on WM tasks. These findings were thought to be an ineffective compensatory mechanism in response to a presumed decrease DLPFC dopamine signaling in schizophrenia (Abi-Dargham, 2003). WM therefore appear to rely heavily on DA transmission in the DLPFC, a process that seems impaired in persons with schizophrenia.

COMT Genotype, Working Memory, and Schizophrenia

First discovered by Julius Axelrod and Robert Tomchick in 1958, catechol-o-methyltrasferase (COMT) has been an important enzyme in understanding the catabolism of DA and other catechols (R. M. Weinshilboum, Otterness, & Szumlanski, 1999). COMT catalyzes the methylation of a hydroxyl group on the catechol nucleus from S-adenosyl-methionine in the presence of Mg^{2+} (Axelrod & Tomchick, 1958) resulting in the DA metabolite 3-methoxytyramine (3-MT; Figure 5; Karoum et al., 1994) . COMT exists in two isoforms, the shorter, soluble form (S-COMT) and the longer, membrane-bound form (MB-COMT) (Tenhunen et al., 1993). While S-COMT is primarily found in the liver and kidneys, MB-COMT is primarily found in the brain (Matsumoto et al., 2003; Tenhunen et al., 1994) located in postsynaptic neurons and glia (Kaakkola,

Mannisto, & Nissinen, 1987; T. Karhunen, Tilgmann, Ulmanen, & Panula, 1995; T. Karhunen, Tilgmann, Ulmanen, & Panul, 1995; Rivett, Francis, & Roth, 1983).

Regional Differences DA Signal Termination

The dopamine active transporter (DAT) is believed to play a primary role in DA signal termination in the striatum where it is widely expressed (Ciliax et al., 1995; D. A. Lewis et al., 2001). In fact, cyclic voltommograms of homozygous DAT knockout mice have demonstrated a 100 fold increase in the amount of time the DA remains in the extracellular space compared to wild-type mice, a similar amount of time as predicted by the rate of neurotransmitter diffusion (Giros et al., 1996). Also, many drugs of abuse increase extracellular dopamine levels in these regions by blocking DAT (Horn, 1990).

In the PFC, catabolism by COMT is believed to play a primary role is DA signal termination rather than pre-synaptic reuptake, as DAT is expressed at low levels in this region and is not localized near synapses (Sesack, Hawrylak, Matus, Guido, & Levey, 1998). Conversely, COMT mRNA is expressed at higher levels in the PFC than in the rat striatum (Matsumoto et al., 2003).

DA catabolism occurs via a two step process with monoamine oxidase (MAO) (Karoum et al., 1994). Pharmacological studies have indicated the relative importance of methylation by COMT as the initial step of DA catabolism in the PFC rather than oxidation, with methylation accounting for more than 60% of DA turnover in the PFC and less than 15% in the striatum and nucleus accumbens (Karoum et al., 1994). More recently, systematic inhibition of MAO and NET resulted in a 2 fold increase in extracellular DA in the PFC of COMT knockout (COMT-ko) mice compared to

MAO/NET inhibited wild type (COMT-wt) mice suggesting that COMT may contribute up to 50% of DA metabolism in the PFC (Kaenmaki et al., 2010). COMT-ko mice also have a twofold longer DA elimination time in the PFC than COMT-wt, adding further evidence for the role of COMT being responsible for about half of DA metabolism in the PFC (Yavich, Forsberg, Karayiorgou, Gogos, & Mannisto, 2007).

COMT Inhibition

Studies of basal extracellular DA levels in COMT-ko mice have yielded conflicting results. While some researchers have demonstrated steady state DA levels to be 2 to 3 times greater in the frontal cortex compared to COMT-wt (Gogos et al., 1998) others have only been able to elicit elevated DA levels after administration of a challenge dose of L-DOPA (Huotari et al., 2002). Pharmacological inhibition of COMT with tolcapone did not significantly elevate basal extracellular DA in the rat medial PFC (Li et al., 1998), however a significant increase was noted when elicited by a challenge dose of clozapine before microdialysis or local perfusion of potassium chloride (Tunbridge, Bannerman, Sharp, & Harrison, 2004). Under basal conditions COMT-ko mice showed no changes in levels of other catecholamines, DA synthesizing enzymes including tyrosine hydroxylase (TH), or other DA deactivating proteins including MAO or DAT despite significantly higher levels of the MAO DA metabolite DOPAC (Huotari et al., 2002). A recent study utilizing a more sophisticated method of microdialysis demonstrated higher basal DA levels in COMT-ko mice PFC compared to COMT-wt mice despite no evident difference when measured with more conventional microdialysis (Kaenmaki et al., 2010). It therefore appears that COMT inactivation, as evidenced by

studies with COMT-ko mice or COMT-wt mice using COMT inhibitors, results in irregular DA transmission and likely higher levels of extracellular DA in the PFC with no effect on other catecholamines or other proteins associated DA transmission.

COMT Allele Activity

Early family studies of COMT activity revealed a strong correlation between siblings of enzymatic activity in red blood cells, suggesting a high degree of heritability (R. M. Weinshilboum, Raymond, Elveback, & Weidman, 1974). Those studies also showed a bimodal distribution of enzymatic activity in the sample suggesting monogenic inheritance of a "low activity" allele (R. M. Weinshilboum & Raymond, 1977) which is autosomal codominant and occurs in equal frequency with the "high activity" allele (R. M. Weinshilboum et al., 1999). The enzyme activity level was hypothesized to be related to the thermostability of the protein product (Scanlon et al., 1979). Cloning and sequencing the gene revealed the presence of a single nucleotide polymorphism (SNP) resulting an amino acid shift from valine (Val) (Lundstrom et al., 1991) to methionine (Met) (Bertocci et al., 1991) at codon 108 of S-COMT (codon 158 of MB-COMT). The functional result of the shift is a protein with significantly less enzymatic activity likely not related to a change in the kinetic properties of the protein itself but to its thermostability at normal physiological temperatures, as incubation of the protein at 37°C results in a linear decrease in activity to 20% that of an equal aliquot kept on ice within 30 minutes (Lotta et al., 1995). This temperature-sensitive decrease in activity is not seen with the COMT-Val allele (Lotta et al., 1995).

Chen and colleagues (2004) demonstrated an approximate 28% decrease in COMT activity in postmortem DLPFC tissue of homozygous Met (Met/Met) carriers compared to homozygous Val (Val/Val) carriers and an approximate 24% decrease in COMT activity in Met/Met lymphoblast cultures compared to Val/Val cultures. No change in COMT mRNA was evident, and the effect was independent of three other SNP's located in the P2 promoter region, intron 1, and 3' flanking region of the gene. No other SNP with the exception of the P2 promoter region in African Americans significantly altered COMT activity (Chen et al., 2004). Also, in healthy controls COMT $Val^{158}Met$ genotype predicted D_1 receptor availability in the DLPFC but not striatum by PET scanning of [^{11}C]NNC112 radiotracer with Val/Val individuals showing significantly higher radiotracer binding that Met/Met individuals, a hypothesized compensatory mechanism for the presumed decrease in DA signal transmission (Slifstein et al., 2008).

COMT-Val and WM Deficits

Based on the body of evidence regarding the role of COMT on DLPFC DA levels and the association of DLPFC DA with WM deficits, many researchers have sought to determine if COMT $Val^{158}Met$ genotype is predictive of WM performance or DLPFC inefficiency. Because individuals heterozygous for the high activity COMT-Val allele presumably have higher DA metabolism in the DLPFC and therefore lower DA transmission, it is consider a potential risk allele for WM dysfunction. Additionally, because WM deficits are evident in schizophrenia, the COMT $Val^{158}Met$ genotype has also been evaluated as a candidate risk gene for schizophrenia.

Two early and influential studies have supported this hypothesis and demonstrated a relationship between COMT genotype and WM in patients with schizophrenia, their unaffected siblings, and healthy controls (Figure 6). Individuals with the COMT Val/Val genotype, which is associated with increased COMT activity, performed worse than those with Val/Met or Met/Met genotype on WM tasks, and the effect was independent of diagnostic group (i.e. patients, siblings, or controls) accounting for about 4% of performance on the Wisconsin Card Sorting Test (Egan et al., 2001) and 3% of performance on the N-Back test (T. E. Goldberg et al., 2003). These effects were not related to general intelligence of participants or demographic factors. As predicted, schizophrenia patients performed worse than controls for all COMT genotypes, but interestingly, both studies demonstrated a near significant effect of diagnostic group in unaffected siblings, whose performance trended between the other two groups, independent of COMT genotype. The trend for poorer performance of siblings than healthy controls may indicate additional genetic factors contributing to DLPFC inefficiency and WM performance. COMT genotype was also predictive of DLPFC inefficiency during other WM tasks for all diagnostic groups (Egan et al., 2001), a finding that has been replicated in healthy controls (de Frias et al., 2010). The relationship between COMT genotype and DLPFC inefficiency may possibly be by way of a DA tuning mechanism mentioned above, as COMT genotype significantly affected DA synthesis in the mesencephalon which receives prefrontal DA afferents and in turn projects back to the PFC (Akil et al., 2003; A. Meyer-Lindenberg et al., 2005).

The authors of recent meta-analyses examining the association between COMT genotype and WM have concluded that there is actually no strong association in a number

of neuropsychological measures (J. H. Barnett, Jones, Robbins, & Muller, 2007; J. H. Barnett, Scoriels, & Munafo, 2008); however, these analyses have been criticized for their methodology (Goldman, Weinberger, Malhotra, & Goldberg, 2009). While not all studies associate COMT genotype and WM (Mata et al., 2008), many do result in some difference in measures of WM performance or DLPFC activation (Bilder et al., 2002; Bruder et al., 2005; Diaz-Asper et al., 2008; Malhotra et al., 2002) even if it is specific to only a certain diagnostic groups (Rosa et al., 2004).

COMT Inhibition and WM

In pharmacological studies the COMT inhibitor, tolcapone altered working memory in healthy controls with a significant interaction with COMT genotype, such that Val/Val individuals taking tolcapone showed better performance compared with placebo, and an associated decrease in blood COMT activity (Apud et al., 2007). Because COMT inactivation would presumably result in increase DA in the DLPFC and increased WM performance, this observation fits with previous hypotheses (Figure 7). Val/Met and Met/Met showed poorer performance compared with their respective placebo controls corresponding with an inverted U-curve model of DA level and task performance, whereby too little or too much DA would result in poor WM performance and moderate DA would result in peak performance. While Val/Val individuals have a presumed deficiency in DA, COMT inhibition would shift performance along the ascending limb of the performance curve, whereas for Val/Met and Met/Met individuals COMT inhibition would shift performance along the descending limb of the curve. While the findings were significant for neuropsychological variables, he authors, however, found no

evidence of interaction between COMT genotype and tolcapone group in fMRI DLPFC activation to confirm this model. They concluded that this may have been due to lack of statistical power.

COMT genotype may impact response to antipsychotic medications for patients with schizophrenia. Bertolino and colleagues (2007) noted a faster time of response to olanzapine for Met/Met individuals. Individuals with the COMT Met/Met and Val/Met genotypes also demonstrated improved attention and verbal fluency from baseline after six months treatment with clozapine, a result not seen in Val/Val participants (Woodward, Jayathilake, & Meltzer, 2007). Others studies, though, have failed to demonstrate any interaction with COMT genotype and treatment response or daily maintenance dosage to various antipsychotic medications (Illi et al., 2007; Nolan et al., 2006).

COMT-Val as a Risk Gene for Schizophrenia

There is strong evidence regarding the contribution of genetics to schizophrenia (Tandon, Keshavan, & Nasrallah, 2008a). The pattern of concordance rates of schizophrenia in twins is consistent across more recent studies with 2-3 times higher concordance rates among monozygotic twins compared to dizygotic twins and heritability estimates between 80% and 85% based on the pooled results multiple studies (Cardno & Gottesman, 2000). Because WM deficits are common to patients with schizophrenia and the COMT Val^{158}Met SNP likely has a small but significant effect on WM performance, research has been conducted to examine if the COMT Val/Val genotype is associated with higher rates of the disease, thereby by making COMT-Val a risk gene for

schizophrenia (H. J. Williams, Owen, & O'Donovan, 2007). Additional evidence supporting the role of COMT as a conveyer of risk comes from observations that the COMT gene maps within the region commonly deleted in 22q11 deletions syndrome, a condition associated with a significantly increased risk of schizophrenia (Murphy, Jones, & Owen, 1999).

A meta-analysis of family based association studies conducted by Glatt and colleagues (2003) found a small but reliable increase in the COMT-Val allele frequency in persons with schizophrenia of European ancestry; however the conclusions of the analysis have been called into question based on statistical methodology (Pittelli, 2004). No association between COMT genotype and a diagnosis of schizophrenia has been determined based on meta-analyses of both population based (Fan et al., 2005) or case-control based (Munafo, Bowes, Clark, & Flint, 2005) association studies, and what few individual studies that do demonstrate a significant association with COMT genotype have been contradictory in terms of determining the risk allele (Egan et al., 2001; Ohmori et al., 1998).

Current Research Questions

Based on 1.) the lack of support for COMT as a risk gene for schizophrenia, 2.) the observation that COMT genotype accounts for a small percentage of the variance in WM performance, 3.) the body of evidence supporting the relationship between schizophrenia, WM, DA transmission in the DLPFC, and 4.) the importance of COMT in DA signal termination in that region, the following research questions were formulated:

1. Does COMT genotype affect DA signaling in the DLPFC? Many studies have sought to answer this question utilizing in vivo microdialysis in COMT-ko mice or radioligand binding to D_1 receptors in the humans (Gogos et al., 1998; Huotari et al., 2002; Slifstein et al., 2008). There have been few studies to date with high-quality postmortem tissue.

2. Is the COMT/DA relationship affected by a diagnosis of schizophrenia? While persons with schizophrenia have obvious WM dysfunction which is dependent on DA transmission in the DLPFC, the effect of COMT genotype has been small and independent of diagnosis (Egan et al., 2001; T. E. Goldberg et al., 2003).

Addressing the Research Questions

In order to address the research questions, the following study will employ real-time polymerase chain reaction (q-PCR) to measure mRNA transcripts of DA receptors and associated proteins in fresh-frozen samples of human postmortem tissue of Brodmann's area 46. Subjects were genotyped for the COMT Val^{158}Met SNP and classified either as having a psychotic illness or not.

Because D_1 and D_2 DA receptor levels vary inversely to the relative amount of DA transmission at the synapse (Ghisi et al., 2009; Giros et al., 1996; Tsukada et al., 2005; Volkow et al., 2001)***, they were used as measures of relative DA transmission. It was hypothesized that individuals who are homozygous for the high activity COMT-Val allele would have decreased levels of DA transmission in the DLPFC, as evidenced by***

an increase in D_1-type and D_2-type DA receptors, due to presumed increased catabolism of DA. It was also hypothesized based on the work of Chen and colleagues (2004) ***that while COMT enzyme would be higher in Val/Val individuals, transcription of the gene would be similar to other genotypes, as increased levels of the enzyme would be due to the thermostability of the protein and not due to transcriptional factors.*** When considering the effect of schizophrenia, it was hypothesized that similar changes in DA receptors compared to healthy controls would be observed due to lower DLPFC DA signaling. Because COMT transcription levels do not appear to alter in response to changes in extracellular DA (Xu, Cawthon, McCastlain, Slikker, & Ali, 2005), no difference in COMT expression was expected between schizophrenic subjects and healthy controls.

Along with D_1 and D_2, the D_5 DA receptor was also measured. D_5 is considered to be D_1-like, as its activation stimulates the cAMP pathway similarly to D_1 (Missale et al., 1998). In the PFC it is frequently co-expressed with D_1 but is localized primarily to dendritic shafts, whereas D_1 is localized primarily to dendritic spines (Bergson et al., 1995). Reduced expression of both D_1 and D_5 receptors is found in the PFC of nonhuman primates chronically treated with antipsychotics, believed to increase extracellular DA in the PFC through D_2 receptor antagnosim (Lidow, Elsworth, & Goldman-Rakic, 1997). We therefore expect increased D_5 expression for the COMT Val/Val genotype compared to the other two and for schizophrenic subjects compared with controls.

In addition to DA receptors, mRNA of other proteins associated with DA signal transmission were measured. These included tyrosine hydroxylase (TH), the rate limiting enzyme of DA synthesis (Kumer & Vrana, 1996), and dopamine and cAMP regulated

phosphoprotein, of 32 kDA (DARPP-32), a downstream target of DA signaling (Svenningsson et al., 2004). Increased TH protein and mRNA was observed after DA depletion with chronic administration of 1-methyl-4-phenyl-1,2,4,6,-tetrahydropyridine (MPTP) (Xu et al., 2005). Because of the hypothesized decrease in DA signaling for the COMT Val/Val genotype and for schizophrenic subjects compared with controls, increased levels of TH expression were expected for the two risk groups.

Reduced levels of DARPP-32 have been observed in DLPFC postmortem tissue from persons with schizophrenia (Albert et al., 2002; Ishikawa, Mizukami, Iwakiri, & Asada, 2007); however, this observation may not result from the presumed decrease in DA signaling in schizophrenia, as levels of DARPP-32 protein and mRNA were unchanged in multiple regions of the rat brain after chronic blockade of D_1 receptors with SCH-23390 (Grebb, Girault, Ehrlich, & Greengard, 1990) or of D_2 receptors by a number of typical antipsychotic medications (Souza et al., 2008). Based on these findings, we predicted that DARPP-32 expression would be lower in schizophrenic subjects compared with controls; however, because its expression seems independent of extracellular DA, no change was expected between the COMT genotypes.

The preceding information can be used to construct a model of a typical DA synapse in the DLPFC and illustrate the working hypothesis for the current studies (Figures 8 & 9). TH activity in the pre-synaptic neuron leads to synthesis of DA which is later released to interact with post-synaptically D_1, D_2, or D_5 receptors or interact pre-synaptically with D_2 autoreceptors. Activation of postsynaptic DA receptors affects the phosphorylation state of DARPP-32 through the cAMP pathway. DA signal deactivation occurs primarily by catabolism by COMT which is located post-synaptically. Figure 8

shows the effect of the COMT Val-Val genotype. Although transcription is not altered, more of the COMT protein is present because of the thermostability. The resulting decreased extracellular DA would lead to upregulation of all DA receptors and TH. Figure 9 shows the similar effect of diagnosis of schizophrenia on the DA receptors and TH with the addition of decreased DARPP-32, which appears to be decreased in persons with schizophrenia but not necessarily related to extracellular DA levels.

CHAPTER THREE

METHODS

Human Tissue

Human brain tissue was provided by the Dallas Brain Collection, collected with consent of the subjects' next of kin and approved by the University of Texas Southwestern Medical Center Institutional Review Board. Next of kin also provided permission to access medical records and interview the primary care giver. At the time of dissection tissue was fresh frozen in a mixture of dry ice and isopentane and stored at -80º C. Brain pH and RNA integrity, measures of postmortem tissue quality, were determined as described previously (Stan et al., 2006). Subjects were screened for drugs of abuse and psychotropic medications including antipsychotics. All information obtained for each subject from medical records, interviews, and blood toxicology reports were reviewed by a panel of three mental health professionals who determined presence of a psychiatric disorders using DSM-IV criteria. All samples were previously characterized for COMT Val^{158}Met genotype and grouped by diagnosis (schizophrenia or control) and COMT genotype (Val [Val/Val] or Met [Val/Met and Met/Met]). Twenty-four subjects with a diagnosis of schizophrenia (Val = 10; Met = 14) and twenty-five healthy controls (Val = 13; Met = 12) were selected. For schizophrenic subjects, exclusionary criteria included presence of another Axis I disorder or history of another neurological disorder.

RNA Extraction and cDNA Synthesis

Tissue samples from the DLPFC (Brodmann's area 46) were removed from storage and pulverized over dry ice. Total RNA was isolated using TRIzol® (Invitrogen Cat. No. 15596-018) according to manufacturer's instructions. Pulverized tissue was homogenized in 500µl TRIzol® reagent and centrifuged at 12,000 rpm for 10 minutes at 4º - 8º C. After 5 minutes incubation at room temperature, 100µl of chloroform was added, followed by 3 minutes incubation at room temperature. Samples were centrifiuged at 12,000 rpm for 10 minutes at 4º - 8º C, and the RNA-containing aqueous layer was transferred to another tube. RNA was precipitated by addition of 250µl of 75% isopropyl alcohol and incubated at room temperature for 10 minutes. Precipitated RNA formed a gel-like pellet after being centrifuged at 12,000 rpm for 10 minutes at 4º - 8º C. The supernatant was discarded, and the RNA pellet was washed in 500µl of 75% ethanol in DEPC water. Samples were again centrifuged at 7,500 rpm for 5 minutes at 4º - 8º C. Supernatant was discarded, and the pellet was allowed to dry for two hours at room temperature.

DNA impurities were removed with TURBO DNA-*free*™ kit (Ambion, Cat. No. AM1907) according to manufacturer's instructions. The RNA pellet was dissolved in 20µl cold DEPC water and heated to 50º C for 2 minutes. To this was added 1.5µl DNase enzyme and 2.5µl 10x buffer. Samples were incubated in a water bath at 37º C for 30 minutes followed by addition of 5µl DNase Inactivation Reagent and incubation for 5 minutes. Samples were centrifuged at 10,000 RPM at room temperature to remove the DNase Inactivation Reagent, and the supernatant was transferred to another tube. RNA concentration was determined with Nanodrop Spectrophotometer (ND-1000).

First stand cDNA was synthesized with SuperScript® III First-Strand Synthesis System (Invitrogen, Cat. No. 18080-051). For each sample 8µl RNA stock (maximum 5000ng RNA) was added to 1µl each of dNTPs and random hexamers. The reactions were placed in a Techne thermocycler (TC-412) at 65º C for 5 minutes followed by 4º C for 1 minute. To this was added 4µl $MgCl_2$, 2µl 10x RT buffer, 2µl 0.1M DTT, 1µl RNaseOUT™, and 1µl SS-III RT™ which cycled for 10 minutes at 25º C, 50 minutes at 50º C, and 5 minutes at 25º C. Samples of cDNA were diluted to 25ng/µl before being stored at -20º C.

Real-Time Polymerase Chain Reaction

Amplification and quantification was conducted with SYBR® Green PCR Master Mix (Applied Biosystems, Cat. No. 4309155) and qPCR with StepOnePlus™ Real-Time PCR System (Applied Biosystems, Part. No. 4376600). Each 10µ reaction contained 75ng cDNA (25ng for housekeeping genes), 5 picomoles of sense and antisense primer, and 5µl PCR Master Mix, run in triplicate in a 96-well plate. PCR cycling conditions were 95º C for 10min and 40 cycles of 95º C for 15s, 60º C for 30s, and 72º C for 35s. The following primer sequences were used:

(1) COMT, GGACAGTGCTACTGGCTGAC (forward)/CAGGAACGATTGGTAGTGTGTG (reverse);

(2) DARPP-32, CCCCCTCTGGATGAGTCCGA (forward)/ GGTTCCTCCCCAGGCTCACT (reverse);

(**3) TH**, GCCGTGCTAAACCTGCTCTT (forward)/ GTCTCAAACACCTTCACAGCTC (reverse);

(4) D_1, AGGTATTGGGCTATCTCCAGC (forward)/ AGATGAGTACAGACAAGGTCCAT (reverse);

(5) D_2, CACTAAAGGGCAACTGTACTCAC (forward)/ GCCTGTTCACTGGGAAACTC (reverse);

(6) D_5, CCGCCAGGTGGAGATGATCC (forward)/ GGGAGGCTTCCTCCTCTGGT (reverse);

(7) HPRT; TGGACAGGACTGAACGTCTTG (forward)/ CCAGCAGGTCAGCAAAGAATTTA (reverse);

(8) GAPDH; TGCACCACCAACTGCTTAGC (forward)/GGCATGGACTGTGGTCATGAG (reverse); and

(9) Cyclophilin; GGAGATGGCACAGGAGGAA (forward)/GCCCGTAGTGCTTCAGTTT (reverse).

PCR data were obtained from Applied Biosystems StepOnePlus™ software. Cycle threshold values (Ct values, the number of cycles before required before signal is detectable above background florescence) were calculated and for each sample at each primer. Ct values for each gene of interest were compared to three common housekeeping genes, which included glyceraldehyde 3-phosphate dehydrogenase (GAPDH, a catalyst of glycolysis), hypoxanthine-guanine phosphoribosyltransferase (HPRT, an enzyme that salvages purines from degraded DNA), and cyclophilin (an enzyme that accelerates the folding of proteins). For each sample the geometric mean of the Ct values of the housekeeping genes were subtracted from Ct value of the gene of interest to generate a ΔCt. For each gene of interest, the ΔCt values of each sample are compared to each other in terms of a single reference sample (Figure 6). This is done by subtracting each ΔCt value from the lowest ΔCt (the reference sample) to create a ΔΔCt. Because each PCR cycle represents a doubling of cDNA transcripts of the gene of interest, the relative fold difference is expressed as a fraction of the reference sample, calculated by $2^{\Delta\Delta Ct}$.

Statistics

Ct averages were discarded if the standard deviation of a minimum of two of the three PCR reaction replications was greater than 0.6 or if a Ct average passed the Grubb's test for outliers. Spearman's Rank Order tests were performed on the dataset to

determine significance of possible covariates include age, postmortem interval (PMI), RNA integrity number (RIN), and postmortem interval (PMI).

The two primary hypotheses sought to determine the effect of COMT genotype or schizophrenia on DA signal transmission as evidenced by increases in DA receptors. Two-tailed *t* tests were used for the two primary outcome analyses comparing differences in gene expression of COMT and the DA receptors. First primary outcome analysis occurred between COMT Val group and COMT Met group in controls to determine effect of genotype. Increased expression of DA receptors was expected for the COMT Val group compared to the Met group, and no change was expected in COMT expression. The second primary outcome analysis occurred between schizophrenics and controls of Val/Val genotype to determine effect of diagnosis. Increased expression of DA receptors was expected for the schizophrenic group, and no change was expected in COMT expression.

The secondary hypotheses sought to determine the effect of COMT genotype or schizophrenia on TH and DARPP-32 expression. Two-tailed *t* tests were again used for secondary outcome analyses which compared differences in gene expression. First secondary outcome analysis occurred between COMT Val group and COMT Met group in controls do determine effect of genotype. An increase in TH expression was expected for the COMT Val group compared with Met group and no change in DARPP-32 was expected. Second secondary outcome analysis occurred between schizophrenics and controls of Val/Val genotype to determine effect of diagnosis. An increase in TH expression and a decrease in DARPP-32 expression was expected for the schizophrenic group compared with controls.

CHAPTER FOUR

RESULTS

Demographic data is summarized in Table 2. Spearman Rank Order Correlations for possible covariates of age, PMI, and RIN on gene expression were significant for an effect of age and TH expression, $\rho(41) = .334$, $p = .026$. The complete data set with fold changes is found in Table 6 and scatter plots of each gene of interest are found in Figures 12-17. Primary and secondary outcome analyses are summarized in Table 3.

Primary Analyses

Primary analyses sought to determine the effect of either COMT Val^{158}Met genotype or schizophrenia on COMT expression and DA transmission in the DLPFC as evidenced by DA receptor expression.

First primary analyses occurred between the COMT Val group and Met group in healthy controls. It was hypothesized that individuals homozygous for the high active COMT-Val allele would have decreased DA transmission as evidenced by decreased DA receptors. No change was expected in COMT gene expression. Two-tailed *t* tests between control COMT Val and Met groups were not significant for any measure. COMT genotype did not significantly affect gene expression in the current study.

Second primary analyses occurred between control and schizophrenic cases in the COMT Val groups. It was hypothesized that individuals with schizophrenia would have decreased DA transmission, again evidenced by decreased DA receptors. No change was expected in COMT gene expression. Between Val/Val schizophrenic and

Val/Val control cases significance was achieved for COMT, t(20) = -2.382, p = 0.028, and D_1 , t(20) = -2.186, p = 0.041, with schizophrenic cases expressing significantly more COMT and D_1. Effect sizes were large in both cases (Cohen's d = -1.02, -0.90 respectively) Gene expression of D_2 and D_5 was not significantly different between the two groups.

Secondary Analyses

Secondary analyses sought to determine the effect of either COMT Val^{158}Met genotype or schizophrenia on TH or DARPP-32 in the DLPFC.

First secondary analyses occurred between the COMT Val group and Met group in healthy controls. It was hypothesized that individuals homozygous for the high active COMT-Val allele would have decreased TH expression and no change in DARPP-32 expression. Two-tailed *t* tests of secondary outcome measures between control COMT Val and Met groups were not significant for an effect of genotype on DARPP-32 or TH expression. COMT genotype did not significantly affect gene expression in the current study.

Second primary analyses occurred between control and schizophrenic cases in the COMT Val groups. It was hypothesized that individuals with schizophrenia would have decreased TH and DARPP-32 expression. Between Val/Val schizophrenic and Val/Val control cases, significance was achieved for TH, t(16) = 2.530, $p = 0.022$, with schizophrenic cases expressing significantly less TH than control cases. Effect size was large (Cohen's d = 1.37) and did not dramatically change when covaried for age. Significance was not achieved for DARPP-32 expression.

Exploratory Analyses

Post hoc exploratory analyses sought to determine the possible significance of treatment with antipsychotic medications on expression of the genes of interest and to confirm the effect of schizophrenia on COMT, D_1, and TH expression in the COMT Met group. For schizophrenic cases for which medication information was available (n = 18 on medication, n = 6 off medication) two-tailed *t* tests were significant only for the D_2 receptor, $t(20) = 2.095$, $p = 0.049$, with schizophrenic cases on medication expressing higher levels of D_2.

Two-tailed *t* tests sought to confirm the finding of increased COMT and D_1 and decreased TH in the COMT Met group. The finding of decreased TH was confirmed in this group, $t(23) = 2.40$, $p = 0.025$. Unfortunately, the finding of increased COMT and D_1 was not confirmed in this group, and additionally, significance was achieved for DARPP-32, $t(22) = 4.29$, $p > 0.001$, and D_2, $t(23) = 2.47$, $p = 0.021$. In the COMT Met groups, schizophrenic cases expressed decreased DARPP-32 and decreased D_2. Although it is unable to be determined in the current study, this finding may be an effect of antipsychotic medication, as all "off medication" cases except one were found in the COMT Met group.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Study Rationale

The purpose of the current study was to determine the effect of catechol o-methyltransferase (COMT) Val158Met genotype and diagnosis of schizophrenia on dopamine (DA) transmission in the dorsolateral prefrontal cortex (DLPFC). Because increased thermostability and protein abundance of the COMT-Val allele, the primary enzyme of DA catabolism in the PFC, has been observed at normal physiological temperatures, it was hypothesized that homozygous individuals for the high activity COMT-Val allele would have less DA transmission as evidenced by increase DA receptor mRNA. Presence of schizophrenia is also believed to be associated with decreased PFC DA signaling based on hypothesized hypofrontality and previous studies' findings of increased D_1 radiotracer binding in the DLPFC of persons with schizophrenia.

To test these research questions, real-time polymerase chain reactions (q-PCR) were conducted on high quality, human postmortem brain tissue from Brodmann's area 46. Because dopamine receptor expression has previously been shown to vary inversely to extracellular DA levels, it was hypothesized that COMT Val group (Val/Val) would have higher expression of DA receptors compared to the COMT Met group (Val/Met, Met/Met) to compensate for their relatively lower DA transmission. Because COMT mRNA levels were not different between these groups in previous studies despite differences in protein abundance (Chen et al., 2004), no difference in expression was

expected. Two other proteins associated with DA signaling, TH and DARPP-32, were also measured.

Effect of COMT Val^{158}Met Genotype

The results of the current study did not support the hypothesis that COMT genotype caused differences in DA signal transmission, as increased DA receptor expression was not observed. This finding is in contradiction to another study that have found increased expression of D_1 receptors for COMT-Val (Slifstein et al., 2008). While COMT genotype has been shown to significantly effect WM tasks independent of schizophrenia, the effect of genotype in the most widely cited studies only accounted for 3% – 4% of the variance in performance (Egan et al., 2001; T. E. Goldberg et al., 2003). Also, one study that found a significant effect of COMT genotype on neuropsychological measures, failed to correlate these findings to neurophysiologic measures (Apud et al., 2007)

In addition to COMT, MAO also participates in DA signal deactivation in the DLPFC (Karoum et al., 1994). Despite the finding of no change in MAO expression in COMT-ko mice, decreased MAO activity may still perhaps serve as a compensatory mechanism for increased COMT activity of the Val allele. Also, the COMT-Val allele may not result in such dramatic changes in DA catabolism in vivo. While some studies estimate a several fold increase in enzymatic activity the COMT-Val enzyme compared to COMT-Met, other studies have demonstrated only modest increases (Chen et al., 2004). WM is likely dependent on complex neural circuits, of which the DLPFC is only one part (D. A. Lewis & Gonzalez-Burgos, 2006). Although unlikely, even if the

decreased performance in these studies was due entirely to decreased DA signaling, current methods may not be able to detect such subtle decreases in DA levels. Secondary analyses showed that expression of TH and DARPP-32 also did not differ significantly by COMT genotype.

Effect of Schizophrenia

It was also hypothesized that in COMT Val group the effect of a diagnosis of schizophrenia would also result in increased DA receptors due to presumed hypofrontality. The results did support the hypothesis that schizophrenia does result in decreased DA transmission in the DLPFC in the COMT Val group, as the D_1 receptor, the most abundant in the PFC, was significantly higher expressed. This finding is supported by previous observations that schizophrenia is associated with increased radioligand binding to DLPFC D_1 receptors (Abi-Dargham et al., 2002).

Unexpectedly, COMT expression was significantly higher in schizophrenic cases. This finding is contradictory to a previous study that showed no difference in gene expression between diagnostic groups (Chen et al., 2004). Tyrosine hydroxylase expression, the rate limiting enzyme in DA synthesis, was also lower in schizophrenic cases. This finding is keeping with other studies that have demonstrated decreased TH after pharmacological manipulation of DA levels and postmortem studies showing decreased TH immunoreactivity in other parts of the PFC of the schizophrenic brain (Akil et al., 1999; Akil, Edgar, Pierri, Casali, & Lewis, 2000). DARPP-32, a downstream target of DA signaling, showed no change in expression; however, it cannot be said that DARPP-32 is therefore unaffected by DA signaling, as the protein activity is dependent

on its phosphorylation state. The current study could not determine the phosphorylation state or protein activity.

Exploratory analyses sought to confirm in the COMT Met group the effect of schizophrenia found in the COMT Val group. While the finding of decreased TH expression was noted, neither D_1 nor COMT expression was altered between healthy controls and schizophrenic cases in this group. In fact, decreased DARPP-32 and D_2 was measured in the COMT Met group.

Although it is possible that COMT genotype and schizophrenia somehow interact to differentially affect expression of these proteins, it seems more likely to be an effect of antipsychotic medication treatment. While 9 of the 10 cases in the COMT Val group were medicated, only 5 of the 14 cases in the COMT Met group were. While increased D_1 is perhaps the most frequent finding in the literature, it is unclear as to the effect of antipsychotic medication in these studies. Although few report significant differences between "on medication" and "off medication" cases, few studies have been conducted purely on schizophrenic patients off medication. The finding of decreased D_2 in the Met group makes sense based on this observation, as antipsychotic medications are known to increase these receptors, and the COMT Val group (with a higher percentage of on medication cases) would be impacted more greatly by this change.

Clinical Significance

The finding of decreased TH and increased COMT in the schizophrenic brain, at least for individuals with COMT Val/Val genotype, demonstrate a multiplied vulnerability to decreased DA signaling, as the effect of lower levels of pre-synaptic DA

synthesis is compounded by increased DA catabolism by the higher levels of COMT. While current medications ameliorate schizophrenia symptoms by increasing extracellular DA by way of D_2 autoreceptor antagonism, this mechanism of action may not be sufficient to raise DA to levels comparable to healthy controls. If persons with schizophrenia suffer from insufficient DA synthesis in the neocortex, future pharmacotherapies designed to increase TH levels specifically in these regions may help to improve cognitive symptoms that still persist even when other symptoms subside. Furthermore, because COMT expression was greater in patients, future antipsychotic medications may be augmented by the addition of tolcapone or other forms of COMT inactivation.

While it is likely that the inability to confirm in the COMT Met group the significant findings in the COMT Val group is due to different proportions of medicated or unmedicated subjects, there is still the possibility of a significant interaction between COMT genotype and schizophrenia on these genes of interest. If future studies indicate that this is actually the case, it will therefore be advisable to consider COMT genotype and tailor medication regiments to the individuals genetic profile.

Study Limitations and Future Direction

The current study suffered from a number of notable limitations. First was the fact that not all of the DA receptors were able to be measured due to problems with receptor primers. Expression levels of the D_3 and D_4 receptors may have provided additional information and should be included in any further studies. Measurement of a number of other genes may also be a future goal of experiments of this type. DARRP-32

was the only measured downstream target of cAMP, a cell signaler with a number of other downstream targets, and future studies may benefit greatly from incorporating them into the experiment along with the other genes of interest.

As with any study assessing the differences between persons with schizophrenia and healthy controls, the effect of treatment with antipsychotic medication is an important fact to consider. While only the D_2 receptor was found to be significantly different between medicated and unmedicated schizophrenic cases in the current study, previous studies have demonstrated a significant effect of antipsychotic medication on mRNA levels of most of the genes of interest. These changes include decreased D_1, D_5, and TH and increased D_2 and COMT (Cheng et al., 2008; Lidow & Goldman-Rakic, 1997; Lidow et al., 1997; Tejedor-Real, Faucon Biguet, Dumas, & Mallet, 2003). In fact, the mechanism of action of most antipsychotic medication is through the increase in D_2, decrease in D_1, and balancing of their opposing actions in the cerebral cortex (Lidow et al., 1998). The current study was also unable to truly parse out the effect of antipsychotic medication and COMT genotype due to the fact that all except one of the unmedicated schizophrenic cases came from the COMT Met group. Future studies could select for cases to better balance antipsychotic treatment condition between groups to better examine the effect on mRNA expression of the genes of interest, as well as look for possible interactions of antipsychotic treatment and COMT genotype.

Demographic characteristics of the selected cases did not match that of the general population, as the dataset was more heavily weighted with Caucasian males. This was due primarily to the cases available for testing. Some of the groups, too, suffered from low sample sizes. The dataset did not represent the allele frequency in the

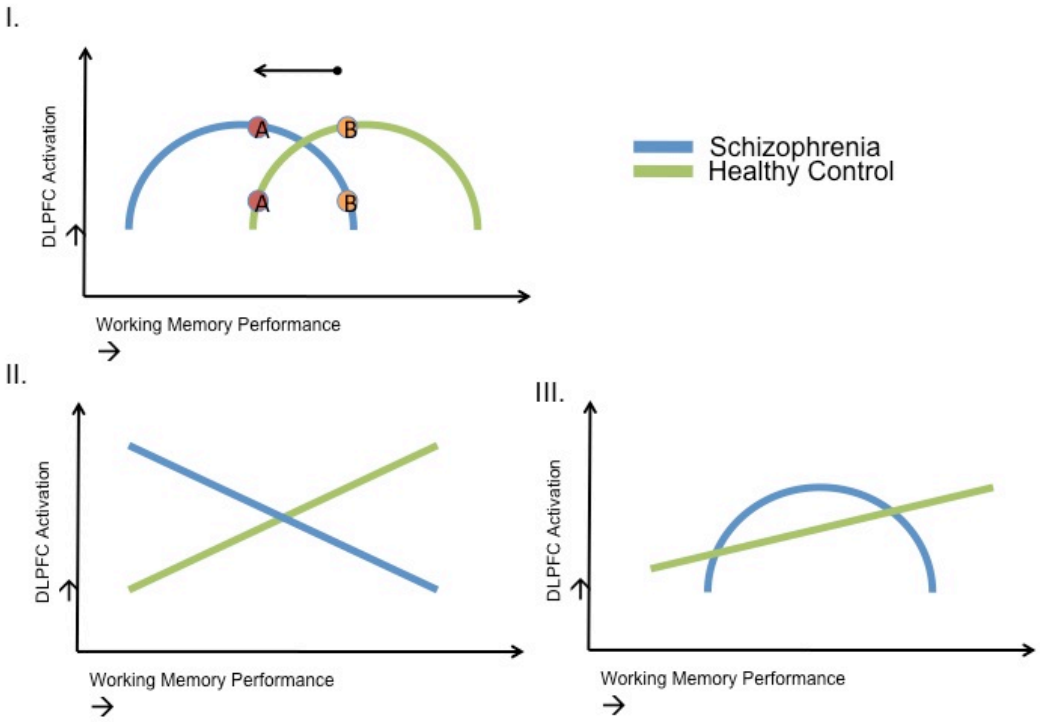
general population, as cases were selected to favor Val/Val and Met/Met genotypes in order to maximize differences between the two groups.

Finally, the most significant limitation to the current study was the fact that rather than measuring DA receptor levels directly through immunoblotting techniques, the peripheral measure of mRNA expression of DA receptors was used. Relative levels of protein expression were assumed based on these measures. While this method represents a first step in addressing the larger research questions posed herein, future studies must incorporate protein measures and determine their correlation with mRNA expression in order more directly assessment of the effect of COMT genotype and schizophrenia on extracellular DA levels in the DLPFC.

Acknowledgements

I would like to take the opportunity to acknowledge a few people for their help in completing this project. First, I'd like to thank my mentor, Dr. Subroto Ghose, for his patience, encouragement, and assistance with this thesis. I would like to thank my other committee members, Dr. Carol Tamminga and Dr. Paula Ulery-Reynolds, for giving up their time and providing helpful feedback. Thanks are in order for the members of the Ghose and Tamminga labs, Kelly Gleason and Brian Potts in particular, who courteously shared their space and took time to teach me laboratory technique. Finally, a very special thanks goes to Abhay Shukla whose training and practical assistance made the data collection possible.

FIGURE 1
Models of DLPFC Activation



I. In order to synthesize these apparent discrepant findings, Manoach (2003) has suggested a model of DLPFC activation in response to WM load that takes the shape of an inverted U-curve. In this model patients with schizophrenia have a leftward shift in their DLPFC activation curve.

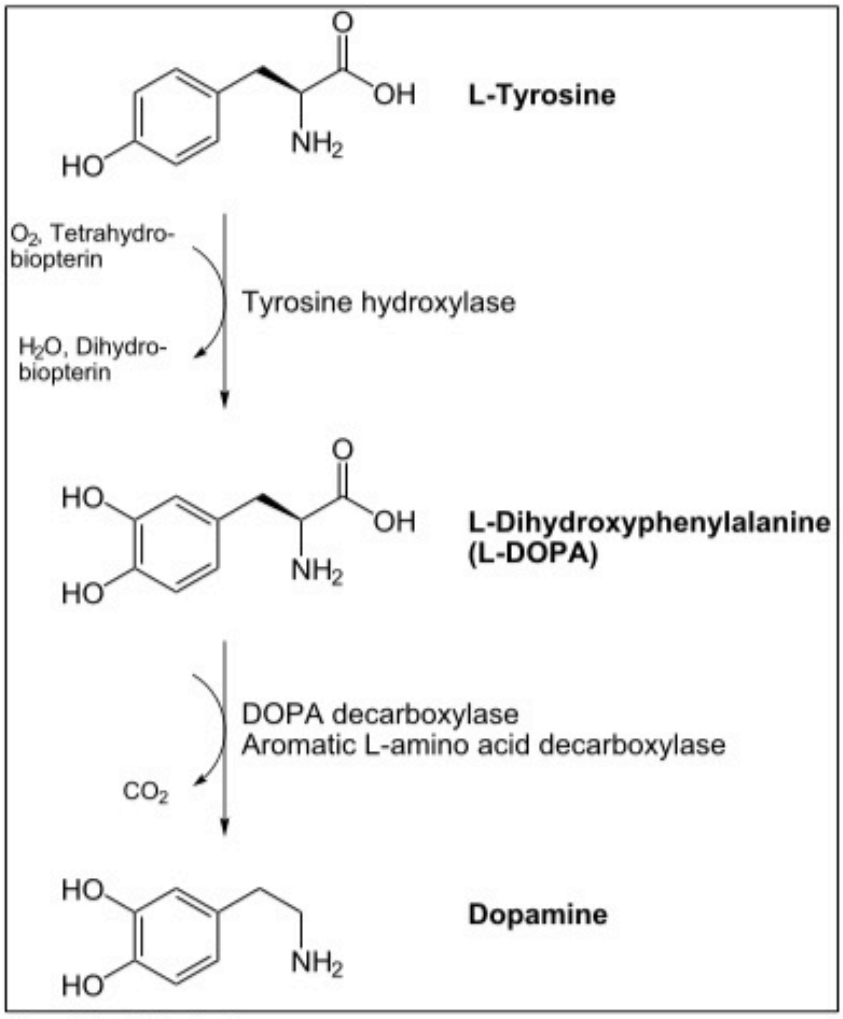
A.) WM load considered low for a healthy control and requiring low DLPFC activation actually would represent moderate WM load for the proband who would have relatively higher DLPFC activation.

B.) WM load considered moderate for a healthy control and requiring high DLPFC activation actually would represent high WM load for the proband who would have relatively low DLPFC activation as cognitive resources reached their capacity.

II. Between cross-over between-subjects model whereby healthy controls demonstrate a linear increase in DLPFC activation with increasing WM load, whereas probands demonstrate a linear decrease in DLPFC activation with increasing WM load Karlsgodt, 2009).

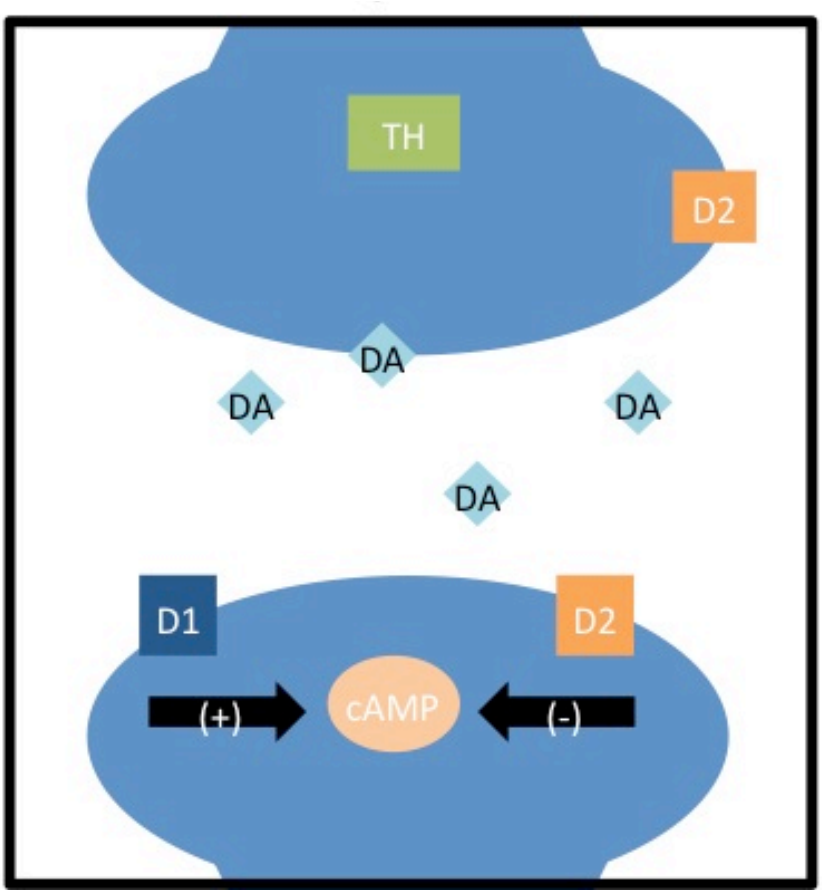
III. More recent studies have argued not for task-related hypo-frontality or hyper-frontality (i.e. increased vs. decreased PFC activation compared to control subjects on a given task), but for inefficient DLPFC functioning that may be manifested in either direction depending on task demands (Kim, 2010; Potkin, 2009).

FIGURE 2
Linear Synthesis of Dopamine



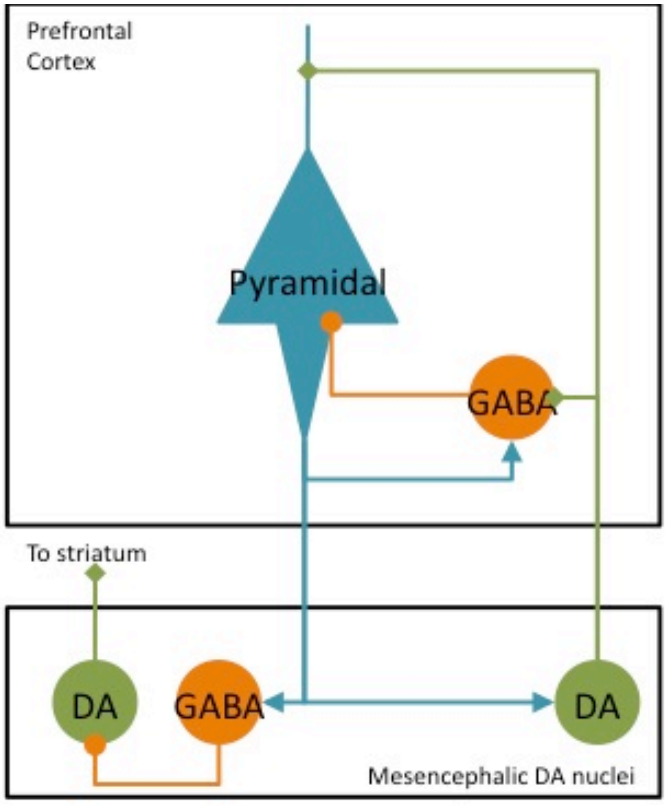
http://chem4513.pbworks.com

FIGURE 3
Typical Dopamine Synapse



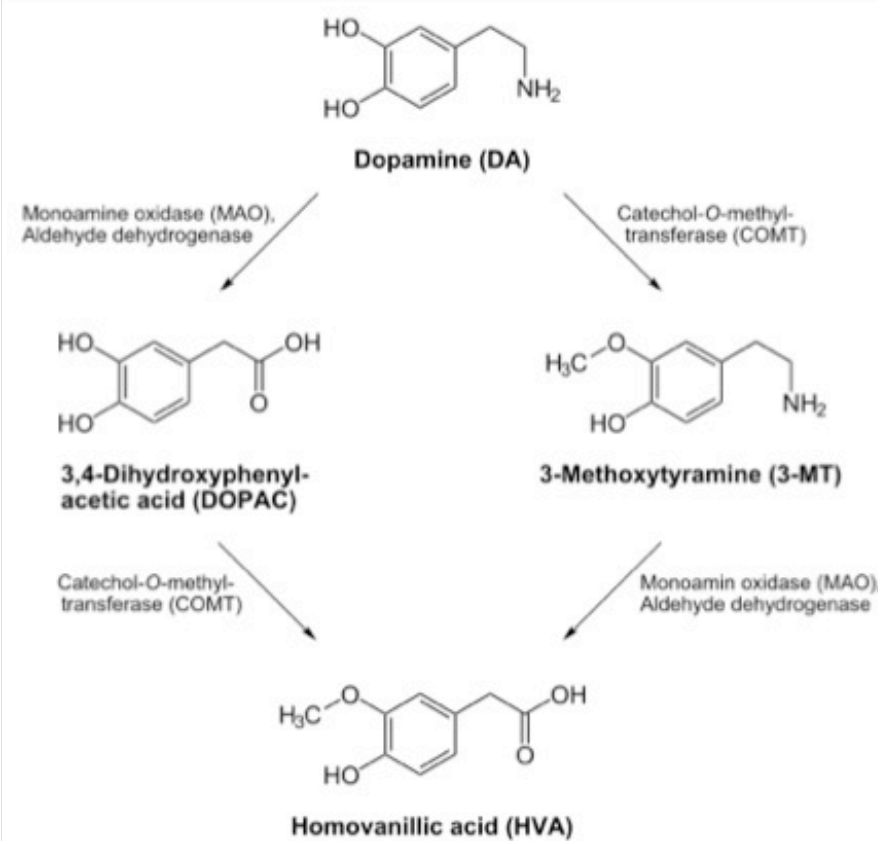
TH activity in the presynaptic neuron leads to synthesis of DA which is later released to interact with postsynaptically D1, D2, and D5 receptors or interact presynaptically with D2 autoreceptors. Activation of postsynaptic DA receptors affects the phosphorylation state of DARPP-32 through the cAMP pathway. DA signal deactivation occurs primarily by catabolism by COMT which is located postsynaptically.

FIGURE 4
Connectivity of DLPFC Pyramidal Neurons
(reproduced From Lewis et al., 2006)



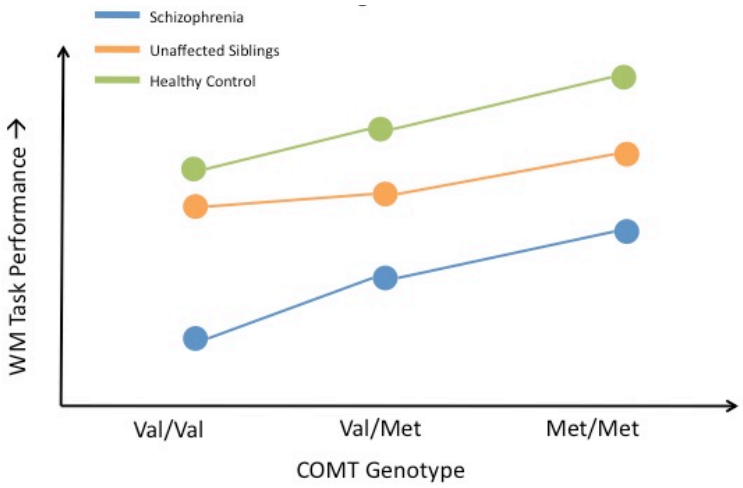
Cortical glutamatergic pyramidal neurons directly excite dopaminergic neurons projecting to the DLPFC and inhibit dopaminergic neurons projecting to the striatum via GABA interneurons. Mesencephalic DA neurons also project to GABA neurons in the DLPFC which in turn synapse in a linear array on pyramidal axon initial segments. GABA activity is believed to give rise to synchronized, oscillating firing of a neurons at 30–80 Hz. Oscillations at this range of frequencies in the human DLPFC increase in proportion to working memory load.

FIGURE 5
Dopamine Catabolism



http://images3.wikia.nocookie.net

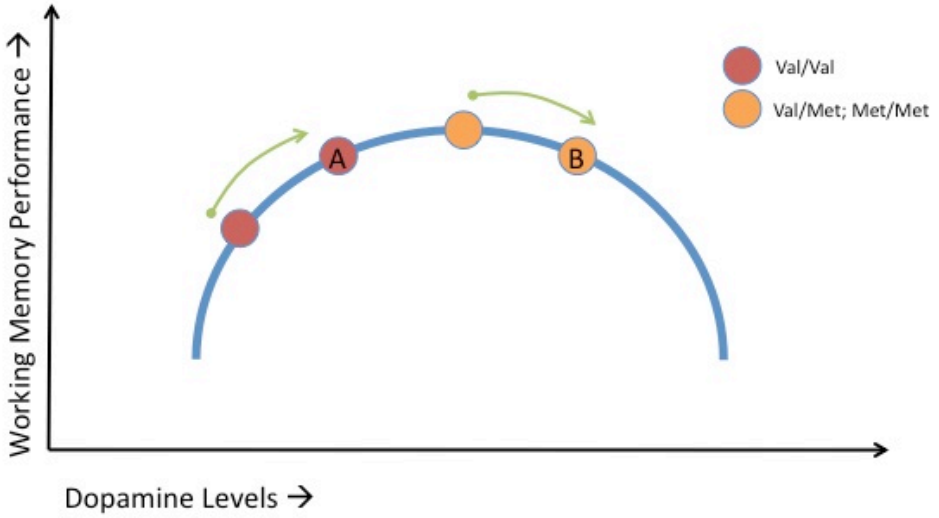
FIGURE 6
Composite Graph of Egan (2001) and Goldberg (2003)



Two early and influential studies have supported this hypothesis and demonstrated a relationship between COMT genotype and WM in patients with schizophrenia, their unaffected siblings, and healthy controls. Individuals with the COMT Val/Val genotype performed worse than those with Val/Met or Met/Met genotype on WM tasks, and the effect was independent of diagnostic group (i.e. patients, siblings, or controls) accounting for about 4% of performance on the Wisconsin Card Sorting Test (Egan, 2001) and 3% of performance on the N-Back test (Goldberg, 2003).

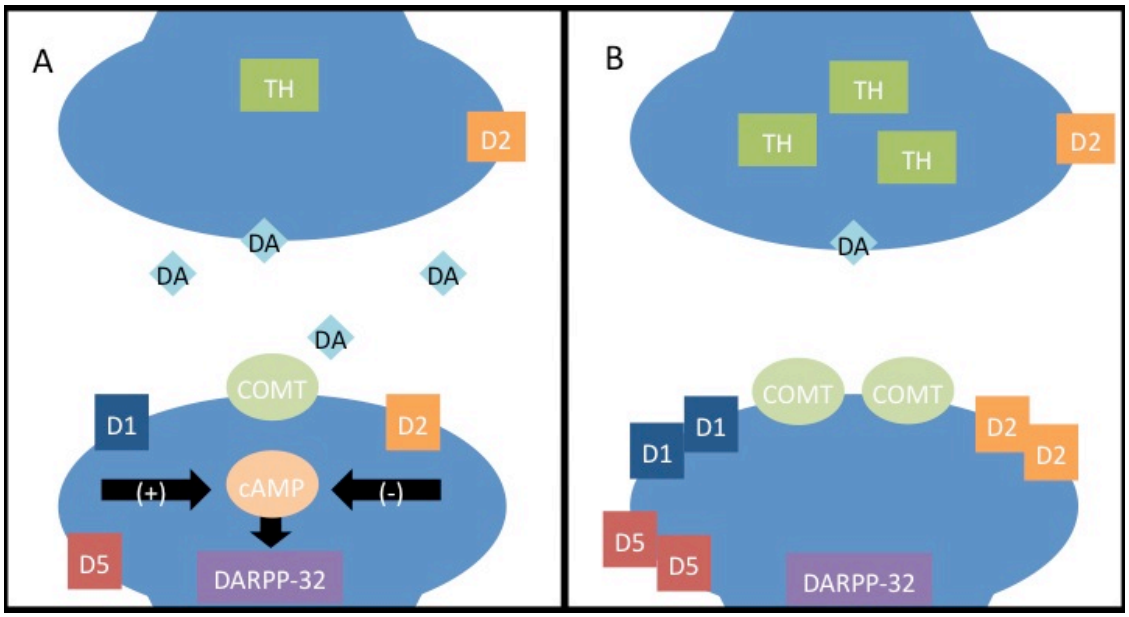
As predicted, patients performed worse than controls for all COMT genotypes, but interestingly, both studies demonstrated a near significant effect of diagnostic group in unaffected siblings independent of COMT genotype whose performance trended between the other two groups. The trend for poorer performance of siblings than healthy controls may indicate additional genetic factors contributing to DLPFC inefficiency and WM performance.

FIGURE 7
Inverted U-curve Model of Working Memory and Dopamine Levels



The COMT inhibitor tolcapone altered working memory in healthy controls with a significant interaction with COMT genotype, such that Val/Val individuals taking tolcapone showed better performance compared with placebo with an associated decrease in blood COMT activity (Apud, 2007). Val/Met and Met/Met showed poorer performance compared with their respective placebo controls corresponding with an inverted U-curve model of DA level and task performance, whereby too little or too much DA would result in poor WM performance and moderate DA would result in peak performance. A.) Val/Val individuals have a presumed deficiency in DA, and COMT inhibition with tolcapone would shift performance along the ascending limb of the performance curve. B.) For Val/Met and Met/Met individuals COMT inhibition would shift performance along the descending limb of the curve.

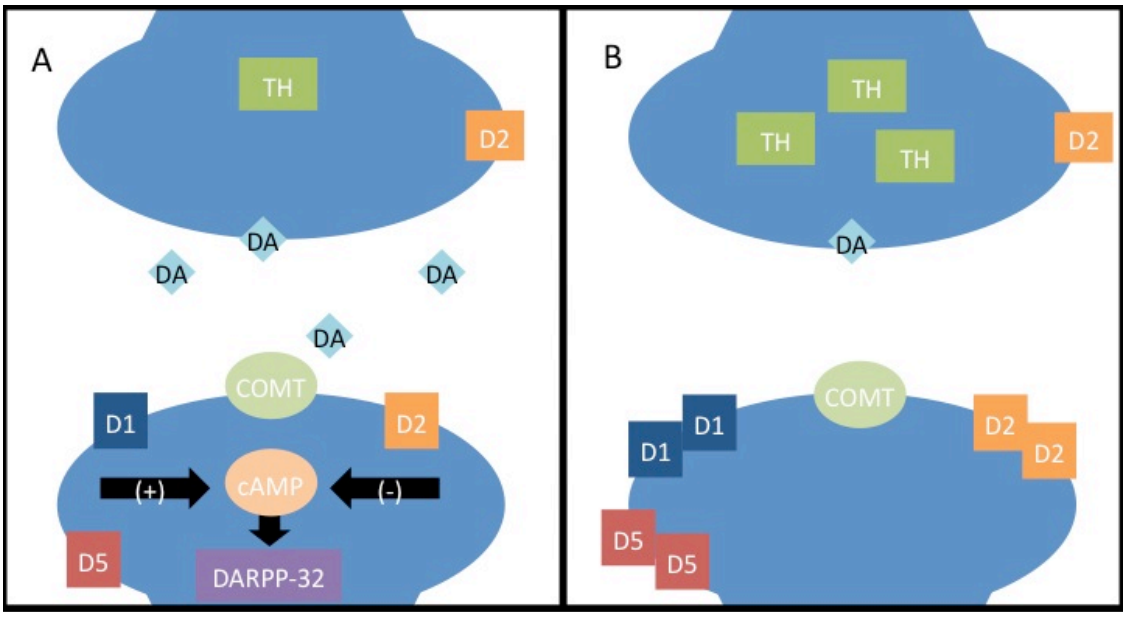
FIGURE 8
Effect of COMT Val/Val Genotype at the DA Synapse



A.) Typical dopaminergic synapse. B.) Effect of COMT Val/Val genotype. Because D_1 and D_2 DA receptor levels vary inversely to the relative amount of DA transmission at the synapse, they were used as measures of relative DA transmission. It was hypothesized that individuals who are homozygous for the high activity COMT-Val allele would have decreased levels of DA transmission in the DLPFC, as evidenced by an increase in D_1-type and D_2-type DA receptors, due to presumed increased catabolism of DA. It was also hypothesized based on the work of Chen and colleagues (2004) that while COMT enzyme would be higher in Val/Val individuals, transcription of the gene would be similar to other genotypes, as increased levels of the enzyme would be due to the thermostability of the protein and not due to transcriptional factors. Increased TH protein and mRNA was observed after DA depletion with chronic administration of

MPTP (Xu,, 2005;). Because of the hypothesized decrease in DA signaling for the COMT Val/Val genotype, increased levels of TH expression were expected. Levels of DARPP-32 protein and mRNA were unchanged in multiple regions of the rat brain after chronic blockade of D_1 receptor (Greb, 1990) or D_2 receptors by a number of typical antipsychotic medications (Souza, 2008). Because DARPP-32 expression seems independent of extracellular DA, no change was expected between the COMT genotypes.

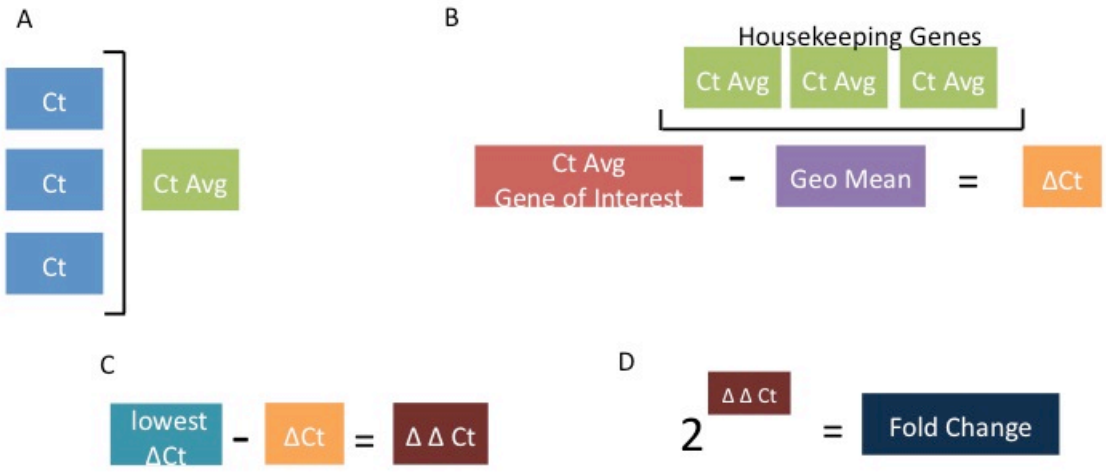
FIGURE 9
Effect of Schizophrenia at the DA Synapse



A.) Typical dopaminergic synapse. B.) Effect of schizophrenia. Because D1 and D2 DA receptor levels vary inversely to the relative amount of DA transmission at the synapse, they were used as measures of relative DA transmission. It was hypothesized that individuals with schizophrenia would have decreased levels of DA transmission in the DLPFC, as evidenced by an increase in D1-type and D2-type DA receptors. Because COMT transcription levels do not appear to alter in response to changes in extracellular DA, no difference in COMT expression was expected between schizophrenic subjects and healthy controls. Increased TH protein and mRNA was observed after DA depletion with chronic administration of MPTP (Xu,, 2005;). Because of the hypothesized decrease in DA signaling for persons with schizophrenia , increased levels of TH expression were expected. Reduced levels of DARPP-32 have been observed in DLPFC

postmortem tissue from persons with schizophrenia (Albert, 2002; Ishikawa,Masanori, 2007), a finding that was expected to be replicated.

FIGURE 10
Fold Change Calculations



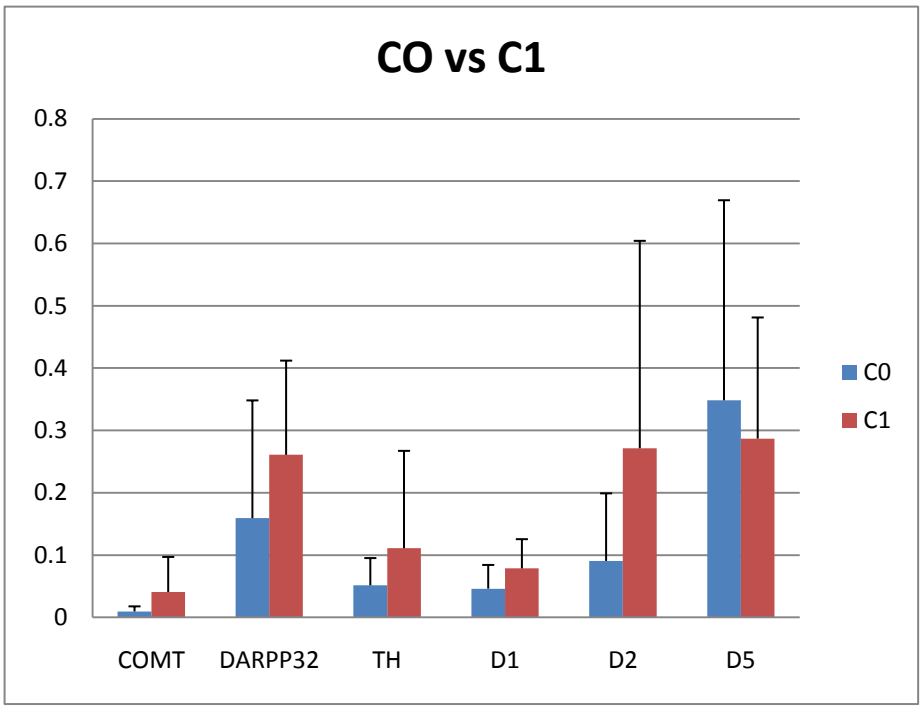
A.) Cycle threshold values (Ct values, the number of cycles before required before signal is detectable above background florescence) were calculated and for each sample at each primer.

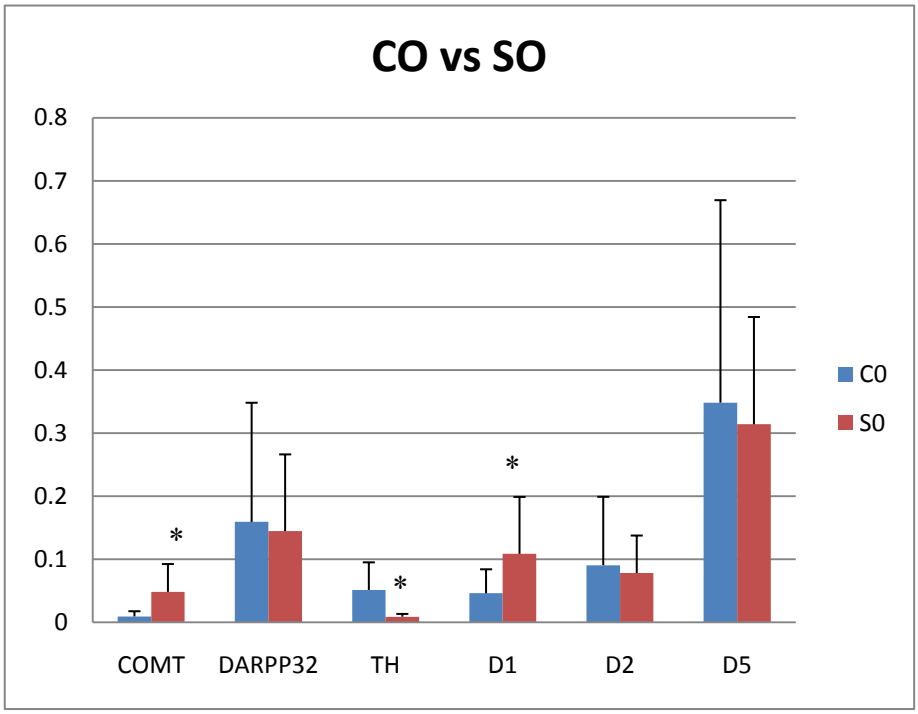
B.) Ct values for each gene of interest were compared to three common housekeeping genes, which included GAPDH, HPRT, and cyclophilin. For each sample the geometric mean of the Ct values of the housekeeping genes were subtracted from Ct value of the gene of interest to generate a ΔCt.

C.) For each gene of interest, the ΔCt values of each sample are compared to each other in terms of a single reference sample , done by subtracting each ΔCt value from the lowest ΔCt (the reference sample) to create a ΔΔCt.

D.) Because each PCR cycle represents a doubling of cDNA transcripts of the gene of interest, the relative fold difference is expressed as a fraction of the reference sample, calculated by $2^{\Delta\Delta Ct}$

FIGURE 11
Fold Changes of Genes of Interest





Panel 1) Two-tailed t tests of primary outcome measures between control COMT Val (C0) and Met (C1) groups were not significant. COMT genotype did not significantly effect gene expression in the current study. Two-tailed t tests of secondary outcome measures between control COMT Val and Met groups were not significant for an effect of genotype on DARPP-32 or TH expression

Panel 2) Between Val/Val schizophrenic (S0) and Val/Val control (C0) cases significance was achieved for COMT, $t(20) = -2.382$, $p = 0.028$, and D1 , $t(20) = -2.186$, $p = 0.041$, with schizophrenic cases expressing more COMT and D1. Gene expression of D2 and D5 was not significantly different between the two groups. Significance for TH did not dramatically change when covaried for age. Between Val/Val schizophrenic and Val/Val control cases, significance was achieved for TH, $t(16) = 2.530$, $p = 0.022$, with schizophrenic cases expressing less TH than control cases. Significance for TH did not dramatically change when covaried for age.

FIGURE 12
COMT Scatter Plot

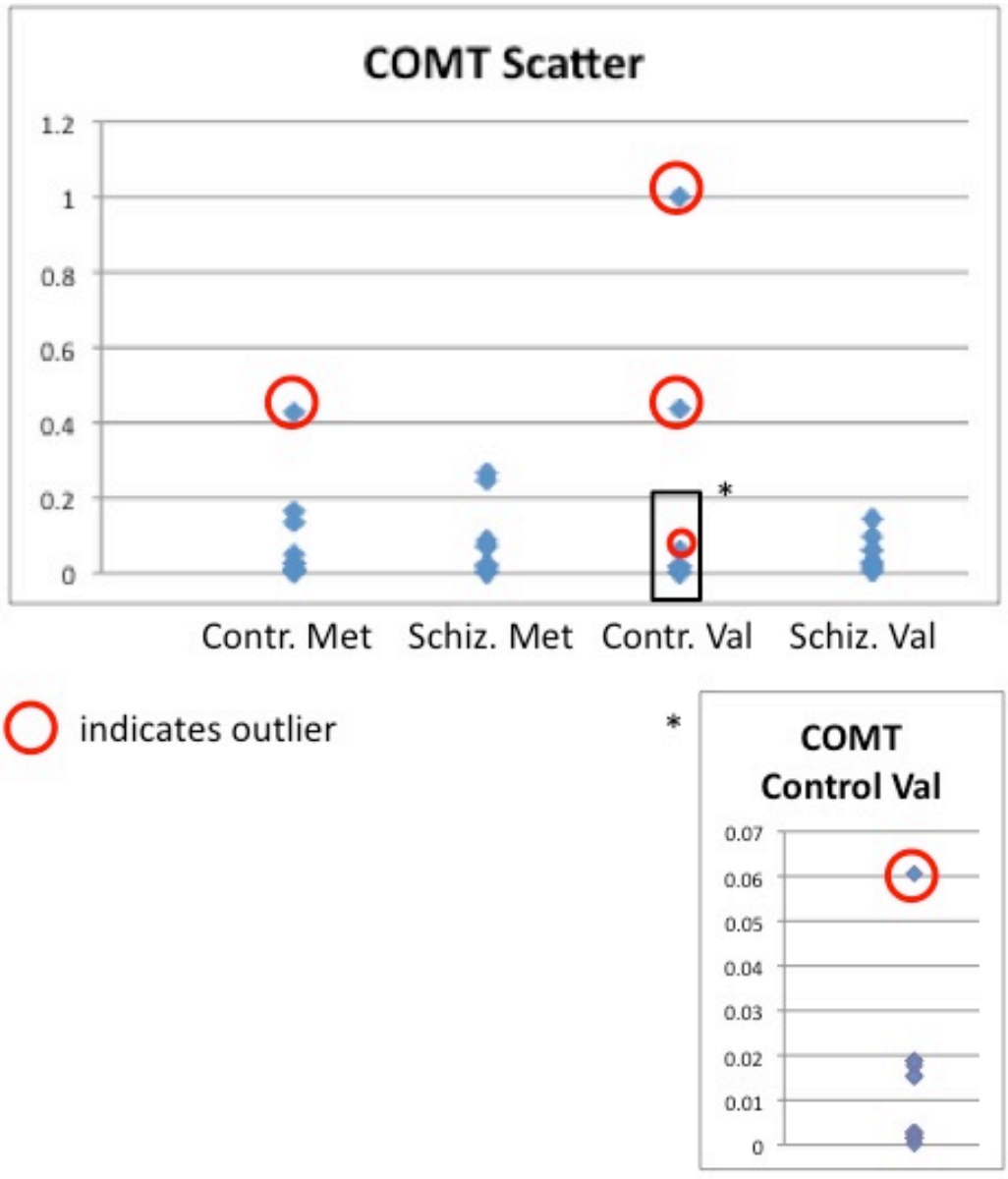


FIGURE 13
DARPP-32 Scatter Plot

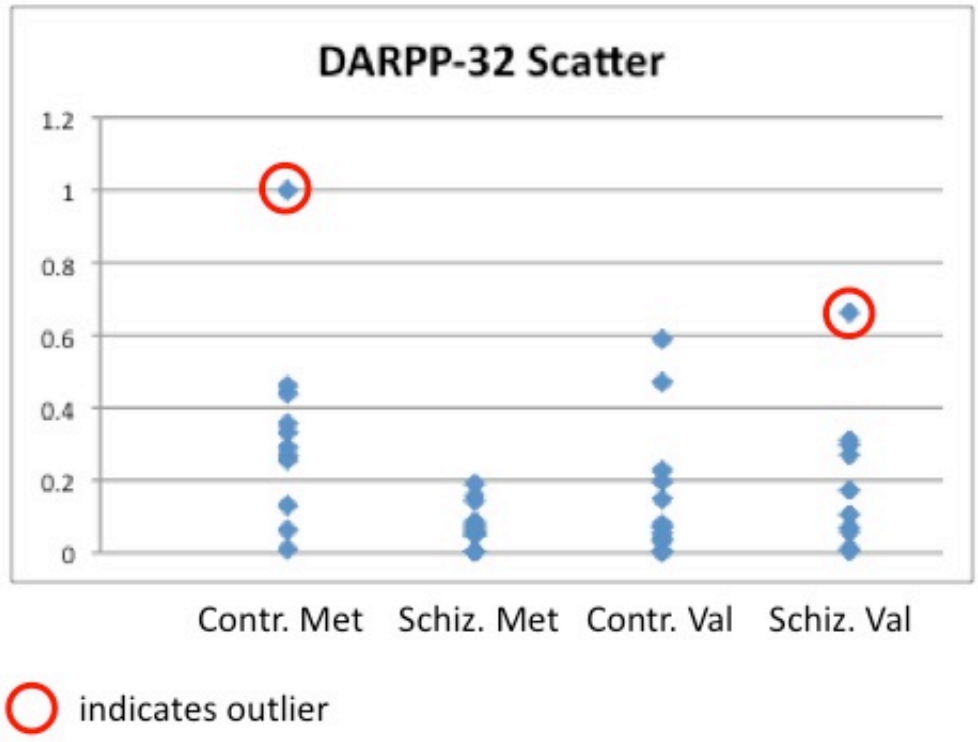
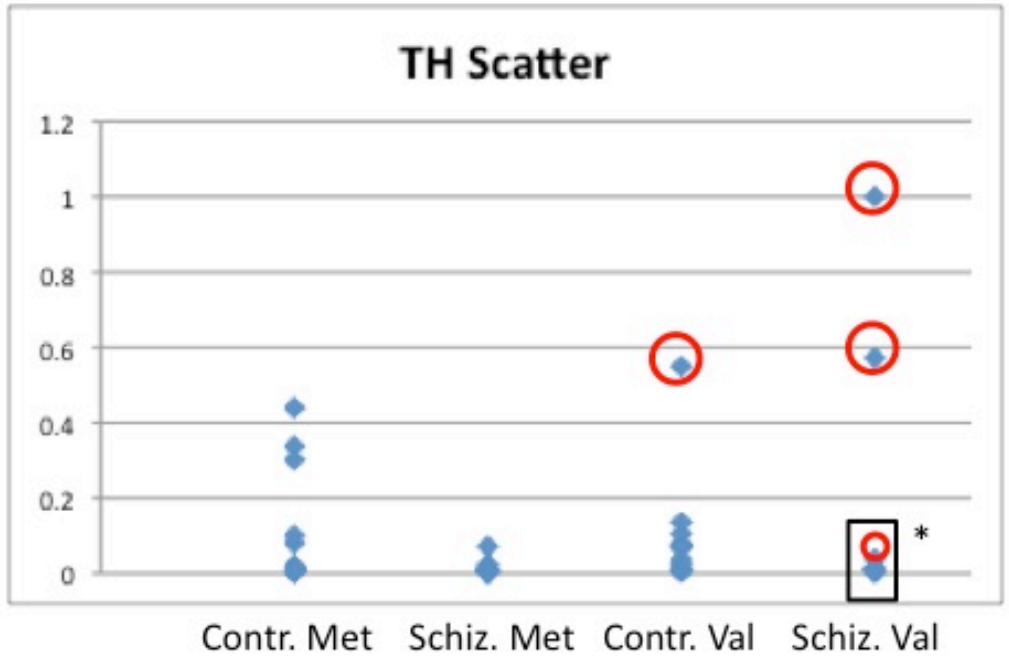


FIGURE 14
TH Scatter Plot



indicates outlier

*

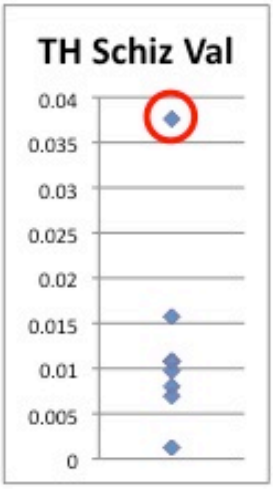


FIGURE 15
D1 Scatter Plot

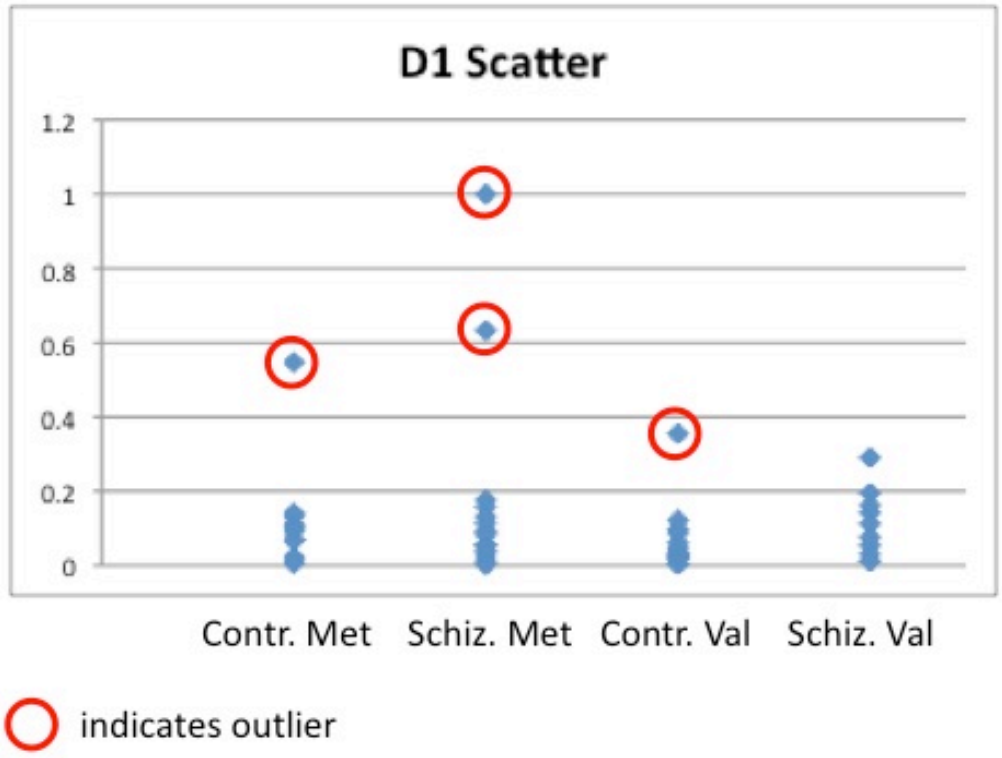


FIGURE 16
D2 Scatter Plot

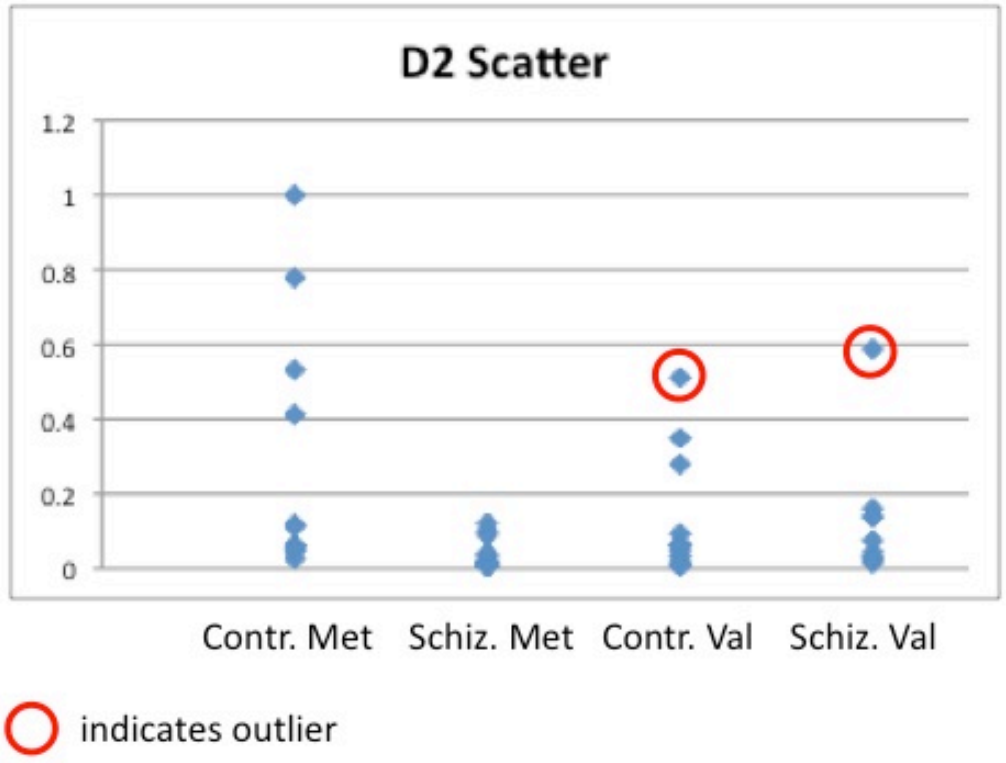


FIGURE 17
D5 Scatter Plot

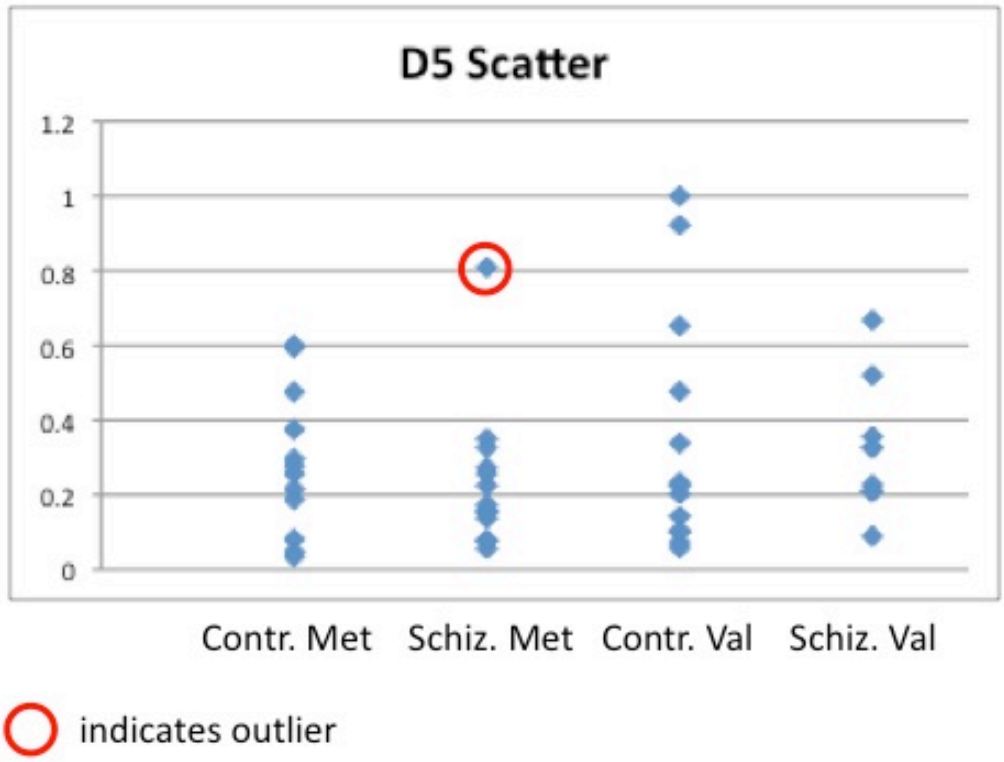


TABLE 1
Frequency of Symptoms of Schizophrenia

Table 1

Frequency of psychotic symptoms in schizophrenia

Symptom	Frequency
Lack of insight	97
Hallucinations (auditory and verbal)	70 - 74
Ideas of reference	70
Suspiciousness	65
Flatness of affect	65
Paranoid state	64
Thought alienation	52
Thoughts spoken aloud	50

(International Pilot Study of Schizophrenia, 1974)

TABLE 2
Subject Demographics

Table 2

Subject Demographics

			Control	Schiz
Val	**Mean Age**		50.9	46.7
	Sex	Male	13	6
		Female	0	4
	Race	Caucasian	12	6
		African American	0	4
		Latino	1	0
Met	**Mean Age**		53.4	44.0
	Sex	Male	8	9
		Female	4	5
	Race	Caucasian	12	13
		African American	0	1
		Latino	0	0

TABLE 3
Spearman Rank Order Correlations of Covariates

Table 3

Spearman Rank Order Correlations of Covariates

	COMT	DARPP-32	TH	D1	D2	D5
Age	0.046	0.149	0.340*	-0.228	0.206	0.035
PMI	0.008	0.197	-0.073	-0.073	0.062	0.011
RIN	0.196	0.017	0.093	-0.060	0.199	0.046

* $p \leq 0.05$

TABLE 4
All Group Means and Standard Deviations

Table 4

All Group Means and Standard Deviations

	Control				Schizophrenia			
	Val		Met		Val		Met	
	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
COMT	0.014	0.017	0.041	0.056	0.048	0.044	0.064	0.090
DRD1	0.046	0.038	0.079	0.047	0.109	0.090	0.079	0.057
DRD2	0.091	0.108	0.205	0.253	0.078	0.059	0.037	0.038
DRD5	0.294	0.266	0.287	0.194	0.314	0.170	0.186	0.094
DARPP-32	0.159	0.189	0.261	0.151	0.145	0.122	0.072	0.057
TH	0.051	0.044	0.111	0.156	0.009	0.004	0.007	0.007

TABLE 5
Primary and Secondary Analyses

Table 5

Primary and Secondary Analyses

Control Val Group vs. Control Met Group

	Val		Met				
	Mean	StDev	Mean	StDev	t-value	df	p
COMT	0.014	0.017	0.041	0.056	-1.508	20	0.147
DRD1	0.046	0.038	0.079	0.047	-1.835	21	0.081
DRD2	0.091	0.108	0.205	0.253	-1.433	21	0.167
DRD5	0.294	0.266	0.287	0.194	0.075	22	0.941
DARPP-32	0.159	0.189	0.261	0.151	-1.371	20	0.185
TH	0.051	0.044	0.111	0.156	-1.218	21	0.253†

Control Val Group vs. Schiz Val Group

	Control		Schiz				
	Mean	StDev	Mean	StDev	t-value	df	p
COMT	0.014	0.017	0.048	0.044	-2.382	19	0.028*
DRD1	0.046	0.038	0.109	0.090	-2.186	20	0.041*
DRD2	0.091	0.108	0.078	0.059	0.295	18	0.772
DRD5	0.294	0.266	0.314	0.170	-0.206	20	0.839
DARPP-32	0.159	0.189	0.145	0.122	0.202	19	0.842
TH	0.051	0.044	0.009	0.004	2.530	16	0.035*†

* indicates significance

† adjusted p value when covaried for age

TABLE 6
Complete Data Set with Fold Changes

Table 6

Complete Data Set with Fold Changes

Diagnosis	Genotype	Race	Sex	Age	PMI	RIN	COMT	DARPP	TH	DRD1	DRD2	DRD5
Control	VAL/VAL	Cauc.	M	16	16	8.3	0.0018	0.0792	0.0722	0.0294	0.0456	0.1429
Control	VAL/VAL	Cauc.	M	49	12	9.5	0.0152	0.0014	0.0675	0.0223	0.0933	0.1038
Control	VAL/VAL	Cauc.	M	74	41	6.8	0.0156	0.1968	0.0275	0.0348	0.0638	0.1008
Control	VAL/VAL	Cauc.	M	63	14	7.7	0.0030	0.4705	0.1360	0.0609	0.2792	0.4768
Control	VAL/VAL	Cauc.	M	47	9.37	8.9	0.0606	0.0708	0.0099			0.6526
Control	VAL/VAL	Cauc.	M	60	20	8.5		0.0317	0.0369	0.0876	0.3489	
Control	VAL/VAL	Cauc.	M	63	12	4.9		0.0531	0.0159	0.0031	0.0059	0.0729
Control	VAL/VAL	Cauc.	M	64	13	8.1	0.0025	0.1481		0.0274	0.0206	0.2333
Control	VAL/VAL	Cauc.	M	66	16	6	0.0015	0.0376	0.0792	0.0182	0.0327	0.2020
Control	VAL/VAL	Cauc.	M	50	16	6.5	0.0003	0.0055	0.1053	0.0047	0.0112	0.0582
Control	VAL/VAL	Cauc.	M	25	19	8.4	0.0177			0.0461	0.0625	0.3382
Control	VAL/VAL	Cauc.	M	36	23	7.4	0.0187	0.2278	0.0026	0.0970	0.0585	0.2243
Control	VAL/VAL	Lat.	M	49	24	9.5	0.0189	0.5887	0.0132	0.1219	0.0662	0.9223
Control	VAL/MET	Cauc.	M	48	13.3	3.3	0.1349		0.0186	0.0689		0.0472
Control	VAL/MET	Cauc.	M	54	15	9.5	0.1654	0.0104	0.3024	0.0926	0.5322	0.0346
Control	VAL/MET	Cauc.	F	68	9	8.2		0.0632	0.3374		0.7786	0.1878
Control	MET/MET	Cauc.	M	31	16.16	8.1	0.0094	0.4394	0.0805	0.1406	0.1177	0.2771
Control	MET/MET	Cauc.	M	60	17.1	9.8	0.0035	0.3321	0.4399	0.0155	0.4126	0.2567
Control	MET/MET	Cauc.	M	20	21.22	8.2	0.0098	0.2685	0.0034	0.1333	0.0619	0.3752
Control	MET/MET	Cauc.	M	43	15	6.1	0.0103	0.2563	0.0093	0.1001	0.0622	0.2963
Control	MET/MET	Cauc.	M	72	17.5	7.8	0.0132	0.4607	0.0089	0.0683	0.0510	0.6003
Control	MET/MET	Cauc.	M	60	30	8.7	0.0277	0.3564	0.0057	0.1097	0.0472	0.2157
Control	MET/MET	Cauc.	F	83	30	8.5	0.0012	0.1307	0.1004	0.0061	0.0265	0.0812
Control	MET/MET	Cauc.	F	52	19.75	5.3	0.0239	0.2907	0.0052	0.1081	0.0536	0.4756
Control	MET/MET	Cauc.	F	50	23	4.6	0.0502		0.0199	0.0221	0.1132	0.5948
Schiz	VAL/VAL	Af. Amer.	M	41	9.45	8.1	0.0160	0.2701	0.0098	0.1419	0.0433	0.5198
Schiz	VAL/VAL	Af. Amer.	M	44	20	9.1	0.0972	0.0580		0.0115		0.3555
Schiz	VAL/VAL	Af. Amer.	M	53	18.3	8.6	0.0263	0.0127	0.0157	0.0126	0.0150	0.0892
Schiz	VAL/VAL	Af. Amer.	F	59	9	2.5	0.0280	0.2969	0.0080	0.1620	0.1382	0.3278
Schiz	VAL/VAL	Cauc.	M	32	27	7.4	0.0603	0.1054	0.0012	0.0541	0.0315	0.2075
Schiz	VAL/VAL	Cauc.	M	52	21	9.3	0.1451	0.1725	0.0069	0.1123	0.0748	0.3253
Schiz	VAL/VAL	Cauc.	M	47	17	8.6	0.0314	0.3102		0.0759	0.1407	0.2110
Schiz	VAL/VAL	Cauc.	F	32	11	6.9	0.0037	0.0053	0.0109	0.2906	0.0225	0.2271
Schiz	VAL/VAL	Cauc.	F	57	16	7.8	0.0133	0.0710		0.0308		0.2100
Schiz	VAL/VAL	Cauc.	F	50	27	8.5	0.0623		0.0107	0.1949	0.1602	0.6676
Schiz	VAL/MET	Cauc.	M	26	24		0.0009	0.0040			0.0139	0.0760
Schiz	VAL/MET	Cauc.	M	52	8	7.5	0.2466	0.0636	0.0051	0.1317	0.1012	0.2726
Schiz	VAL/MET	Cauc.	M	51	14	9.1	0.0736	0.0586	0.0029	0.0298	0.0099	0.1738
Schiz	VAL/MET	Cauc.	M	22	15	7.9	0.0180	0.0785	0.0021	0.0931	0.0289	0.1606
Schiz	VAL/MET	Cauc.	F	43	26	9.2		0.0026	0.0101		0.0047	0.0800
Schiz	VAL/MET	Cauc.	F	46	23	9.2	0.0882	0.0709	0.0007	0.0534	0.0173	0.2565
Schiz	VAL/MET	Cauc.	F	43	11	6.9	0.0014	0.0033	0.0235	0.1762	0.0062	0.0560
Schiz	VAL/MET	Cauc.	F	22	12	4.3	0.0705	0.0501	0.0014	0.0416	0.0126	0.1482
Schiz	VAL/MET	Cauc.	F	71	17.2	8.6	0.2667	0.0866	0.0031	0.1578	0.0890	0.2252
Schiz	MET/MET	Af. Amer.	M	57	12	8.1	0.0073	0.1900	0.0049	0.0827	0.0132	0.3501
Schiz	MET/MET	Cauc.	M	43	19	8	0.0075	0.0505	0.0190	0.0136	0.0409	0.1553
Schiz	MET/MET	Cauc.	M	59	11	3.7	0.0238	0.0517	0.0048	0.0554	0.0347	0.3265
Schiz	MET/MET	Cauc.	M	33	20	8.4	0.0068	0.1435	0.0036	0.1139	0.1210	0.1374

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