Imaging and Genetics Investigations in Schizophrenia and Aging: A Focus on White Matter

by

Aristotle Nicholas Voineskos

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Medical Science
University of Toronto

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University of Toronto

2010

Abstract

Schizophrenia has long been considered a disorder of impaired brain connectivity, and such disconnectivity might be due to disruption of white matter tracts that connect brain regions. This thesis investigates the oligodendrocyte/myelin/white matter pathway in schizophrenia in vivo, and also considers aging effects, as similar substrates are affected during the healthy aging process. In study one, association of oligodendrocyte/myelin genes is examined with schizophrenia, and in study two association of a myelin gene is examined with basic MRI volumetric phenotypes. Then, in study three, diffusion tensor tractography, a technique that can visualize and measure white matter is applied, and is shown to be reliable in healthy controls and schizophrenia patients using a novel clustering segmentation method. In study four, this method is then used to examine interaction of schizophrenia and aging with respect to white matter, where fronto-temporal disconnectivity is demonstrated in younger chronic schizophrenia patients, but not in elderly community dwelling schizophrenia patients compared to age-matched controls. In study five, relationships among age, white matter tract integrity, and cognitive decline in healthy aging are demonstrated using diffusion tensor tractography and structural equation modeling. Genetics and neuroimaging are then combined using the intermediate phenotype approach in study six to demonstrate a key role for the BDNF gene across adult life in...
healthy aging. In these individuals, the BDNF val66met variant influenced neural structures and cognitive functions in a pathological aging risk pattern. Finally, in study seven, complex relationships are then demonstrated among oligodendrocyte gene variants, white matter tract integrity and cognitive performance in both healthy controls and schizophrenia patients. The combination of genetics and neuroimaging can parse out heterogeneity of disease phenotypes, and characterize the effects of gene variants on at-risk neural structures and cognitive functions in healthy and disease populations.
Acknowledgments

I would like to thank my PhD Supervisor, Dr. James Kennedy, and my PhD Committee Members, Drs. Nancy Lobaugh, Albert Wong, and Bruce Pollock for all their help, time, and commitment. In particular, Dr. Kennedy has been extremely supportive, and a wonderful mentor over many years, and has helped me realize my vision of doing imaging-genetics research. I am also very thankful to Dr. Pollock for his mentorship and unwavering support, and his encouragement to study individuals across the adult lifespan. Dr. Lobaugh has provided a degree of scientific rigour to my work and guidance that I am very thankful for. I would also like to thank Dr. Benoit Mulsant for his supervision and support, and for making recruitment possible in the first place. Dr. Martha Shenton has also been very supportive, and a great mentor, and I appreciate the time in her lab. Dr. Randy McIntosh and Natasa Kovacevic have also been very helpful with PLS, and I appreciate their generosity and support. Dielle Miranda played a critical role in recruitment and study management, and I genuinely appreciate her tremendous efforts. I would also like to thank Dr. Jeff Daskalakis for his mentorship, friendship, and exchange of scientific ideas.

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<td>DTI</td>
<td>diffusion tensor imaging</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>DSM-IVTR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Classification of Disease</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-amino-butyric acid</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-d-aspartic acid</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>NRG</td>
<td>neuregulin</td>
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<tr>
<td>ErbB4</td>
<td>tyrosine kinase erbb4 receptor</td>
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<td>ZNF804A</td>
<td>zinc finger 804a</td>
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<tr>
<td>CNV</td>
<td>copy number variants</td>
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<tr>
<td>BA</td>
<td>brodmann area</td>
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<td>MAG</td>
<td>myelin associated glycoprotein</td>
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<tr>
<td>CNP</td>
<td>2’,3’, cyclic nucleotide – 3’ - phosphodiesterase</td>
</tr>
<tr>
<td>ErbB3</td>
<td>tyrosine kinase erbb3 receptor</td>
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<tr>
<td>QKI</td>
<td>quaking</td>
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<tr>
<td>TF</td>
<td>transferrin</td>
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<tr>
<td>PLP-1</td>
<td>preteolipid protein1</td>
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<tr>
<td>MOG</td>
<td>myelin oligodendrocyte glycoprotein</td>
</tr>
<tr>
<td>OLG2</td>
<td>oligodendrocyte transcription factor-2</td>
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<tr>
<td>OMR</td>
<td>oligodendrocyte and myelin related</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>RTN4R</td>
<td>Nogo receptor</td>
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<tr>
<td>Pos</td>
<td>positive</td>
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<tr>
<td>Neg</td>
<td>negative</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>CT</td>
<td>computer assisted tomography</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>FA</td>
<td>fractional anisotropy</td>
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<tr>
<td>MD</td>
<td>mean diffusivity</td>
</tr>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
</tr>
<tr>
<td>$D_L$</td>
<td>axial diffusivity</td>
</tr>
<tr>
<td>$D_R$</td>
<td>radial diffusivity</td>
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<tr>
<td>VBM</td>
<td>voxel based morphometry</td>
</tr>
<tr>
<td>TBSS</td>
<td>tract based spatial statistics</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
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<tr>
<td>FACT</td>
<td>fiber assessment by continuous tracking</td>
</tr>
<tr>
<td>CB</td>
<td>cingulum bundle</td>
</tr>
<tr>
<td>UF</td>
<td>uncinate fasciculus</td>
</tr>
<tr>
<td>IFOF</td>
<td>inferior occipitofrontal fasciculus</td>
</tr>
<tr>
<td>ILF</td>
<td>inferior longitudinal fasciculus</td>
</tr>
<tr>
<td>AF</td>
<td>arcuate fasciculus</td>
</tr>
<tr>
<td>SLF</td>
<td>superior longitudinal fasciculus</td>
</tr>
<tr>
<td>CST</td>
<td>corticospinal tract</td>
</tr>
<tr>
<td>FH</td>
<td>fornix of hippocampus</td>
</tr>
<tr>
<td>SOFF</td>
<td>superior occipitofrontal fasciculus</td>
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<tr>
<td>ATR</td>
<td>anterior thalamic radiation</td>
</tr>
<tr>
<td>CC</td>
<td>corpus callosum</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>SCP</td>
<td>superior cerebellar peduncle</td>
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<tr>
<td>MPFC</td>
<td>medial prefrontal cortex</td>
</tr>
<tr>
<td>STG</td>
<td>superior temporal gyrus</td>
</tr>
<tr>
<td>ITG</td>
<td>inferior temporal gyrus</td>
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<tr>
<td>MTG</td>
<td>medial temporal gyrus</td>
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<tr>
<td>OPS</td>
<td>occipitoparietal sulcus</td>
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<td>ACC</td>
<td>anterior cingulate cortex</td>
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<td>MTL</td>
<td>medial temporal lobe</td>
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<td>STS</td>
<td>superior temporal sulcus</td>
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<td>FMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>mini mental status examination</td>
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<td>APOE4</td>
<td>apolipoprotein epsilon 4</td>
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<td>BDNF</td>
<td>brain derived neurotrophic factor</td>
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<tr>
<td>5HTTLPR</td>
<td>serotonin transporter polymorphism</td>
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<tr>
<td>FDR</td>
<td>false discovery rate</td>
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<tr>
<td>PLS</td>
<td>partial least squares</td>
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<td>ICA</td>
<td>independent components analysis</td>
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<tr>
<td>SVD</td>
<td>singular value decomposition</td>
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<td>latent variable</td>
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<tr>
<td>LD</td>
<td>linkage disequilibrium</td>
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<td>transmission disequilibrium test</td>
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<td>family based association test</td>
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<td>fmrif software library</td>
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<td>montreal neurologic institute</td>
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<td>PANSS</td>
<td>positive and negative syndrome scale</td>
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<tr>
<td>Acronym</td>
<td>Description op</td>
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<tr>
<td>RBANS</td>
<td>repeated battery for the assessment of neuropsychological status</td>
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<td>alzheimer’s disease</td>
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<td>orientation distribution function</td>
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<td>magnetization transfer ratio</td>
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<td>fasciculation and elongation protein zeta-1</td>
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Chapter 1

1 Introduction

1.1 Schizophrenia: A Brief History

Early descriptions of schizophrenia may date back to ancient Hebrew times in the Bible, in Deuteronomy. However, others have suggested that mental illness, including schizophrenia was documented first during ancient Egyptian times. Despite these early accounts, descriptions of schizophrenia in ancient times are puzzlingly sparse, at least in comparison to what appear to be descriptions of mood disorders (e.g. melancholia, well-described in ancient Greek times). It has been suggested that this discrepancy was due to explanations of symptoms and mental phenomena that were contextualized in religious terms (Stone 2006). In addition, cultural beliefs were likely used to explain mental illness, for instance, through beliefs in lycanthropy (Alexander and Selesnick 1966). Perhaps one of the earliest, least ambiguous, descriptions of what might have been schizophrenia occurred during the height of the Byzantine empire in the sixth century A.D., when a personal physician of the Emperor Justinian, described a dementia in young people, who previously had ‘modest, but intact minds’, who did not appear delirious, and showed deterioration of mental function without clouding of consciousness (Zilboorg 1941). Centuries later, descriptions of severe mental illness emerged from medieval Europe in afflicted European Royal Families. Some time later, Thomas Willis, known better for his discovery of the ‘Circle of Willis’, described what was almost certainly schizophrenia, particularly as it pertained to negative symptoms. He wrote about young persons, often intelligent in childhood, who ended up in adolescence ‘enfeebled and dull, who became, duller, foolish, and insipid’(Stone 2006).
Descriptions of patients with schizophrenia began to abound by the nineteenth century from the Bethlem hospital in England, the Charite hospital in Berlin, and in other European countries as well (Stone 2006). However, it was a challenge for some to describe schizophrenia as a unitary entity, and Heinrich Neumann wrote: ‘there is but one type of mental disturbance and we call it insanity’ (Neumann 1859; Stone 2006). Benedict Morel, described a more phenotypically homogenous group of patients who suffered what he termed ‘demence precoce’, the French term for dementia praecox, utilized by Emil Kraepelin to describe schizophrenia patients at the end of the nineteenth century. Morel observed breakdown of mental compartments of feeling, understanding and acting (affect, thought, and behaviour), and in addition, noted that these patients often had a ‘hereditary weakness’ and had parents who were afflicted with addiction or other psychiatric conditions (Morel 1860; Stone 2006). Therefore, Morel provided one of the first descriptions of the significant genetic component of severe mental illness.

The modern ‘fathers’ of descriptions of schizophrenia are most certainly Emil Kraepelin and Eugen Bleuler. Kraepelin was the first person to characterize the prognosis of schizophrenia in detail. He suggested that dementia praecox began in early adolescence or adulthood, and ended in chronic mental deterioration (Kraepelin 1893; Stone 2006). Bleuler changed the term dementia praecox to schizophrenia. He did so in part both because he was more optimistic about prognosis, and he believed that the term schizophrenia was less pessimistic. Furthermore, he described the primary symptoms of schizophrenia as autism, ambivalence, loosening of associations, and inappropriate affect, and believed that delusions, hallucinations, and thought disorder were secondary symptoms (Bleuler 1911; Stone 2006). In his conceptualization of schizophrenia as a brain disorder, Kraepelin considered schizophrenia as a disconnection syndrome. Wernicke took that conceptualization one step further, and suggested that fronto-
temporal disconnection was present (Kubicki, Westin et al. 2005), and such disconnection was
due to disruption of the large fronto-temporal white matter tracts that connected brain regions
However, neither Kraepelin nor Wernicke discovered any evidence for their theories, despite the
postmortem investigations by Alois Alzheimer, who Kraepelin hired to help investigate the
neuropathology of schizophrenia. Without any biological evidence for their theories, the
conceptualization of schizophrenia moved toward the psychological domain in the mid 20th
century, and was even labeled as a ‘reaction’. However, others maintained that schizophrenia
was a biological entity, and continued to attempt to refine the description of schizophrenia as the
20th century progressed. The advent of in vivo neuroimaging techniques in the 1970s in humans
turned schizophrenia studies back toward the biological domain, and a return to investigations
examining the underlying pathology of the disease (Harrison 1999).

Since the beginning of last century, the same challenges facing neurobiologically oriented
investigators remain, i.e. prognostic identification (progressive vs. static course of disease),
phenotypic heterogeneity and genetic pleiotropy (vulnerability within families to different mental
disorders) are all still not well-understood. Although there has been an explosion of
neurobiological investigation in schizophrenia over the past 30 years, some of which will be
reviewed herein, identification of consistent pathophysiologic markers of disruption in this
disorder remains elusive. A major challenge and source of potential progress, is the identification
of the genetic risk factors of disease as they pertain to the brain structures or circuitry vulnerable
in this illness (Akil, Brenner et al.).
1.2 Epidemiology, Diagnosis and Prognosis

The currently utilized diagnostic criteria for schizophrenia, present in the DSM-IVTR or the ICD-10, are neo-Kraepelinian, based in considerable part on positive symptoms (i.e. delusions and hallucinations) although some of the negative symptoms described by Bleuler are incorporated. These criteria include two or more of the following, each present for a significant portion of time during a one month period (or less if successfully treated): (1) delusions, (2) hallucinations, (3) disorganized speech, (4) grossly disorganized or catatonic behavior, (5) negative symptoms (i.e. affective flattening, alogia, or avolition. There must also be social or occupational dysfunction for a significant portion of the time since the onset of the disturbance, and continuous signs of the disturbance must persist for at least 6 months, with at least one month of active phase symptoms (AmericanPsychiatricAssociation 2000). The DSM criteria are fairly reliable, and permit epidemiologic investigation. Epidemiologic studies have shown that the range in point prevalence of schizophrenia is from 2.7 to 8.3 per 1000 individuals (Eaton and Chen 2006). The approximate lifetime prevalence estimate in the U.S. and Canada is one per one hundred. The greatest peak of schizophrenia onset is in the decade of 15-24 years of age (Eaton and Chen 2006). However, women tend to have a later age of onset than men, and also have a small second peak of onset in the 55-64 years of age decade. Furthermore, men may be more impaired by negative symptoms and tend to have worse outcome and poorer social functioning than women (Sadock and Sadock 2007). Overall, patients with schizophrenia have a much shorter life expectancy (15-20 years less) than the general population, even when those that die from suicide are removed from analysis (Tiihonen, Lonnvqvist et al. 2009). Living in a developed country has also been associated with poorer prognosis, as have social variables such as socioeconomic position and marital status, as well as substance abuse (Eaton and Chen 2006). An important
predictor of relapse is the phenomenon of ‘expressed emotion’ in families, essentially serving as a stressor that may accentuate or worsen symptomatology, or trigger relapse (Sadock and Sadock 2007). Finally, cognitive status (or performance) is a well-known predictor of outcome and real-world function (Green 1996). Cognitive performance has become an area of intense research focus in schizophrenia over the last ten to twenty years, and cognitive impairment is now considered a core component of the illness, although it is not specifically a diagnostic criterion for the disease itself (Rajji and Mulsant 2008).

1.3 Risk Factors

Few risk factors have been conclusively identified for schizophrenia. The most certain and important common risk factor is family history. Monozygotic twin concordance rate for schizophrenia is approximately 50 percent, whether twins are reared together or apart, suggesting that ‘environment’ is much less important than biology in this disease (Wong, Gottesman et al. 2005). In addition, the more genetically proximal an affected relative, or the greater the number of affected relatives, the greater the likelihood an individual will develop schizophrenia (Sadock and Sadock 2007). More recently, however, certain environmental risk factors have gained prominence. Cannabis use may increase risk of developing schizophrenia, although it might be more true to assert that there is a link between cannabis use and psychosis (van Os, Bak et al. 2002). Studies examining individuals of Caribbean origin in western countries, such as the United Kingdom, suggest that being of African or Caribbean descent in these countries is a risk factor. Thus, some researchers suggest that social discrimination may increase risk for schizophrenia, since other immigrant groups do not demonstrate increased risk in the U.K. in the same manner (Eaton and Chen 2006). Relative risk for those born in urban areas has been shown to be two to four times higher. Other risk factors may include winter birth, birth complications,
maternal malnutrition, and maternal influenza or other infections during pregnancy. Mechanisms of disease development (i.e. altering neurodevelopment) via these risk factors may occur through hypoxia-ischemia, or through infection or inflammation via cytokines, generated in response to maternal infection (Eaton and Chen 2006). Finally, in terms of rare events, the 22q11 deletion syndrome that occurs one in 4000 births, confers a 20-25 fold increased risk of developing schizophrenia (Bassett and Chow 1999).

1.4 Treatment

Antipsychotic medications form the mainstay of treatment for schizophrenia. The discovery of the antipsychotic property of chlorpromazine paved the way for the design of antipsychotic, or neuroleptic medications (Lopez-Munoz, Alamo et al. 2005). Current antipsychotic medications share one feature: their affinity for the dopamine D2 receptor (Kapur 2003). The seminal paper by Seeman et al (Seeman, Lee et al. 1976), demonstrated that antipsychotic medication affinity for the D2 receptor was directly related to the treating dose for antipsychotic efficacy. There was initial enthusiasm for the ‘atypical’ or ‘second generation’ antipsychotics that were characterized by affinity for the 5HT2A receptor along with some D2 affinity for their potential to treat negative and even possibly cognitive symptoms. However, recent clinical effectiveness trials have dampened enthusiasm for any initial claims of greater efficacy (Lieberman, Stroup et al. 2005). Furthermore, despite early claims, atypical antipsychotic medications may be no better at improving negative or cognitive symptoms compared to the older first generation antipsychotics (Lieberman, Stroup et al. 2005). Even newer antipsychotics such as ziprasidone and aripiprazole, that agonize other dopamine receptors, provide no added benefit in terms of efficacy. Clozapine stands alone in its improved efficacy compared to other antipsychotic medications, at least in
treatment refractory cases (Remington 2003). Preliminary studies examining clozapine in a first episode population that failed one antipsychotic medication trial suggest that clozapine may even have benefit in the first episode population (Agid, Remington et al. 2007). However, a study that examined clozapine in never medicated patients, suggested no improved efficacy in this population (Woerner, Robinson et al. 2003). The finding that D2 receptor occupancy in vitro was related to clinically effective doses contributed to a wealth of investigations in neurogenetics, receptor-ligand imaging in positron emission tomography, and in receptor interactions in biochemistry, examining the dopaminergic system (Kapur, Zipursky et al. 2000). However, despite an improved understanding of the relationship between D2 receptor occupancy, treatment response, and side effects, there has been no concomitant improvement in therapeutics. As a result, other medications have been developed that target other neurochemical systems, such as the glutamatergic system (e.g. glycine), particularly for the purpose of treating negative and cognitive symptoms. However, no consistent therapeutic effect of these medications has been demonstrated either (Buchanan, Javitt et al. 2007). Other investigators are currently focusing on treatments that may be ‘neuroprotective’ (Lee, Pei et al. 2007), and yet others are utilizing brain stimulation, to investigate possible effects on cognitive symptoms, possibly via GABA-ergic inhibitory mechanisms (Barr, Farzan et al. 2009). In summary, dopamine D2 antagonists provide relief primarily for positive symptoms, and new treatment targets and drug development are required in order to more effectively target negative symptoms and cognitive deficits.

1.5 Neuropathology of Schizophrenia

Despite the known dopaminergic mechanisms of antipsychotic medication, dopamine-related findings in schizophrenia pathophysiology are often inconsistent and in fact, are not what stand
out in post-mortem schizophrenia brain. In fact, schizophrenia was famously described as the graveyard of neuropathologist in 1972 by Plum (Harrison 1999). It was the *in vivo* findings of enlarged lateral ventricles in schizophrenia using computer assisted tomography imaging in 1976 by Johnstone and Crow (Johnstone, Crow et al. 1976) that re-galvanized the search for the underlying neurobiology and neuropathology of schizophrenia. The review by Harrison provides an outstanding summary of findings until 1999, and highlights the common and disparate findings in schizophrenia neuropathology (Harrison 1999).

Schizophrenia is thought to be, for the most part, a neurodevelopmental disorder. Although neurodegenerative findings have been shown (e.g. gliosis), they are not commonly found, and do not align with most common clinical presentations, where a minority of patients demonstrates a deteriorating course of illness. Early findings of gliosis in schizophrenia, supported a number of etiopathogenic possibilities, including infective, ischemic, autoimmune or neurodegenerative processes. However, the majority of subsequent studies did not find gliosis, although gliosis was observed in demented schizophrenics (Arnold, Franz et al. 1996). The absence of gliosis has been taken as evidence for an early neurodevelopmental origin of schizophrenia, though one cannot make such a conclusion with absolute certainty. More consistent findings include enlarged ventricles, decreased brain volume, and decreased neuropil (i.e. axons and dendrites). Key macroscopic features suggest a decrease in brain weight, brain length, and volume of the cerebral hemispheres (Pakkenberg 1987). Reduced size of the temporal lobe (Falkai, Bogerts et al. 1988), decreased thalamic volume (Pakkenberg 1990) and increased volume of basal ganglia (Heckers, Heinsen et al. 1991) are also replicated findings.

By 1999, MRI had replaced neuropathology in eliciting macroscopic features of brain change in schizophrenia, and investigators began to focus more on microscopic changes. The increasingly
sophisticated measurement techniques of cortical cytoarchitecture produced a wealth of findings at the more microscopic level. However, it is important to note that there are caveats to nearly all of these findings, and none have been consistently replicated. Studies of neurons find cytoarchitectural abnormalities in entorhinal cortex (Jakob and Beckmann 1986) (disturbance in location, clustering and/or size). Also, disarray of hippocampal pyramidal cell neurons and abnormal distribution of cortical subplate neurons (Akbarian, Bunney et al. 1993; Akbarian, Vinuela et al. 1993) (i.e. neurons distributed more deeply in frontal and temporal cortical white matter in schizophrenics than controls) were found, although these findings have been criticized (Dwork 1997). Loss of hippocampal neurons has been reported, though refuted on several occasions, and increased neuronal density in DLPFC has also been reported (Selemon, Rajkowska et al. 1995). Smaller neuronal size in hippocampus (Benes, McSparren et al. 1991) and DLPFC (Rajkowska, Selemon et al. 1998) and lower numbers of neurons in dorsal thalamic nuclei (Pakkenberg 1990) have also been reported. In examinations of synapses and dendrites, in hippocampal formation, synaptic proteins are found in reduced levels (Harrison and Eastwood 1998). Synaptophysin is also reduced in DLPFC (Glantz and Lewis 1997), though increased in cingulate gyrus. The direction of the synaptic alterations in hippocampus and DLPFC were taken to support the hypothesis of excessive synaptic pruning in schizophrenia (Keshavan, Anderson et al. 1994).

Neurochemical pathology studies in schizophrenia have not produced consistent results, and more recent in vivo findings using positron emission tomography do not demonstrate consistent findings in receptor density alterations. Dopaminergic, serotonergic, glutamatergic, and GABA-ergic systems have been investigated (Javitt and Laruelle 2006), with particular focus on D1, D2, D3, and D4 receptors. However, only upregulation of D2 receptors in striatum (likely an effect of
prolonged exposure to antipsychotic medication) holds up as a consistent finding (Seeman, Bzowej et al. 1987). Even dopamine D2 receptor binding potential in vivo is not consistently increased or decreased. A more consistent dopaminergic finding is elevated dopamine release in response to amphetamine, which is also seen clinically in patients, and in animal models (Laruelle 2000), suggesting a dopaminergic sensitivity. Lowered 5HT2A expression and elevated 5HT1A expression are replicated findings; however, PET studies do not demonstrate consistency in vivo regarding serotonin receptor binding potentials (Laruelle, Kegeles et al. 2003) in schizophrenia.

Postmortem studies show changes in glutamate receptor binding, transcription, and subunit protein expression in the prefrontal cortex, thalamus, and hippocampus of subjects with schizophrenia (Clinton and Meador-Woodruff 2004; Clinton and Meador-Woodruff 2004), supporting possible NMDA receptor dysfunction. If NMDA receptors in certain circuits are hypofunctional, GABAergic inhibitory control over the activity of corticolimbic neurons is impaired, potentially allowing unmodulated stimulatory activity to flood corticolimbic brain regions, thus producing psychotic symptoms. Thus, the GABAergic system is implicated in schizophrenia and GABAergic findings align with the NMDA receptor hypofunction hypothesis. These findings include reduced expression of GAD67, the GABA transporter, GAT, and the co-expressed calcium binding protein, parvalbumin. Evidence also exists for the loss of GABAergic chandelier cell terminal (cartridges), on pyramidal initial axon segments. There is compensatory upregulation of postsynaptic GABA-A receptors in the same regions associated with downregulation of presynaptic GABAergic markers (Lewis, Hashimoto et al. 2005).

Rather than focusing on neurochemical receptors, per se, studies examining genes that are essential for neurodevelopment may be more effective in demonstrating a mechanism via which
receptor activity in schizophrenia may be altered. For instance, studies examining neuregulin1 (Nrg1), and the effects of Nrg1 signaling, have been particularly illuminating. Particularly compelling evidence from postmortem brain of decreased NMDA receptor function, came from a study examining the effects of signaling of neuregulin1 through its tyrosine kinase receptor erbB4 (Hahn, Wang et al. 2006). Despite the fact that Nrg1 and erbB4 levels were no different than controls in postmortem PFC samples, the authors found that when they added exogenous Nrg1, twice as much phosphorylated erbB4 could be detected in PFC slices from schizophrenia patients. When the authors stimulated PFC slices with NMDA, classic signs of NMDAR activation (such as increased phosphorylation of subunit 2A), were decreased in schizophrenia brain. The addition of Nrg1 attenuated NMDAR activation even further. Therefore, although expression of the genes in question were no different in patients compared to controls, careful experimental manipulation revealed disrupted signaling in this pathway in schizophrenia.

1.6 Schizophrenia Genetics

Meta-analyses of whole genome linkage scans, generally conducted in multiply affected families have implicated a number of chromosomal regions in schizophrenia, most prominently at 8p, 22q, and 1q (Badner and Gershon 2002). Many investigators have utilized the rationale from whole genome linkage studies to search for genes within linked regions that might have biological plausibility for a role in schizophrenia. One such example is the COMT gene in the 22q11 region. This gene is responsible for dopamine metabolism in the prefrontal cortex. Other candidate genes that have been seriously considered include the Neuregulin1 gene, on chromosome 8p, the DISC1 gene on chromosome 1q, the dysbindin gene on chromosome 6p, dopamine system genes, NMDA receptor genes, and other genes involved in glutamatergic
and/or GABA-ergic signaling, such as G72/DAO-A (13Q34), RGS4 (1Q21), and GAD1 (Harrison and Weinberger 2005). Although several of these genes have replicated evidence for association with schizophrenia, a major criticism of the field has been the difficulty in replicating findings. Postulated reasons for such lack of replication have included low power, ethnic stratification in samples, ethnic stratification between samples (i.e. different risk alleles may operate in different ethnic groups), interactions with environmental factors, disease heterogeneity, and the disease classification system (Cardon 2006). Other findings have occurred in association studies that are not easily explained, such as association of different variants of the same gene, and even association of opposite alleles at the same allelic locus with disease, called the ‘flip-flop’ phenomenon (Lin, Vance et al. 2007). Candidate gene studies are most commonly conducted using the case-control approach, where schizophrenia cases are matched to healthy controls, which depending on the study, are done with varying degrees of rigour: e.g. ethnicity, gender, age, etc. In these studies allelic or genotypic frequencies are compared, in order to determine whether the putative disease variant occurs at a statistically significant greater rate in patients than in controls.

Family-based association studies represent another manner in which to conduct genetic association studies, and eliminate one major confound present in case-control association studies: ethnic stratification. In this approach, transmitted vs. non transmitted alleles from parent to proband are compared, to determine whether the proband (who has schizophrenia) has the disease or risk allele transmitted from the parent at a higher rate than the non-risk allele. Far more case-control studies have been published compared to family-based association studies, likely due to the challenges involved in recruiting a large number of nuclear families.
The challenges of genetic studies in schizophrenia were illustrated by a recent study that examined 14 ‘strong’ candidate genes for schizophrenia using a case-control approach with a large European sample. No positive associations were found following multiple comparison correction (Sanders, Duan et al. 2008). Increasingly large sample sizes have been used over the past few years, in order to overcome potential heterogeneity, in line with the notion that many of the previous genetic studies were inadequately powered for positive association. The whole-genome association approach, made possible by DNA microarray technology, can now examine hundreds of thousands (and now over one million) genetic polymorphisms simultaneously for association with disease. With such an extraordinarily large number of comparisons, significance thresholds have been stringently applied, and power required to achieve such thresholds is difficult, and thus thousands of individuals must be recruited into such studies. A recent success for schizophrenia genetics was that three genome-wide studies all found association at chromosome 6p22 (Purcell, Wray et al. 2009; Shi, Levinson et al. 2009; Stefansson, Ophoff et al. 2009), a region containing human leukocyte antigen (HLA) genes. One of these studies also replicated the first significant genome-wide study that implicated the Znf804A gene (O'Donovan, Craddock et al. 2008). However, odds ratios were very small (1.1-1.2); that is an individual’s odds of having disease if the risk variant were present would increase only 1.1-1.2 fold. Therefore, much of the genetic risk in schizophrenia, a disease with heritability estimates of 50-80% is still unexplained. Another recent development in schizophrenia genetics research has occurred thanks to technological developments that now easily permit examination of rare variants across the genome. Copy number variation in the genome occurs due to copy number variants (CNVs), which span from 1 kb to several megabases. CNVs can be inherited or can arise de novo, and are generally caused by genomic rearrangements, such as deletions, duplications,
inversions, or translocations. Copy number variants are classified as rare variants, that also include single-gene mutations or other cytogenetic abnormalities. Such mutations are typically highly penetrant, and have been shown to increase risk for a number of neuropsychiatric disorders in addition to schizophrenia, such as autism (Cook and Scherer 2008). One study, for instance, demonstrated an increased rate of CNVs in childhood onset schizophrenia patients that increased risk for schizophrenia, and also occurred in genes related to brain structure and function such as ErbB4 and Neurexin1 (Walsh, McClellan et al. 2008). Another study analyzed a population based sample to uncover de novo CNVs. The rate of these CNVs was then examined in schizophrenia patients compared to controls, and CNVs in chromosomal regions 1q21.1, 15q11.2, and 15q13.3 were shown to occur more frequently in schizophrenia patients. Although these variants occurred less than 1% of the time, odds ratios ranged from approximately 3-15 depending on the CNV. Therefore, while these rare variants occur very infrequently, they are highly penetrant and considerably increase risk for schizophrenia (Stefansson, Rujescu et al. 2008). These findings provide evidence for the rare variant – common disease hypothesis, as compared to the common variant – common disease hypothesis, investigated using candidate gene or whole genome approaches. Risk is likely conferred in both ways (Purcell, Wray et al. 2009), and further investigation is required. Other approaches to understand the effects of risk variants in schizophrenia, and to improve penetrance of these risk variants, such as combining genetics and neuroimaging are discussed later in this Chapter.
1.7 Microarray technology and downregulation of myelin genes in postmortem schizophrenia brain

Nearly ten years ago, the surprising result of a study using novel microarray technology found that the most downregulated genes in schizophrenia postmortem brain, compared to postmortem brain of healthy controls, were myelin genes (Hakak, Walker et al. 2001). This study was novel at the time, since microarray analysis permitted examination of gene expression of thousands of genes at once, with no *a priori* hypothesis. The authors examined dorsolateral prefrontal cortex (BA 46), in severely ill, institutionalized, elderly patients who had schizophrenia. Among these downregulated genes were the myelin associated glycoprotein (MAG) gene, the 2',3',-cyclic nucleotide 3’-phosphodiesterase (CNP) gene, and the receptor tyrosine-protein kinase erbB-3 (ErbB3) gene. Shortly thereafter, a study examining postmortem brains of schizophrenia and bipolar patients, obtained from the Stanley Brain Foundation examined prefrontal cortex (BA 9) (Tkachev, Mimmack et al. 2003). These schizophrenia patients were different than those investigated by Hakak et al (Hakak, Walker et al. 2001), since they were between 25-62 years of age, and many had not been chronically institutionalized. The authors (Tkachev, Mimmack et al. 2003) utilized both quantitative PCR and microarray analyses, and again found that myelin related genes were considerably downregulated in schizophrenia. In particular the MAG gene showed greatest downregulation of all genes. Other downregulated genes included myelin oligodendrocyte glycoprotein (MOG) and ErbB3. Both studies partially controlled for medication effects on gene expression by comparing patients who had not been taking antipsychotic medications in the weeks prior to death compared to those on antipsychotics at the time of death. These groups did not show any difference in downregulation of these genes.
Furthermore, such downregulation did not correlate with alcohol use. Myelin-related genes were also downregulated in bipolar disorder brains in the same study (Tkachev, Mimmack et al. 2003), providing further indirect evidence that the effects on gene expression were not due to antipsychotic medications. Demonstration of diagnostic overlap for myelin gene downregulation raises also provided evidence for the intriguing possibility that myelin and white matter may be a common line of pathophysiology between schizophrenia and bipolar disorder.

Several other postmortem gene expression studies in schizophrenia have now replicated these findings in oligodendrocytes and myelin related genes (Aston, Jiang et al. 2004; Sugai, Kawamura et al. 2004; Katsel, Davis et al. 2005; Katsel, Davis et al. 2005). Some investigators used both microarray and qPCR approaches to cross-validate their findings (Haroutunian, Katsel et al. 2007). Others have focused on single genes e.g. (Flynn, Lang et al. 2003; Peirce, Bray et al. 2006) or a small group of genes, e.g. (Aberg, Saetre et al. 2006; McCullumsmith, Gupta et al. 2007). Several cortical and subcortical regions have been studied. Frontal cortex, cingulate gyrus, temporal cortex, and hippocampus are brain regions where this group of genes is most consistently downregulated (Haroutunian, Katsel et al. 2007). Studies have primarily utilized grey matter tissue, although more recently some investigators have begun to investigate expression in white matter. For instance McCullumsmith et al (McCullumsmith, Gupta et al. 2007) investigated anterior cingulate white matter, and found reduced expression of MAG, CNP, quaking (QKI), and transferrin (TF) using in situ hybridization. Mitkus et al (Mitkus, Hyde et al. 2008) investigated expression of MAG, myelin basic protein (MBP), CNP, and oligodendrocyte transcription factor-2 (OLIG2) in DLPFC gray and white matter. While reduced expression of CNP and OLIG2 were not found in schizophrenia, allelic variants correlated with expression levels of these genes. As shown in studies by Georgieva (Georgieva, Moskvina et al. 2006) and
McCullumsmith (McCullumsmith, Gupta et al. 2007), coordinated downregulation in OMR genes may be present in schizophrenia. In particular, Aberg et al. (Aberg, Saetre et al. 2006) demonstrated that the QKI gene coordinated oligodendrocyte and myelin-related (OMR) gene expression for several genes, including the major myelin protein proteolipid protein-1 (PLP1), MAG, MBP, and TF. In this study, QKI accounted for 47 percent of the interindividual variation in expression of these genes. Furthermore, one specific QKI splice variant, QKI7b was downregulated. QKI7b levels explained a high percentage of variation in MAG, PLP1 and TF, and putative QKI binding elements were found in these genes. The authors concluded that alteration of levels of QKI7b might explain downregulation of OMR genes in schizophrenia.

The CNP, OLIG2 and receptor tyrosine-protein kinase erbB4 (ErbB4) genes share coordinated expression (Georgieva, Moskvina et al. 2006). OLIG2 is a basic helip-loop-helix transcription factor and maps to the 22q22.11 chromosomal region, very near the 22q11 region, deleted in velocardiofacial syndrome (Bassett and Chow 1999). The investigation by Georgieva et al showed that OLIG2 and CNP may mutually regulate each other’s expression (Georgieva, Moskvina et al. 2006). Furthermore, OLIG2 variants showed significant interaction effects with CNP and ErbB4 variants in conferring increased risk for disease in schizophrenia patients compared to controls. ErbB4 is a tyrosine kinase receptor, and is a binding site for neuregulin1, a major susceptibility gene for schizophrenia (Stefansson, Sigurdsson et al. 2002). An erbB4 transgenic mouse model, demonstrated disrupted oligodendrocyte development, and altered morphology, in addition to dopaminergic abnormalities and behavior (Roy, Murtie et al. 2007). A recent study in zebrafish showed that Neuregulin1 and disrupted in schizophrenia 1 (DISC1) are both essential for oligodendrocyte development, and are required for specification of oligodendrocytes in zebrafish brain (Wood, Bonath et al. 2009). Both Neuregulin1 and DISC1
play critical roles for oligodendrocytes by acting through OLIG2, and knockdown of NRG1 and DISC1 caused near or total loss of OLIG2 positive neurons.

Reductions of oligodendrocyte number in prefrontal cortex provide further support for oligodendrocyte and myelin pathophysiology in schizophrenia. For instance Hof et al (Hof, Haroutunian et al. 2003), reported a 28% decrease in total numbers of cortical layer III oligodendrocytes and a 27% decrease in the white matter in schizophrenia vs. control cases. Uranova et al. (Uranova, Orlovskaya et al. 2001) reported apoptotic oligodendrocytes and damaged myelin sheath lamellae, and other investigators (Byne, Buchsbaum et al. 2002) found reduced oligodendrocyte number in the anterior thalamic nucleus, particularly in men with schizophrenia. One accurate criticism of the Uranova study was that the authors did not clarify whether they were blinded to diagnosis (Dwork, Mancevski et al. 2007). Nevertheless, Uranova et al., in a follow up study (Uranova, Vostrikov et al. 2007) found signs of oligodendrocyte degeneration, from damage to necrosis, and apoptosis in the PFC and head of the caudate nucleus. Findings were characterized by chromatin condensation, few swollen mitochondria, and cytoplasmic inclusions of electron-dense material. These types of ultrastructural changes were found in greater than 50 percent of the schizophrenia brains in perineuronal, pericapillary, and interfascicular oligodendrocytes. From the same study, investigation of Stanley Brains using light microscopy revealed reduced oligodendrocyte number in both layer VI and layer III of PFC in schizophrenia. Such ultrastructural changes have been noted in mouse models of myelin genes. MAG deficient mice have morphologically abnormal myelin sheaths, lack a well-developed cytoplasmic collar, contain redundant myelin and noncompact areas of myelin and have periaxonal areas of degeneration and dystrophy of oligodendrocyte processes (Li, Tropak et al. 1994; Bartsch 1996; Lassmann, Bartsch et al. 1997), which are similar to ultrastructural changes reported in schizophrenia brains (Uranova,
Orlovskaya et al. 2001; Uranova, Vostrikov et al. 2007). These mice demonstrate a relatively mild phenotype with slight developmental deficits, and some mild sensorimotor abnormalities, but no differences in spatial learning or memory. Also, alteration of PFC layer III pyramidal cells, particularly with respect to basal dendritic integrity is observed (Segal, Koschnick et al. 2007). The authors speculated that such alterations may lead to abnormalities of specific white-matter tracts, and may affect the prefrontal circuitry.

The Cnp mouse model reveals normal myelin ultrastructure; however, the mice develop axonal swellings, diffuse neurodegeneration, enlarged ventricles and reduced corpus callosum size (Lappe-Siefke, Goebbels et al. 2003). Qki mice display CNS dysmyelination that has been noted in schizophrenia, but the clinical model is of a mouse with severe body tremors and decreased lifespan (Sidman, Dickie et al. 1964). Both Cnp and Qki provide useful models in terms of studying the relationship between oligodendrocytes and axons and dysmyelination in the CNS (QKI), but are not necessarily representative of clinical symptoms or cognitive deficits observed in schizophrenia.

1.8 Genetic Association Studies Implicating Myelin System Genes in Schizophrenia

Several genetic association studies of OMR genes with schizophrenia have been performed. Much like other gene systems, both positive and negative studies have been published for OMR genes, with generally modest sample sizes. These studies have generally been completed in either Caucasian or Asian populations. A small number of studies have utilized larger sample sizes and/or complementary molecular biology approaches in order to provide a more in-depth understanding of positively associated gene variants.
### Table 1-1 Summary of OMR Gene Association Studies with Schizophrenia

<table>
<thead>
<tr>
<th>Author</th>
<th>Number</th>
<th>Result</th>
<th>Further Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnic Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Wan, Yang et al. 2005)</td>
<td>470/470 C.C.</td>
<td>MAG – pos.</td>
<td>none</td>
</tr>
<tr>
<td>Han Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Yang, Qin et al. 2005)</td>
<td>413 Family</td>
<td>MAG – pos.</td>
<td>none</td>
</tr>
<tr>
<td>Han Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Qin, Gao et al. 2005)</td>
<td>487 Family</td>
<td>PLP1 – pos</td>
<td>none</td>
</tr>
<tr>
<td>Han Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Zai, King et al. 2005)</td>
<td>111 Family</td>
<td>MOG – neg</td>
<td>none</td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Liu, Qin et al. 2005)</td>
<td>532 Family</td>
<td>MOG – pos</td>
<td>none</td>
</tr>
<tr>
<td>Han Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Peirce, Bray et al. 2006)</td>
<td>708/711 C.C.</td>
<td>CNP – pos</td>
<td>CNP expression</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Aberg, Saetre et al. 2006)</td>
<td>176 families</td>
<td>QKI – pos</td>
<td>QKI expression</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Georgieva, Moskvina et al. 2006)</td>
<td>673/716 C.C.</td>
<td>OLIG2/CNP/</td>
<td>Expression/Epistasis</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Usui, Takahashi et al. 2006)</td>
<td>759/757 C.C.</td>
<td>OLIG2/CNP</td>
<td>none</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Sample Size</td>
<td>Genes Tested</td>
<td>Results</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Tang, Qu et al. 2007)</td>
<td>426/439 C.C.</td>
<td>CNP – neg</td>
<td>none</td>
</tr>
<tr>
<td>Han Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Meng, Shi et al. 2007)</td>
<td>707/689 C.C.</td>
<td>RTN4R – neg</td>
<td>none</td>
</tr>
<tr>
<td>Chinese</td>
<td>and 372 families</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Hsu, Woodroffe et al. 2007)</td>
<td>312 families</td>
<td>RTN4R – neg</td>
<td>Sequencing and mouse</td>
</tr>
<tr>
<td>Chinese</td>
<td></td>
<td></td>
<td>model</td>
</tr>
<tr>
<td>(Maeno, Takahashi et al. 2007)</td>
<td>915/927 C.C.</td>
<td>SOX10 - pos</td>
<td>Mutational search</td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Maeno, Takahashi et al. 2007)</td>
<td>384/384 C.C.</td>
<td>TF – neg</td>
<td>none</td>
</tr>
<tr>
<td>Caucasian/African</td>
<td>1122 trios;621/501</td>
<td>RTN4R – pos</td>
<td>Sequencing; mouse</td>
</tr>
<tr>
<td>(Aleksic, Ikeda et al. 2008)</td>
<td>1640; 487 trios</td>
<td>PLP1 –mainly pos</td>
<td>none</td>
</tr>
<tr>
<td>Chinese</td>
<td></td>
<td></td>
<td>neg</td>
</tr>
<tr>
<td>(Qu, Yue et al. 2008)</td>
<td>326/344 C.C.</td>
<td>TF – pos</td>
<td>none</td>
</tr>
<tr>
<td>Chinese Han</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Voineskos, Lang et al. 2008)</td>
<td>41/43 C.C.</td>
<td>MAG-pos</td>
<td>Neuroimaging</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Certain themes emerge from these studies. First, like other genetic association studies, there is no consistently replicated finding for any one gene, nor for any one gene variant. Furthermore, phenomena such as the ‘flip-flop’ phenomenon, whereby different allelic variants at the same locus confer risk in different populations e.g. Voineskos et al (Voineskos, de Luca et al. 2008) vs Georgieva et al (Georgieva, Moskvina et al. 2006), or even within the same population e.g. Wan et al (Wan, Yang et al. 2005) vs. Yang et al (Yang, Qin et al. 2005), cast uncertainty on findings. However, the addition of convergent techniques which can provide insight into possible mechanistic explanations for these variants, and in the process confer a degree of added confidence to some results. Thus, the study by Georgieva et al (Georgieva, Moskvina et al. 2006), which examined risk variants at OLIG2, CNP, ErbB3, and NRG1, but also epistatic interactions between risk variants, and then coordinated expression of these genes in post mortem human and mouse brain is an example of one such study. That is, confidence in OLIG2 rs1059004, and CNP rs2070106 as schizophrenia risk variants is higher, since these variants are associated with schizophrenia, confer epistatic risk, and are associated with expression of each gene respectively. The study by Budel et al (Budel, Padukkavidana et al. 2008) provided converging evidence for a role for the Nogo-66 receptor in schizophrenia that is a good example
of a study that used additional lines of enquiry to support a genetic association finding. The Nogo-66 receptor 1 (NgR1) is the axonal receptor encoded for by the Nogo-66 receptor (NGR) gene located in the chromosomal region 22q11. MOG, MAG, and Nogo-A proteins share this common axonal receptor. Activation of NgR1 initiates a cascade whereby these proteins together form a myelin-mediated complex of inhibition of axonal growth. The authors first performed a genetic association study for the Nogo-66 receptor gene with schizophrenia in three ethnically divergent samples. Then, they investigated the frequency of novel NGR variants in their Caucasian sample (by sequencing) and then performed detailed functional analyses \textit{(in vitro)} of these rare variants that they found present only in individuals with schizophrenia. Finally, they examined behavioural paradigms in mice lacking NgR1. By utilizing convergent lines of neuroscientific enquiry, the authors were able to provide more convincing evidence for a role for NgR1 in schizophrenia than via any one line of evidence alone.

1.9 Neuroimaging of White Matter in Schizophrenia

Findings of white matter disruption in schizophrenia \textit{in vivo} provide critical supporting evidence for postmortem findings of reduced oligodendrocyte number, downregulation of myelin genes, and findings of association of genes with schizophrenia that are related to the oligodendrocyte/myelin system. Furthermore, localization of white matter disruption to frontal and temporal lobes, and to white matter structures connecting frontal and temporal lobes provide
a degree of neuroanatomic specificity that aligns with postmortem data (Voineskos, Lobaugh et al. 2010).

The application of MRI as a tool to investigate brain structure in vivo in schizophrenia initially provided a considerable degree of information regarding gray matter changes, but less so regarding white matter changes. Following an MRI study in schizophrenia by Smith et al in (Smith, Calderon et al. 1984), several other MRI studies were published that supported findings of lateral ventricle enlargement originally found using computed tomography (CT) (Johnstone, Crow et al. 1976). MRI provided an opportunity to delineate gray matter regions, which was a major advance over CT approaches. As documented in a meticulous review (Shenton, Dickey et al. 2001), an explosion of MRI research in schizophrenia took place in the 1990s, where between 1988 and 2000, approximately 200 peer-reviewed studies were published. That review paper provides a detailed summary of the findings of each of those MRI schizophrenia papers, and also provides cumulative evidence for each brain region studied during that time. In brief summary, findings included ventricular enlargement (80% of studies reviewed) and third ventricle enlargement (73% of studies reviewed). Medial temporal lobe structure reductions (amygdala, hippocampus, parahippocampal gyrus) were found in 74% of studies reviewed. Superior temporal gyrus reductions were found in 100% of studies reviewed (when superior temporal gyrus was separately evaluated). Moderate evidence for frontal lobe structures (59% of studies reviewed) and for parietal lobe abnormalities (60%) was found, with the inferior parietal lobule containing supramarginal gyrus and angular gyrus as particularly susceptible. An important overarching theme is that abnormalities were demonstrated more on the left side of the brain than on the right, particularly in regards to temporal cortical structures, suggesting a possible disruption of the normal asymmetry seen in healthy human brain. Other structures, namely the
subcortical structures of the basal ganglia (e.g. caudate, putamen) tend to demonstrate enlargement in schizophrenia, likely due to long-term antipsychotic exposure, presumably through upregulation of dopamine D2 receptor density. The thalamus, which is part of an important relay network in the brain demonstrated equivocal volumetric differences, and it is likely that measurement here was limited due to insufficient resolution and methods, since particular thalamic nuclei may be more susceptible than others. Finally cerebellar abnormalities were also demonstrated (Andreasen, Nopoulos et al. 1999; Wassink, Andreasen et al. 1999), although these findings require further replication since they were predominantly shown by one research group.

Studies using MRI at the first episode of schizophrenia provide an opportunity to study the effects of the disease at illness-onset, without many of the confounds that accumulate over the lifetime of a patient with schizophrenia. A systematic review and meta-analysis of brain volumes in first episode schizophrenia studies in 2006 (Steen, Mull et al. 2006) summarized the major findings as ventricular enlargement and cortical gray matter volume reductions. Other studies report changes in similar regions to those seen in chronic schizophrenia, e.g. superior temporal gyrus, and other temporal lobe structures (Kasai, Shenton et al. 2003; Kasai, Shenton et al. 2003). Prospective, i.e. longitudinal studies in schizophrenia demonstrate conflicting results – both absence (Lieberman, Chakos et al. 2001) and presence (DeLisi, Sakuma et al. 1997) of progressive changes are found, and in some cases such changes were associated with poorer clinical outcome (Mathalon, Sullivan et al. 2001), though not in others (DeLisi, Sakuma et al. 2004). In studies examining risk of conversion from prodromal states to first episode psychosis, prospective studies tend to demonstrate changes in medial temporal lobe, and it has been demonstrated that those individuals who did ultimately develop psychosis had significantly
different changes compared to non-converters during the prodromal state (Pantelis, Velakoulis et al. 2003).

White matter is harder to define and to evaluate using conventional MRI as it appears uniform and homogeneous. Several studies have evaluated the corpus callosum, the largest midline brain structure, and main connection between the two hemispheres, since it is relatively easy to define on a mid-sagittal slice. Postmortem studies have shown a thicker corpus callosum in schizophrenia patients. Of the 27 MRI studies reviewed in Shenton et al(Shenton, Dickey et al. 2001), 17 reported positive findings with the corpus callosum. The first study, in 1986, by Nasrallah et al(Nasrallah, Andreasen et al. 1986) reported increased thickness in middle and anterior regions. Another study by Hauser et al found no difference (Hauser, Dauphinais et al. 1989), and yet others found reduced volumes in these same regions (Woodruff, Pearlson et al. 1993). As in previous MRI studies, comparability of these studies is quite limited given different methods of ascertainment of callosal volume, as well as considerably different acquisition paradigms. However, volumes of other white matter structures in the brain are not easily delineated, and thus conventional MRI studies have generally been limited to reporting white matter volumes in cortical regions. Reviews have concluded that white matter volumes are either increased in schizophrenia (Lawrie and Abukmeil 1998), or decreased in schizophrenia (Bartzokis 2002), while others have shown no volume change, but have correlated reduced white matter volume in frontal regions with negative symptoms (Sanfilipo, Lafargue et al. 2000). The inability to visualize or measure properties of white matter tracts are major limitations of conventional MRI research, and the advent of diffusion tensor imaging (DTI) provided an exciting opportunity to overcome these limitations.
1.10 Diffusion Tensor Imaging: Visualizing and Measuring White Matter in a Manner Not Possible with Conventional MRI

1.10.1 Diffusion as a Physical Process

Diffusion was first described by Robert Brown, following suspension of a sample of pollen grains in water, and subsequent observation under the light microscope, and is now also known as ‘Brownian motion’. Brown actually observed water molecules undergoing random thermal fluctuations (Jones 2008). It was Einstein, however, who operationalized the process of diffusion via his diffusion equation:

\[ (\Delta r^2) = 2nD\Delta t \]

Einstein stated that the mean squared displacement \((\Delta r^2)\) from diffusion is proportional to the diffusivity, \(D\) (in \(\text{mm}^2/\text{s}\)), over the diffusion time \((\Delta t)\) (Alexander and Lobaugh 2007).

In living, human brain, the behaviour of water diffusion is modulated by cytoplasmic currents and the interactions with cellular membranes, subcellular organelles, and cells (Alexander and Lobaugh 2007), in addition to random thermal fluctuations. Using diffusion weighted MRI, measurement of diffusion of water in the living brain has the potential to provide insight into cell physiology, cell structure, and white matter connections.

1.10.2 Diffusion Weighted Image Acquisition

A diffusion weighted MR sequence sensitizes the MR signal to diffusion by imposing a given phase to a molecule that is dependent on its overall displacement (Stejskal and Tanner 1965). By
applying a pair of gradient pulses, the MR signal can be sensitized to water diffusion. There is a
distribution of displacements and thus a distribution of phases. This spread of phases means a
loss of signal coherence and therefore a reduction in signal amplitude. As a result, an image
appears darker. In the presence of diffusion gradients, each diffusing molecule will accumulate a
different amount of phase. The phase dispersion from diffusion will cause destructive
interference, which causes signal attenuation (Mori and Zhang 2006; Jones 2008). The diffusion
weighted signal is then created by summing the magnetization from all water molecules in a
voxel.

1.10.3 The Diffusion Tensor

The current model of characterizing diffusion of water within a voxel is via the diffusion tensor
(described by Basser et al in 1994 (Basser, Mattiello et al. 1994)). The diffusion tensor can be
represented by a 3 x 3 symmetric matrix of numbers that characterizes three-dimensional
displacements, and takes the shape of a 3D ellipsoid. In this model, the diagonal elements
correspond to diffusivities along three orthogonal axes. The off-diagonal elements correspond to
the correlation between displacements along the same orthogonal axes (Jones 2008). In tissue,
the displacement profile can be described by an ellipsoid with the long axis parallel to the long
axis of the anisotropic medium. The principal axes of the ellipsoid are given by the eigenvectors
(v₁, v₂, v₃) and the lengths are given by the diffusion distance over a given time. Since the
displacement in a given time is proportional to the square root of the diffusivity, the ellipsoid
axes are scaled according to the square root of the eigenvalues (λ₁, λ₂, λ₃). When the diffusion
eigenvalues are approximately equal (λ₁=λ₂=λ₃), the diffusion tensor is nearly isotropic (i.e.
diffusion occurs evenly in all directions). When the eigenvalues are significantly different in
magnitude, the diffusion tensor is anisotropic (i.e. diffusion occurs primarily in one direction).

Changes in local tissue microstructure with tissue injury, disease, or normal physiologic changes
(i.e. aging) will cause changes in the eigenvalue magnitudes. Thus, the diffusion tensor is a
sensitive probe for characterizing both normal and abnormal tissue microstructure. At current
field strength (1.5T and 3T), in the CNS, water diffusion is typically anisotropic in white matter
regions, and isotropic in gray matter and cerebrospinal fluid (CSF).

One of the most widely used metrics of diffusion anisotropy is fractional anisotropy (FA)(Basser
and Pierpaoli 1996).

\[
FA = \frac{(1/2)^{1/2} [(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2]^{1/2}}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}^{1/2}
\]

FA measures the fraction of the tensor that is due to anisotropic diffusion. The FA index is
normalized, so that it takes values from 0 (diffusion is isotropic) to 1 (completely anisotropic).

FA maps can then be created, such that voxels containing white matter fibers that run together in
parallel appear bright, whereas gray matter and CSF appear dark. The trace of the diffusion
tensor is equal to the sum of the three eigenvalues, and provides a rotationally invariant index of
the overall amount of diffusivity within each image voxel. The mean diffusivity within a voxel is
the trace divided by three.

Axial (D_L) and radial diffusivity (D_R) are also diffusion indices of interest. D_L = (\lambda_1) (Song,
Yoshino et al. 2005) and D_R = ([\lambda_2 + \lambda_3]/2) (Song, Sun et al. 2002). D_R may be specific to
myelination (Song, Yoshino et al. 2005) and D_L to axonal degeneration (Song, Sun et al. 2002),
as suggested by DTI performed in mice where damage to myelin and to axons was modeled.
1.10.4 Tissue Microstructure and Diffusion

Tissue microstructure fundamentally affects the apparent diffusion properties of water and diffusion should therefore act as a sensitive probe to any changes in cellular structures that alter the displacement per unit time. In fibrous tissues, such as white matter tracts in the brain, water diffusion is less hindered, or restricted in the direction parallel to the fiber orientation. Myelin, the axonal membrane, microtubules, and neurofilaments are all longitudinally oriented structures that could hinder water diffusion perpendicular to the length of the axon. Although it was initially thought that this directional dependence (or anisotropy) was due to myelin, studies in unmyelinated nerve (e.g. of the garfish, see Beaulieu et al, 2002 for a review) (Beaulieu 2002)) demonstrated that myelin was not necessary for diffusion anisotropy. The series of studies by Beaulieu and colleagues demonstrated that the primary determinant of anisotropy was axonal membranes, and myelin was deemed to modulate anisotropy. Other possible determinants of anisotropy, e.g. the effects of susceptibility induced gradients, axonal cytoskeleton, and fast-axonal transport were mainly ruled out with respect to anisotropy (Beaulieu 2002).

1.10.5 Diffusion Tensor Tractography

There are several types of analysis that can be conducted on diffusion data. Anatomical methods for selecting white matter for study include drawing regions of interest on specific slices within or encompassing white matter tracts, or by reconstructing white matter tracts using tractography approaches. Other studies have used voxel-based analyses, although this approach is problematic, and thus has fallen out of favour. For voxel-based morphometry (VBM), the non-
diffusion weighted images (b=0) are often used to register subject data to a common space (Jones, Griffin et al. 2002) but this does not guarantee that the underlying fiber architecture is in register. Many published results of voxel-based assessment of group FA differences or FA correlations have identified significant effects in regions of more variable FA, which are often located at interfaces of white matter with gray matter, or CSF, or in regions of complex architecture. The question of whether to smooth DTI data further complicates matters, and the size of the smoothing filter can dramatically affect residual errors and the sensitivity to detect group-wise differences (Jones, Symms et al. 2005). Therefore, conventional VBM approaches are problematic for DTI data, and other analysis methods are now primarily used.

A newer voxel-based method that successfully contends with certain difficulties in applying conventional VBM to DTI uses non linear registration as a first step in aligning all subjects’ FA images together; peak FA ridges are found on the group-averaged FA template, creating a skeleton of white matter tracts. Subject-specific FA values are then derived by finding the location in each subject’s data that most closely matches the spatial location of the ridge. This approach, known as tract-based spatial statistics (TBSS) is robust against residual misregistration and ensures better alignment of tracts across subjects (Smith, Jenkinson et al. 2006). Aside from TBSS, the other major paradigm currently applied to DTI data is diffusion tensor tractography, an approach that provides exceptional visualization of neuroanatomy, can provide diffusion measures along white matter tracts, and where analysis can be performed in the subject’s native space.

There are two main types of tractography: deterministic (or streamline) tractography and probabilistic (or stochastic) tractography. Fiber tracking, or tractography, reconstructs the three-dimensional trajectories of anisotropic structures in tissue by piecing together voxel-based
estimates of the continuous fiber orientation field (Conturo, Lori et al. 1999; Jones, Simmons et al. 1999; Mori, Crain et al. 1999). For probabilistic algorithms, multiple trajectories are generated from the seed points with random perturbations to the trajectory directions (Alexander and Lobaugh 2007). Various probabilistic algorithms are currently in use. Some methods involve devising an *ad hoc* relationship between the uncertainty in fibre orientation and the shape of the diffusion tensor. Others derive the distribution in possible estimates of fiber orientation using Bayesian methods (Behrens, Johansen-Berg et al. 2003), while others draw samples from this distribution by employing bootstrap methodologies (Lazar and Alexander 2005). The aim of probabilistic tractography is to create a map that attempts to quantify how confident one can be that a pathway can be found, through the data, between each voxel and the seedpoint (Jones 2008). A major shortcoming, however, is that if there are systematic errors in the data, one may obtain highly reproducible but inaccurate tract reconstructions. Thus, high likelihood of a connection through data between a seedpoint and a given voxel on a probabilistic tract map does not necessarily mean that a white matter pathway actually exists connecting the two points in space. Furthermore, there is the problem of ‘accumulated error’. That is, there is uncertainty in fiber orientation at each stage in the propagation of the tract, and the longer the tract the greater the accumulated error. These issues are reviewed in greater detail elsewhere (Jones 2008), and are in part resolved by Morris et al (Morris, Embleton et al. 2008).

The implicit underlying assumption in deterministic tractography is that the principal eigenvector is parallel to the underlying dominant fiber orientation in each voxel (Basser, Mattiello et al. 1994), and forms a tangent to the space curve traced out by the white matter tract (Basser, Pajevic et al. 2000). The evolution of the space curve is performed by propagating a single pathway bidirectionally from a seedpoint (usually the centre of an imaging voxel) by moving in a
direction that is parallel with the principal eigenvector. It is assumed that the underlying tensor field is continuous, and therefore as the step size is usually fixed, subvoxel estimates of the tensor are required for this approach. Continuous integration methods such as 2\textsuperscript{nd} or 4\textsuperscript{th} order Runge-Kutta enable more accurate estimates of curved tracts (Basser, Pajevic et al. 2000).

Another approach to deterministic tractography is via the FACT method (fiber assignment by continuous tracking) (Mori, Crain et al. 1999). Instead of assuming that the underlying fiber trajectory is continuous, this method applies the principle that the fiber orientation is uniform everywhere within a voxel, and changes abruptly at the boundary of a voxel. Here, the step length is no longer constant since the path is propagated from the seedpoint, parallel to the principal eigenvector until the boundary of the voxel is encountered. At this point the algorithm traverses the next voxel in a direction parallel to the eigenvector at the centre of this new voxel.

Most deterministic approaches involve seeding a region of interest (ROI), from which to initiate tracking. This ROI, based on prior anatomical knowledge, intersects the white matter tract of interest. In some cases, a single ROI is sufficient (the user must be absolutely certain that no other fasciculi are included). Single ROIs are problematic, however, for white matter tracts that run together. A solution to this problem is to define additional regions of interest and apply Boolean logic operators to the tract reconstruction. However, this ROI approach is a weakness of deterministic tractography. A reliance on accurate placement of seed and deflection point ROIs making them susceptible to generating highly errant results arising from small errors at a single step. This process can be susceptible to operator bias, and is heavily dependent on the skill and neuroanatomical knowledge of the operator. Furthermore, no reliability information during this process is generated. A recently developed automated clustering segmentation method (O'Donnell, Kubicki et al. 2006) eliminates some of these biases. Following whole brain
tractography, this method groups fibers together in a scheme based on their shape and similarity, and their direction of travel. While this method also requires \textit{a priori} neuroanatomical knowledge to combine clusters into tract segments, the operator visualizes a 3-D whole brain model of white matter tracts, rather than single slices. The operator can introduce bias at one step only: by selecting additional clusters that may or may not comprise that tract of interest following selection of the cluster that clearly comprises the neuroanatomic tract of interest. Published work to date suggests that both the multiple ROI method (Wakana, Caprihan et al. 2007) and the clustering method are highly reliable for several large white matter tracts (Voineskos, O'Donnell et al. 2009).

Other diffusion based analysis methods such as high angular resolution diffusion imaging, q ball imaging and diffusion spectrum imaging are emerging and may provide specific advantages over commonly-used diffusion approaches. However, these approaches have yet to gain significant traction in the neuropsychiatric field (Alexander and Lobaugh 2007).

1.10.6 DTI and Schizophrenia

As described earlier, considerable evidence exists from postmortem and genetic studies supporting oligodendrocyte dysfunction in schizophrenia. DTI can infer the microstructural integrity of white matter structure (large bundles of myelinated axons) since axonal membranes, myelin, and other microstructural elements collectively influence diffusion of water within white matter. Therefore, the application of DTI to schizophrenia is an opportunity to measure possible disruption of microstructural integrity of these white matter tracts in the brain. There are several theories regarding how the brain may change in neurodevelopment in a maladaptive fashion that may ultimately lead to schizophrenia. One such theory is that of overaggressive synaptic pruning (Keshavan, Anderson et al. 1994) (i.e. over and above the normal cortical pruning that occurs
during adolescence), which was supported by a series of dynamic modeling studies (Thompson, Giedd et al. 2000) measuring cortical gray matter volumes in childhood onset or early onset schizophrenia patients (Thompson, Vidal et al. 2001; Gogtay, Lu et al. 2008). However, an alternative hypothesis has been postulated (Paus 2005) suggesting that during adolescence frontal and temporal cortex (which undergoes peaks of myelination at that stage) is not losing gray matter but rather gaining myelinated white matter. These peaks of myelination, occurring in late adolescence (Benes 1989) may be disrupted in some individuals, and in the process contribute to the onset of schizophrenia. DTI studies of healthy neurodevelopment support increases in myelination during this time, via tract-specific increases in FA (Lebel, Walker et al. 2008), and decreases in radial diffusivity through adolescence and into the early 20s (Asato, Terwilliger et al. 2010). Therefore, the neurodevelopmental trajectory of myelination may be a critical component of pathology in schizophrenia (Bartzokis 2002).

Different investigators provided early reviews of DTI findings in schizophrenia (Kubicki, McCarley et al. 2007), but have differed (Kanaan, Kim et al. 2005; Konrad and Winterer 2008) in their levels of optimism regarding the consistency and reproducibility of DTI findings in schizophrenia. However, none of those reviews included DTI tractography or tract-based spatial statistics studies. Therefore, those reviews were limited (2006 and previously) to voxel based morphometry and region of interest studies using DTI in schizophrenia. VBM carries with it a host of unresolved biases and limitations in DTI (Kanaan, Shergill et al. 2006). ROI based studies are difficult to compare, since slice selection and ROI size (both area, and number of slices drawn) are rarely the same between studies, and thus the results are highly dependent on the portion of the tract being studied. However, tractography studies, where investigators report mean FA or other scalar indices of the tensor along a given tract may be more easily compared,
since the neuroanatomic structure in question is the same. Although differences between studies such as magnet strength, and acquisition parameters differ, these differences are common to all types of DTI studies, and are only overcome by multicentre collaboration. There is little argument (particularly when investigators provide figures of the tracts generated by their tractography algorithm) of which neuroanatomic tract they are comparing. Furthermore, reliability studies have now been published for deterministic tractography approaches, and several white matter tracts can be measured with high reliability (Wakana, Caprihan et al. 2007) (Voineskos, O'Donnell et al. 2009). TBSS studies are more easily compared as well. Therefore, DTI tractography (both deterministic and probabilistic) and TBSS studies in schizophrenia are summarized below:

**Table 1-2 Summary of DTI Tractography and TBSS Studies in Schizophrenia**

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Population</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Jones, Catani et al. 2006)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>↓ FA differences with age</td>
</tr>
<tr>
<td>UF, SLF, IFOF, CB</td>
<td>(14,14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kanaan, Shergill et al. 2006)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>(-)FA with ROIs; ↓ FA</td>
</tr>
<tr>
<td>Genu CC</td>
<td>(33,40)</td>
<td></td>
<td>with tractography ROIs</td>
</tr>
<tr>
<td>(Price, Cercignani et al. 2007)</td>
<td>Probabilistic</td>
<td>FE</td>
<td>↓ FA; (-) tract volumes</td>
</tr>
<tr>
<td>Genu, Splenium CC</td>
<td>(18,21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Miyata, Hirao et al. 2007)</td>
<td>FACT</td>
<td>chronic</td>
<td>↓ anterior CC length</td>
</tr>
<tr>
<td>CC</td>
<td>(40,36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Type</td>
<td>Time</td>
<td>Changes</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------------</td>
<td>--------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Ashtari, Cottone et al. 2007)</td>
<td>Deterministic</td>
<td>Adolescent</td>
<td>↓ FA, ↑ Rd, ↑ Tr (L ILF); (-)</td>
</tr>
<tr>
<td>ILF, Genu of CC</td>
<td>(23,21)</td>
<td></td>
<td>genu</td>
</tr>
<tr>
<td>(Price, Cercignani et al. 2008)</td>
<td>Probabilistic</td>
<td>FE</td>
<td>↑ FA variance in left UF</td>
</tr>
<tr>
<td>UF</td>
<td>(19,23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Zhou, Shu et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↓ FA</td>
</tr>
<tr>
<td>FH</td>
<td>(17,14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kim, Kim et al. 2008)</td>
<td>Probabilistic</td>
<td>Chronic</td>
<td>↓ FA, ↑ Ax, ↑ Rd</td>
</tr>
<tr>
<td>Thalamocortical paths</td>
<td>(30,22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nestor, Kubicki et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↓ FA in CB, (-) in UF</td>
</tr>
<tr>
<td>CB, UF</td>
<td>(25,28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Takei, Yamasue et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↓ FA, ↑ MD</td>
</tr>
<tr>
<td>FH</td>
<td>(31,65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Rosenberger, Kubicki et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>FA(CB/UF) differences</td>
</tr>
<tr>
<td>CB, UF, IFOF</td>
<td>(27,34)</td>
<td></td>
<td>↑ with age</td>
</tr>
<tr>
<td>(Magnotta, Adix et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>(-); but ↓ FA in SCP</td>
</tr>
<tr>
<td>Cerebello-thalamic</td>
<td>(12,10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Carpenter, Tang et al. 2008)</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↑ FA differences</td>
</tr>
<tr>
<td>Genu, Splenium CC; pyramidal</td>
<td>(76,76)</td>
<td></td>
<td>with age in genu of CC</td>
</tr>
<tr>
<td>(McIntosh, Maniega et al. 2008)</td>
<td>Probabilistic</td>
<td>Chronic</td>
<td>↓ FA in UF and ATR</td>
</tr>
<tr>
<td>UF, ATR</td>
<td>(25,49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Methodology</td>
<td>Status</td>
<td>Change</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>(Kubicki, Styner et al. 2008)</td>
<td>Probabilistic</td>
<td>chronic</td>
<td>↓ FA in anterior segments of CC</td>
</tr>
<tr>
<td>CC subdivisions</td>
<td>(32,42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kunimatsu, Aoki et al. 2008)</td>
<td>Probabilistic</td>
<td>chronic</td>
<td>↓ FA, ↑ ADC</td>
</tr>
<tr>
<td>SOFF</td>
<td>(19,20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Phillips, Nuechterlein et al. 2009)</td>
<td>FACT</td>
<td>chronic</td>
<td>↓ FA in AF and ILF</td>
</tr>
<tr>
<td>AF, ILF</td>
<td>(23, 22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fitzsimmons, Kubicki et al. 2009)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>↓ FA in AF and ILF</td>
</tr>
<tr>
<td>FH</td>
<td>(36,35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gasparotti, Valsecchi et al. 2009)</td>
<td>Deterministic</td>
<td>FE</td>
<td>↓ FA splenium; (-) FA genu</td>
</tr>
<tr>
<td>CC</td>
<td>(21,21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kito, Jung et al. 2009)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>(-) FA; ↓ area L ATR</td>
</tr>
<tr>
<td>ATR</td>
<td>(20,20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Oh, Kubicki et al. 2009)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>↓ FA projections to DLPFC,</td>
</tr>
<tr>
<td>thalamo-frontal paths</td>
<td>(18,21)</td>
<td></td>
<td>ACC, Broca’s area</td>
</tr>
<tr>
<td>(Takei, Yamasue et al. 2009)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>↓ FA dorsal, pregenual CB</td>
</tr>
<tr>
<td>CB</td>
<td>(31,65)</td>
<td></td>
<td>↑ MD dorsal CB</td>
</tr>
<tr>
<td>(Kanaan, Borgwardt et al. 2009)</td>
<td>Deterministic</td>
<td>chronic</td>
<td>↓ FA main effect; (-) MD</td>
</tr>
<tr>
<td>cerebellar tracts</td>
<td>(33,33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pomarol-Clotet, Canales-Rodriguez et al. 2010)Probabilistic</td>
<td>chronic</td>
<td>↓ FA in anterior CC;</td>
<td></td>
</tr>
<tr>
<td>whole brain</td>
<td>babilistic (32,32)</td>
<td></td>
<td>↓ FA in tracts to MPFC</td>
</tr>
<tr>
<td>Study</td>
<td>Method</td>
<td>Age Group</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------</td>
<td>-----------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Voineskos, Lobaugh et al. 2010</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↓ FA: UF,CB;</td>
</tr>
<tr>
<td>UF, IFOF, ILF, CB, AF, CC</td>
<td>(50,50)</td>
<td></td>
<td>↓ differences age all tracts</td>
</tr>
<tr>
<td>Luck, Malla et al.</td>
<td>Deterministic</td>
<td>FE</td>
<td>↓ FA in FH</td>
</tr>
<tr>
<td>FH</td>
<td>(32,25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whitford, Kubicki et al.</td>
<td>Deterministic</td>
<td>Chronic</td>
<td>↓ FA and ↑RD in frontal CC</td>
</tr>
<tr>
<td>CC subdivisions</td>
<td>(19,19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Douaud, Smith et al. 2007</td>
<td>TBSS</td>
<td>Adolescent</td>
<td>↓ FA in left AF; CC</td>
</tr>
<tr>
<td>whole brain</td>
<td>(25,25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlsgodt, van Erp et al. 2008</td>
<td>TBSS</td>
<td>FE</td>
<td>↓ FA esp. in L SLF</td>
</tr>
<tr>
<td>SLF</td>
<td>(12,17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miyata, Hirao et al. 2009</td>
<td>TBSS</td>
<td>Chronic</td>
<td>↓ FA left prefrontal/occipital</td>
</tr>
<tr>
<td>whole brain</td>
<td>(27,33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karlsgodt, Niendam et al. 2009</td>
<td>TBSS</td>
<td>Ultra high risk</td>
<td>↓ FA in ILF, MTL</td>
</tr>
<tr>
<td>six tracts</td>
<td>(36,25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jeong, Wible et al. 2009</td>
<td>TBSS</td>
<td>Chronic</td>
<td>↓ FA in STS, IFG,</td>
</tr>
<tr>
<td>whole brain</td>
<td>(10,10)</td>
<td></td>
<td>MTG semantic network</td>
</tr>
<tr>
<td>Miyata, Yamada et al. 2010</td>
<td>TBSS</td>
<td>Chronic</td>
<td>↓ FA in bilateral deep white</td>
</tr>
<tr>
<td>whole brain</td>
<td>(26,27)</td>
<td></td>
<td>matter, corona radiata</td>
</tr>
</tbody>
</table>

Note: ‘FE’ = first episode; (-) = negative finding
Although the first DTI study in schizophrenia was published in 1998 (Buchsbaum, Tang et al. 1998), the first DTI tractography study in schizophrenia was published in 2006 (Jones, Catani et al. 2006). Of the tractography studies reviewed here, nearly all found differences between schizophrenia patients and healthy controls. Unlike the earlier studies that used VBM and ROI based approaches, a more consistent pattern is evident from tractography studies. In particular the uncinate fasciculus, cingulum bundle, fornix of hippocampus, anterior thalamic radiation, inferior longitudinal fasciculus, and the arcuate fasciculus/superior longitudinal fasciculus are found to have reduced FA in schizophrenia. For instance, for left uncinate fasciculus, the significant FA findings in schizophrenia patients compared to controls occurred in 4 out of 6 studies. For the cingulum bundle, significant findings occurred in 5 out of 6 studies. Limbic system tracts (uncinate fasciculus, cingulum bundle, inferior longitudinal fasciculus, fornix), semantic system tracts (AF/SLF), and tracts that may play a role in higher cognitive functions (cingulum, anterior thalamic radiation) were likely to show reduced FA in schizophrenia patients. The genu and splenium of the corpus callosum were somewhat likely to show reduced FA, with more anterior segments of the corpus callosum (i.e. genu) implicated in several studies. There is likely insufficient evidence at this point to implicate cerebellar system tracts in schizophrenia, although one study demonstrated a main effect of FA difference in schizophrenia patients compared to controls (Kanaan, Borgwardt et al. 2009). Therefore, overall, fronto-temporal disconnectivity is strongly supported by these findings, interhemispheric disconnectivity is potentially supported, and it may be too early to draw conclusions regarding the cerebello-thalamo-cortical system, although connections from thalamus to frontal cortical regions in 3 of 4 studies demonstrated reduced FA in schizophrenia.
One question raised by these findings is: at what point do these disruptions in white matter occur? Might they be integral to development of the illness itself, viewed perhaps as part of neurodevelopment gone wrong? Or are they progressive, perhaps occurring during the chronic phase of the disease. A large ROI-based study examined both first episode and chronic schizophrenia patients and demonstrated differences in chronic patients compared to healthy controls, but not in first episode patients (Friedman, Tang et al. 2008). Furthermore, this study showed that differences in left ILF and left forceps minor of the corpus callosum were greatest in late-life. Others showed that these differences are present at the onset of illness. A recent study using TBSS, examining patients at ultra high risk for psychosis, demonstrated differences in FA compared to healthy controls. Furthermore, the patients with lower FA values demonstrated poorer social function (Karlgodt, Niendam et al. 2009). Many of the reviewed studies attempted to correlate FA with positive symptoms, negative symptoms, or cognitive performance. Here the data were not as consistent. Studies have associated positive symptoms, such as auditory hallucinations with increased FA in left CB (Hubl, Koenig et al. 2004), while the study by Ashtari et al, associated visual hallucinations with reduced FA in left ILF (Ashtari, Cottone et al. 2007). Negative symptoms have generally been correlated with lower FA. The study by Voineskos et al (Voineskos, Lobaugh et al. 2010) correlated negative symptom burden with increased asymmetry between left and right CB FA. It may be that finer subdivisions of white matter tracts may be required to uncover reliable correlations with symptomatology.

DTI studies in schizophrenia have attempted to correlate white matter tract integrity with cognitive performance. Association of FA with cognitive performance in healthy individuals is a common finding, and is also demonstrated fairly consistently in studies of healthy aging. In schizophrenia, like in healthy aging, a wide range of cognitive domains may be impaired.
Cognitive impairments in schizophrenia have been reviewed in detail elsewhere (Heinrichs and Zakzanis 1998). In brief, domains of verbal memory and learning, working memory, visuospatial ability, executive function, semantic verbal fluency, attention, set-shifting, response-inhibition, perceptual motor speed, and sensorimotor function are all impaired to some degree in schizophrenia. However, like in healthy aging populations, considerable heterogeneity exists among schizophrenia patients regarding the nature (i.e. which domains) and the extent (i.e. the severity) of cognitive impairment. These deficits are generally considered to be relatively stable across the lifespan, and present from illness onset (and in some cases present before the onset of psychosis) (Palmer, Dawes et al. 2009). However, there may be a subset of patients (likely those who are institutionalized long-term) who experience some degree of cognitive decline in conjunction with disease progression (Rajji and Mulsant 2008).

In some schizophrenia studies, association between FA and cognitive function is demonstrated in schizophrenia patients only (e.g. L SLF FA with working memory (Karlsgodt, van Erp et al. 2008)). The study by Nestor et al demonstrated a clear dissociation of executive function and episodic memory with CB FA and UF FA respectively, but in schizophrenia patients only (Nestor, Kubicki et al. 2008). It is not clear why cognitive performance was not tied to FA in healthy controls. One possible explanation may be that controls have a generally weaker relationship between white matter FA and cognitive performance due to a higher cognitive reserve. However, in schizophrenia patients, if white matter is susceptible, and cognitive reserve is already lower, a modest reduction in FA may lead to a higher than expected (compared to controls) impact on cognitive performance. Despite this important question, no study has yet investigated the relationship between integrity of white matter tract networks and a cognitive
performance battery in schizophrenia, in order to obtain a more comprehensive understanding of how white matter and cognition are related in this disorder.

1.10.7 DTI, Aging, and Schizophrenia

In typical development across the first years of life, FA values increase until adulthood. However, the pattern of FA increase may differ between tracts, and maturation of certain white matter tracts continues through adolescence, and in some tracts, into the twenties. The largest cross-sectional study of white matter development in healthy individuals (n=202) (Lebel, Walker et al. 2008), revealed that fronto-temporal tracts tend to mature more slowly than other regions. While the fornix does not demonstrate FA increases after age 5, fronto-temporal association fibers such as the SLF and IFOF began to plateau (i.e. reached the 90% milestone) between 13-20 years of age. Furthermore, the uncinate fasciculus and cingulum bundle do not reach 90% development (as measured by FA) until 25 years of age. Generally the pattern of growth was exponential, and non-linear. Postmortem studies have described a general pattern of posterior to anterior myelination (Benes 1989), while anatomical MRIs showed a rostral to caudal wave of growth in the corpus callosum (Thompson, Giedd et al. 2000) in development in childhood and adolescence. Fronto-temporal peaks of myelination occur in late adolescence, corresponding with age of onset of schizophrenia (Davis, Stewart et al. 2003).

Based on the cognitive reserve hypothesis (Dwork, Mancevski et al. 2007), one might expect schizophrenia patients to demonstrate worsening cognitive performance and possibly worse white matter indices with age, in a manner greater than healthy individuals. However, the data in this regard are mixed. Rosenberger et al (Rosenberger, Kubicki et al. 2008), and Carpenter et
al (Carpenter, Tang et al. 2008), demonstrated greater differences in FA with age when comparing schizophrenia patients to healthy controls (although the patients in Rosenberger’s study ranged from 25-56). On the other hand, Jones et al (Jones, Catani et al. 2006), and Voineskos et al (Voineskos, Lobaugh et al. 2010) demonstrated that differences were less apparent with age. These latter two studies had superior DTI acquisition protocols (i.e. > 20 orientation directions) in order to measure FA and conduct tractography. Another explanation for such different findings might be that patient selection (i.e. disease severity) influences white matter findings in schizophrenia. For instance, the elderly patients in the study by Voineskos et al, (ranging from 56-80 years of age) were community dwelling outpatients with MMSE scores in the non demented range (25-30). FA reductions may be greater in more severely ill, institutionalized patients, whose disease course and cognitive performance progressively worsens over time. Furthermore, elderly patients with schizophrenia may already be resilient in a manner different than other schizophrenia patients given that the average lifespan of someone with schizophrenia is approximately 15-20 years less than in the general population (Tiihonen, Lonnqvist et al. 2009). A more conclusive study regarding the question of progression of white matter deficits would require a prospective design over a 5 to 10 year time period with scans every 1-2 years. However, the practical issues associated with successful completion of such a study, particularly in severely mentally ill patients with schizophrenia are difficult to overcome.

1.10.8 Findings of White Matter Disruption in Healthy Aging Using DTI

The later phases of adult life are also a dynamic time for white matter. In healthy aging, changes in oligodendrocytes and myelin occur. Specifically, there is breakdown of myelin, certain constituents of cytoskeleton and axonal density, along with decline in the number and length of myelinated fibers (Sullivan and Pfefferbaum 2006). Age-related myelin breakdown has been
shown by electron microscopy and includes alterations of the myelin sheath, either by splits in the lamellae of the myelin sheaths or ballooned sheaths (Peters and Sethares 2002; Bartzokis, Sultzer et al. 2004). Postmortem data align with in vivo data obtained using DTI in healthy aging populations (Sullivan and Pfefferbaum 2006). These DTI studies show fairly dramatic reductions in white matter integrity in later adult life. The pattern of such reduction tends to be more pronounced in anterior or frontal based white matter.

A considerable value of studies of healthy aging that have examined both DTI and cognitive parameters in the same subjects is the consistent finding that white matter integrity is related to cognitive performance. While there is some divergence in findings regarding exactly which tracts mediate which higher order cognitive functions, individuals with lower FA tend to demonstrate poorer cognitive performance. That is, the functional ramifications of DTI metrics have been regularly verified with observations of correlations between regionally specific low FA or high diffusivity and poor cognitive or motor test performance in humans, and even in a monkey model of aging (Sullivan and Pfefferbaum 2007). Bartzokis et al has described a pattern of retrogenesis of white matter changes during aging (Bartzokis 2004; Bartzokis, Sultzer et al. 2004). That is, the last tracts to develop (and myelinate) are the first susceptible to age-related damage. The review by Sullivan et al (Sullivan, Adalsteinsson et al. 2006) describes an antero-posterior gradient of age-related decline in white matter tract integrity, that is similar to that gradient described for gray matter by Raz (Raz, Gunning et al. 1997) and others. This pattern of decline has been linked to the decline in cognitive functions that have been shown to be frontally based that occur in healthy aging (Hedden and Gabrieli 2004). However, only now are tractography measures in healthy aging samples being combined with cognitive measures from the same individuals. These studies have partly confirmed the link between decline in frontally
based tracts and higher order frontally based cognitive functions (e.g. executive function, working memory, etc.). Other studies (Charlton, Landau et al. 2008; Sullivan, Rohlfing et al. 2008; Zahr, Rohlfing et al. 2009) have also examined this question. All of these studies demonstrated relationships between decline in white matter integrity and decline in cognitive performance in healthy aging; however support for the frontally based tract – higher order cognitive function model from these studies is not consistent. Another conceptualization of the neuroanatomic pattern of white matter decline in healthy aging is that there is a steep gradient of decline in commissural and association fibers connecting frontal, temporal, parietal or occipital cortical regions as compared to tracts connecting motor or sensory regions (e.g. commissural fibers connecting left and right motor cortex), or to motor projection fibers such as the corticospinal tract (Voineskos, Rajji et al. 2010).

To date, conclusions regarding white matter decline in healthy aging, and its relationship to decline in cognitive performance have been largely drawn from cross-sectional studies. In order to understand progression of white matter decline and its relationship with progression of cognitive decline, longitudinal studies over several years are required. However, no such study has yet been published.

1.11 Imaging-Genetics Strategies: Reducing phenotypic heterogeneity and understanding gene effects on the brain and behaviour

Imaging genetics strategies should be useful in reducing phenotypic heterogeneity in aging and schizophrenia. In aging for instance, considerable heterogeneity is demonstrated in both neuroimaging and cognitive studies. FA is lower in certain white matter tracts in elderly individuals compared to younger individuals, and considerable variability is present amongst
older healthy individuals in both imaging and cognitive measures (Sullivan, Adalsteinsson et al. 2006). The same is true in schizophrenia: considerable heterogeneity exists amongst patients, both in terms of their symptoms and cognitive performance, and even in neuroimaging findings (Gottesman and Gould 2003; Meyer-Lindenberg and Weinberger 2006). The combination of neuroimaging and genetics may also improve our understanding of disease risk. Such a combination (i.e. imaging-genetics) offers the potential to characterize the effects of genetic risk variants on at-risk neural structures relevant to disease, via the intermediate phenotype approach. Such an approach may evince greater penetrance of the effects of the gene on the vulnerable neural structure or function (Meyer-Lindenberg and Weinberger 2006). Genotype to brain phenotype associations can be shown in carriers of risk alleles even if the carriers do not exhibit the clinical phenotype. Findings may be most robust at the level of brain structure, and less robust at the level of observable behavior (i.e. cognition), consistent with the intermediate phenotype concept (Tan, Callicott et al. 2008). For instance, for sporadic or late-onset Alzheimer’s disease, APOE4 allele status is the most prominent genetic risk factor, and studies have demonstrated patterns of risk in APOE4 allele carriers many years before any onset of disease symptoms. One study (Shaw, Lerch et al. 2007) demonstrated a neuroanatomic signature of E4 carriers in childhood and adolescence, where a thinner entorhinal cortex was shown compared to non E4 carriers. Also distinct patterns of brain activity have been shown in younger adult E4 carriers (Filippini, McIntosh et al. 2009) during an episodic memory task, where no differences in episodic memory were yet present. Therefore, imaging genetics studies can demonstrate patterns of effect on the brain of gene variants that may confer risk for disease, long before clinical expression of the disease itself. Furthermore, effects of these gene variants can be characterized in a neuroanatomic specific manner, and illuminate where and possibly how risk is
conferred. Other such examples have been demonstrated for depression (serotonin transporter polymorphism gene variant - 5HTTLPR on cingulate cortex – (Pezawas, Meyer-Lindenberg et al. 2005), BDNF val66met on hippocampus (Pezawas, Verchinski et al. 2004), 5HTTLPR on amygdala reactivity for anxiety(Hariri, Mattay et al. 2002), and the catechol-O-methyl-transferase val158met variant on dorsolateral prefrontal cortex activation for schizophrenia(Meyer-Lindenberg, Nichols et al. 2006). More recently, the genome-wide significant Zn804A risk variant was examined in healthy individuals using the intermediate phenotype approach to demonstrate effects on regional connectivity between frontal and temporal brain regions using fMRI, in a schizophrenia susceptibility pattern (Esslinger, Walter et al. 2009).

It is also possible, however, that gene systems may predict neural variation and behaviour (cognition) in a different manner in a disease population compared to a healthy population. That is to say that utilization of the intermediate phenotype approach in a healthy population does not conclusively prove how a gene variant might predict brain structure or function in an individual with disease. Several studies have now combined imaging and genetics approaches in schizophrenia research, using the imaging genetics approach in both schizophrenia patients and healthy controls. For instance Szeszko et al (Szeszko, Lipsky et al. 2005) examined BDNFval66met genotype and its effect on hippocampal volume in schizophrenia patients and healthy controls. They discovered that the BDNF gene variant predicted hippocampal volume in both groups, but greater variance in hippocampal volumes was predicted in schizophrenia patients compared to healthy controls.

Regarding DTI and genetics, four studies relevant to schizophrenia have been published so far, all of which have indexed the Neuregulin1-ErBb4 system. Three studies examined NRG1 gene
SNPs (all from the core risk haplotype) on white matter, and one study examined ErbB4 gene
SNPs. All were positive, where these variants predicted FA in medial prefrontal regions, anterior
thalamic radiation, and cingulum (for the three NRG1 studies) and FA at the uncinate fasciculus
(for ErbB4). Despite the compelling evidence for myelin gene involvement in schizophrenia, and
the evident fact that oligodendrocytes and myelin modulate diffusion anisotropy, no study has
yet examined the relationship between oligodendrocyte and myelin gene variants with DTI
measures of tissue microstructure.

1.11.1 More than one SNP and more than one Brain Measure: Moving
Toward Multivariate Statistical Approaches in Imaging-Genetics

Generally, imaging genetics studies have used univariate statistical approaches to examine the
relationship between gene variants and neuroimaging (and cognition). However, it is likely that a
set of gene variants (acting in a system of genes) may be related to a series of brain structures
(acting as part of a network or circuit), particularly as they relate to cognitive performance.
Multivariate statistical modeling can better contend with the presence of several independent and
dependent variables simultaneously. In addition the problem of multiple comparison testing, that
has polarized opinion in the psychiatric genetics field (see different opinions in Meyer-
Lindenberg et al(Meyer-Lindenberg, Nicodemus et al. 2008), and Sullivan et al(Sullivan 2007) is
eliminated. Regarding multiple comparison testing, the false discovery rate (FDR) (Genovese,
Lazar et al. 2002) has been used, particularly in fMRI studies, when a gene variant is examined
in relation to the BOLD signal in hundreds of thousands of voxels. Simulations have been
completed demonstrating that a 5% FDR is an acceptable method of controlling for false positive
findings(Meyer-Lindenberg, Nicodemus et al. 2008). It has been argued that the Bonferroni
method is overly conservative, and leads to ‘the baby being thrown out with the bathwater’ – that
is, valuable information can be lost with overly-stringent corrections for multiple comparisons. Such decisions become potentially even more difficult with the recent explosion of bioinformatics tools, whereby 500,000 or 1,000,000 SNPs can be compared (extracted from gene chips with microarray technology) with 500,000 voxels in the brain. Multivariate approaches remove some of the pressure from the ‘p value chase’. Emerging multivariate approaches in imaging genetics include the independent components analysis (ICA) (Meda, Jagannathan et al. 2009; Sui, Adali et al. 2009) and partial least squares (PLS) (McIntosh, Bookstein et al. 1996; McIntosh and Lobaugh 2004) approaches.

1.11.2 Partial least squares analysis

More detailed explanations of the PLS method can be found elsewhere (McIntosh, Bookstein et al. 1996; McIntosh and Lobaugh 2004). PLS is an approach that can assess a large-covariance matrix in multivariate neuroimaging (McIntosh, Bookstein et al. 1996; McIntosh and Lobaugh 2004) and genetics data (Raadsma, Moser et al. 2008; Opiyo and Moriyama 2009). PLS has several advantages over conventional univariate approaches (McIntosh and Lobaugh 2004), including: (i) greater power, (ii) the capability to deal with data sets where the dependent measures within a block are highly correlated, and (iii) the capability to evaluate the reliability of the findings over and above tests of significance. The use of resampling algorithms to evaluate reliability enables a degree of certainty in the analysis that conventional parametric statistics cannot provide. PLS uses permutation analysis to derive measures of significance for latent variables obtained from the data. Each latent variable, or LV, is similar to a factor in principal components analysis (PCA), and serves to relate groups of variables. Permutation sampling involves randomly reassigning participants across groups. The stability of these results is then
determined by bootstrap resampling, which involves sampling the dataset with replacements to
derive estimates of standard errors of the LV. Such reliability estimates ensure that the data are
not driven by a subsample of data points, a key issue in imaging-genetics studies.

1.12 Outline of Experiments
Chapter 2 will provide a background and rationale for the seven manuscripts included in this
PhD thesis. In the subsequent chapter, the objectives and hypotheses for each study will be
presented. Studies one through five (Chapters 3-7) have been published in peer-reviewed
journals: *Psychiatric Genetics, Brain Imaging and Behavior, NeuroImage, Brain*, and
*Neurobiology of Aging* respectively. Study six (Chapter 8) has been accepted for publication by
the *Archives of General Psychiatry*. Study seven (Chapter 9) is about to be submitted to a peer-
reviewed journal. Because these studies exist as stand alone articles, material contained in each
of these articles may overlap with material presented in other articles as well as material
presented in the Introduction.
Chapter 2

2 Overview of Experiments, and Hypotheses

This thesis consists of seven papers that advance and bring together schizophrenia, aging, neuroimaging, and genetics, with a particular focus on the oligodendrocyte/myelin/white matter pathway.

2.1 Study One: A family-based association study of the myelin associated glycoprotein and 2’,3’-cyclic nucleotide 3’-phosphodiesterase genes with schizophrenia

2.1.1 Background

Recent postmortem investigations highlighted the downregulation of myelin system genes in schizophrenia. Therefore genetic variation at polymorphisms within these genes might increase risk for schizophrenia. This first study was an examination of association of MAG and CNP gene variants with schizophrenia using a family based association approach, that is resistant to ethnic stratification.

2.1.2 Hypotheses

We hypothesized that variants at the MAG and CNP genes would be associated with schizophrenia. In addition, in a secondary analysis, we looked for gene-gene interactions and parent of origin effects at each gene. We hypothesized that an interaction would be present conferring increased risk for schizophrenia, and preferential parental transmission of MAG gene risk variants might occur, given MAG’s location in a known imprinted locus.
2.2 Study Two: MAG Gene Variation and Cortical Gray Matter Volume in First Episode Schizophrenia

2.2.1 Background

The MAG gene has been shown to be downregulated in both cortical gray matter, and white matter. While genetic association studies provide some evidence for association of the MAG gene with schizophrenia, MAG gene risk variants may be more penetrant at the level of brain phenotypes rather than disease phenotypes, and may provide a better understanding of the manner in which the MAG gene confers risk in schizophrenia.

2.2.2 Hypotheses

Since the MAG gene has been shown to be downregulated in both cortical gray matter, and white matter, it was hypothesized that MAG gene risk variants might be associated with cortical gray matter volume and white matter volume in schizophrenia.

2.3 Study Three: Quantitative Examination of a Novel Clustering Method Using Diffusion Tensor Tractography

2.3.1 Background

Diffusion tensor imaging, an MRI approach that can infer properties of white matter microstructure in vivo requires reliable approaches to segment and measure white matter tracts for studies in human diseases. Streamline (or deterministic) tractography is an excellent way to visualize and measure certain white matter fiber tracts in the brain. However, in order to segment these tracts, investigators have typically seeded brain regions, created by drawing regions-of-interest, which is a process where bias can be introduced in several ways. With the recent development of a novel clustering method, an opportunity arose to demonstrate reliability of this method, which potentially affords less opportunity for user-related bias, in segmenting white
matter tracts. This method is automatic, unguided, and takes advantage of the similarity of shape and direction of travel of white matter tracts, and clusters related tracts together, which in turn facilitates the user’s tract selection process.

2.3.2 Hypotheses
It was hypothesized that the clustering segmentation method would demonstrate high reliability for a large number of white matter tracts, both in terms of voxel overlap and scalar indices of diffusion, in both healthy controls and schizophrenia patients. Furthermore, it was hypothesized that the clustering method would be as reliable as the multiple ROI method in segmenting these tracts.

2.4 Study Four: Diffusion Tensor Tractography Findings in Schizophrenia Across the Adult Lifespan

2.4.1 Background
Fronto-temporal disconnectivity is a core theory of schizophrenia. Disruption of white matter tracts connecting frontal and temporal brain regions may underlie this disconnectivity in schizophrenia. Perturbation of oligodendrocytes in frontal and temporal brain regions has also been shown in schizophrenia, and these oligodendrocytes form the myelin sheath of the white matter tracts that can be studied using DTI. Late-life is a particularly dynamic time of white matter change in healthy individuals. However, there has been no investigation of fronto-temporal white matter tracts in elderly patients with schizophrenia.

2.4.2 Hypotheses
Fronto-temporal and interhemispheric white matter tracts were examined, consistent with fronto-temporal and interhemispheric theories of disconnectivity in schizophrenia. The main hypotheses were that age-related decline in white matter integrity would be observed at each white matter
tract in schizophrenia patients and healthy controls, but that schizophrenia patients would experience similar or accelerated age-related decline (rather than less decline) compared to healthy controls. Further, it was hypothesized that older schizophrenia patients would demonstrate differences at white matter tracts compared to healthy controls in a similar or greater manner to differences observed in younger individuals. These differences were hypothesized to occur particularly at fronto-temporal white matter tracts, consistent with the theory of fronto-temporal disconnectivity in schizophrenia.

2.5 Age-related Decline in White Matter Integrity and Cognitive Performance: A DTI Tractography and Structural Equation Modeling Study

2.5.1 Background
Neuroimaging studies of healthy aging provide an opportunity to improve our understanding of the neural correlates of cognitive decline. Although healthy aging is a heterogeneous process, various cognitive domains are subtly affected in later life. At the same time, neuropathologic and neuroimaging studies demonstrate various brain changes associated with aging. More recently, using DTI, reduced white matter integrity, noted in the elderly, has been associated with reduced performance on cognitive tasks. No study had examined the relationship of white matter tract integrity and a cognitive battery across the adult lifespan, and structural equation modeling had not yet been used to examine the relationship between age, microstructural integrity of white matter tracts, and cognitive performance.

2.5.2 Hypotheses
SEM was used to examine the relationship of aging, white matter tract integrity, and cognitive performance. Based on the literature, we hypothesized that we would primarily observe
associations among age, microstructural integrity of cortico-cortical white matter tracts (in particular those connecting to frontal cortical regions), and specific cognitive functions, primarily reflecting the frontally based model of cognitive decline in normal aging.

2.6 Study 6: The BDNF val66met polymorphism: Genetic susceptibility for an intermediate phenotype related to Alzheimer’s disease in healthy individuals

2.6.1 Background
The BDNF gene has been implicated in several neuropsychiatric illnesses including schizophrenia. BDNF also appears to play a key role in healthy aging and disorders of pathological aging. Imaging-genetic strategies may clarify heterogeneity in cognitive and/or neuroimaging data in healthy aging populations. In particular, the risk for late-onset Alzheimer’s disease increases with age, and clarifying who might be at-risk for pathological aging, is of critical importance. Furthermore, determining genetic variants that may confer susceptibility to neural structures or cognitive functions at-risk in pathological aging, can be of important prognostic and preventive value. A gene that may be of particular importance in healthy aging and pathological aging is the BDNF gene, and recent evidence suggests that BDNF may rescue cognitive performance in animal models of aging and Alzheimer’s disease. Therefore, the BDNF val66 met polymorphisms was examined in relation to cortical thickness, white matter tract integrity, and episodic performance in healthy individuals across the adult lifespan.

2.6.2 Hypotheses
The functional BDNF val66met variant was examined in relation to cortical thickness, white matter tract integrity, and episodic memory performance in order to determine whether the BDNF gene predicts risk for an intermediate phenotype related to healthy versus pathological
aging. It was hypothesized, that val/val individuals would demonstrate an at-risk pattern in
cortical thickness of structures at medial and lateral temporal lobe, reduced white matter integrity
in tracts connecting to medial temporal lobe, and reduced episodic memory performance.

2.7 Study 7: Oligodendrocyte Genes, White Matter, and
Cognition in Schizophrenia: An Imaging-Genetics Study

2.7.1 Background
Oligodendrocyte and myelin related genes, and white matter tract disruption have been
separately implicated in schizophrenia. The relationship of such disruption to clinical
presentation is not well-understood. However, DTI studies in other fields, such as healthy aging,
have demonstrated that white matter integrity is related to cognitive performance. Therefore, it is
possible that white matter integrity may be related to cognitive performance in schizophrenia.
Furthermore, such investigations generally have a considerable degree of phenotypic
heterogeneity. By combining information from oligodendrocyte gene variants with white matter
integrity and cognitive measures, it may be possible to parse out such heterogeneity to identify
which individuals are at particularly elevated risk for cognitive disruption. The aim here was to
bring together data from molecular genetics studies of schizophrenia implicating oligodendrocyte
and myelin related genes with data from DTI studies implicating disruption of white matter tracts
in the context of impaired cognitive performance in schizophrenia. This final study examined
myelin gene variants, white matter integrity, and cognitive performance in schizophrenia patients
and healthy controls using the multivariate PLS approach.

2.7.2 Hypotheses
Variants from oligodendrocyte genes, and those from Neuregulin1-ErbB4 (a gene system that
influences oligodendrocyte development) were hypothesized to be related to white matter
integrity in both schizophrenia patients and healthy controls. In light of impaired cognitive performance in schizophrenia, and potentially lower cognitive reserve, white matter tract integrity was hypothesized to be related to cognitive performance in schizophrenia. Finally, myelin gene variants were hypothesized to mediate white matter – cognition relationships in schizophrenia. Variants from four myelin genes (all had replicated evidence for association with schizophrenia, and for downregulation in postmortem schizophrenia brain): MAG, CNP, OLIG2, QKI, as well as variants from the NRG1-ErbB4 system were examined in conjunction with DTI phenotypes (fractional anisotropy and radial diffusivity of fronto-temporal and interhemispheric white matter tracts: UF, ILF, CB, AF, IFOF, and genu and splenium of the corpus callosum) and cognitive performance.
Chapter 3

3 A family-based association study of the myelin associated glycoprotein (MAG) and 2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNP) genes with schizophrenia

Contents of this chapter have been published as:

Voineskos AN et al. A family based association study of the myelin associated glycoprotein (MAG) and 2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNP) genes with schizophrenia. *Psychiatric Genetics*. 2008 Jun 18(3); 243-6.

A link to the published paper can be found at:
http://journals.lww.com/psychgenetics/Abstract/2008/06000/A_family_based_association_study_of_the.8.aspx

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3.1 Abstract

There has been a recent surge of evidence implicating myelin abnormalities in the etiology of schizophrenia. The present study is a family-based genetic association analysis examining the MAG and CNP genes in schizophrenia. Two hundred forty six families of primarily European Caucasian origin were genotyped for MAG rs2301600, rs720308, rs720309, rs756796, and CNP rs2070106 single nucleotide polymorphisms (SNPs). The FBAT program (v1.7.2) and TRANSMIT were used to analyze individual SNPs as well as haplotypes, respectively. The CNP SNP (rs2070106) was potentially associated with schizophrenia (p=0.027). MAG variants were not associated with disease transmission based on single marker or haplotype analysis. A
significant maternal parent of origin effect for the CNP risk allele for schizophrenia was found (p=0.003). There was no CNP-MAG gene gene interaction conferring increased risk for schizophrenia. Our finding provides support for potential association of the CNP gene but not the MAG gene in schizophrenia in a Caucasian population.

3.2 Introduction

Recent investigations have shown extensive myelin and oligodendrocyte changes in schizophrenia (Flynn, Lang et al. 2003). Postmortem gene expression studies in schizophrenia show significant downregulation of myelin associated and oligodendrocyte related genes in the brain (Hakak, Walker et al. 2001), as well as a decrease in oligodendrocyte density (Hof, Haroutunian et al. 2003). Several myelin associated genes have been linked with schizophrenia (Qin, Gao et al. 2005) or appear to play a role in regulating oligodendrocyte differentiation and maturation (Aberg, Saetre et al. 2006). Therefore, accumulating evidence points to myelin related and oligodendrocyte dysfunction as potentially key pathophysiological pathways in schizophrenia.

Significantly reduced levels of myelin associated glycoprotein (MAG) mRNA in prefrontal cortex of schizophrenia subjects and a 3-fold decrease of MAG expression in schizophrenia (Hakak, Walker et al. 2001; Tkachev, Mimmack et al. 2003) provide strong rationale for further investigation into MAG’s role in schizophrenia. Two studies (one family study and one case-control study) in the Chinese Han population showed a core risk haplotype (rs720309-rs720308) of the MAG gene associated with schizophrenia (Wan, Yang et al. 2005; Yang, Qin et al. 2005). However, the family study found different alleles that conferred the risk haplotype compared to the case control study.
Another important gene which may play a role in oligodendrocyte function and myelination is the 2’,3’-cyclic nucleotide 3’-phosphodiesterase (CNP) gene. Several studies show significantly reduced expression of the CNP gene in postmortem brain of schizophrenia patients (Hakak, Walker et al. 2001; Davis, Stewart et al. 2003; Tkachev, Mimmack et al. 2003). A combined gene expression/gene association study of the CNP gene showed that the rs2070106 A allele was associated with lower CNP expression in postmortem schizophrenia brain, and the same A allele was more common in affected individuals in the case-control sample (Peirce, Bray et al. 2006). In recent Chinese and Japanese case-control samples, CNP was not associated with schizophrenia (Usui, Takahashi et al. 2006; Tang, Qu et al. 2007).

The discrepancy between the risk alleles in the core risk haplotype for the MAG gene within the Chinese Han population, the lack of a family-based association study for the CNP gene, and the accumulating body of evidence for pathophysiological implication of these two genes in schizophrenia, all point to the need for further investigation of the MAG and CNP genes.

Here we present the results of a family-based association study of the MAG and CNP genes in a primarily European Caucasian population. The two principal aims of this study are to: 1) replicate the findings of association of the MAG rs720309-720308 core risk haplotype and 2) of association of the A allele of the CNP rs2070106 SNP in schizophrenia.

3.3 Methods

3.3.1 Recruitment and Diagnosis

This study was approved by the local research ethics board at the Centre for Addiction and Mental Health (CAMH). Informed written consent was obtained from all subjects in the study after explanation of the procedure. We recruited 790 individuals from 245 nuclear families. All
probands had a DSM-IV diagnosis of schizophrenia or schizoaffective disorder. DSM-IV diagnoses were made by the subject’s primary clinician, and confirmed using a SCID-I completed by a trained research analyst.

3.3.2 Demographics
Two hundred twenty of the families were mixed Caucasian (European ancestry), and the remainder were Asian, African-American or Native. Of the three hundred probands, two hundred eighty five had a diagnosis of schizophrenia and fifteen had schizoaffective disorder. Probands showed a 2:1 male to female ratio.

3.3.3 SNP Selection
The MAG rs720309 - rs720308 haplotype has been identified as a core risk haplotype associated with schizophrenia (Wan, Yang et al. 2005; Yang, Qin et al. 2005). We chose to genotype one additional marker on either side of the rs720308-rs720309 haplotype block to help delineate the genomic region associated with the disorder. Since one of our original hypotheses was that the rs720309-720308 haplotype would be associated with schizophrenia, and the original work contained Chinese subjects for the MAG gene, the additional SNPs were selected from haplotype blocks on either side of the core risk haplotype block identified in the Chinese population (Yang, Qin et al. 2005), based on Caucasian data from the HapMap project (HapMap.org) that had a minor allele frequency > 20%. Rs2301600 is located upstream of the fine-scale Caucasian block and served as a 5’ flanking SNP. However, due to the tight linkage disequilibrium (LD) within and downstream of the MAG gene, and the presence of an additional gene (CD22 antigen) within 15 kb of the MAG 3’ end, we could not select a 3’ flanking SNP completely outside the core risk
haplotype block. Accordingly, we selected rs756796, which lies between MAG and CD22, and meets our original SNP selection criteria.

The CNP rs2070106 SNP was chosen based on its significant downregulation in schizophrenia and association in a large Caucasian case-control sample in the same study (Peirce, Bray et al. 2006). UCSC Genome Browser reference numbers for all SNPs are provided (Table 1).

3.3.4 Genotyping Protocol

Genotyping of the SNPs was performed using a standard ABI 5' nuclease Taqman® assay-on-demand protocol in a total volume of 10 µl. Post-amplification products were analyzed on the ABI 7500 Sequence Detection System (Applied Biosystems Inc, CA, USA) and genotype calls were performed manually. Results were verified independently by two laboratory personnel blind to demographic or diagnostic information.

3.3.5 Statistical Analysis

FBAT v1.7.2 was used to analyze transmission disequilibrium (TD) for the single SNPs and haplotypes. FBAT has the advantage over the conventional TDT of being able to use data from all family members, not just case-parent trios (Horvath, Xu et al. 2001; Horvath, Xu et al. 2004). Haploview v3.32 was used to calculate pair-wise D’ prime values as a measure of LD (Barrett, Fry et al. 2005). TRANSMIT was used post-hoc, incorporating a bootstrap procedure, for the analysis of haplotype transmission (Clayton and Jones 1999). Hardy-Weinberg equilibrium calculations and calculation of p values from the chi-square results of TRANSMIT were performed using the program Linkage Utilities (Ott, 2006).

Post-hoc parent-of-origin effect (POE) analyses were performed for all five markers using UNPHASED v2.0 (Dudbridge 2003) since the MAG gene lies very near a known imprinted
region on 19q13. Finally, a MAG-CNP gene-gene interaction in schizophrenia was examined using PedSplit (Lanktree, VanderBeek et al. 2004), since the MAG and CNP genes are both regulated by the Quaking (QKI) gene (McCullumsmith, Gupta et al. 2007).

The MAG SNPs tested are highly correlated (www.hapmap.org), and thus cannot be considered independent, making the Bonferroni correction too conservative within this gene. Therefore, we corrected for multiple testing of the MAG SNPs by running 10,000 permutations using FBAT v1.7.2. We did, however, consider the CNP gene as independent of the MAG gene, and, therefore, set our threshold for significance to \( \alpha = 0.025 \), as per Bonferroni correction.

### 3.4 Results

All SNPs were in Hardy Weinberg equilibrium. We found no association of any of the individual MAG SNPs with schizophrenia (Table 2). The linkage disequilibrium was very high among all the MAG markers with LD ranging from \( D' = 0.986 \) to 1.0. MAG haplotypes were also not significantly associated with schizophrenia. Of particular interest, the core haplotype identified in previous studies (rs720309-rs720308) showed no association with schizophrenia. The rs2301600 - rs720309 - rs720308 C-T-A haplotype showed a trend toward association with schizophrenia (\( p = .085 \)) (permuted p value = 0.095). Post-hoc analysis of the haplotypes with TRANSMIT, despite providing more power, yielded no increased significance of the risk C-T-A haplotype identified (Table 2).

We found potential overtransmission of the rs2070106 G allele (Table 2) of the CNP gene. Since our threshold of significance was set at \( \alpha = 0.025 \) (as per Bonferroni correction) we consider this result as indicative of a potential association of the CNP rs2070106 G allele with schizophrenia.
The single CNP marker showed biased maternal transmission of allele G to affected offspring (T: 23, UT: 7; relative risk: 3.29; LRS = 8.992; DF = 1 p = 0.003). No POE was observed for any of the four MAG SNPs. There was no significant interaction between CNP and MAG SNPs that conferred increased risk for schizophrenia.

3.5 Discussion

Our *a priori* hypotheses in this study were that: 1) we would find the same common core risk MAG gene haplotype (rs720309-rs720308) associated with schizophrenia as in previous reports, and 2) the CNP rs2070106 A allele would be associated with schizophrenia. However, there was no association between MAG haplotypes and schizophrenia in our study, and no single MAG SNP was associated with schizophrenia either. While we did find a possible association of the CNP rs2070106 SNP with schizophrenia, it was with the G allele rather than the A allele.

LD values for the MAG SNPs were very high, close to 1.0. This indicates our study population had differences in the block structure from the Chinese populations studied (Wan, Yang et al. 2005; Yang, Qin et al. 2005), that may be responsible, in part, for our different results. Interestingly, the Chinese family study identified a common risk haplotype T-A (rs720309-rs720308) (Yang, Qin et al. 2005), while the case-control study identified risk haplotype A-G (rs720309-rs720308) (Wan, Yang et al. 2005) that conferred increased risk for schizophrenia. Associations of opposite alleles at the same biallelic locus can be perplexing, particularly in the same ethnic group. This is known as the ‘flip-flop’ phenomenon (Lin, Vance et al. 2007). This phenomenon occurred in our study when comparing our positive CNP finding and the positive association for the CNP gene by Peirce et al (2006). The G allele conferred risk for schizophrenia in our study, while the A allele was significant in a previous study (Peirce, Bray et al. 2006). Possible explanations for this ‘flip-flop’ phenomenon include another locus acting in
concert with the CNP locus to cause disease, a false-positive, or differences in LD between the population of each study (Lin, Vance et al. 2007), though the most likely explanation is a false positive result.

Our study was limited by the low minor allele frequencies of the MAG SNPs in our population. Our considerably lower than expected minor allele frequency for the MAG rs720309 and rs720308 SNPs (13 per cent in our study vs 22 per cent on the HapMap database for the European Caucasian population), reduced our power by decreasing the number of informative families.

We examined POE because of the proximity of the MAG gene to the imprinted chromosomal region 19q13.4, but found no POE for MAG. Surprisingly, we did find maternal POE for CNP rs2070106 risk allele G, indicating possible epigenetic regulation of the CNP gene. Our POE finding at this locus requires replication with a larger sample size and the 17q21.2 chromosomal site of the CNP gene is not currently known as imprinted in the human genome. However, further examination of sex differences in studies of myelin genes may be useful, since it has been shown in rodents that females have lower levels of myelin protein gene expression, and, estrogen and progesterone are regulators of oligodendrocyte precursor cells (Cerghet, Skoff et al. 2006).

Our analysis of a CNP-MAG gene-gene interaction was negative. Both the CNP and MAG genes are regulated by the QKI gene and are each known to have functional interaction with QKI (McCullumsmith, Gupta et al. 2007). Though we did not find a gene-gene interaction, a case control design may provide more information about such a mechanism. Furthermore, looking at the effect of the QKI gene in concert with MAG and CNP may help elucidate the process of disruption of oligodendrocyte development and signaling pathways. In the broader perspective,
large, high powered, hypothesis-driven studies examining a small number of genes that act in concert, may be an optimal approach to better understand dysregulation of oligodendrocytes and the myelin system in schizophrenia.

**Table 3-1. UCSC Genome Browser Reference Numbers**

<table>
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<tr>
<th>SNP</th>
<th>Absolute Location</th>
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<tr>
<td>MAG rs2301600</td>
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</tr>
<tr>
<td>MAG rs720308</td>
<td>4048990</td>
</tr>
<tr>
<td>MAG rs720309</td>
<td>40489756</td>
</tr>
<tr>
<td>MAG rs756796</td>
<td>40501640</td>
</tr>
<tr>
<td>CNP rs2070106</td>
<td>37379390</td>
</tr>
</tbody>
</table>

*The absolute location of the SNPs reported were taken from the March 26, 2007 University of California Santa Cruz (UCSC) Genome Browser annotation.*
Table 3-2. Results of the TDT for both the MAG and CNP genes and TRANSMIT haplotype analysis for the MAG gene

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Allele Freq.</th>
<th>Informative families</th>
<th>z score</th>
<th>p value</th>
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</thead>
<tbody>
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<td>MAG rs2301600</td>
<td>C</td>
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<td>MAG rs720309</td>
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<td>0.869</td>
<td>28</td>
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<td>A</td>
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<td>1.134</td>
<td>0.256</td>
</tr>
<tr>
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<td>1.280</td>
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<td>CNP rs2070106</td>
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</table>

<table>
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<th>Experimental</th>
<th>Variance (O-E)</th>
<th>Chi square</th>
<th>df</th>
<th>p value</th>
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<tr>
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<td>22.062</td>
<td>2.708</td>
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<td>0.099</td>
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<tr>
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<td>34.039</td>
<td>12.409</td>
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<td>32.057</td>
<td>11.237</td>
<td>1.698</td>
<td>1</td>
<td>0.193</td>
</tr>
</tbody>
</table>
Chapter 4

4 MAG gene variation and cortical gray matter volume in first episode schizophrenia

Contents of this chapter have been published as:


A link to the published paper can be found at:
http://www.springerlink.com/content/a158152q65126707/

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4.1 Abstract

**Background:** Evidence implicating myelin related genes in the pathophysiology of schizophrenia is accumulating. Abnormalities of brain structure at the onset of psychosis may be related to variation in genes such as myelin associated glycoprotein (MAG).

**Methods:** Subjects with first episode schizophrenia (n=30) or schizoaffective disorder (n=11), and healthy comparison subjects (n=43) participated in an MRI scan. Two single nucleotide polymorphisms (rs720309, rs720308) in the MAG gene were genotyped.

**Results:** MAG genotype variation predicted cortical gray matter volume in first episode schizophrenia patients (p=0.039), but not in controls (p=0.827). Cortical gray matter, total gray matter, total white matter, and ventricular cerebrospinal fluid volumes did not differ between groups.
Conclusions: Genetic variation in the MAG gene may predict cortical gray matter volume differences in patients in the first episode of schizophrenia or schizoaffective disorder.

4.2 Introduction

Altered neural connectivity may be present from the first episode of schizophrenia. At a cellular level, oligodendrocytes, myelin, and myelinated axons are critical for development and proper function of neuronal connectivity. Recent studies indicate extensive myelin and oligodendrocyte changes in schizophrenia (Flynn, Lang et al. 2003; Tkachev, Mimmack et al. 2003). Gene expression studies show significant downregulation of myelin associated genes and oligodendrocyte related genes (Hakak, Walker et al. 2001; Katsel, Davis et al. 2005), as well as lower oligodendrocyte density in schizophrenia (Hof, Haroutunian et al. 2003). These studies primarily found downregulated myelin genes in cortical gray matter regions. Furthermore, several myelin related genes have demonstrated association with schizophrenia (Qin, Gao et al. 2005; Peirce, Bray et al. 2006) or appear to play a role in regulating oligodendrocyte differentiation and maturation (Aberg, Saetre et al. 2006).

The myelin associated glycoprotein (MAG) gene has perhaps the most compelling evidence for involvement in schizophrenia, among the myelin genes implicated in schizophrenia to date. Two studies (one family study and one case control) in the Chinese Han population have shown
significant association of two intron 8 markers in the MAG gene with schizophrenia (Wan, Yang et al. 2005; Yang, Qin et al. 2005). MAG is a likely a cellular adhesion molecule, and, interestingly, also blocks axon regeneration in vivo (Davis, Stewart et al. 2003). MAG is implicated in mediating axo-glial interactions during myelination. MAG deficient mice have morphologically abnormal myelin sheaths, periaxonal areas of degeneration and dystrophy of oligodendrocyte processes, similar to the ultrastructural changes observed in post mortem schizophrenia brains (Davis, Stewart et al. 2003). MAG mRNA levels are lower in gray matter of prefrontal cortex and other regions in schizophrenia (Hakak, Walker et al. 2001; Katsel, Davis et al. 2005). Studies by independent groups using divergent methodological platforms have consistently identified MAG as highly downregulated in schizophrenia in cortical gray matter (Haroutunian, Katsel et al. 2007).

Genetic variation in MAG could contribute to structural brain abnormalities visualized with magnetic resonance imaging (MRI) in schizophrenia. A recent metanalysis of first episode schizophrenia MRI studies showed a small number of consistent changes across studies in gray matter, including cortex (Steen, Mull et al. 2006). Decreases in cortical gray matter have not been consistently found, however, and negative results exist as well (Morgan, Dazzan et al. 2007). Volumetric findings of white matter in schizophrenia have been inconsistent at best, with different studies showing reductions, increases, or no change in white matter in schizophrenia (Mitelman, Brickman et al. 2007). Medication effects are frequent confounders in imaging studies of schizophrenia, especially affecting gray matter. One recent review of the effects of antipsychotics on brain structure in schizophrenia (Scherk and Falkai 2006), identified 7 studies that examined the effects of antipsychotics on cortical gray matter volume. Patients treated with
typical antipsychotics showed no change or decrease in cortical gray matter volume, and patients treated with atypical antipsychotics showed no change or increase in cortical gray matter volume.

Examining the effects of genetic variation on volumetric measurements in the brain may help reconcile some of the varying results seen in published MRI studies of schizophrenia. Studies of first episode schizophrenia in which subjects have received little or no medication provide a better opportunity to more directly examine the effect of genetic variation on brain structure. The present study examines the association of the MAG gene with brain structure in subjects with a first episode of schizophrenia or schizoaffective disorder (SSA) and healthy controls.

4.3 Methods and Materials

Participants were recruited from consecutive admissions to a catchment area-based early psychosis program, the sole source of specialized care for a population of 603,300 in a geographically defined area near Vancouver, Canada. Written informed consent was obtained, and studies were approved by the Clinical Research Ethics Boards of the University of British Columbia, and the Centre for Addiction and Mental Health in Toronto. Inclusion criteria for the research study were a first episode of psychosis, age 14-45, and less than 12 weeks of treatment with antipsychotic medication. We chose less than 12 weeks as a standard approach that was used in previous studies of first episode psychosis (Robinson, Woerner et al. 2006) to minimize the effects of medications, ensure subjects were stable enough to be scanned, and not be overly restrictive for inclusion purposes. Subjects with substance induced psychosis were excluded, as were individuals with neurological disorders including head injury. We also excluded major Axis I disorders that were not schizophrenia or schizoaffective disorder (but only in this analysis- our overall program of research includes other forms of functional psychosis). There was no history of psychosis in healthy comparison participants or in their first degree relatives. Clinical
information including a Structured Clinical Interview for DSM-IV was collected at baseline, and subjects were followed for 9-12 months after entry to the research protocol, at which time a consensus diagnosis (WGH, LCK, GWM) was made based on all available information.

The MAG gene spans 21.67 kb on chromosome 19q13.1, and the 19q chromosome site has been previously identified as a susceptibility locus in schizophrenia in a study of Scottish families. Blood was drawn from each subject and the two single nucleotide polymorphisms (SNPs) for MAG (rs 720309, rs720308) were genotyped using standard protocols as previously published (Wan, Yang et al. 2005; Zai, King et al. 2005). Briefly, genotyping of the SNPs was performed using a standard AB 5' nuclease Taqman® assay-on-demand protocol in a total volume of 10 µl. Post-amplification products were analyzed on the AB 7000 Sequence Detection System (Applied Biosystems, CA, USA) and genotype calls were performed manually. Results were verified independently by two laboratory personnel blind to demographic or diagnostic information. The haplotype of these two MAG markers was significantly associated with schizophrenia in two previous studies (Wan, Yang et al. 2005; Yang, Qin et al. 2005).

Inversion recovery prepped axial 3D fast spoiled gradient echo (FSPGR) images were acquired on a 1.5T GE Signa scanner for all subjects (slice thickness 1.5mm, TR: 11.4 ms, TE: 5.0 ms). Brain tissue volumes were assessed with FSL software (v4.0, www.fmrib.ox.ac.uk). FSL SIENAX was used to extract total brain from skull with BET initially, then affine-registration to MNI152 space was carried out with FLIRT, followed by further exclusion of non-brain tissue using the standard brain mask from the MNI template. Tissue segmentation with partial volume estimation was done with FAST to calculate total brain, gray matter, white matter, cortical gray matter and ventricular cerebrospinal fluid (CSF) volumes.
The association between MAG genotypes and diagnostic group was tested using a chi-square test. For segmented brain volumes, normal distributions were confirmed by Shapiro-Wilks tests except for the ventricular CSF which was then subject to logarithmic transformation. Equal variances between groups were confirmed using Levene’s test for equality of variances. Segmented brain volumes were compared between the SSA group and healthy subjects using analysis of covariance (ANCOVA), with the additional variables sex, age and total brain volume as covariates. The addition of genotype and genotype-by-diagnosis interaction terms were also included in the model. Since the MAG gene may be regulated differently in schizophrenia, the effect of MAG genotype on segmented brain volumes was examined separately in the SSA group and then in the control group using ANCOVA. Statistical software, SPSS 14.0, was used for statistical analyses.

4.4 Results

The combined group of subjects with schizophrenia (n=30) or schizoaffective disorder (n=11) did not differ from the comparison group (n=43, controls) in gender distribution (SSA: 28 men, 13 women; controls: 23 men, 20 women) or ethnicity (SSA: 35 Caucasian, 5 Asian, 1 African; controls: 36 Caucasian, 7 Asian). The two groups did differ significantly in age (SSA 19.5, SD 2.8; controls 23.2, S.D. 2.8) (p<0.01). Mean duration of antipsychotic treatment at time of scan for the 30 subjects taking medication (28 on atypicals, 2 on typicals) was 5.2 SD 3.4 weeks.

The distribution of MAG genotypes was consistent with Hardy-Weinberg equilibrium ($X^2=2.567$, df=1, 2-sided p=0.109), and did not show statistically significant differences between the SSA group and controls ($X^2=0.145$, df=1, 2-sided p=0.703). The two markers for MAG were in full linkage disequilibrium in this sample (D’=1.0); thus results were reported for one marker: rs720308 (SSA: AA/GA n=28/13; controls: AA/GA n=31/12).
Cortical gray matter volume was modeled as a function of diagnosis, age, gender and total brain volume. The overall model was significant (F=153.58, df=4, 83, p<0.0001). There was no statistically significant effect related to diagnosis (F=1.41, p=0.240). There were significant effects of total brain volume (F=363.8, p<0.0001) and age (F=40.24, P<0.0001), but not of gender. There was no difference in cortical gray matter volume (least squares mean, i.e. adjusted mean in ANCOVA) in the SSA group compared with controls. Total brain volume, total gray and total white matter volumes, and ventricular cerebrospinal fluid volume did not differ between groups.

We next investigated the potential contribution of MAG genotype to cortical gray matter volume at the onset of psychosis, controlling for effects of age, gender and total brain volume. The overall model was statistically significant (F=152.00, df=4,83, p<0.0001). Total cortical gray matter volume was not affected by MAG genotype (F=0.67, p=0.42) in the overall sample. No interaction between diagnosis and genotype was observed.

Cortical gray matter was then modeled as a function of age, gender and total brain volume separately within the SSA group and within the control group. In the SSA group, there was a significant contribution of MAG rs720308 genotype to cortical gray matter volume (F=4.601, p=0.039). The AA genotype predisposed SSA participants to lower cortical gray matter volume (Figure 1). In the healthy control group, MAG genotype did not predict cortical gray matter volume difference (F=0.048, p=0.827). MAG genotype accounted for a larger proportion of the variance in the SSA group, (partial eta squared = 0.11), than in the control group, (partial eta squared = 0.001). Gender was not used as a covariate since it did not contribute to cortical gray matter volume within either group (SSA: F=0.158, p=0.693), (controls: F=0.103, p=0.750).

Since MAG might also be expected to contribute to white matter volumes, we performed a
secondary analysis of the relationship between MAG genotypes and total white matter volume. As seen (Figure 2), white matter volume for AA genotype in the SSA group was not significantly different (p=0.17) than for GA genotype in the SSA group.

Since the two groups were significantly different in terms of age (p<0.01), we removed the eight eldest individuals from our control group thereby creating two groups with no age difference (p=0.12), (mean age control group = 21.2 ± 3.8); (mean age SSA group = 19.5 ± 2.8), and recalculated our measures of interest. There was still no difference in cortical gray matter between the two groups (p=0.304), and the MAG gene did not influence cortical gray matter volume in the control group (p=0.578). Finally, there was no change of the effect of the MAG gene on the overall model.

4.5 Discussion

The results suggest that variation in the MAG gene may act as a risk factor for low cortical gray matter volume in first episode schizophrenia or schizoaffective disorder. Our finding complements the increasing body of evidence implicating myelin genes in general, and the MAG gene specifically, in the pathophysiology of schizophrenia.

We did not find a difference in cortical gray matter in the first episode schizophrenia group compared to the healthy control group. There are relatively few neuroimaging studies that specifically examine only patients with first episodes of schizophrenia, and these studies often have small sample sizes and findings have not always been consistent. Our retrospective power calculation showed that we were underpowered to detect a difference (21.7%) in cortical gray matter, but it is unclear whether such calculation is useful retrospectively (Hoenig and Heisey 2001; Lenth 2001). Interestingly, several MRI studies of first episode schizophrenia have found
decreased cortical gray matter volume measures compared to controls, with similar or smaller sample sizes (Lim, Tew et al. 1996; Fannon, Chitnis et al. 2000; Chua, Cheung et al. 2007) than in the present study, and, studies larger than ours (Morgan, Dazzan et al. 2007) have shown no difference between such measures. The variance of cortical gray matter volume measurements in the SSA group and control group was similar in our study to previously published studies in the first episode population (Fannon, Chitnis et al. 2000).

Atypical antipsychotics may diminish the extent of gray matter volume changes that may occur during a first psychotic episode (Lieberman, Tollefson et al. 2005), that perhaps prevented us from detecting a difference in cortical gray matter volume between groups. The effects of atypical antipsychotics have even been shown to reverse changes observed in cortical gray matter in schizophrenia (Keshavan, Haas et al. 1998).

Genetic variation and its contribution to volumetric differences in individuals with schizophrenia may help explain certain disparate findings. We showed that MAG genotype accounts for a greater proportion of the variance in the SSA group than the control group. An intronic SNP, such as rs720308, could influence alternative splicing of the MAG gene, which is a powerful way to regulate gene expression at the posttranscriptional level and generate protein diversity. The MAG gene is known to undergo alternative splicing leading to different MAG isoforms. In schizophrenia, the most compelling evidence for MAG’s role comes from post-mortem gene expression studies, suggesting different regulation of the MAG gene in schizophrenia. Different protein products of the MAG gene generated by alternative splicing could explain in part gray matter volume differences in schizophrenia. Intronic variations may alter gene expression by other mechanisms, such as cis-trans activations, or by being in linkage disequilibrium with a not yet investigated, but relevant variation.
An important contributor to the variation seen in this study could be the reduction in the total number of oligodendrocytes demonstrated in post mortem schizophrenia brain (Dwork, Mancevski et al. 2007). The MAG protein enhances the survival and maintenance of oligodendrocytes (Davis, Stewart et al. 2003). Variation in the MAG gene could alter protein levels and contribute to the differences in oligodendrocyte number previously shown in cortical gray matter.

While it may inherently make sense that the MAG gene may contribute to variation in white matter volume, the large body of literature implicating MAG in schizophrenia is, in fact, based on studies of cortical gray matter in postmortem schizophrenia patients (Hakak, Walker et al. 2001; Tkachev, Mimmack et al. 2003; Katsel, Davis et al. 2005; Katsel, Davis et al. 2005; Aberg, Saetre et al. 2006). Furthermore, schizophrenia studies describing loss and altered spatial distribution of oligodendrocytes (Hof, Haroutunian et al. 2003) and electron microscopy studies of oligodendrocytes (Uranova, Orlovskaya et al. 2001) were in cortical gray matter as well. This work has been replicated again very recently and has been the subject of special attention in schizophrenia research (Haroutunian and Davis 2007; Haroutunian, Katsel et al. 2007). We were, however, somewhat surprised that MAG did not play a role in determining white matter volume. The QKI gene, a major regulator of MAG, is downregulated in both gray matter and white matter (McCullumsmith, Gupta et al. 2007). Qki may act as a possible common pathway for myelin gene downregulation via differential regulation of the QKI gene in schizophrenia (Aberg, Saetre et al. 2006). These recent findings lead us to suspect that MAG may indeed play a role in white matter pathology in schizophrenia, and further investigation is necessary, perhaps with a different white matter imaging phenotype, such as diffusion tensor imaging.
Limitations of this study exist in both genetics and imaging. First, there is potential population stratification, since the SSA group and control group include individuals of both southeast Asian and European descent. However, this potential confound is mitigated somewhat by the nearly equivalent distribution of individuals of southeast Asian descent and European Caucasian descent in each group, and the very similar allelic frequencies between Asians and Caucasians for the MAG rs720309 and rs720308 SNPs (www.hapmap.org). To date, the MAG gene has not been fully investigated in studies of its association with schizophrenia. Over 20 tag SNPs exist, and a more thorough investigation of the MAG gene and its potential association with schizophrenia is worthwhile. Interestingly, the rs720309-720308 findings were different between the two previous positive studies for the MAG gene in schizophrenia. The Chinese family study identified a common risk haplotype T-A (rs720309-rs720308) (Yang, Qin et al. 2005), while the case-control study identified risk haplotype A-G (rs720309-rs720308) (Wan, Yang et al. 2005) that conferred increased risk for schizophrenia. Therefore, further work is needed to elucidate risk alleles or haplotypes for the MAG gene. Second, there are common problems in measuring gray matter and in MRI scanning, such as ‘machine drift’ and partial volume effects that can affect volumetric measurements. A third limitation is potential effects of antipsychotic medication on volumetric brain measurements. Average length of medication exposure was 5.2 weeks in this study, though this length of exposure is far less than most published MRI studies in schizophrenia (Steen, Mull et al. 2006).

Overall, our study is relatively free of many of the additional confounds present in imaging studies of schizophrenia. Imaging studies in schizophrenia are often complicated by the potential effects of the illness on the brain, as well as by effects of medication. Our study had antipsychotic medication use greater than 12 weeks as an exclusion criterion. An early psychosis
sample also allows for less environmental and epigenetic influence on the brain as these subjects have been scanned relatively early in their lifespan. Our finding, potentially relating genetic variation in MAG to cortical gray matter volume at illness onset creates a new link in converging genetic and imaging evidence for myelin and oligodendrocyte dysfunction in schizophrenia.

Figure 4-1 Volumes of cortical gray matter based on least squares means from model used to test effect of MAG rs720308 genotype.

As described in the text, mean cortical gray matter in the SSA group was lower with MAG-AA (n=28) genotype than MAG-GA (n=13) genotype, but there was no such difference in controls (MAG-AA, n=31) vs (MAG-GA, n=12).
Figure 4-2 Volumes of white matter based on least squares means from model used to test effect of MAG rs720308 genotype

MAG genotype did not play a significant role in determining white matter volume in either the SSA group or controls.
5 Quantitative Examination of a Novel Clustering Method using Magnetic Resonance Diffusion Tensor Tractography

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5.1 Abstract

Background: MR diffusion tensor imaging (DTI) can measure and visualize organization of white matter fibre tracts in vivo. DTI is a relatively new imaging technique, and new tools developed for quantifying fibre tracts require evaluation. The purpose of this study was to compare the reliability of a novel clustering approach with a multiple region of interest (MROI) approach in both healthy and disease (schizophrenia) populations.

Methods: DTI images were acquired in 20 participants (n=10 patients with schizophrenia: 56 ± 15 years; n=10 controls: 51 ± 20 years) (1.5 Tesla GE system) with diffusion gradients applied in 23 non-collinear directions, repeated three times. Whole brain seeding and creation of fibre
tracts were then performed. Interrater reliability of the clustering approach, and the MROI approach, were each evaluated and the methods compared.

Results: There was high spatial (voxel-based) agreement within and between the clustering and MROI methods. Fractional anisotropy, trace, and radial and axial diffusivity values showed high intraclass correlation (p<0.001 for all tracts) for each approach. Differences in scalar indices of diffusion between the clustering and MROI approach were minimal.

Conclusions: The excellent interrater reliability of the clustering method and high agreement with the MROI method, quantitatively and spatially, indicates that the clustering method can be used with confidence. The clustering method avoids biases of ROI drawing and placement, and, not limited by a priori predictions, may be a more robust and efficient way to identify and measure white matter tracts of interest.

5.2 Introduction

Magnetic resonance diffusion tensor imaging (DTI) is the most powerful and currently the only way to measure and to visualize the organization of white matter fibre tracts in vivo (Alexander and Lobaugh 2007). Diffusion tensor tractography takes advantage of underlying large scale features of the diffusion data to estimate the direction of white matter fibre tracts in the brain (Basser, Pajevic et al. 2000). In addition, tractography permits the calculation of quantitative measures of the tensor along white matter fibre tracts. For DTI, particularly tractography, new tools and applications are being developed that require evaluation. Our group has published an innovative method (O'Donnell, Kubicki et al. 2006; O'Donnell and Westin 2007) that automatically clusters white matter tracts together based on tract shape and similarity. This
method thus offers an alternative to the multiple region of interest method (MROI) that is commonly used in streamline tractography (Wakana, Caprihan et al. 2007).

Most common methods for isolating fibre bundles based on streamline tractography require the manual placement of multiple regions of interest. These MROI methods include: 1) an approach that starts from seed points within a predefined region of interest, and then calculates and preserves only traces that touch other predefined ROIs (Mori and van Zijl 2002) or 2) an alternative approach that creates seed points throughout the entire brain (whole brain tractography). Only tracts that pass through the ROIs are then included (Wakana, Caprihan et al. 2007). Our recently published method eliminates the need to manually place ROIs to identify fibre bundles. Importantly, this method is able to differentiate fibre bundles which would be difficult to define and separate using MROI approaches. This clustering approach is fully automatic, unguided, and takes advantage of the similarity of fibre paths. Collections of fibre trajectories are also analyzed in 3D and separated into bundles or clusters that contain paths with similar shape and spatial position. Fibre paths are thus grouped on the basis of shape and location using spectral clustering (O'Donnell, Kubicki et al. 2006). We have also recently created a high dimensional white matter atlas using automatic tractography segmentation whereby whole brain tractography was performed (O'Donnell and Westin 2007). In that study, the entire brain was seeded and a collection of several hundred clusters were produced that represent white matter fibre tracts of the brain (O'Donnell and Westin 2007).

Our clustering method is an efficient and high throughput approach that produces a whole brain model of white matter tracts. White matter tracts of interest can then be visualized in their correct anatomic location and selected to evaluate tract-specific diffusion parameters. For this study, diffusion parameters included fractional anisotropy (FA), related to fibre density and/or
myelination and reflecting the degree to which diffusion is directionally dependent, and trace (diffusion magnitude). Axial (D_L) and radial diffusivity (D_R) were also included, as they may be specific to myelination (Song, Yoshino et al. 2005) and axonal degeneration (Song, Sun et al. 2002), respectively, and therefore may provide useful information in neuropsychiatric disorders.

Fibre tracking algorithms may behave differently in disease populations (Ciccarelli, Catani et al. 2008). Therefore, we believe that it is important to perform methodological studies in both healthy and disease populations. The importance of successful segmentation of corresponding white matter tracts across subjects and across hemispheres is that neuroscientific hypotheses can be tested regarding group differences and questions of symmetry (O'Donnell and Westin 2007). Since our group is particularly interested in the study of schizophrenia, where white matter changes using DTI have been shown (Kubicki, McCarley et al. 2007), we examined our clustering method both in healthy controls and in individuals with schizophrenia. Evaluation of this method in both a healthy and schizophrenia population, if successful, will allow for greater confidence in future studies in both populations.

The present study seeks to establish: 1) that the clustering method is reliable, and 2) comparable to the MROI approach both spatially (voxel overlap), and quantitatively, using four scalar indices of the diffusion tensor (fractional anisotropy, FA; trace, axial diffusivity, D_L; radial diffusivity, D_R).

5.3 Methods

5.3.1 Image Acquisition

DTI images were acquired using an eight-channel head coil on a 1.5 Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, WI), which permits maximum gradient
amplitudes of 40 mT/m. A single shot spin echo planar sequence was used with diffusion gradients applied in 23 non-collinear directions and $b=1000 \text{ s/mm}^2$. Two $b=0$ images were obtained. Fifty seven to sixty two slices were acquired for whole brain coverage oblique to the axial plane. Slice thickness was 2.6 mm, and voxels were isotropic. The field of view was 330 mm and the size of the acquisition matrix was 128 x 128 mm, with echo time (TE) = 85.5 ms, and repetition time (TR) = 15,000 ms. To improve the signal to noise ratio, the entire sequence was repeated three times. Inversion recovery prepped spoiled gradient recall and fast spin echo T2 weighted images were also acquired in the event of need for registration and to ensure anatomical accuracy.

5.3.2 Study Participants

Participants were recruited at the Centre for Addiction and Mental Health. All participants had DSM-IV Structured Clinical Interview for Diagnosis performed (First M 1995; First MB 1995), and were interviewed by a psychiatrist to ensure diagnostic accuracy. Any subject with a recent history of substance abuse or dependence was excluded, as were those subjects with previous head trauma with loss of consciousness, or neurological disorders. All subjects were between the ages of 25 and 80, with verbal IQ greater than 71. Study participants included (n=10) patients with schizophrenia: $56 \pm 20$ years, 6M, 4F; and (n=10) healthy controls: $51 \pm 15$ years, 6M, 4F; all right handed, as assessed by the Edinburgh handedness inventory (Oldfield 1971). All subjects provided informed, written consent, and the study was approved by the Centre for Addiction and Mental Health Ethics Review Board.

5.3.3 Image Analysis and Tractography

DTI data were transferred to a workstation and reconstructed. A diffusion-weighted image was created for each of the three repetitions. The three DTI repetitions were co-registered to the first
b=0 image in the first repetition using FSL (v. 4.0) [www.fmrib.ox.ac.uk](http://www.fmrib.ox.ac.uk) to produce a new averaged image, with gradients re-oriented. Registration corrects eddy current distortions and subject motion, important artifacts that can affect the data, and averaging improves the signal to noise ratio. A brain ‘mask’ was then generated. Points were seeded throughout each voxel of the brain.

Whole-brain tractography was performed with a deterministic (streamline) approach (Runge-Kutta order two tractography with a fixed step size of 0.5 mm). The three threshold parameters for tractography were: Tseed, Tstop, and Tlength. Tseed and Tstop are anisotropy thresholds based on the linear anisotropy measure $C_L$ (Westin, Maier et al. 2002), where $C_L = (\lambda_1 - \lambda_2)/\lambda_1$ and, $\lambda_1$ and $\lambda_2$ are the two largest eigenvalues of the diffusion tensor sorted in descending order. The goal of the anisotropy thresholds is to limit tractography to the white matter. Thresholds were based on the $C_L$ rather than on FA, because FA can be relatively high in regions of planar anisotropy which may indicate tract crossings or branching (Ennis and Kindlmann 2006). The Tlength threshold is used to eliminate very short fibres from being generated. The parameters chosen for this study were: Tseed = 0.3, Tstop =0.15, and Tlength = 20 (in mm). Tractography and creation of white matter fibre tracts using both the clustering and multiple region of interest approach were performed using 3D Slicer (open source software [www.slicer.org](http://www.slicer.org)) and Matlab 7.0 (www.mathworks.com).

1. **Clustering approach:** As previously described (O'Donnell, Kubicki et al. 2006), pairwise fibre trajectory similarity was quantified by first computing a pairwise fibre distance. The mean closest point distance was employed, which is defined as the mean distance between pairs of closest points on two fibres. The directed distances between fibres ‘A’ and ‘B’ are converted to a
symmetric pairwise fibre distance by taking the mean of the distance from A to B and from B to A. Each distance is then converted to an affinity measure suitable for spectral clustering via a Gaussian kernel \((W_{ij}) = e^{-d_{ij}^2/\sigma^2}\), a method that is employed in the clustering literature (Shi and Malik 2000). The role of \(\sigma (\sigma=60 \text{ mm used in the present study})\) is to define the size scale of the problem by setting the distance over which fibres can be considered similar (O'Donnell and Westin 2007).

A spectral embedding of fibres is then created based on the eigenvectors of the fibre affinity matrix. In our clustering application, we used the top 15 eigenvectors of the fibre similarity matrix to calculate the most important shape similarity information for each fibre. The clustering algorithm used was k-way normalized cuts, as it produces clusters with high within-cluster similarity and low between-cluster similarity (Ng, Jordan et al. 2002).

Once the whole brain cluster model is produced, the operator combines the clusters that correspond to a given fibre tract. Clusters of the same anatomical tract tend to have similar weights, thus facilitating selection (Figure 1). In this study, the left and right uncinate fasciculus, left and right inferior occipitofrontal fasciculus, left and right cingulum bundle left and right inferior longitudinal fasciculus, left and right arcuate fasciculus, left and right corticospinal tract, and genu of corpus callosum were selected. The remainder of the corpus callosum was segmented using the clustering method, and selection of neuroanatomical subdivisions were made according to a previously demonstrated DTI based topographical study of the corpus callosum (Hofer and Frahm, 2006). Specifically, following selection of the genu, premotor and supplementary motor projections (CC2), motor projections (CC3), sensory projections (CC4), and finally parietal, temporal, and occipital projections (CC5) were selected. Correct selection of
tracts was verified by superimposing clusters on both the FA and T1 images (Mori, Wakana et al. 2005). Two individuals, blind to participant information, performed the entire clustering procedure to generate two complete sets of cluster models for each dataset.

2. Multiple Region of interest approach: Regions of interest were drawn on the baseline image of the DTI scans. All ROIs were placed bilaterally. For uncinate fasciculus, two ROIs were drawn on each side on the same coronal slice, one just anterior to the temporal stem, where the frontal cortex begins and one in the temporal pole. For inferior occipitofrontal fasciculus, the same ROI on the coronal slice anterior to the temporal stem for uncinate fasciculus was used, and one coronal ROI was drawn in temporal cortex, just anterior to the occipital cortex. For the cingulum bundle, three ROIs were drawn in the antero-posterior direction on consecutive coronal slices around the cingulum on each side, five slices posterior to where the genu joins at the midline, and three ROIs were drawn on consecutive coronal slices starting five slices anterior to where the splenium joined at the midline. For the inferior longitudinal fasciculus, two ROIs were drawn on coronal slices. The anterior ROI was drawn in temporal cortex on the slice where the corticospinal tract is seen to decussate. The posterior ROI was drawn in occipital cortex, below the inferior occipitofrontal fasciculus, one slice posterior to where the forceps major separates. For the arcuate fasciculus, three ROIs were drawn on consecutive axial slices. These ROIs were placed on the FA image. Since there is great interindividual variability in the anatomy of the AF (Catani and Mesulam 2008), it was necessary to visualize the tract cross sectionally for accurate ROI placement. The first ROI was drawn once the cingulum bundles were visible. Placement of the ROI was lateral to where the corticospinal tract was visible and was made around the visible part of the arcuate fasciculus. The second and third ROIs followed on successive descending axial slices where the arcuate fasciculus was visible. The first ROI includes the shorter fibres of
the anterior indirect segment, the second ROI includes the long fibres, and the third ROI includes the shorter fibres of the posterior indirect segment (Catani and Mesulam 2008). For the corticospinal tract, three ROIs were drawn on axial slices. One ROI was drawn around the border of the midbrain. The other ROI was drawn just lateral to the corpus callosum, beside the motor projections of the corpus callosum. In order to ensure no crossing fibres from other tracts were obtained, an ROI was placed liberally in the contralateral hemisphere at the level of the anterior commissure. Any fibre penetrating this ROI was removed. For the genu of the corpus callosum, one ROI was drawn on the midsagittal slice on the corpus callosum. The remainder of the corpus callosum using an MROI approach was not segmented, since reliable segmentation is not easily achieved using this approach (Wakana, Caprihan et al. 2007), and identifying a specific protocol to reproducibly identify 2D slices for ROI drawing is challenging. Tracts generated from whole brain seeding had to pass through all ROIs that corresponded to those drawn for each fasciculus or bundle. Two individuals, blind to participant information, drew ROIs on the dataset to generate two complete sets of ROIs and white matter fibre tracts for each individual scanned.

5.3.4 Quantifying the Tensor

Four tensor measures were obtained for each white matter fibre tract: FA and trace (Basser & Pierpaoli 1996), $D_L(\lambda_1)$ (Song, Yoshino et al. 2005) and $D_R[(\lambda_2+\lambda_3)/2]$ (Song, Sun et al. 2002). These values were calculated for each of the thirteen tracts for each operator and fibre segmentation method (clustering, MROI). Matlab (v. 7.0) was used to make the calculations. Data presented represent the mean values along the selected tracts.

5.3.5 Statistical Analysis

Spatial agreement. Cohen’s kappa ($k$) was used to compare pixels covered by the selected white matter tracts generated by each operator for the two fibre segmentation methods (Landis and
Koch 1977). This metric measures the proportion of voxels that are covered between two white matter tracts. A recent evaluation of the MROI method used this measure as their primary measure of reliability as described by Wakana et al. (Wakana, Caprihan et al. 2007). Pixels occupied by the tracts were assigned a value of 1 and non-occupied pixels a value of 0. Four pixel categories were created: 1) pixels that did not contain the tract in both trials (nn), 2) pixels that contained the tract in only one of the two trials (pn, np), and 3) pixels that contained the tracts in both trials (pp). For the calculation, only pixels with FA>0.2 were included. Expectation values (Enn, Enp, Epn, and Epp) for each class were then calculated as follows:

\[
\text{Expected } nn \ (Enn) = \frac{(nn + np)(nn + pn)}{N} \\
\text{Expected } np \ (Enp) \text{ or } Epn = \frac{(nn + np)(np + pp)}{N} \text{ or } \frac{(nn + pn)(pn + pp)}{N} \\
\text{Expected } pp \ (Epp) = \frac{(pn + pp)(np + pp)}{N}
\]

Where \( N = nn + np + pn + pp \) is the total number of pixels for that particular fibre.

\[
\text{Observed agreement} = \frac{(nn + pp)}{N} \times 100
\]

\[
\text{Expected agreement} = \frac{(Enn + Epp)}{N} \times 100
\]

\[
k = \frac{\text{observed agreement} - \text{expected agreement}}{(100 - \text{expected agreement})}
\]
Higher values of $k$ indicate stronger agreement between the two methods. The Landis and Koch (Landis and Koch 1977) criteria for interpretation of Cohen’s kappa ($k$) coefficient was employed, where $k = 0.61-0.8$ indicates ‘substantial’ agreement, and $k = 0.81-1.0$ ‘almost perfect’ agreement.

Inter-rater reliability (defined as comparison of identical methods between two operators) was first calculated within each fibre identification method for each of the thirteen tracts for each participant. Mean and standard deviation of inter-rater reliability values were then calculated. Inter-method reliability (defined as comparison between fibre identification methods by the same operator) was then calculated for each of the thirteen tracts for each participant, followed by mean and standard deviation calculations. Only inter-rater reliability was calculated for the subdivisions of the corpus callosum (except for the genu, where inter-rater and inter-method reliability were calculated).

Scalar indices of the tensor. Intraclass correlation coefficients were calculated for each of the scalar indices of diffusion (FA, trace, D_L, D_R) for each fibre identification method using SPSS vr 15. To compare the clustering and MROI methods, the percent difference of the clustering method from the MROI method was calculated for each tract for each study participant. Mean percent difference (of all study participants) for each tract was then calculated.

5.4 Results
The result of whole brain clustering and the process of selection of a white matter tract (left uncinate fasciculus) is shown in Figure 1, where similar colours indicate tract similarity. Results for each anatomical tract are overlaid on a FA map in Figure 2. Tracts generated using the clustering method (Fig 2, left) and the MROI method (Fig 2, centre) were quite similar, and the
high spatial agreement is apparent in Fig 2 (right), where the tracts are superimposed. Successful segmentation of the corpus callosum, and its subdivisions, as previously explained, is also shown (Fig 3).

**Spatial agreement.** Mean $k$ criterion results across the sample are presented in Table 1. The clustering method had excellent reliability, with ‘substantial’ or ‘almost perfect’ agreement for each white matter tract studied. The MROI method also had excellent reliability. The two fibre identification methods were also similar within rater. That is, for each rater, the clustering method showed either ‘substantial’ or ‘almost perfect’ agreement with the MROI method, indicating a high degree of voxel overlap. When the schizophrenia group and healthy control group were separated, both the clustering and MROI method still showed high reliability for each group (Table 2). For the remainder of the corpus callosum (where only the clustering method was used) there was high reliability for each subdivision across all participants, and, high reliability for both the schizophrenia group and healthy control group (Table 3).

**Scalar indices of the tensor.** Scalar indices of the diffusion tensor (FA, trace, axial diffusivity, radial diffusivity) had high intraclass correlation coefficients for both the clustering and MROI methods. The intraclass correlation coefficient for all tracts using the clustering method was 0.93 ($p<.001$), and for the MROI method was 0.91 ($p<.001$). Percent differences between the clustering method and the MROI method for the four measures were small, as can be seen in Table 4 (shown for one operator).

### 5.5 Discussion

Our results indicated that the clustering method has excellent reliability. The present study also confirmed the reliability of the MROI method, that has recently been shown (Wakana, Caprihan
et al. 2007). For all of the tracts studied, only minimal differences between the clustering method and the MROI method were found when comparing quantitative measures of diffusion and in the specific voxels included in the segmented tracts. We have previously shown that the clustering method is useful for grouping fibres based on their shape and similarity (O'Donnell, Kubicki et al. 2006) and for constructing a white matter atlas (O'Donnell and Westin 2007). The present study extends that work to demonstrate the suitability of the clustering method for the study of a wide range of quantitative measures of the diffusion tensor in white matter tracts in both healthy and diseased populations (schizophrenia).

The clustering method and the MROI method for segmenting whole-brain streamline tractography represent a significant advance over other approaches for quantifying diffusion, such as drawing ROIs on individual brain slices and taking measurements from the voxels covered by the ROIs alone (Alexander and Lobaugh 2007). Due to their construction via streamline tractography based methods, the clustering and MROI methods allow for calculation of quantitative properties of the diffusion tensor along white matter tracts in the brain.

Reproducibility values ($k$) of the clustering method exceeded 0.75 for all white matter tracts studied. The clustering method may present an advantage over the MROI method in segmenting those tracts that require the placement of several ROIs. It is possible that the placement of each successive ROI increases the potential for error. The $k$ values for reproducibility of the MROI method for the right and left cingulum bundle were less than 0.8. However, in the case of the genu of the corpus callosum, or the UF or IFOF, where only one, two, and two ROIs were placed respectively, $k$ values for the MROI method fell in the highest range of reproducibility (i.e. greater than 0.8). Variability in the clustering method is due to the operator’s decision regarding which clusters to choose following initial selection of the main cluster(s) that clearly comprise
the bulk of the neuroanatomic tract of interest. A small number of clusters, usually consisting of short fibres, can be subsequently selected by the operator. When both operators do not select the same short fibres, voxel overlap drops below 100%, and variability is introduced. For instance, the arcuate fasciculus, consists of short as well as long fibres (Catani and Mesulam 2008), and voxel overlap between operators in our study show, as expected, more variability than in the uncinate fasciculus, where fewer clusters of short fibres are present. Superimposing the clusters on the FA and T1 image allows for confirmation that the correct clusters are chosen.

Less opportunity for user bias exists with the clustering method since it is impossible for the user to visualize the properties of the tensor of that tract when selecting clusters corresponding to the white matter tract of interest. Conversely, using the MROI method, bias can occur in several situations, such as deciding on the size of the ROI, the number of ROIs drawn, and the slice(s) on which the ROIs are drawn. Limiting user bias is especially important for studies comparing disease populations to healthy populations, where investigators are usually interested in finding a difference between groups. In addition, ROI analyses of DTI data may be more sensitive to bias created by ROI placement in the presence of disease or atrophy (Alexander and Lobaugh 2007). The clustering method may also be more suitable for longitudinal studies, since opportunities for interrater discrepancies are minimized.

A limitation of streamline fibre tractography that has an impact on both the clustering and MROI methods is that it can be difficult to accurately map white matter pathways in regions with crossing or converging fibres (such as the limbic projection of the cingulum bundle). Streamline (deterministic) tractography, in particular, poses challenges in resolving fibres in such regions (Alexander and Lobaugh 2007). Limbic system segmentation can produce challenges for the clustering method, since there is a general ‘noisiness’ in this area. For example, the fibres in the
fornix region may, at times, cross structures to follow part of the corona radiate. Image resolution affects the size scale of tracts that may be traced (both tract width and radius of curvature should be larger than the voxel scale). Therefore, smaller tracts such as the posterior commissure, fimbria, and stria terminalis may not be appropriate for standard streamline tractography techniques (Wakana, Caprihan et al. 2007). The reliability of the clustering method for the fornix is not high, due to errors of tractography (trajectories crossing into corpus callosum and anterior commissure for example) and due to the fact that trajectories are often "broken" along the course of the structure. These issues are better addressed by improvements in acquisition, tractography, and/or fiber distribution modeling (such as multiple tensors, etc) rather than at the clustering stage. A potential limitation of the clustering method is that it may include small, short, white matter fibre tracts that the MROI method may not. For instance, the inferior occipito-frontal fasciculus was created by drawing two ROIs using the MROI method. Any white matter fibres not sufficiently long to pass through both ROIs would not have been included. However, our data (not shown) indicate that the clustering method might include such shorter fibres, particularly if they are similar in shape and location to the longer fibres and meet the selected distance threshold. As a result, mean fibre length for each tract is slightly shorter in the clustering method, but mean fibre number slightly larger. While it is unclear if these additional fibres create a more or less ‘anatomically correct’ white matter tract, the virtually identical scalar measures of the diffusion tensor between the clustering and MROI method, and the strong spatial overlap results, indicate that there is little to no effect of these additional smaller fibres on average values across the whole bundle.

In conclusion, our clustering algorithm presents an alternative to the more commonly used MROI method. Since it eliminates some forms of user bias, it may be especially useful when
characterizing multiple white matter fibre tracts or studying disease populations. It is a robust method in both a healthy and disease population and produces easily visualized and highly reliable quantifiable white matter tracts with diffusion tensor tractography.
Table 5-1 Mean spatial (voxel) agreement using $k$ criterion for all participants$^{a,b,c}$

<table>
<thead>
<tr>
<th></th>
<th>CLUST 1 MROI 1</th>
<th>CLUST 1 MROI 2</th>
<th>CLUST 2 MROI 1</th>
<th>CLUST 2 MROI 2</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(n=20)</td>
<td>(n=20)</td>
<td>(n=20)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>L CB</td>
<td>0.86 ± 0.09</td>
<td>0.72 ± 0.10</td>
<td>0.76 ± 0.12</td>
<td>0.74 ± 0.14</td>
</tr>
<tr>
<td>R CB</td>
<td>0.90 ± 0.05</td>
<td>0.78 ± 0.12</td>
<td>0.82 ± 0.11</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td>Genu</td>
<td>0.88 ± 0.05</td>
<td>0.81 ± 0.07</td>
<td>0.82 ± 0.07</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td>L IFOF</td>
<td>0.83 ± 0.11</td>
<td>0.88 ± 0.10</td>
<td>0.77 ± 0.09</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>R IFOF</td>
<td>0.81 ± 0.13</td>
<td>0.86 ± 0.07</td>
<td>0.72 ± 0.14</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>L UF</td>
<td>0.90 ± 0.08</td>
<td>0.87 ± 0.08</td>
<td>0.69 ± 0.11</td>
<td>0.72 ± 0.12</td>
</tr>
<tr>
<td>R UF</td>
<td>0.92 ± 0.06</td>
<td>0.88 ± 0.07</td>
<td>0.72 ± 0.12</td>
<td>0.70 ± 0.14</td>
</tr>
<tr>
<td>L ILF</td>
<td>0.87 ± 0.12</td>
<td>0.80 ± 0.08</td>
<td>0.72 ± 0.08</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>R ILF</td>
<td>0.92 ± 0.08</td>
<td>0.76 ± 0.06</td>
<td>0.74 ± 0.06</td>
<td>0.71 ± 0.11</td>
</tr>
<tr>
<td>L AF</td>
<td>0.76 ± 0.15</td>
<td>0.69 ± 0.06</td>
<td>0.65 ± 0.12</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>R AF</td>
<td>0.81 ± 0.08</td>
<td>0.74 ± 0.08</td>
<td>0.69 ± 0.09</td>
<td>0.74 ± 0.10</td>
</tr>
<tr>
<td>L CST</td>
<td>0.84 ± 0.15</td>
<td>0.71 ± 0.09</td>
<td>0.71 ± 0.09</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>R CST</td>
<td>0.77 ± 0.15</td>
<td>0.73 ± 0.06</td>
<td>0.68 ± 0.11</td>
<td>0.67 ± 0.10</td>
</tr>
</tbody>
</table>

$^a$ $k = 0.61$-$0.80$ is considered ‘substantial agreement and $k = 0.81$-$1.0$ is considered ‘almost perfect’ agreement.

$^b$ CLUST 1 = clustering result by operator 1; CLUST 2 = clustering result by operator 2; MROI 1 = MROI result by operator 1; MROI 2 = MROI result by operator 2

$^c$ CB = cingulum bundle, Genu (of corpus callosum), IFOF = inferior occipitofrontal fasciculus, UF= uncinate fasciculus, ILF = inferior longitudinal fasciculus, AF = arcuate fasciculus, CST = corticospinal tract
Table 5-2 Spatial (voxel) agreement using $k$ criterion\textsuperscript{a} by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>\textsuperscript{b}SZ CLUST1</th>
<th>HC CLUST1</th>
<th>\textsuperscript{b}SZ MROI1</th>
<th>HC MROI1</th>
<th>\textsuperscript{b}SZ CLUST</th>
<th>HC CLUST</th>
<th>\textsuperscript{c}(n=10)</th>
<th>\textsuperscript{c}(n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L CB</td>
<td>0.88 ± 0.07</td>
<td>0.84 ± 0.10</td>
<td>0.69 ± 0.13</td>
<td>0.74 ± 0.08</td>
<td>0.76 ± 0.16</td>
<td>0.73 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R CB</td>
<td>0.88 ± 0.07</td>
<td>0.90 ± 0.04</td>
<td>0.79 ± 0.11</td>
<td>0.76 ± 0.12</td>
<td>0.81 ± 0.10</td>
<td>0.83 ± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genu</td>
<td>0.89 ± 0.05</td>
<td>0.87 ± 0.05</td>
<td>0.80 ± 0.05</td>
<td>0.82 ± 0.09</td>
<td>0.80 ± 0.07</td>
<td>0.85 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L IFOF</td>
<td>0.73 ± 0.14</td>
<td>0.91 ± 0.11</td>
<td>0.86 ± 0.09</td>
<td>0.90 ± 0.10</td>
<td>0.81 ± 0.13</td>
<td>0.84 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R IFOF</td>
<td>0.80 ± 0.10</td>
<td>0.83 ± 0.15</td>
<td>0.85 ± 0.05</td>
<td>0.86 ± 0.08</td>
<td>0.76 ± 0.15</td>
<td>0.76 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L UF</td>
<td>0.82 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.85 ± 0.08</td>
<td>0.89 ± 0.07</td>
<td>0.75 ± 0.14</td>
<td>0.67 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R UF</td>
<td>0.94 ± 0.05</td>
<td>0.91 ± 0.07</td>
<td>0.90 ± 0.09</td>
<td>0.85 ± 0.06</td>
<td>0.70 ± 0.10</td>
<td>0.75 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L ILF</td>
<td>0.93 ± 0.09</td>
<td>0.82 ± 0.15</td>
<td>0.79 ± 0.08</td>
<td>0.80 ± 0.08</td>
<td>0.73 ± 0.08</td>
<td>0.70 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R ILF</td>
<td>0.96 ± 0.05</td>
<td>0.87 ± 0.10</td>
<td>0.74 ± 0.07</td>
<td>0.78 ± 0.05</td>
<td>0.71 ± 0.10</td>
<td>0.72 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L AF</td>
<td>0.78 ± 0.17</td>
<td>0.74 ± 0.12</td>
<td>0.70 ± 0.05</td>
<td>0.69 ± 0.07</td>
<td>0.68 ± 0.06</td>
<td>0.63 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R AF</td>
<td>0.79 ± 0.11</td>
<td>0.82 ± 0.06</td>
<td>0.77 ± 0.05</td>
<td>0.70 ± 0.10</td>
<td>0.71 ± 0.07</td>
<td>0.75 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L CST</td>
<td>0.86 ± 0.14</td>
<td>0.82 ± 0.15</td>
<td>0.75 ± 0.11</td>
<td>0.67 ± 0.08</td>
<td>0.69 ± 0.10</td>
<td>0.72 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R CST</td>
<td>0.70 ± 0.17</td>
<td>0.84 ± 0.13</td>
<td>0.76 ± 0.04</td>
<td>0.71 ± 0.07</td>
<td>0.70 ± 0.11</td>
<td>0.68 ± 0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} $k = 0.61-0.80$ is considered ‘substantial agreement and $k = 0.81-1.0$ is considered ‘almost perfect’ agreement. \textsuperscript{b} Inter rater reliability for each method calculated separately in schizophrenia (SZ) and healthy control (HC) groups. \textsuperscript{c} Averaged values for rater 1 and rater 2; intermethod reliability calculated separately for each rater initially for each subject, then averaged.
Table 5-3. Segmentation and Reliability of Corpus Callosum

<table>
<thead>
<tr>
<th></th>
<th>CLUST1</th>
<th>SZ CLUST1</th>
<th>HC CLUST1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLUST1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLUST2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>aCC2</td>
<td>0.88 ± 0.09</td>
<td>0.84 ± 0.12</td>
<td>0.92 ± 0.07</td>
</tr>
<tr>
<td>bCC3</td>
<td>0.76 ± 0.18</td>
<td>0.76 ± 0.15</td>
<td>0.75 ± 0.20</td>
</tr>
<tr>
<td>cCC4</td>
<td>0.81 ± 0.12</td>
<td>0.72 ± 0.11</td>
<td>0.79 ± 0.12</td>
</tr>
<tr>
<td>dCC5</td>
<td>0.74 ± 0.11</td>
<td>0.76 ± 0.11</td>
<td>0.71 ± 0.11</td>
</tr>
</tbody>
</table>

- a premotor and supplementary motor projections
- b motor projections
- c sensory projections
- d parietal, temporal, and occipital projections

For genu, please see Tables 1 and 2.
Table 5-4. Impact of Tract Segmentation Method on Diffusion Measures*

<table>
<thead>
<tr>
<th>Region</th>
<th>FA</th>
<th>Trace</th>
<th>Radial</th>
<th>Axial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diffusivity</td>
<td>Diffusivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L CB</td>
<td>1.19 ± 1.2</td>
<td>0.49 ± 1.2</td>
<td>0.05 ± 0.3</td>
<td>0.03 ± 0.8</td>
</tr>
<tr>
<td>R CB</td>
<td>1.73 ± 2.3</td>
<td>0.67 ± 1.7</td>
<td>0.11 ± 0.4</td>
<td>0.21 ± 0.9</td>
</tr>
<tr>
<td>Genu</td>
<td>1.57 ± 1.8</td>
<td>0.25 ± 1.3</td>
<td>0.49 ± 0.7</td>
<td>0.48 ± 0.8</td>
</tr>
<tr>
<td>L IFOF</td>
<td>0.22 ± 1.6</td>
<td>0.84 ± 1.6</td>
<td>0.11 ± 0.4</td>
<td>0.76 ± 1.8</td>
</tr>
<tr>
<td>R IFOF</td>
<td>0.89 ± 1.4</td>
<td>0.36 ± 2.8</td>
<td>0.15 ± 0.7</td>
<td>0.88 ± 2.9</td>
</tr>
<tr>
<td>L UF</td>
<td>2.37 ± 2.3</td>
<td>1.22 ± 2.0</td>
<td>0.72 ± 0.5</td>
<td>1.87 ± 1.7</td>
</tr>
<tr>
<td>R UF</td>
<td>0.28 ± 1.3</td>
<td>0.87 ± 0.7</td>
<td>0.21 ± 0.4</td>
<td>0.62 ± 0.8</td>
</tr>
<tr>
<td>L ILF</td>
<td>1.72 ± 2.1</td>
<td>1.13 ± 2.7</td>
<td>0.69 ± 0.8</td>
<td>1.29 ± 1.3</td>
</tr>
<tr>
<td>R ILF</td>
<td>2.61 ± 2.9</td>
<td>1.20 ± 2.9</td>
<td>0.91 ± 0.9</td>
<td>1.65 ± 0.9</td>
</tr>
<tr>
<td>L AF</td>
<td>2.13 ± 2.4</td>
<td>0.50 ± 1.7</td>
<td>0.75 ± 1.3</td>
<td>1.07 ± 2.7</td>
</tr>
<tr>
<td>R AF</td>
<td>2.42 ± 1.8</td>
<td>0.68 ± 2.3</td>
<td>0.86 ± 1.7</td>
<td>0.97 ± 2.1</td>
</tr>
<tr>
<td>L CST</td>
<td>0.69 ± 1.7</td>
<td>0.03 ± 1.9</td>
<td>0.10 ± 0.4</td>
<td>0.08 ± 1.1</td>
</tr>
<tr>
<td>R CST</td>
<td>0.82 ± 1.9</td>
<td>1.80 ± 1.6</td>
<td>0.12 ± 0.6</td>
<td>0.29 ± 0.8</td>
</tr>
<tr>
<td>All regions**</td>
<td>1.43 ± 2.2</td>
<td>0.77 ± 1.9</td>
<td>0.41 ± 0.7</td>
<td>0.78 ± 1.3</td>
</tr>
</tbody>
</table>

* Mean and SD of the percent difference between the clustering and MROI methods.

** The value for ‘All regions’ is simply the mean of all the regional means.
Figure 5-1. (A,B,C). Whole brain clustering result and example of how white matter tract is selected.

Tracts are grouped into clusters according to similarity of shape and location and are colour coded accordingly. This facilitates neuroanatomical selection of tracts of interest (left uncinate fasciculus).
Figure 5-2. Selection of Tracts of Interest

(A,B,C,D,E,F,G). Genu of corpus callosum (A), Right inferior occipitofrontal fasciculus (B), Left cingulum bundle (C), Left uncinate fasciculus (D), Left arcuate fasciculus (E), Right corticospinal tract (F), Right inferior longitudinal fasciculus (G)

For each tract output of clustering method (on left), MROI method (centre), and both methods superimposed (right) each displayed on a sagittal slice
Figure 5-3. Segmentation of corpus callosum, and selection of subdivisions.

Tracts are grouped into clusters according to similarity of shape and location and are colour coded accordingly (A). Clusters are then selected (B), red highlighted from left to right, as genu, premotor and supplementary motor projections, motor projections, sensory projections, and finally parietal temporal and occipital projections.
6 Diffusion Tensor Tractography Findings in Schizophrenia Across the Adult Lifespan

Contents of this chapter have been published as:


A link to the published paper can be found at:
http://brain.oxfordjournals.org/cgi/content/abstract/133/5/1494

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6.1 Abstract

In healthy adult individuals, late life is a dynamic time of change in microstructural integrity of white matter tracts. Yet, elderly individuals are generally excluded from diffusion tensor imaging studies in schizophrenia. Therefore, we examined microstructural integrity of fronto-temporal and interhemispheric white matter tracts in schizophrenia across the adult lifespan.

Diffusion tensor imaging data from 25 younger schizophrenia patients ($\leq 55$ years), 25 younger controls, 25 older schizophrenia patients ($\geq 56$ years), and 25 older controls were analyzed. Schizophrenia patients in each group were individually matched to controls. Whole brain tractography and clustering segmentation were employed to isolate white matter tracts. Groups were compared using repeated measures ANOVA with 12 within group measures of fractional anisotropy: (left and right) uncinate fasciculus, arcuate fasciculus, inferior longitudinal
fasciculus, inferior occipito-frontal fasciculus, cingulum bundle, and genu and splenium of the corpus callosum. For each white matter tract, fractional anisotropy was then regressed against age in patients and controls, and correlation coefficients compared.

Main effect of group ($F_{3,92} = 12.2$, $p < 0.001$), and group by tract interactions ($F_{26,832} = 1.68$, $p = 0.018$) were evident for fractional anisotropy values. Younger patients had significantly lower fractional anisotropy than younger controls (Bonferroni corrected alpha = 0.0042) in the left uncinate fasciculus ($t_{48} = 3.7$, $p = 0.001$) and right cingulum bundle ($t_{48} = 3.6$, $p = 0.001$), with considerable effect size but the older groups did not differ. Schizophrenia patients did not demonstrate accelerated age-related decline compared to healthy controls in any white matter tract.

To our knowledge, this is the first study to examine microstructural integrity of fronto-temporal white matter tracts across the adult lifespan in schizophrenia. The left uncinate fasciculus and right cingulum bundle are disrupted in younger chronic patients with schizophrenia compared to matched controls, suggesting that these white matter tracts are related to fronto-temporal disconnectivity. The absence of accelerated age-related decline or differences between older community-dwelling patients and controls, suggests that these patients may possess resilience to white matter disruption.

6.2 Introduction

The theory that disrupted oligodendrocyte structure/function (i.e. white matter) is a core underlying pathophysiological mechanism in schizophrenia is compelling: converging evidence from electron microscopy, postmortem gene expression, animal models, gene association, neurophysiology and neuroimaging has repeatedly pointed to myelin and oligodendrocyte
abnormalities as a core feature of, and possible common pathway for impaired connectivity in schizophrenia (Davis, Stewart et al. 2003; Dwork, Mancevski et al. 2007; Kubicki, McCarley et al. 2007; Voineskos 2009).

Myelinated bundles of axons that form white matter tracts can be visualized and characterized using diffusion tensor imaging (DTI), a clinical neuroimaging technique sensitive to alterations in brain white matter microstructure. Such alterations in microstructure can be indexed as fractional anisotropy (FA) (Basser and Pierpaoli 1996), the degree to which diffusion of water molecules is restricted by microstructural elements such as cell bodies, axons, myelin, and other constituents of cytoskeleton (Beaulieu 2002). Use of methods not optimally suited to DTI (e.g. voxel based morphometry) (Jones, Symms et al. 2005), underpowered samples, and limited reporting of effect size, have limited interpretability and have created challenges for replication of findings in DTI studies of schizophrenia (Konrad and Winterer 2008). More recent DTI tractography approaches allow for measurement of tissue integrity along white matter tracts, provide an anatomically based sample of the target fiber (Catani, Howard et al. 2002), and permit reconstruction of white matter tracts consistent with known neuroanatomy (Mori, Crain et al. 1999). However, most common methods for isolating fiber bundles based on streamline tractography still require some manual placement of multiple regions of interest (ROIs), which can introduce bias. We have developed a white matter tract ‘clustering’ segmentation method that eliminates the need to manually place ROIs to identify fiber bundles (O'Donnell, Kubicki et al. 2006), and is successful in measuring fronto-temporal and interhemispheric white matter tracts (Voineskos, O'Donnell et al. 2009).

Disruption in fronto-temporal and interhemispheric white matter tracts may underlie fronto-temporal and interhemispheric disconnectivity respectively in schizophrenia. However, much
less is known about the relationship of such deficits with age. A recent DTI study (Friedman, Tang et al. 2008) demonstrated accelerated age-related decline in the inferior longitudinal fasciculus, an occipito-temporal tract, and in certain subdivisions of the corpus callosum, but in contrast, such deficits were not present at the first episode of psychosis. To our knowledge, the possible progression across the life-span of fronto-temporal white matter tract deficits has not yet been studied. Rather, age-related studies of fronto-temporal white matter tracts have been limited to mid-life patients with chronic schizophrenia (i.e. 20-55 years) with conflicting results (Jones, Catani et al. 2006; Rosenberger, Kubicki et al. 2008).

The absence of studies comparing DTI white matter tract measures in older patients with schizophrenia and older healthy individuals is striking. Late-life is a particularly dynamic time of white matter change, where healthy individuals are susceptible to decline in white matter integrity (Salat, Tuch et al. 2005; Sullivan and Pfefferbaum 2006), and such decline may explain cognitive changes in normal ageing (Hedden and Gabrieli 2004; Sullivan and Pfefferbaum 2007; Ziegler, Piguet et al. 2008). Similarly, in patients with schizophrenia, late-life may also be a time when significant changes in white matter may occur, at least in a subset of individuals. Clinical studies suggest that some, but not all, patients undergo progressive decline in cognition, and function in late-life (Rajji and Mulsant 2008). It is possible that white matter changes may represent the neurobiologic correlates of such divergent outcomes in schizophrenia. The increasing numbers of older individuals with schizophrenia and the finding that schizophrenia in late-life is one of the most expensive of medical disorders (Jeste and Nasrallah 2003) underscore the need for understanding the synergies between schizophrenia and ageing with respect to white matter changes.
Using whole brain tractography and our clustering segmentation approach, we examined intrahemispheric fronto-temporal association fiber tracts (left and right): the uncinate fasciculus, cingulum bundle, arcuate fasciculus, inferior occipito-frontal fasciculus; the main occipito-temporal association fiber tract (left and right), the inferior longitudinal fasciculus; and interhemispheric fiber tracts comprising the genu and splenium of the corpus callosum. We focused on patients with chronic schizophrenia, since the bulk of evidence suggests that white matter abnormalities in schizophrenia may not be present at illness onset (Friedman, Tang et al. 2008; Konrad and Winterer 2008). Our study had three main hypotheses: 1) that differences in fronto-temporal white matter tracts would be present in younger patients with chronic schizophrenia compared to healthy controls; 2) that similar or even greater differences would be present in older patients with schizophrenia and age-matched controls; and 3) that schizophrenia patients would demonstrate similar or greater age-effects on white matter tract integrity across adult life compared to healthy controls.

6.3 Methods

6.3.1 Study Participants

Participants were recruited at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada, via referrals, study registries, and advertisements. All clinical assessments occurred at CAMH while DT-MRI scans were performed at a nearby general hospital in Toronto. All participants were administered the Mini Mental Status Exam to screen for dementia (Folstein, Folstein et al. 1975), the Structured Clinical Interview for DSM-IV Disorders (First MB 1995), and were interviewed by a psychiatrist to ensure diagnostic accuracy. The Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein et al. 1987) was administered to further characterize illness symptoms. Comorbid illness burden was measured by administration of the Clinical
Information Rating Scale for Geriatrics (CIRS-G) (Miller, Paradis et al. 1992). Medication histories were recorded via self-report, and verified when necessary from the patient’s treating psychiatrist and chart review. Patients or controls with current substance abuse or any history of substance dependence were excluded, and urine toxicology screens were performed on all subjects. Individuals with previous head trauma with loss of consciousness, or neurological disorders were also excluded. A history of a primary psychotic disorder in first-degree relatives was also an exclusion criterion for controls. Criteria used to match controls with patients were: age within 5 years, gender, handedness (Edinburgh handedness inventory) (Oldfield 1971), and IQ (Wechsler Test for Adult Reading) (WTAR)(Wechsler 2001). Initially, we attempted to match based on parental socioeconomic status (Hollingshead four-factor index of social position) (Hollingshead 1975); however, several older subjects were unable to confidently report parental education and occupation. After complete description of the study to the subjects written, informed consent was obtained. The study was approved by the Centre for Addiction and Mental Health Ethics Review Board.

Although 143 subjects met initial screening criteria, nineteen were unable to complete all protocols (e.g., due to failure to return for DT-MRI procedure, claustrophobia in MRI scanner, request to withdraw from study). Thus, 124 subjects completed all DT-MRI and clinical procedures. Three DTI scans were deemed unusable due to excessive artifact and DTI data from 21 subjects were not used in the present study since no suitable match was found. Thus, 100 subjects are included in this report. They were distributed in the following four groups, each including 25 subjects: patients with chronic schizophrenia 55 years and younger; younger matched healthy control participants; patients with schizophrenia 56 years and older; and older healthy controls. The choice of 55 years as the dividing point between age groups was intended
to reflect a commonly chosen upper age limit in neuroimaging studies of patients with chronic schizophrenia that, typically, have excluded older patients e.g. (Kubicki, Westin et al. 2002; Jones, Catani et al. 2006; Rosenberger, Kubicki et al. 2008).

6.3.2 Image Acquisition

DTI images were acquired using an eight-channel head coil on a 1.5 Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, WI), which permits maximum gradient amplitudes of 40 mT/m. A single shot spin echo planar sequence was used with diffusion gradients applied in 23 non-collinear directions and $b=1000$ s/mm$^2$. Two $b=0$ images were obtained. Whole brain coverage was obtained (no gap), oblique to the axial plane. Slice thickness was 2.6 mm, and voxels were isotropic. The field of view was 330 mm and the size of the acquisition matrix was 128 x 128 mm, with echo time (TE) = 85.5 ms, and repetition time (TR) = 15,000 ms. To improve the signal to noise ratio, the entire sequence was repeated three times. Inversion recovery prepped spoiled gradient recall and fast spin echo T2 weighted images were also acquired in the event of need for registration and to ensure anatomical accuracy.

6.3.3 Image Analysis and Tractography

Diffusion weighted images were transferred to a workstation for analysis. The three repetitions were co-registered to the first $b=0$ image in the first repetition using FSL (v. 4.0) [www.fmrib.ox.ac.uk](http://www.fmrib.ox.ac.uk) to produce a new averaged image, with gradients re-oriented according to the registration transformation. A final diffusion tensor was then estimated based on all 75 aligned volumes using a weighted least squares approach. Registration corrects eddy current distortions and subject motion, important artifacts that can affect the data, and averaging improves the signal to noise ratio. A brain ‘mask’ was then generated. Points were seeded throughout each voxel of the brain. Whole-brain tractography was performed with a
deterministic (streamline) approach (Runge-Kutta order two tractography with a fixed step size of 0.5 mm). More detailed descriptions of our tractography approach and our clustering segmentation algorithm have been recently published (O'Donnell, Kubicki et al. 2006; Voineskos, O'Donnell et al. 2009), and are summarized here. Threshold parameters for tractography were based on the linear anisotropy measure $C_L$, where $C_L = (\lambda_1 - \lambda_2)/\lambda_1$ and, $\lambda_1$ and $\lambda_2$, the two largest eigenvalues of the diffusion tensor sorted in descending order. Thresholds were based on the $C_L$ rather than on FA, because FA can be relatively high in regions of planar anisotropy which may indicate tract crossings or branching (Ennis and Kindlmann 2006). The three threshold parameters for tractography were $T_{\text{seed}}$, $T_{\text{stop}}$, and $T_{\text{length}}$. The $T_{\text{seed}}$ and $T_{\text{stop}}$ are anisotropy thresholds based on $C_L$. The $T_{\text{length}}$ threshold was 20 mm to prevent very short fibers from being generated (O'Donnell and Westin 2007) in order to permit reliable selection of clusters that comprise the major neuroanatomic tracts of interest (Voineskos, O'Donnell et al. 2009). Tractography and creation of white matter fiber tracts were performed using 3D Slicer (www.slicer.org) and Matlab 7.0 (www.mathworks.com).

A pairwise fiber trajectory similarity was quantified by first computing a pairwise fiber distance, and a mean closest point distance was then employed. The directed distances between fibers ‘A’ and ‘B’ were converted to a symmetric pairwise fiber distance. Each distance was then converted to an affinity measure suitable for spectral clustering via a Gaussian kernel $W_{ij} = e^{(-d^2_{ij}/\sigma^2)}$ (Shi and Malik 2000). The role of $\sigma$ ($\sigma=60$ mm) is to define the size scale of the problem by setting the distance over which fibers can be considered similar. A spectral embedding of fibers was then created based on the eigenvectors of the fiber affinity matrix. We used the top 15 eigenvectors of the fiber similarity matrix to calculate the most important shape similarity
information for each fiber, using a k-way normalized cuts clustering algorithm (O'Donnell, Kubicki et al. 2006).

Once the whole brain cluster model was produced, a trained operator (ANV) combined the clusters that correspond to a given fiber tract. Left and right uncinate fasciculus, inferior occipitofrontal fasciculus, cingulum bundle, inferior longitudinal fasciculus, and arcuate fasciculus, and genu and splenium (parietal, temporal, occipital fibers) of the corpus callosum were selected (Voineskos, O'Donnell et al. 2009) (Figure 1). As reported elsewhere (Voineskos, O'Donnell et al. 2009), two individuals, blind to participant information, performed separately the entire clustering procedure on ten individuals with schizophrenia, and ten healthy controls and achieved excellent spatial and quantitative reliability using this clustering method (i.e., both voxel overlap and scalar measures of the tensor showed high agreement). Matlab (v. 7.0) was then used to calculate FA (Basser and Pierpaoli 1996). Presented data represents the mean values along the selected tracts. With the exception of participants included in the reliability study, this is a new cohort of subjects, with data reported for the first time.

### 6.3.4 Statistical Analysis

Data were analyzed using SPSS vr. 15.0. For groupwise comparisons, a repeated measures ANOVA model was used. The four ‘diagnosis-age’ groups were compared: younger controls, younger schizophrenia patients, older controls, older schizophrenia patients. Some reports have indicated that gender may affect microstructural integrity of white matter (Schmithorst, Holland et al. 2008; Huster, Westerhausen et al. 2009). Therefore, gender was also included as a between group factor. There were twelve within group factors: mean FA for each of twelve white matter tracts studied. Post hoc t tests comparing the younger control to the younger schizophrenia groups and the older control to the older schizophrenia groups were used to discover where
significant differences in tract FA were present between age-specific diagnostic groups. For individual tract analyses, Bonferroni correction for twelve comparisons ($p < 0.05/12$) (i.e. $p < 0.0042$) was applied. When a significant difference was found, tract volume and chlorpromazine-equivalents of medication dosage were regressed against FA for that fiber tract, to correct for any influence of these variables.

Rather than conduct post hoc within diagnostic group comparisons of the younger schizophrenia to older schizophrenia groups and the younger control to older control groups, we sought to examine age-related change in each white matter tract within diagnostic group by using correlational analysis across adult life separately for all schizophrenia patients and then for all controls. We then compared correlations of age-related FA decline between the schizophrenia group and the control group for each white matter tract across adult life. Significance of the differences between correlations (to compare age-related FA changes for each white matter tract in schizophrenia patients vs healthy controls) was calculated by dividing the difference between Fischer’s z-score transformation of Pearson’s $r$ by the standard error of difference between the two correlations (Blalock 1972).

Since previous studies have found evidence of potential differences in asymmetry (Kubicki, Westin et al. 2002) in schizophrenia, a laterality index was calculated for each bilateral hemispheric tract (i.e. uncinate fasciculus, arcuate fasciculus, inferior longitudinal fasciculus, inferior occipitofrontal fasciculus, cingulum bundle), as in Catani et al.(Catani, Allin et al. 2007): laterality index = $(\text{left tract FA} – \text{right tract FA}) / [(\text{left tract FA} + \text{right tract FA})] / 2$. A repeated measures ANOVA was performed, with five tract laterality index within group measures and diagnosis-age group as the between group measure. To examine for age related effects, laterality index for each tract pair was regressed against age.
Exploratory correlational analyses for tract FA values were performed with positive and negative symptom subscale scores from the PANSS in each schizophrenia group (younger schizophrenia group, older schizophrenia group). For each laterality index, correlations with PANSS scores were performed for all schizophrenia patients, since age was not related to laterality.

The relationship of Mini Mental Status Exam scores to tract FA was also explored using separate Pearson correlational analyses for schizophrenia patients and controls.

### 6.4 Results

Demographic and clinical characteristics of the 100 subjects are presented in Table 1.

Figure 2 illustrates mean FA values for each ‘diagnosis-age’ group at each white matter tract. There was a significant main effect of group ($F_{3,92} = 12.2, p < 0.001$) and a group by tract interaction ($F_{33,1012} = 1.68, p = 0.010$) (Greenhouse-Geiser correction, $F_{26, 832} = 1.68, p = 0.018$) (observed power $= 0.989$). There was no significant main effect of gender ($F_{1, 92} = 0.53, p = 0.47$), nor was there a significant group by gender ($F_{3, 92} = 1.68, p = 0.18$) or group by gender by tract interaction ($F_{33, 1012} = 1.17, p = 0.26$).

Three individuals in the schizophrenia group experienced ‘late-onset schizophrenia’ (i.e. onset of illness at age 45 and older) (Jeste, Harris et al. 1995), which may represent a milder version of illness (Almeida, Howard et al. 1995), resulting in a significant difference in age-of-onset in the two schizophrenia groups ($t_{48} = 2.5, p = 0.02$); thus, the analysis was repeated without these three individuals (age-of-onset was no longer different between the schizophrenia groups $t_{45} = 1.4, p = 0.16$). Significant results were unchanged following this new analysis: a significant main effect of group ($F_{3,89} = 14.1, p < 0.001$) and a group by tract interaction ($F_{33,979} = 1.68, p = 0.010$).
(Greenhouse-Geiser correction, $F_{26,784} = 1.68, p=0.018$) were still present, while no significant main effects or interaction effects of gender were present.

The post-hoc comparisons of the younger control and the younger schizophrenia groups demonstrated statistically significant differences (threshold set at $p < 0.0042$) with relatively large effect size for the left uncinate fasciculus ($t_{48} = 3.7, p = 0.001$) (Cohen’s $d = 1.05$), and right cingulum bundle ($t_{48} = 3.6, p = 0.001$) (Cohen’s $d = 1.02$). There was no relationship between FA and fiber tract volume for the left uncinate fasciculus and right cingulum bundle, nor with FA and mean medication dose. No differences in FA were present between the older groups for any white matter tract ($p > 0.10$ for all tracts).

Significant inverse correlations between age and FA were found in healthy controls ($p \leq 0.001$ for all white matter tracts), and in schizophrenia patients, except for left uncinate fasciculus ($r = -0.26, p = 0.07$), right uncinate fasciculus ($r = -0.32, p = 0.02$), left arcuate fasciculus ($r = -0.24, p = 0.10$), right arcuate fasciculus ($r = -0.35, p = 0.01$), and right cingulum bundle ($r = -0.36, p =0.01$), which did not reach significance after applying the $p$ value corrected for the 12 comparisons (Figure 3). There was no statistically significant difference in correlation coefficients measuring age related decline of FA between diagnostic groups for any white matter tract (all $p > 0.05$).

For the laterality index, there were no main effects of group ($F_{3,96} = 1.05, p=0.37$), nor group by tract interaction ($F_{12,384} = 1.10, p = 0.36$). No relationship was present between age and laterality index for any bilateral tract in either the controls or schizophrenia samples.
No significant relationships were found for PANSS scores with FA in either schizophrenia group. A significant relationship between cingulum bundle laterality index and negative symptoms ($r = 0.47, p = 0.001$) across all schizophrenia subjects was found.

No relationship between Mini Mental Status Exam score and tract FA was found for schizophrenia patients, and no such relationship was found for healthy controls.

6.5 Discussion

We conducted a comprehensive DTI study of the microstructural integrity of fronto-temporal and interhemispheric white matter tracts in 50 patients with schizophrenia stratified in a younger and an older group and 50 individually matched controls. This study has three main findings: 1) the left uncinate fasciculus and right cingulum bundle showed a significant decrease in microstructural integrity (FA) in younger patients compared to younger controls; 2) no differences in FA were observed/detected between older patients and older controls, and 3) age related FA decline occurs in both patients and controls; however, no exaggerated ageing effects in schizophrenia were found.

The decreased FA we observed in the left uncinate fasciculus in younger patients with schizophrenia is consistent with the literature suggesting fronto-temporal disconnectivity in schizophrenia (McIntosh, Maniega et al. 2008), although there have been negative studies (Jones, Catani et al. 2006). Reduced oligodendrocyte number and gene expression in frontal and temporal cortex in schizophrenia have been reported (Hakak, Walker et al. 2001; Uranova, Orlovskaya et al. 2001; Katsel, Davis et al. 2005), that align with our finding of reduced FA in the left uncinate fasciculus. Reduced oligodendrocyte number in schizophrenia may lead to disrupted myelin, or insufficient myelin mediated inhibition of neuritic sprouting (Budel,
Padukkavidana et al. 2008), which in turn may contribute to axonal disorganization, and hence reduced FA in the left uncinate fasciculus (Voineskos 2009). The UF may also have disrupted asymmetry in schizophrenia (Kubicki, Westin et al. 2002); however, we found no difference in our laterality index. A role for the UF in self-regulation, self-awareness and goal directed behaviour is suggested by the specific fronto-temporal regions it connects (Kubicki, Westin et al. 2002) and by results from lesion studies. Therefore, disrupted UF integrity in schizophrenia may be related to impaired social cognition, characterized mainly by theory of mind and empathy deficits (Benedetti, Bernasconi et al. 2009). However, these dimensions of the disease are not measured by the PANSS scale, or conventional neuropsychological testing.

We also observed decreased FA of the right cingulum bundle in younger schizophrenia patients compared to controls, consistent with fronto-temporal disconnectivity. Increased white matter volume in the right cingulate but not the left (Mitelman, Shihabuddin et al. 2005) has been shown, and right cingulate metabolism has shown preferential decrease during the Stroop task in schizophrenia (Nordahl, Carter et al. 2001). Glial cell loss has been demonstrated in cingulate cortex in schizophrenia, and oligodendrocyte related genes are downregulated in three regions connected only by the cingulum bundle, the cingulate, frontal, and temporal cortex (Davis, Stewart et al. 2003). Recent work has also demonstrated downregulation of oligodendrocyte genes in cingulate white matter, namely the quaking gene, myelin associated glycoprotein gene, 2’;3’-cyclic nucleotide 3’-phosphodiesterase gene, and the transferrin gene (McCullumsmith, Gupta et al. 2007). Downregulation of such genes in cingulate white matter in schizophrenia may be related to decreased FA in the cingulum bundle measured with DTI. However, previous DTI studies have shown decreases in FA in both left and right cingulum bundles, while others have not (Konrad and Winterer 2008). Disrupted cingulum integrity in schizophrenia may be related
to negative symptoms and impaired executive function (Kubicki, McCarley et al. 2007). In our analysis, we found no relationship with negative symptoms in left or right cingulum. However, a significant correlation between the cingulum laterality index and negative symptom burden was present across both schizophrenia age groups (though this should be viewed as preliminary given the large number of comparisons made with tract FA and the PANSS). Nevertheless, it is possible that greater asymmetry of the cingulum may represent a pathophysiological mechanism underlying negative symptoms in schizophrenia. Others have also shown that increasing asymmetry may be disadvantageous: while asymmetry of the arcuate fasciculus is normal, healthy individuals with the greatest asymmetry performed most poorly on the California Verbal Learning Task (Catani, Allin et al. 2007).

We found no differences in FA in the arcuate fasciculus, inferior longitudinal fasciculus, inferior occipitofrontal fasciculus, or corpus callosum. For the left inferior longitudinal fasciculus, recent tractography findings in early onset schizophrenia adolescents have shown reduced FA, where individuals with visual hallucinations showed further reduced FA (Ashtari, Cottone et al. 2007). On the other hand, higher FA in both the arcuate fasciculus (Shergill, Kanaan et al. 2007) and cingulum bundle (Hubl, Koenig et al. 2004) has been associated with auditory hallucinations in schizophrenia. Despite our negative findings, continued investigation of the arcuate fasciculus, inferior longitudinal fasciculus, inferior occipitofrontal fasciculus, and corpus callosum in schizophrenia is important, particularly given their crucial roles in language, visuo-emotional processing, visuo-spatial function, and interhemispheric communication respectively (Gazzaniga 2000; Catani, Jones et al. 2003; Kier, Staib et al. 2004; Kubicki, McCarley et al. 2007; Catani and Mesulam 2008).
To our knowledge, this is the first report examining fronto-temporal white matter tracts in schizophrenia across the adult lifespan. Our data showed no excess in the expected age-related decline in older patients compared to healthy controls. While it is possible that we were insufficiently powered to detect differences between the older groups, the detectable differences, and large effect sizes observed between the younger groups make such a possibility less likely. Other studies using DTI tractography have also found differences between groups with similar or smaller sample sizes compared to ours (Ashtari, Cottone et al. 2007; McIntosh, Maniega et al. 2008). Another DTI tractography study (Jones, Catani et al. 2005), examined elderly patients with ‘very-late-onset schizophrenia-like’ psychosis compared to healthy elderly controls and found no differences in fractional anisotropy measured along fronto-temporal white matter tracts. Though the patients in that study did not have schizophrenia, and had a much shorter duration of illness (since onset of illness was 60 years or greater) one important similarity with our elderly patients was that all individuals had Mini Mental Status Exam scores of 25 or greater. Our findings are unlike the recent finding of exaggerated age-related decline in schizophrenia in the forceps minor of the corpus callosum and the inferior longitudinal fasciculus (Friedman, Tang et al. 2008). One explanation for this difference might be due to different methodological approaches, whereby Friedman et al. used an ROI based approach, and thus sampled specific anatomic portions of the corpus callosum and ILF, whereas via our tractography approach, our FA results represent microstructural integrity measured along each fiber tract. In addition, Friedman et al. included long-term institutionalized patients, who have been shown to experience dramatic cognitive decline, with cognitive scores consistent with severe dementia (Rajji and Mulsant 2008). Our patients were community-dwelling with cognitive scores in the non-demented range. Unlike institutionalized patients, older community-dwelling patients may be
more resilient, given the otherwise dramatically decreased life-expectancy in individuals with schizophrenia (Marder, Essock et al. 2004; Tiihonen, Lonnqvist et al. 2009). Our lack of finding of a relationship between Mini Mental Status Exam score and tract FA was not surprising given the narrow range of Mini Mental Status Exam scores in our sample, and the simplified nature of the Mini Mental Status Exam as a neurocognitive assessment. Efforts are underway by our group to comprehensively investigate the relationship between white matter integrity and cognition in schizophrenia across the adult lifespan. Further characterization of such a relationship, particularly in late-life, may provide insight into neurobiological underpinnings of cognitive and functional outcomes, and neural correlates of successful ageing in schizophrenia.

The absence of differences between our older groups provides some insight regarding the potential confounding effects of medication on FA. Like ours, most studies demonstrate no relationship between medication and FA. These cross-sectional findings do not prove that reduced FA in chronic schizophrenia samples are not medication-related. However, they help mitigate such concerns. If medication had a significant effect on FA, the group with the longer medication exposure (i.e. the elderly group) might have had a greater reduction in FA than the control group. Furthermore, antipsychotic medications may lead to white matter repair, possibly reflected by increased FA (Garver, Holcomb et al. 2008), and, in mice, the atypical antipsychotic, quetiapine, has been shown to facilitate oligodendrocyte development (Xiao, Xu et al. 2008).

Our study has several limitations. First, even though they may provide a protective effect for oligodendrocytes, antipsychotic medication effects on FA are still not well-understood. Second, ageing is associated with a larger cumulative burden of exposure to environmental factors that may change white matter integrity, such as smoking, alcohol, or comorbid physical disorders. In patients with schizophrenia these factors are present at a higher rate than in the general
population (Marder, Essock et al. 2004). Third, postmortem studies of schizophrenia patients that demonstrate reduced myelin gene expression have been shown with both younger chronic (Torrey, Webster et al. 2000; Tkachev, Mimmack et al. 2003) and older chronic subjects (Hakak, Walker et al. 2001), though the older subjects had ‘chronic intractable schizophrenia, each with at least 35 years of hospitalization’ (Hakak, Walker et al. 2001). Since these postmortem studies do not include elderly community-dwelling subjects, conclusions regarding the relationship of postmortem myelin gene downregulation reported in the literature to lack of FA differences between our elderly groups cannot be drawn. Regarding study design, a longitudinal DTI study is needed to provide information about white matter decline in each individual, and would protect against a survivor selection bias, that can be present in a cross-sectional design, such as ours. Fourth, we did not study institutionalized patients, who may have a more severe form, or different variant of illness. They may experience progressive deterioration, and accelerated decrease in white matter integrity. Recently, prominent FA changes in schizophrenia have emerged from datasets that include individuals who have severe disease phenotypes, such as early-onset schizophrenia patients (Ashtari, Cottone et al. 2007), institutionalized inpatients (Friedman, Tang et al. 2008), and deficit syndrome patients (Rowland, Spieker et al. 2009).

Finally, while we are confident in our measurement of fractional anisotropy given that previous work has demonstrated at least 20 unique sampling orientations as necessary for a robust measurement of anisotropy (Jones 2004) (we obtained 23 unique sampling orientations), for robust estimations of tensor orientation and mean diffusivity at least 30 unique sampling orientations are required (Jones 2004).

In summary, we investigated twelve susceptible cortico-cortical white matter tracts in schizophrenia across the adult lifespan. We studied a relatively large, carefully matched group,
assessing both age- and group-effects for each white matter tract. Statistically significant reductions in FA with large effect sizes were observed in the left uncinate and right cingulum of younger patients with chronic schizophrenia. However, these differences were not present in older patients. Future DTI investigations of these elderly patients in conjunction with more detailed cognitive measures may ultimately reveal specific mechanisms or biomarkers of resilience in schizophrenia. At the same time, a focus on white matter in individuals with schizophrenia who have a severe, deteriorating course of illness may reveal which patients experience age related decline that exceeds that of healthy ageing, thus providing increased capability to discover biological substrates of prognosis in schizophrenia across adult life.
Table 6-1. Demographic and Clinical Characterization of Subjects

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<th>Old Controls (n=25)</th>
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<td>13 (3)</td>
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<td>29 (1)</td>
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<td>16M, 9F</td>
<td>13M, 12F</td>
<td>13M, 12F</td>
</tr>
<tr>
<td>Handedness</td>
<td>24R, 1L</td>
<td>24R, 1L</td>
<td>24R, 1L</td>
<td>24R, 1L</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>18 C, 3 As, 4 Af</td>
<td>21 C, 4 As</td>
<td>22 C, 1 As, 2 Af</td>
<td>23 C, 2 As</td>
</tr>
<tr>
<td>Currently Smoking</td>
<td>8 12</td>
<td>10 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotic treatment</td>
<td>NA 2 1º, 22 2º</td>
<td>NA 1 1º, 23 2º</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- a: Education
- b: CIRS-G
- c: Age of Onset
- d: Antipsychotic treatment
- N: Not applicable

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SZ = Schizophrenia, SA = Schizoaffective, NA = Not Applicable, N = Number
1º = first generation antipsychotic, 2º = second generation antipsychotic
M = Male, F = Female   WTAR – Wechsler Test for Adult Reading
MMSE – Minimental State Examination
CIRS-G – Clinical Information Rating Scale, Geriatrics
PANSS – Positive and Negative Syndrome Scale
Chlorpr. Equiv. – Chlorpromazine Equivalent
C = Caucasian, As = Asian, Af = African (based on self – report)

a,b Young Controls (YC) were compared to Young Schizophrenia Patients (YS) and Old Controls (OC) to Old Schizophrenic Patients (OS) using t tests on Age, Education, WTAR, MMSE, and CIRS-G. Significant differences (i.e. p < .05) were present on Education (t_{48}=2.8, p=.008) (YS<YC and OS<OC), and CIRS-G (t_{48}=5.5, p<.001) (YC<YS), and (t_{48}=4.0, p<.001) (OC<OS)

cAge of onset is higher in the older schizophrenia group; however, when the three late-onset (i.e. > 45 years of age) individuals were removed from the older schizophrenia group, the schizophrenia groups were no longer different in age of onset, and all analyses of group FA differences were unchanged.

dIn each schizophrenia group, one individual was not on antipsychotic medication
Figure 6-1. White Matter Tracts of Interest Superimposed on Fractional Anisotropy Gray Scale Images

A. Left to Right: left uncinate fasciculus, left inferior occipitofrontal fasciculus, left arcuate fasciculus

B. Left to Right: left inferior longitudinal fasciculus, right cingulum bundle, genu and splenium of corpus callosum (in same panel, both colored red)
Figure 6-2. Fractional anisotropy by diagnostic-age group for twelve white matter tracts

*Significant differences (that survived Bonferroni correction) between young controls and young schizophrenia patients are noted on the figure: L UF (left uncinate fasciculus) $t_{48} = 3.7$, $p = 0.001$, and R CB (right cingulum bundle) $t_{48} = 3.6$, $p = 0.001$.

R UF (right uncinate fasciculus), L IFOF (left inferior occipitofrontal fasciculus), R IFOF (right inferior occipitofrontal fasciculus), L AF (left arcuate fasciculus), R AF (right arcuate fasciculus), L ILF (left inferior longitudinal fasciculus), R ILF (right inferior longitudinal fasciculus), L CB (left cingulum bundle), R CB (right cingulum bundle), G of CC (genu of corpus callosum), S of CC (splenium of corpus callosum)
Figure 6-3. Relationship between age and fractional anisotropy for each white matter tract in both schizophrenia patients and healthy controls

No significant differences (when comparing correlation coefficients) in age-related decline of fractional anisotropy were present in any white matter tract between schizophrenia patients and controls.
Chapter 7

7 Age-Related Decline in White Matter Tract Integrity and Cognitive Performance: A DTI Tractography and Structural Equation Modeling Study

Contents of this chapter have been published as:


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http://www.neurobiologysfaging.org/article/S0197-4580(10)00087-4/abstract

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7.1 Abstract

Age-related decline in microstructural integrity of certain white matter tracts may explain cognitive decline associated with normal aging. Whole brain tractography and a novel clustering segmentation method were used to examine white matter tract integrity and cognitive performance in 48 healthy individuals across the adult lifespan. White matter tracts studied were interhemispheric (corpus callosum), intrahemispheric association (cingulum, uncinate, arcuate, inferior longitudinal, inferior occipito-frontal), and projection (cortico-spinal) fibers. Principal components analysis reduced cognitive tests into six meaningful factors (1. memory and executive function, 2. visuomotor dexterity, 3. motor speed, 4. attention and working memory, 5. set-shifting/flexibility, and 6. visuospatial construction). Using theory-based structural equation
modeling, relationships among age, white matter tract integrity and cognitive performance were investigated. Parsimonious model fit demonstrated relationships where decline in white matter integrity may explain age-related decline in cognitive performance: inferior longitudinal fasciculus (ILF) with visuomotor dexterity; the inferior occipito-frontal fasciculus with visuospatial construction; and posterior fibers (i.e. splenium) of the corpus callosum with memory and executive function. Our findings suggest that decline in the microstructural integrity of white matter fibers can account for cognitive decline in normal aging.

7.2 Introduction

In normal aging, a wide range of cognitive functions experience age-related decline (Hedden and Gabrieli 2004). Improved understanding of the neurobiological substrates of cognitive decline in normal aging has been facilitated by the advent of diffusion tensor imaging (DTI). DTI is a clinical neuroimaging technique quite sensitive to alterations in brain white matter microstructure (Le Bihan 2003), and provides an opportunity to study white matter in vivo in a manner not previously possible with conventional MRI. DTI has emerged as an important tool in the study of neuropsychiatric and neurologic disorders (Kubicki, McCarley et al. 2007; Ciccarelli, Catani et al. 2008), as well as in the study of healthy and pathologic aging (Sullivan and Pfefferbaum 2006). In normal (or healthy) aging, DTI studies have begun to demonstrate that microstructural integrity of cerebral white matter exhibits age related decline (Salat, Tuch et al. 2005; Sullivan and Pfefferbaum 2006; Sullivan, Rohlfing et al. 2008). Thus, combining DTI approaches with examination of cognitive functions susceptible to age-related decline should help further elucidate mechanisms of cognitive decline in normal aging.
White matter changes in normal aging likely play an important role in contributing to age-related cognitive decline. Early volumetric MRI studies were equivocal in their assessment of white matter changes with age (Sullivan and Pfefferbaum 2006). In contrast, DTI studies have shown widespread age-related declines in fractional anisotropy (FA) in white matter (Sullivan and Pfefferbaum 2003; Salat, Tuch et al. 2005; Charlton, Barrick et al. 2006). FA measures the degree to which diffusion of water molecules is restricted by microstructural elements such as cell bodies, axons, myelin, and other constituents of cytoskeleton (Beaulieu 2002); thus, reduced FA associated with normal aging may be related to a number of age-related changes in white matter demonstrated in postmortem studies of aging: change in the axon’s cytoskeleton, reduction in axon density (Sullivan, Adalsteinsson et al. 2006), decline in number and length of myelinated fibers (Marner, Nyengaard et al. 2003), breakdown in the myelin sheaths (Bartzokis 2004; Bartzokis, Sultzer et al. 2004; Sullivan and Pfefferbaum 2006), trapping of fluid between thin or lysed sheaths, or bulbous swelling of oligodendrocytes (Peters, Sethares et al. 2001; Peters and Sethares 2002; Sullivan, Rohlfing et al. 2008). These studies support that DTI findings in normal aging align with histopathological findings in normal aging in white matter.

Age effects demonstrated with DTI are regionally diverse and typically show an antero-posterior gradient of age-related decline (Sullivan and Pfefferbaum 2006; Sullivan, Rohlfing et al. 2008). This gradient has been hypothesized by some investigators as underlying cognitive decline of frontally based functions (Salat, Tuch et al. 2005; Sullivan and Pfefferbaum 2006; Kochunov, Thompson et al. 2007). In quantifying age-related decline, most DTI studies have utilized region of interest or voxel-based morphometry approaches, where white matter integrity in focal brain regions were examined. For a review please see (Sullivan and Pfefferbaum 2006).
More recently, investigators have used DTI tractography-based approaches in their examination of age-related decline of white matter fiber tracts (Sullivan, Adalsteinsson et al. 2006; Stadlbauer, Salomonowitz et al. 2008; Sullivan, Rohlfing et al. 2008; Zahr, Rohlfing et al. 2009). White matter tracts serve as connections between brain regions, and likely play an important role in coordinating complex cognition. Information is required to transfer quickly between different brain regions and age-related damage to any part of these white matter connections could lead to changes in cognitive performance (Mesulam 2000; Charlton, Landau et al. 2008). However, an important limitation of tractography is that the output is only a mathematical representation of underlying structure. In certain cases it may not necessarily reflect brain anatomy e.g. (Sullivan, Adalsteinsson et al. 2006). In particular, false positive and false negative results can be produced due to noise, partial volume effects, and complex fiber architecture within a voxel (Pierpaoli, Barnett et al. 2001; Wakana, Caprihan et al. 2007). Nevertheless, several studies have shown that streamline tractography can produce anatomically faithful reconstructions of white matter fasciculi that agree with anatomic definitions based on postmortem studies (Catani, Howard et al. 2002; Mori and van Zijl 2002; Schmahmann, Pandya et al. 2007; Catani and Thiebaut de Schotten 2008; Jones 2008).

Few studies have combined tractography approaches with cognitive testing in normal aging. Comprehensive measurement of cognitive functions that may decline in normal aging requires administration of a battery of cognitive tests that include measures of processing speed, working memory (and executive function), episodic memory, mental flexibility (Hedden and Gabrieli 2004), set shifting, attention, visuospatial performance, and visual processing (Davis, Dennis et al. 2008), among others. To our knowledge, only one published tractography study has applied a relatively comprehensive cognitive battery (Zahr, Rohlfing et al. 2009): in a sample of 12
younger and 12 older adults, performance in the working memory and problem solving domains correlated with the microstructural integrity of the genu and the fornix and several fiber systems were also correlated with motor performance.

Structural equation modeling (SEM) in DTI (Charlton, Landau et al. 2008; Fonteijn, Norris et al. 2008) can provide a framework to examine the complex relationship among age, microstructural integrity of white matter fiber tracts, and cognitive performance. However, no published study to our knowledge has used diffusion tensor tractography and SEM to examine how age and microstructural integrity of white matter fiber tracts might influence cognition. Therefore, in this study, we used whole brain tractography and a novel clustering segmentation method, to measure microstructural integrity of white matter fiber tracts, and their relationship with cognitive performance in healthy individuals whose age-range spans the adult lifespan. We first investigated the relationship between age and specific white matter fiber tracts that may be susceptible to age-related decline, and for which we demonstrated high reliability of FA measurement, using our whole brain tractography, clustering segmentation approach (Voineskos, O'Donnell et al. 2009). These tracts include intrahemispheric association fibers (cingulum bundle (CB), inferior longitudinal fasciculus (ILF), uncinate fasciculus (UF), arcuate fasciculus (AF), inferior occipito-frontal fasciculus (IFOF)), interhemispheric or commissural fibers of the corpus callosum (segmented into five subdivisions), and, for comparative purpose, projection fibers of the corticospinal tract (CST). Then, following principal components analysis of all cognitive tests (chosen to index a wide range of cognitive functions susceptible to age-related decline, e.g. executive function, working memory, motor speed, visuospatial function, set shifting etc. (Hedden and Gabrieli 2004), we used SEM to examine the relationship of aging, white matter tract integrity, and cognitive performance. Based on the literature, we hypothesized that we
would primarily observe associations between microstructural integrity of cortico-cortical white matter tracts (in particular those connecting to frontal cortical regions) with specific cognitive functions, primarily reflecting the frontally based model of cognitive decline in normal aging.

7.3 Methods

7.3.1 Recruitment and Characterization of Study Participants

This study was conducted from March 2007 to March 2009 in Toronto, Canada. Sixty-four individuals volunteered via registries and advertisement. All participants were assessed with the Edinburgh handedness inventory (Oldfield 1971), interviewed by a psychiatrist, completed the Structured Clinical Interview for DSM-IV Disorders (First MB 1995) and the Mini Mental Status Exam (Folstein, Folstein et al. 1975). All participants completed a urine toxicology screen. Fifty-three healthy participants met the inclusion criteria (age between 18 and 85; right handedness) and none of the exclusion criteria (any history of a mental disorder; current substance abuse or a history of substance dependence, positive urine toxicology, a first degree relative with a history of psychotic mental disorder, a dementia, a history of head trauma with loss of consciousness, seizure, or another neurological disorder). Participants were characterized using the following instruments (see Table 1): Wechsler Test for Adult Reading (WTAR); Hollingshead index (Hollingshead 1975); Clinical Illness Rating Scale for Geriatrics (CIRS-G) (Miller, Paradis et al. 1992); weight, height, and blood pressure. Three individuals did not complete all DTI and cognitive protocols and two individuals had artifacts on their DTI scan that prevented reliable analysis, leaving 48 participants for final analyses. The study was approved by the Review and Ethics Board of the Centre for Addiction and Mental Health (Toronto, Canada) and all participants provided informed, written consent.
7.3.2 Neuropsychological Assessment

Participants underwent a battery of cognitive tests (see Table 2) that was administered over approximately one and one-half hours. This battery assessed a wide range of cognitive domains, with a focus on domains that are most likely to be affected by age, primarily using tests validated in elderly populations: executive function, working memory, immediate memory, delayed or episodic memory, attention, set-shifting, response inhibition, mental flexibility, visuospatial construction, processing speed, fine visuomotor, and motor skills.

7.3.3 Image Acquisition

Diffusion weighted images were acquired using an eight-channel head coil on a 1.5 Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, WI), which permits maximum gradient amplitudes of 40 mT/m. A single shot spin echo planar sequence was used with diffusion gradients applied in 23 non-collinear directions and b=1000 s/mm². Two b=0 images were obtained. Fifty seven to sixty two slices, with no gap, were acquired for whole brain coverage oblique to the axial plane. Slice thickness was 2.6 mm, and voxels were isotropic. The field of view was 330 mm and the size of the acquisition matrix was 128 x 128 mm, with Echo time was 85.5 ms, and repetition time was 15,000 ms. To improve the signal to noise ratio, the entire sequence was repeated three times. Inversion recovery prepped spoiled gradient recall images, fast spin T2 weighted images, and fluid attenuated inversion recovery images were also acquired.

7.3.4 Image Analysis and Tractography

Diffusion weighted images were then transferred to a workstation for analysis. The three repetitions were co-registered to the first b=0 image in the first repetition using FSL (v. 4.0) www.fmrib.ox.ac.uk to produce a new averaged image, with gradients re-oriented according to
the registration transformation. A final diffusion tensor was then estimated using a weighted least squares approach. Registration corrects eddy current distortions and subject motion, important artifacts that can affect the data, and averaging improves the signal to noise ratio. A brain ‘mask’ was then generated. Points were seeded throughout each voxel of the brain.

Whole-brain tractography was performed with a deterministic (streamline) approach (Runge-Kutta order two tractography with a fixed step size of 0.5 mm). The three threshold parameters for tractography were: $T_{\text{seed}}$, $T_{\text{stop}}$, and $T_{\text{length}}$. $T_{\text{seed}}$ and $T_{\text{stop}}$ are anisotropy thresholds used to limit the tractography to white matter. The linear anisotropy measure $C_L$ (Westin, Maier et al. 2002) was used, where $C_L = (\lambda_1 - \lambda_2) / \lambda_1$ and, $\lambda_1$ and $\lambda_2$ are the two largest eigenvalues of the diffusion tensor sorted in descending order (where the goal of the anisotropy thresholds is to limit tractography to the white matter). Thresholds were based on the $C_L$ rather than on fractional anisotropy (FA), because FA can be relatively high in regions of planar anisotropy which may indicate tract crossings or branching (Ennis and Kindlmann 2006). Advantages of using $C_L$ as the tracking threshold have been previously demonstrated (Westin, Maier et al. 2002). Using $C_L$ thresholds will avoid seeding in planar regions that happen to have reasonably high FA. This is an advantage because in planar regions, the major eigenvector is unlikely to correspond to an actual axon direction, and rather it is some average of multiple tracts (Westin, Maier et al. 2002). Using $C_L$ for tractography also facilitates fibre clustering (below) because it reduces the likelihood of fiber jumping from one structure to another, a side effect of streamline methods in planar partial-volume regions. So by somewhat reducing partial-volume tractography errors, it improves the ability of the clustering to separate different structures (O'Donnell and Westin 2007). The $T_{\text{length}}$ threshold is used to eliminate very short fibers from being generated. The parameters chosen for this study were: $T_{\text{seed}} = 0.3$, $T_{\text{stop}} = 0.15$, and $T_{\text{length}} = 20$ (in mm).
Tractography and creation of white matter fiber tracts was performed using 3D Slicer (open source software www.slicer.org) and Matlab 7.0 (www.mathworks.com).

As previously described by O’Donnell and coworkers (O’Donnell, Kubicki et al. 2006), pairwise fiber trajectory similarity was quantified by first computing a pairwise fiber distance. The mean closest point distance was employed, defined as the mean distance between pairs of closest points on two fibers. The directed distances between fibers ‘A’ and ‘B’ are converted to a symmetric pairwise fiber distance by taking the mean of the distance from A to B and from B to A. Each distance is then converted to an affinity measure suitable for spectral clustering via a Gaussian kernel \( W_{ij} = e^{-d_{ij}^2/\sigma^2} \), a method employed in the clustering literature (Shi and Malik 2000). The role of \( \sigma \) (\( \sigma = 60 \) mm used in the present study) is to define the size scale of the problem by setting the distance over which fibers can be considered similar (O’Donnell and Westin 2007). A spectral embedding of fibers is then created based on the eigenvectors of the fiber affinity matrix. In our clustering application, we used the top 15 eigenvectors of the fiber similarity matrix to calculate the most important shape similarity information for each fiber. The use of the top 15 eigenvectors has been shown to produce excellent spatial and quantitative reliability of the selected white matter tracts (Voineskos, O’Donnell et al. 2009), as well as good reproducibility (O’Donnell and Westin 2007). The clustering algorithm used was k-way normalized cuts, as it produces clusters with high within-cluster similarity and low between-cluster similarity (Ng, Jordan et al. 2002). In the k-way normalized cuts algorithm, ‘k’ represents the number of clusters. In our reliability study (Voineskos, O’Donnell et al. 2009) and in the present study white matter fibers were automatically segmented into 200 clusters. Following this step, groups of clusters were manually combined to comprise each neuroanatomical tract of interest by a trained operator (ANV) with neuroanatomical knowledge (a priori neuroanatomical
knowledge is required for this step). Clusters of the same anatomical tract tend to have similar weights, thus facilitating selection (O'Donnell, Kubicki et al. 2006). Our white matter tract ‘clustering’ segmentation method eliminates the need to manually place regions of interest (ROIs) to identify fiber bundles, following whole brain tractography (O'Donnell, Kubicki et al. 2006; O'Donnell and Westin 2007), thus eliminating some forms of user bias inherent in ROI approaches. White matter tracts of interest can then be visualized in their correct anatomic location and selected to evaluate tract-specific diffusion parameters (Voineskos, O'Donnell et al. 2009). In this study (see Figure 1), ten association fibers (five on each side): the left and right uncinate fasciculus (UF), left and right inferior occipitofrontal fasciculus (IFOF), left and right cingulum bundle (CB), left and right inferior longitudinal fasciculus (ILF), left and right arcuate fasciculus (AF), two projection fibers (left and right corticospinal tracts) (CST) were selected. Five clusters within the corpus callosum (CC) were selected: genu (CC1), premotor and supplementary motor projections (CC2), motor projections (CC3), sensory projections (CC4), and finally parietal, temporal and occipital projections (CC5). The CC was segmented using the clustering method, and selection of neuroanatomical subdivisions were made according to a previously demonstrated DTI based topographical study of the corpus callosum (Hofer and Frahm 2006), confirmed in more recent studies (Wahl, Lauterbach-Soon et al. 2007; Voineskos, O'Donnell et al. 2009). Correct selection of all tracts was verified by superimposing clusters on both the FA and T1 images (Mori, Wakana et al. 2005). Tract variability can be introduced when the operator selects additional cluster(s) to add to the main cluster initially selected for the neuroanatomic tract of interest. Overall, this approach is at least as reliable as the multiple ROI approach (Voineskos, O'Donnell et al. 2009), and was consistently reliable for all white matter tracts in the present study. Two individuals, blind to participant information, performed the entire
clustering procedure on ten individuals: as reported elsewhere (Voineskos, O'Donnell et al. 2009), reliability was demonstrated both spatially and quantitatively (i.e., both voxel overlap and scalar measures of the tensor showed high agreement). Matlab (v. 7.0) was then used to calculate FA (Basser and Pierpaoli 1996). Presented data represents the mean values along the selected tracts.

7.3.5 Statistical Analysis

Age related decline of white matter is generally considered equivalent in men and women (Sullivan, Adalsteinsson et al. 2001; Sullivan and Pfefferbaum 2006); however, others have reported that gender differences in FA may be present (Hsu, Leemans et al. 2008). Similarly there are reports suggesting the presence (Kubicki, Westin et al. 2002) and absence (Nestor, Kubicki et al. 2008) of hemispheric asymmetry with respect to bilateral tract FA. Thus, to test for effects of gender and hemisphere on all white matter tracts in the present study, a repeated measures ANCOVA was performed for bilateral tracts with gender as the between group factor, hemisphere and tract as within group factors, and age as a covariate. A separate repeated measures ANCOVA for the corpus callosum was conducted with gender as the between group factor, corpus callosum subdivision as the within group factor, and age as a covariate. FA for tracts not showing hemispheric effects were averaged as follows: (left side FA + right side FA)/2, an approach previously used (Sullivan, Rohlfing et al. 2008; Zahr, Rohlfing et al. 2009). FA for each tract was then regressed against age.

Second, Pearson product moment correlations comparing cognitive performance with age were computed. Raw scores were converted to z-scores. The z-scores were then multiplied by (-1) for tests in which a high score reflected poor performance. Where available (namely for all tests of the RBANS), age-normed scores of each cognitive test were regressed against age to ensure that
there was no stratification within the sample. Z-scores of all cognitive tests were then submitted to principal components analysis (PCA) using SPSS 15.0 (PCA; orthogonal transformation varimax solution). Principal components were retained if eigenvalues were greater than one, and factor scores were then calculated for each principal component for each participant for SEM.

Finally, SEM was used to simultaneously estimate relationships among age, white matter integrity, and the factor scores for the six retained cognitive principal components: memory and executive function; attention and working memory; set shifting/flexibility; visuomotor speed and dexterity; visuospatial construction. SEM was conducted using AMOS software (Arbuckle 1999). A literature-derived model was built, emphasizing frontally based white matter tracts and their relationship with age and executive function (O'Sullivan, Barrick et al. 2005; Sullivan, Adalsteinsson et al. 2006; Sullivan and Pfefferbaum 2006; Zahr, Rohlfing et al. 2009). Thus paths from the genu (CC1) to ‘memory and executive function’, and ‘attention and working memory’, and from the CB to ‘set shifting/flexibility’ (O'Sullivan, Barrick et al. 2005; Zahr, Rohlfing et al. 2009) were drawn. A path from posterior fibers of the CC (CC5) to ‘memory and executive function’ was also included based on previous reports (Ziegler, Piguet et al. 2008). White matter tracts that might contribute to motor speed, namely premotor and supplementary motor fibers of CC (CC2), motor fibers of the CC (CC3), (Sullivan and Pfefferbaum 2006), and UF (Zahr, Rohlfing et al. 2009) were included The ILF was hypothesized to contribute to ‘visuomotor speed and dexterity based on a previous report that the ILF may subserve a ‘fast or direct stream’ of visual processing (Catani, Jones et al. 2003), and that the ILF in a previous study demonstrated a potential relationship with a motor speed component that included the Grooved Pegboard task (Zahr, Rohlfing et al. 2009), a task that is part of our ‘visuomotor speed and dexterity’ component. Paths from both the IFOF, given its occipito-parieto-temporal-frontal
connection (Kier, Staib et al. 2004; Karnath, Rorden et al. 2009), and from CC5 (given its interhemispheric parietal, temporal, and occipital connections), to visuospatial construction ability were also included. A path from the AF (hypothesized function related to language(Catani, Allin et al. 2007) and working memory (Aboitiz and Garcia 1997) was drawn to the ‘attention and working memory’ component. The CST was not included since it was not hypothesized to be involved in any of the cognitive domains from the PCA. After the path coefficients were derived, the paths were thresholded to achieve a second, more parsimonious model, by eliminating paths with p values > 0.10 (a threshold of p = 0.10, rather than p=0.05 was chosen to ensure that relevant paths were not eliminated due to low power). Path elimination was also monitored via successive improvement of the chi-squared statistic, comparative fit index (CFI), and root mean square error of approximation (RMSEA). Good model fit can be reflected by a chi square to degrees of freedom ratio of less than 2.0 (Ullman 2001), a CFI of greater than .90 (Bentler 1990), and an RMSEA of less than .06 (Hu and Bentler 1999) or .05 (Schumacker and Lomax 2004). The CFI and RMSEA are among the measures least affected by sample size (Fan, Thompson et al. 1999), and in fact, the CFI performs very well at all sample sizes (Bentler 1990). As a robustness check, the final model fitting was repeated using bootstrapping. 95 percent confidence intervals of the path parameters of the final model were estimated using this non-parametric re-sampling method. Two thousand replications were used in the bootstrapping (the maximum number possible using AMOS software) for all 48 participants.

7.4 Results

7.4.1 Age and DTI Measures

Following the repeated measures ANCOVA for bilateral tracts, no gender by tract \( (F_{5, 225} =0.46, \ p=0.80) \), hemisphere by tract \( (F_{5, 225} =1.28, \ p=0.27) \), or gender by hemisphere by tract
interactions were found ($F_{5, 225} = 0.71, p=0.61$). The repeated measures ANCOVA examining the effect of gender on subdivisions of the corpus callosum also revealed no gender by callosal subdivision interaction ($F_{4, 180} = 1.42, p=0.23$). Therefore, a mean tract FA was calculated for all intrahemispheric (i.e. bilateral) tracts, and gender was not treated as a separate variable. All intrahemispheric tracts (UF, AF, CB, IFOF, ILF) displayed significant age-related decline except for the corticospinal tract (CST) (see Figure 2). Within the corpus callosum, the genu callosal region (CC1) connecting left and right prefrontal cortex, experienced greater age related decline than callosal regions connecting primary motor cortex (CC3) (CC1, $r = -0.69$ vs. CC3, $r = -0.34$, $t_{45}=3.9$, $p<0.01$) or primary sensory cortex (CC4) (CC1, $r = -0.69$ vs. CC4, $r = -0.39$; $t_{45}=3.6$, $p<0.01$), using a test of significance of the difference between dependent correlations from the same sample (Blalock 1972) $t = (r_{xy} - r_{yz}) \sqrt{\frac{(n - 3)(1 + r_{xz})}{2(1 - r_{xy}^2 - r_{xz}^2 - r_{yz}^2 + 2r_{xy}r_{xz}r_{yz})}}$, where ‘n’ represents sample size, ‘x’ represents age, ‘y’ and ‘z’ are FA values for two white matter tracts of interest, and ‘r’ represents the Pearson product moment correlation of the two variables. The most posterior callosal subdivision (CC5) containing interhemispheric parietal, temporal, and occipital fibers also experienced significant age-related decline, but not to the same extent as the genu (CC1, $r = -0.69$ vs. CC5, $r = -0.49$; $t_{45}=3.4$, $p <0.01$).

7.4.2 Age and Cognitive Performance

Table 3 shows the relationship of age with scores on each cognitive test. There was no correlation of age with age-normed scores, suggesting that there was no age-related stratification in performance in our sample. The principal components analysis of the cognitive data yielded six factors with eigenvalues $\geq 1$ (see supplementary Table). The six factors (or principal components) were theoretically meaningful, collectively accounting for 73.9 % of the variance in
test scores. The six principal components were: 1. memory and executive function ($\lambda=6.9$, 32.8 percent of variance), 2. visuomotor speed and dexterity ($\lambda=3.1$, 15.0 percent of variance), 3. motor speed and coordination ($\lambda=1.6$, 7.4 percent of variance), 4. attention and working memory ($\lambda=1.4$, 6.7 percent of variance), 5. set shifting/flexibility ($\lambda=1.3$, 6.2 percent of variance), and 6. visuospatial construction ($\lambda=1.2$, 5.8 percent of variance).

### 7.4.3 Structural Equation Modeling

The original literature derived model did not provide a good fit (chi squared = 1880, df=84, $p<.001$, CFI = .000, RMSEA = .679 (see Supplementary Figure). To modify the model, each path that did not meet the $p = .10$ significance threshold was systematically removed. Thus, paths from CC1 to ‘memory and executive function’ and ‘attention and working memory’ were removed, from CC2 and CC3 to ‘motor speed’, from CB and AF to ‘attention and working memory’, from CB to ‘set shifting’, and from CC5 and ILF to ‘visuospatial construction’ were also removed. Then, variables representing white matter tracts (CC1, CC2, CC3, CB, UF) that had no remaining relationships with any cognitive component were removed, and cognitive components (motor speed, working memory and attention, set shifting) that had no remaining relationship with any white matter tract were removed. Thus, the remaining tracts were the ILF, IFOF, and CC5. The remaining cognitive components were ‘memory and executive function’, ‘visuomotor dexterity’, and ‘visuospatial construction’). Finally, direct paths from age to ‘memory and executive function’, ‘visuomotor dexterity’, and ‘visuospatial construction’ were removed, since these paths also did not meet the significance threshold. By removing these paths, a new statistically significant model was created where age and white matter tract integrity did determine cognitive performance. For this final model (see Figure 3), there was excellent model fit (chi squared = 5.0, df=12, $p=.96$, CFI=1.00, RMSEA = .000) (remainder of data listed
below Figure 3 in Table 4). As explained, good model fit can be measured by a chi-squared to degree of freedom ratio of < 2 (here it is 0.42), a CFI of greater than 0.91 (here it is 1.0), and an RMSEA of <.05 (here it is .000) (Bentler 1990; Ullman 2001; Schumacker and Lomax 2004). This new model contains age, white matter tracts (CC5, ILF, IFOF), and principal cognitive components (memory and executive function, visuomotor dexterity, and visuospatial construction). This model suggests that CC5 integrity broadly predicted memory and executive function, ILF integrity predicts performance on tasks requiring visuomotor dexterity, and IFOF integrity predicts performance on tasks requiring visuospatial construction. Finally, effects of age on these three cognitive components occurred via the three white matter tracts in the model.

In this model, all standardized regression weights were significant (See Table 4). The bootstrap confidence intervals (2000 replications, the maximum permissible with AMOS software) suggested a robust model. The 95th percentile confidence intervals derived by bootstrapping demonstrated a p value of ≤ .05 (see Table 4).

7.5 Discussion

This study demonstrated age-related decline in FA all cortico-cortical white matter fiber tracts. An antero-posterior gradient of age related decline was evident in corpus callosum fibers. No gradient was evident in the intrahemispheric association fibers studied (CB, AF, UF, IFOF, and ILF). As expected, the projection fiber studied, the CST, demonstrated no age-related decline. Following PCA of a cognitive battery, and construction of an SEM based on existing theories of cognitive aging, each path was tested systematically to produce a final SEM. In our final model, age-related change in integrity of the CC5 subdivision (i.e., posterior projections/splenium) of the corpus callosum predicted ‘memory and executive function’, of the IFOF predicted
‘visuospatial construction’ performance, and of the ILF predicted ‘visuomotor dexterity’ performance.

Our finding for potential ILF function is novel. The role of the ILF in cognitive performance has been unknown, and direct approaches to characterize ILF function such as peri-operative stimulation have not been successful (Mandonnet, Nouet et al. 2007). In our study, age-related disruption of the ILF predicted performance in tasks that require visuomotor dexterity and fast visual processing. Tests that loaded high on this cognitive factor include the grooved pegboard task, letter cancellation task, and the digit-symbol coding task. Using DTI, virtual in vivo dissection has provided important evidence for the ILF, where reconstructions closely matched those of early anatomical descriptions (Catani, Howard et al. 2002), and it was suggested that the ILF may subserve a ‘direct short-latency pathway’ of visual processing, i.e. a fast stream of visual processing, whereby the parahippocampal gyrus (in the temporal lobe) and visual cortex (in the occipital lobe) communicate (Catani, Jones et al. 2003). Our results provide in vivo evidence that the ILF may subserve this ‘direct short-latency pathway’ of visual processing. Evidence for ILF function in this direct pathway was suggested in a case report on a patient who had sustained a lesion restricted to this direct pathway and was unable to learn novel, non-verbalizable visual stimuli. The conclusion was that the direct pathway serves to prime medial temporal structures to facilitate the processing of visual information (Ross 1980). Such a “priming” role could be critical, for instance, during the performance of the digit-symbol coding task. Performance on the digit-symbol coding task depends on the speed and accuracy of the task, whereby the participant must visually process the symbol, link the symbol to the digit, and then transcribe the digit. Although the digit-symbol associations key is always available for the participant to refer to during the task, the participant’s performance is likely to improve if the
medial temporal structures are “primed” and active representation of the digit-symbol associations are readily available to be matched with direct information from the visual cortex.

Our SEM demonstrated a significant association of the IFOF with visuospatial construction ability (cognitive tasks loading high on this factor included the figure-copy task, and the figure-recall task). The inferior occipito-frontal fasciculus (IFOF) is a large association bundle of fibers connecting the occipital and frontal lobes, and also connects the frontal lobe with the posterior part of the parietal and temporal lobes (Kier, Staib et al. 2004). The IFOF is an ideal candidate to mediate interactions between visual, spatial, and executive functions considering its occipito-parietal and occipito-frontal connections. Performance on a complex visuospatial construction task is likely to depend on the involvement of more than one cognitive function. In particular, the abilities to process visual and spatial abilities combined with executive function are likely mediated by the IFOF’s connection to anterior frontal regions, from occipital and parietal regions, which may allow for better organization and planning of the “construction” part of the task. Furthermore, the fronto-temporal connection of the IFOF may play a role in figure-recall, which would also require the aforementioned visual and spatial abilities. Direct (i.e. invasive) investigations have demonstrated a potential language function for the IFOF via electrostimulation of this pathway intra-operatively, whereby semantic paraphasias were induced (Duffau, Gatignol et al. 2005). Nevertheless, studies where damage to the IFOF is known (Urbanski, Thiebaut de Schotten et al. 2008; Karnath, Rorden et al. 2009), suggest a role for the IFOF in visuospatial function, and we have demonstrated such a relationship in our normal aging population.

Posterior fibers of the corpus callosum (i.e. occipital, parietal, temporal) were associated with the ‘memory and executive function’ factor. Most notably, tests of episodic memory, loaded
particularly high on this factor, such as Story Recall and List Recall. Considerable evidence from functional neuroimaging studies implicates temporoparietal areas in episodic memory e.g. (Buckner and Wheeler 2001; Rugg, Otten et al. 2002). Furthermore, white matter integrity underlying temporal and posterior parietal areas, but not frontal areas, was associated with episodic memory performance in an elderly population (Ziegler, Piguet et al. 2008). Diminishing integrity in occipital fibers of the corpus callosum, may also be related to perceptual processing declines that may explain worsening executive function in elderly individuals (Davis, Dennis et al. 2008), which aligns with our finding relating posterior callosal fibers to executive function. Other cognitive tasks that loaded on this factor of ‘memory and executive function’ included the Stroop color-word task and the Letter Number Span task. Therefore, our findings add to previous findings, namely, association of integrity of the splenium of the corpus callosum with the Stroop task in a small elderly sample (Sullivan, Adalsteinsson et al. 2006), association of integrity of the splenium with working memory performance in a chronic alcoholism population (Pfefferbaum, Sullivan et al. 2000), and association of white matter integrity of temporal and posterior parietal areas with episodic memory performance in an elderly population (Ziegler, Piguet et al. 2008).

Surprisingly, we found that the genu of the corpus callosum, which connects left and right dorsolateral prefrontal cortex, did not contribute significantly to any one cognitive domain. Others have found that the genu predicts executive function test performance, particularly working memory (Zahr, Rohlfing et al. 2009). Despite our negative findings here, the genu remains an important candidate in executive function processes given that it connects left and right DLPFC, cortical regions with critical roles in executive function and working memory. Age related compensatory mechanisms may explain the lack of significance between genu microstructure and overall executive function performance found in our study. Some elderly
subjects may recruit compensatory brain regions or networks, maintaining their level of executive function, that may compensate for age related decline in a specific region or white matter tract. Such compensatory strategies are particularly relevant to frontal brain regions and the genu of the corpus callosum, and have been demonstrated in fMRI studies, where different patterns of recruitment of brain regions predicted executive function performance in elderly individuals (Cabeza, Anderson et al. 2002). Some have suggested that subtle compromise of the genu may actually enable bilateral engagement of the hemispheres, thus enabling compensatory mechanisms (Buckner 2004) and influencing resource allocation in executive function processes (Banich 1998; Reuter-Lorenz and Stanczak 2000; Sullivan and Pfefferbaum 2006). It is possible that in a more homogeneous sample (i.e. smaller age range), where age effects do not contribute to the variance in performance, such as in younger healthy adults, the genu of the corpus callosum may play a more prominent role in overall executive function.

Some limitations of this study should be considered. We did not study all white matter tracts in the brain. Such an approach might have elicited relationships between other white matter tracts and cognitive performance, thus accounting more comprehensively for widespread declines in cognitive function. However, we focused primarily on cortico-cortical white matter tracts for which we have previously shown high reliability, both spatially and quantitatively (Voineskos, O'Donnell et al. 2009). Others have studied the fornix of the hippocampus (Fitzsimmons, Kubicki et al. 2009) a white matter tract that has been demonstrated to play a role in working memory function (Zahr, Rohlfing et al. 2009). However, highly reliable segmentation of the fornix using streamline tractography can be a challenge with either clustering or region of interest segmentation methods (O'Donnell and Westin 2007; Wakana, Caprihan et al. 2007), and thus it was not included in our study. Healthy elderly subjects often have white matter
hyperintensities (Wen and Sachdev 2004), generally not accounted for in DTI studies of healthy aging. In our sample, seven subjects (all age 55 and greater, data not shown) had indications of white matter disease on fluid attenuated inversion recovery images (FLAIR). Two had extensive confluent deep white matter hyperintensities (WMH), and five had either small or medium sized focal WMH in deep white matter. WMH may affect white matter microstructural integrity (FA), though a relationship between these measures is only just beginning to be understood (Zhan, Zhang et al. 2009). It is also possible that relationships between white matter tract integrity and specific cognitive tests were present, but not detected. Reduction of individual cognitive tests into principal components was necessary in order to reduce the complexity of the data. A larger sample size might have provided us with more power for our model, which in turn might have permitted retention of more paths in our model, and further insight into the relationship of other white matter tracts with cognitive components in the model. A larger sample might have also given a narrower range of confidence intervals in bootstrapping procedures. However, the white matter tracts in our current model demonstrated strong association with the cognitive tasks included, and reasonable bootstrap estimates. Finally, our cross-sectional design is a limitation of our study. Cross-sectional studies examining aging are potentially confounded by cohort differences and might therefore overestimate age-related differences, particularly in cognitive decline. Yet others have suggested that cross-sectional studies where some demands were placed on participants (e.g. volunteering to come to a university hospital for testing), as required in our study, might over-represent higher performing elderly adults, (Hedden and Gabrieli 2004). Given the design of our study, it is possible that our findings may not reflect the true evolution of white matter integrity or cognition with age. This would require a longitudinal study lasting several years and an analysis of individual trajectories of white-matter integrity and cognition.
However, in the absence of any such extended longitudinal DTI study of healthy aging (to our knowledge), our findings of an age effect on both white-matter integrity and cognition support that the two processes are related.

In summary, we demonstrated significant age-related decline in the great majority of white matter tracts studied. Our findings emphasize the relationship between age-related decline in white matter integrity and cognitive function, and verify the functional ramifications of DTI metrics. We identified potential cognitive functions for two white matter tracts, the ILF and IFOF, consistent with their neuroanatomical connections, and provide supporting evidence for the role for posterior projections of the CC in memory and executive function. The investigation of functional ramifications of white matter measures remain an important area of enquiry that will likely continue to offer fascinating insight into relationships between brain structure and function. Future combinations of DTI and functional neuroimaging and/or neurophysiological tools in vivo may yield even more powerful conclusions about the mechanisms of age-related cognitive decline in the brain.
<table>
<thead>
<tr>
<th>Demographic</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 17</td>
<td>22-81</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15 ± 2</td>
<td>12-20</td>
</tr>
<tr>
<td>Socioeconomic Status (four factor*)</td>
<td>49 ± 11</td>
<td>27-66</td>
</tr>
<tr>
<td>IQ (WTAR)</td>
<td>118 ± 7</td>
<td>92-127</td>
</tr>
<tr>
<td>MMSE</td>
<td>29 ± 1</td>
<td>26-30</td>
</tr>
<tr>
<td>BMI</td>
<td>25 ± 5</td>
<td>19-36</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>122 ± 12</td>
<td>105-153</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>75 ± 8</td>
<td>59-91</td>
</tr>
<tr>
<td>CIRS-G (total score)</td>
<td>2 ± 2</td>
<td>0-11</td>
</tr>
</tbody>
</table>

WTAR – Wechsler Test of Adult Reading
MMSE – Mini-mental state examination
BMI – Body Mass Index
BP – Blood Pressure
CIRS-G – Cumulative Illness Rating Scale – Geriatrics

*Four factors are education, occupation, sex, and marital status
Table 7-2. Cognitive Battery of Neuropsychological Tests Administered

<table>
<thead>
<tr>
<th>Neuropsychological Tests</th>
<th>Domain</th>
<th>Test Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive Interview (EXIT)</td>
<td>Executive function</td>
<td>High = poor performance</td>
</tr>
<tr>
<td>(Royall, Mahurin et al. 1992)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finger Tapping</td>
<td>Fine motor speed</td>
<td>Number of finger taps</td>
</tr>
<tr>
<td>(Halstead 1947)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grooved Pegboard</td>
<td>Fine visual-motor coordination</td>
<td>Time to completion</td>
</tr>
<tr>
<td>(Matthews and Klove 1964)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>Semantic memory</td>
<td>Total words for F+A+S</td>
</tr>
<tr>
<td>(Ruff, Quayhagen et al. 1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letter-Number Span</td>
<td>Working memory</td>
<td>Total numbers and letters</td>
</tr>
<tr>
<td>(Wechsler 1997)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letter Cancellation Test</td>
<td>Visuospatial attention/scanning</td>
<td>Time to completion</td>
</tr>
<tr>
<td>(Geldmacher, Fritsch et al. 2000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trail Making Test (A &amp; B)</td>
<td>Flexibility</td>
<td>Ratio TrailsB/TrailsA</td>
</tr>
<tr>
<td>(Reitan and Wolfson 1985)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroop Neuropsychological Screening Test</td>
<td>Set shifting/response suppression</td>
<td>Ratio score</td>
</tr>
<tr>
<td>(Trenerry 1989)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)  

All RBANS scores are total scores  

(Gold, Queern et al. 1999; Hobart, Goldberg et al. 1999)

<table>
<thead>
<tr>
<th>Task</th>
<th>Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>List Learning</td>
<td>Verbal memory/encoding</td>
</tr>
<tr>
<td>Story Memory</td>
<td>Verbal memory/encoding</td>
</tr>
<tr>
<td>Figure Copy</td>
<td>Visuospatial constructional</td>
</tr>
<tr>
<td>Line Orientation</td>
<td>Visuospatial/constructional</td>
</tr>
<tr>
<td>Picture Naming</td>
<td>Language</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>Language</td>
</tr>
<tr>
<td>Digit Span</td>
<td>Attention</td>
</tr>
<tr>
<td>Digit-Symbol Coding</td>
<td>Attention/Visual processing and encoding</td>
</tr>
<tr>
<td>List Recognition</td>
<td>Delayed Memory</td>
</tr>
<tr>
<td>Story Recall</td>
<td>Delayed Memory</td>
</tr>
<tr>
<td>Figure Recall</td>
<td>Delayed Memory/Visuospatial</td>
</tr>
</tbody>
</table>
Table 7-3. Correlations between age and cognitive scores

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ± SD</th>
<th>Correlation with Age</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXIT*</td>
<td>2.8 ± 2.4</td>
<td>.48</td>
<td>.001</td>
</tr>
<tr>
<td>Finger Taps (DH)</td>
<td>44.4 ± 10.6</td>
<td>-.63</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Finger Taps (NDH)</td>
<td>39.6 ± 9.3</td>
<td>-.59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Grooved Pegboard (DH) *</td>
<td>72.3 ± 16.8</td>
<td>.35</td>
<td>.014</td>
</tr>
<tr>
<td>Grooved Pegboard (NDH) *</td>
<td>82.7 ± 20.0</td>
<td>.32</td>
<td>.029</td>
</tr>
<tr>
<td>Letter Fluency</td>
<td>14.7 ± 4.3</td>
<td>-.22</td>
<td>.13</td>
</tr>
<tr>
<td>Letter Number Span</td>
<td>16.2 ± 4.0</td>
<td>-.42</td>
<td>.003</td>
</tr>
<tr>
<td>Letter Cancellation*</td>
<td>63.2 ± 24.8</td>
<td>.43</td>
<td>.002</td>
</tr>
<tr>
<td>TrailsB/TrailsA *</td>
<td>2.5 ± 1.0</td>
<td>.34</td>
<td>.019</td>
</tr>
<tr>
<td>Stroop Ratio *</td>
<td>2.2 ± 0.5</td>
<td>.44</td>
<td>.002</td>
</tr>
<tr>
<td>RBANS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>List Learning</td>
<td>30.4 ± 4.4</td>
<td>-.44</td>
<td>.002</td>
</tr>
<tr>
<td>Story Memory</td>
<td>19.7 ± 3.1</td>
<td>-.29</td>
<td>.045</td>
</tr>
<tr>
<td>Figure Copy</td>
<td>17.6 ± 2.7</td>
<td>-.19</td>
<td>.19</td>
</tr>
<tr>
<td>Task</td>
<td>Score (Mean ± SD)</td>
<td>Correlation Value</td>
<td>p-Value</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Line Orientation</td>
<td>17.6 ± 1.9</td>
<td>-.50</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Picture Naming</td>
<td>9.5 ± 0.9</td>
<td>-.24</td>
<td>.10</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>23.0 ± 5.1</td>
<td>-.40</td>
<td>.005</td>
</tr>
<tr>
<td>Digit Span</td>
<td>12.2 ± 2.6</td>
<td>-.42</td>
<td>.003</td>
</tr>
<tr>
<td>Coding</td>
<td>50.3 ± 12.9</td>
<td>-.59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>List Recall</td>
<td>7.2 ± 2.2</td>
<td>-.39</td>
<td>.007</td>
</tr>
<tr>
<td>Story Recall</td>
<td>10.3 ± 1.6</td>
<td>-.38</td>
<td>.009</td>
</tr>
<tr>
<td>Figure Recall</td>
<td>12.5 ± 5.3</td>
<td>-.50</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Tests for which higher scores are indicative of poorer performance

DH= Dominant Hand, NDH = Non-dominant Hand

EXIT – Executive Interview

RBANS – Repeatable Battery for the Assessment of Neuropsychological Status
Table 7-4. Regression coefficients for final model, along with 95% confidence intervals and significance

<table>
<thead>
<tr>
<th>Paths</th>
<th>Standardized Regression</th>
<th>P value</th>
<th>95% C.I.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age → IFOF FA</td>
<td>-.57</td>
<td>&lt;.001</td>
<td>-.421 to -.707</td>
<td>.001</td>
</tr>
<tr>
<td>Age → ILF FA</td>
<td>-.56</td>
<td>&lt;.001</td>
<td>-.343 to -.735</td>
<td>.001</td>
</tr>
<tr>
<td>Age → CC5 FA</td>
<td>-.49</td>
<td>&lt;.001</td>
<td>-.236 to -.677</td>
<td>.001</td>
</tr>
<tr>
<td>IFOF FA → Visuospat. Const.</td>
<td>.37</td>
<td>.007</td>
<td>.144 to .548</td>
<td>.001</td>
</tr>
<tr>
<td>ILF FA → Visuomot. Dext.</td>
<td>.38</td>
<td>.005</td>
<td>.001 to .604</td>
<td>.050</td>
</tr>
<tr>
<td>CC5 FA → Mem/Exec. Function</td>
<td>.34</td>
<td>.014</td>
<td>.082 to .531</td>
<td>.016</td>
</tr>
</tbody>
</table>

P values of the 95% C.I. (confidence interval) were derived by 2000 bootstrap replications

IFOF – Inferior Occipitofrontal fasciculus,

ILF – Inferior Longitudinal Fasciculus

CC5 – occipital, parietal, temporal fibers of corpus callosum

Visuospat Const – Visuospatial Construction

Visuomot Dext – Visuomotor Dexterity,

Mem/Exec. Function – Memory/Executive Function
Figure 7-1. Segmented White Matter Fiber Tracts Superimposed on Fractional Anisotropy Images

A. from left to right: uncinate fasciculus (UF), arcuate fasciculus (AF), cingulum bundle (CB)

B. from left to right: inferior longitudinal fasciculus (ILF), inferior occipito-frontal fasciculus (IFOF), corticospinal tract (CST)
C. from left to right: corpus callosum (whole structure with fibers colour coded according to similarity of shape and location), genu of corpus callosum (CC1) selected in red, premotor and supplementary motor fibers of corpus callosum (CC2) selected in red

D. from left to right: motor fibers of corpus callosum (CC3) selected in red, sensory fibers of corpus callosum (CC4) selected in red, parietal, temporal and occipital fibers of corpus callosum (CC5) selected in red
Figure 7-2. Relationship between Age and Fractional Anisotropy of White Matter Tracts

**Uncinate Fasciculus**
- $r = 0.49$
- $p < 0.001$

**Arcuate Fasciculus**
- $r = 0.56$
- $p < 0.001$

**Cingulum Bundle**
- $r = 0.59$
- $p < 0.001$

**Inferior Longitudinal Fasciculus**
- $r = 0.56$
- $p < 0.001$

**Inferior Occipitofrontal Fasciculus**
- $r = 0.57$
- $p < 0.001$

**Corticospinal Tract**
- $r = 0.07$
- $p = 0.66$
Figure 7-3. Final Parsimonious Model Illustrating Relationships Among Age, White Matter Tract Integrity, and Cognitive Performance

Curved two way arrows represent covariance terms between each variable
One way arrows represent the impact of one variable on another

e3,e5,e6,e7,e8,e9 – error variance terms associated with each variable

ILFFA – Fractional Anisotropy of Inferior Longitudinal Fasciculus

IFFOFFA – Fractional Anisotropy of Inferior Occipitofrontal Fasciculus

CC5 FA – Fractional Anisotropy of parietal, temporal, occipital fibers of corpus callosum
Table 7-5. Supplementary. Principal Components Analysis (Following Varimax Rotation) of Cognitive Data (only principal components with eigenvalues > 1 included)

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXIT*</td>
<td>.519</td>
<td>.259</td>
<td>.356</td>
<td>.320</td>
<td>-.162</td>
<td>-.178</td>
</tr>
<tr>
<td>FT_DH_AVG</td>
<td>.034</td>
<td>.280</td>
<td>.883</td>
<td>.071</td>
<td>.174</td>
<td>-.045</td>
</tr>
<tr>
<td>FT_NDH_AVG</td>
<td>.121</td>
<td>.292</td>
<td>.839</td>
<td>-.094</td>
<td>.056</td>
<td>.057</td>
</tr>
<tr>
<td>GPD*</td>
<td>.078</td>
<td>.945</td>
<td>.184</td>
<td>.061</td>
<td>.036</td>
<td>.079</td>
</tr>
<tr>
<td>GPND*</td>
<td>.010</td>
<td>.949</td>
<td>.118</td>
<td>.091</td>
<td>.046</td>
<td>.133</td>
</tr>
<tr>
<td>LetterFluency</td>
<td>.499</td>
<td>.252</td>
<td>.037</td>
<td>.236</td>
<td>.364</td>
<td>-.219</td>
</tr>
<tr>
<td>LNS</td>
<td>.449</td>
<td>.093</td>
<td>.119</td>
<td>.631</td>
<td>.290</td>
<td>.011</td>
</tr>
<tr>
<td>LCT*</td>
<td>-.004</td>
<td>.876</td>
<td>.239</td>
<td>.030</td>
<td>.122</td>
<td>.121</td>
</tr>
<tr>
<td>RatioTrailsB/TrailsA*</td>
<td>-.147</td>
<td>.070</td>
<td>.296</td>
<td>.162</td>
<td>.731</td>
<td>.205</td>
</tr>
<tr>
<td>StroopRatio*</td>
<td>.464</td>
<td>-.029</td>
<td>.260</td>
<td>-.434</td>
<td>.468</td>
<td>-.141</td>
</tr>
<tr>
<td>ListLearning</td>
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Scores followed by * are those z scores multiplied by (-1), where a high score indicated a poor result.

Principal components: 1. memory/executive function

2. visuomotor speed and dexterity

3. motor speed and coordination

4. attention/working memory

5. set shifting/flexibility

6. visuospatial construction

EXIT = Executive Interview
GPD = Grooved Pegboard Dominant Hand
GPND = Grooved Pegboard Nondominant Hand
FTDH = Finger Taps Dominant Hand
FTNDH = Finger Taps Nondominant Hand
LNS = Letter Number Span
LCT = Letter Cancellation Test
Figure 7-4. Supplementary. Theory-Derived Structural Equation Model

Curved two way arrows represent covariance terms between each variable. One way arrows represent the impact of one variable on another. e1 - e15 represent error variance terms associated with each variable. Path coefficients from error terms to endogenous variables are set to 1. Means of error terms are not estimated and remain zero.

CC1FA – Fractional Anisotropy of genu of corpus callosum
CC5 FA – Fractional Anisotropy of parietal, temporal, occipital fibers of corpus callosum
CC2FA – Fractional Anisotropy of prefrontal, supplementary motor fibers of corpus callosum
CC3FA – Fractional Anisotropy of motor fibers of corpus callosum
AFFA – Fractional Anisotropy of arcuate fasciculus
UFFA – Fractional Anisotropy of uncinate fasciculus
CBFA – Fractional Anisotropy of cingulum bundle
IFOFFA – Fractional Anisotropy of inferior occipitofrontal fasciculus
ILFFA – Fractional Anisotropy of inferior longitudinal fasciculus
Chapter 8

8 The BDNFval66met polymorphism: Genetic susceptibility for an intermediate phenotype related to Alzheimer’s disease in healthy individuals

Contents of this manuscript are accepted for publication in the Archives of General Psychiatry

8.1 Abstract

The brain derived neurotrophic factor (BDNF) val66met polymorphism may predict risk for healthy vs. pathologic aging (Alzheimer’s disease). However, genetic association studies of the BDNF gene with Alzheimer’s disease have produced equivocal results. Imaging-genetics strategies may clarify the manner in which BDNF gene variation predicts risk for Alzheimer’s disease via characterization of its effects on at-risk structures or neural networks susceptible in this disorder. The objective was to determine whether the BDNF val66met gene variant interacts with age to predict brain and cognitive measures in healthy volunteers across the adult lifespan in an intermediate phenotype pattern related to Alzheimer’s disease by examining: (i) cortical thickness, (ii) fractional anisotropy of white matter tracts (i.e. white matter integrity) and (iii) episodic memory performance.

A cross-sectional study using genetics, high resolution magnetic resonance imaging and diffusion tensor imaging, and cognitive testing in healthy individuals spanning the adult lifespan was performed in 69 healthy individuals ranging in age from 19-82 years.

The BDNF val66met interacted with age to predict: i) cortical thickness (prominently at entorhinal cortex and temporal gyri), ii) fractional anisotropy of white matter tracts, prominently
at white matter tracts connecting to medial temporal lobe, and iii) episodic memory performance. For each of these findings, the pattern was similar: val/val individuals in late-life were susceptible, and in early adult life, met-allele carriers demonstrated susceptibility.

The BDNF gene confers risk in an age-dependent manner on the brain structures and cognitive functions that are consistent with the neural circuitry vulnerable in the earliest stages of Alzheimer’s disease. Our novel findings provide convergent evidence \textit{in vivo}, for a BDNF genetic mechanism of susceptibility in an intermediate phenotype related to Alzheimer’s disease.

\section*{8.2 Introduction}

A substantial proportion of cases of pathologic aging are due to sporadic or late-onset Alzheimer’s disease (AD). Late onset AD constitutes 90 - 95 percent of AD cases and is a complex, heterogeneous disorder, with increasing prevalence (Harman 2006). While some notable examples of genetic risk in AD have been established (Corder, Saunders et al. 1993), genetic investigations in this disorder have been fraught with many of the same complexities and conundra as those of other neuropsychiatric disorders (Kennedy, Farrer et al. 2003). The brain derived neurotrophic factor (BDNF) gene represents an intriguing potential genetic mechanism for risk for healthy vs. pathologic aging, and specifically for late-onset AD (Zuccato and Cattaneo 2009). BDNF is critical for neuronal plasticity, and facilitates hippocampal and cortical long-term potentiation (Figurov, Pozzo-Miller et al. 1996), processes that are especially important for learning and memory. Learning and memory processes are affected in both healthy aging and AD, arising in large part from impaired neuronal plasticity(Tapia-Arancibia, Aliaga et al. 2008). In AD patients, BDNF expression is prominently reduced in hippocampus, and in entorhinal cortex(Narisawa-Saito, Wakabayashi et al. 1996), and these regions are consistently affected in the earliest stages of the disease(Gomez-Isla, Price et al. 1996; Price, Ko et al. 2001).
Variation in the BDNF val66met polymorphism has been shown to be related to episodic memory performance in younger adults via the hippocampal formation (Egan, Kojima et al. 2003). In addition, this polymorphism predicts cognitive performance in the elderly (Harris, Fox et al. 2006) and may confer risk for AD (Ventriglia, Bocchio Chiavetto et al. 2002). Recent animal model findings suggest a compelling potential role for BDNF as a therapeutic agent in AD (Nagahara, Merrill et al. 2009). Taken together, these findings suggest that BDNF gene variation may be a genetic susceptibility mechanism for AD in aging individuals.

The combination of neuroimaging and genetics (i.e. imaging-genetics) offers the potential to characterize the effects of BDNF risk variants on at-risk neural structures relevant to AD, via the intermediate phenotype approach. Such an approach may evince greater penetrance of the effects of the gene on the vulnerable neural structure or function in healthy individuals, and is not subject to confounds present in disease populations (Meyer-Lindenberg and Weinberger 2006). In AD, a prominent at-risk neural feature in gray matter is reduced cortical thickness in temporal lobe structures, demonstrated via structural MRI. Reduced thickness is most prominent in entorhinal cortex (Lerch, Pruessner et al. 2005; Desikan, Cabral et al. 2009), a finding present at the earliest stages of disease, which aligns directly with neuropathological studies that show the earliest and greatest neurodegenerative changes occur in entorhinal cortex, and then in hippocampus (Price, Ko et al. 2001). However, structural brain changes in AD are not limited to gray matter -- more recently, white matter abnormalities have become a focus of investigation (e.g. (Zhang, Schuff et al. 2007; Damoiseaux, Smith et al. 2009) ).

Diffusion tensor imaging (DTI) is a powerful tool that can differentiate between normal and abnormal white matter (Alexander and Lobaugh 2007). In patients with AD, DTI has demonstrated disruption of white matter fibers in AD in cortico-cortical association fiber tracts
(Stricker, Schweinsburg et al. 2009). Recent work (Villain, Desgranges et al. 2008) has identified that disruption of the cingulum bundle is highly correlated with hippocampal atrophy, represents the source of disconnection between hippocampus and posterior cingulate cortex, and is the primary factor in posterior cingulate cortex hypometabolism, a characteristic feature of this disorder (Chetelat, Desgranges et al. 2003). White matter findings in AD align with neuropathological studies, where individuals with AD exhibit more severe oligodendroglial loss and myelin breakdown (Bartzokis, Sultzer et al. 2004), as well as axonal loss (Brun and Englund 1986) compared to matched controls. BDNF plays a role in mediating myelination (Ng, Chen et al. 2007), provides trophic support for oligodendrocytes, and influences levels of myelin basic protein (Djalali, Holtje et al. 2005), the major protein in the myelin sheath.

We conducted a study in healthy volunteers spanning the adult lifespan to assess the impact of the BDNF gene and age on neural structures and cognitive functions that are disrupted in AD. We hypothesized that the BDNF val66met polymorphism would interact with age to predict variation in: (1) cortical thickness in temporal lobe structures, (2) microstructural integrity of white matter tracts that connect to medial temporal lobe, and (3) episodic memory performance.

8.3 Methods

8.3.1 Subjects

Sixty-nine healthy volunteers (44 men and 25 women), (46 ± 18 years of age) (range 19-82 years of age) met the inclusion criteria (age between 18 and 85; right handedness) and none of the exclusion criteria (any history of a mental disorder, including dementia; current substance abuse or a history of substance dependence; positive urine toxicology, a history of head trauma with loss of consciousness, seizure, or another neurological disorder; a first degree relative with a
history of psychotic mental disorder). Ethnic distribution was 67 Caucasian and 2 Asian. All participants were assessed with the Edinburgh handedness inventory (Oldfield 1971), interviewed by a psychiatrist, and completed the DSM-IV Structured Clinical Interview for Diagnosis (First MB 1995) and the Mini Mental Status Exam (Folstein et al. 1975). They also completed a urine toxicology screen. Participants were characterized using the following instruments (Table 1): Wechsler Test for Adult Reading (WTAR); Hollingshead index (Hollingshead 1975); Clinical Illness Rating Scale for Geriatrics (CIRS-G) (Miller, Paradis et al. 1992); weight, height, and blood pressure. The study was approved by the Review and Ethics Board of the Centre for Addiction and Mental Health (Toronto, Canada) and all participants provided informed, written consent.

8.3.2 Neuroimaging

8.3.2.1 Image Acquisition

High resolution magnetic resonance images were acquired as part of a multi-modal imaging protocol using an eight-channel head coil on a 1.5 Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, WI), which permits maximum gradient amplitudes of 40 mT/m. Axial inversion recovery prepared spoiled gradient recall images were acquired: (echo time (TE): 5.3, repetition time (TR): 12.3, time to inversion (TI): 300, flip angle 20, number of excitations (NEX) = 1 (124 contiguous images, 1.5 mm thickness). For DTI, a single shot spin echo planar sequence was used with diffusion gradients applied in 23 non-collinear directions and b=1000 s/mm². Two b=0 images were obtained. Fifty-seven slices were acquired for whole brain coverage oblique to the axial plane. Slice thickness was 2.6 mm, and voxels were isotropic. The field of view was 330 mm and the size of the acquisition matrix was 128 x 128 mm, with TE =
85.5 ms, TR = 15,000 ms. The entire sequence was repeated three times to improve signal to noise ratio.

8.3.2.2 Image Processing

8.3.2.2.1 Cortical Thickness Mapping

All MRIs were submitted to the CIVET pipeline (version 1.1.9, http://wiki.bic.mcgill.ca/index.php/CIVET; ). T1 images were registered to the ICBM152 nonlinear sixth generation template with a 9-parameter linear transformation, inhomogeneity corrected (Sled, Zijdenbos et al. 1998) and tissue classified (Zijdenbos, Forghani et al. 2002; Tohka, Zijdenbos et al. 2004). Deformable models were then used to create white and gray matter surfaces for each hemisphere separately, resulting in 4 surfaces of 40,962 vertices each (MacDonald, Kabani et al. 2000; Kim, Singh et al. 2005). From these surfaces, the t-link metric was derived for determining the distance between the white and gray surfaces (Lerch and Evans 2005). The thickness data were subsequently blurred using a 20-mm surface based diffusion blurring kernel in preparation for statistical analyses. Unnormalized, native-space thickness values were used in all analyses owing to the poor correlation between cortical thickness and brain volume. Normalizing for global brain size when it has little pertinence to cortical thickness risks introducing noise and reducing power (Ad-Dab’bagh, Singh et al. 2005) (Figure 1).

8.3.2.2.2 DTI Image Analysis, Whole Brain Tractography, and Clustering Segmentation

The three repetitions were co-registered to the first b=0 image in the first repetition using FSL (v. 4.0) www.fmrib.ox.ac.uk to produce a new averaged image, with gradients re-oriented using a weighted least squares approach. Registration corrects eddy current distortions and subject
motion, important artifacts that can affect the data, and averaging improves the signal to noise ratio. A brain mask was then generated. Points were seeded throughout each voxel of the brain. Whole-brain tractography was performed with a deterministic (streamline) approach (Runge-Kutta order two tractography with a fixed step size of 0.5 mm). More detailed descriptions of our tractography approach and our clustering segmentation algorithm have been recently published (O'Donnell, Kubicki et al. 2006; Voineskos, O'Donnell et al. 2009), and are summarized here. Threshold parameters for tractography were based on the linear anisotropy measure $C_L\$, which provides specific advantages over thresholding using $\text{FA}$ (Ennis and Kindlmann 2006)(Westin, Maier et al. 2002). The parameters chosen for this study were: $T_{\text{seed}} = 0.3$, $T_{\text{stop}} = 0.15$, and $T_{\text{length}} = 20$ (in mm). Tractography and creation of white matter fiber tracts were performed using 3D Slicer (www.slicer.org) and Matlab 7.0 (www.mathworks.com).

A pairwise fiber trajectory similarity was quantified and the directed distances between fibers ‘A’ and ‘B’ were converted to a symmetric pairwise fiber distance. A spectral embedding of fibers was then created based on the eigenvectors of the fiber affinity matrix, and shape similarity information for each fiber was calculated, using a k-way normalized cuts clustering algorithm (O'Donnell, Kubicki et al. 2006).

Once the whole brain cluster model was produced, a trained operator (ANV) combined clusters corresponding to a given fiber tract. Left and right association fiber tracts connecting to the temporal lobe were selected (Voineskos, O'Donnell et al. 2009): uncinate fasciculus, inferior occipitofrontal fasciculus, cingulum bundle, inferior longitudinal fasciculus, and arcuate fasciculus. The genu of the corpus callosum was selected for comparative purposes, since this structure is highly susceptible to age-related FA change in healthy aging populations (Voineskos, Rajji et al. 2010), and is not preferentially disrupted in AD (Head, Buckner et al. 2004) (Figure

174
2). As reported elsewhere (Voineskos, O'Donnell et al. 2009), excellent spatial and quantitative reliability using this clustering method (i.e., both voxel overlap and scalar measures of the tensor showed high agreement) has been demonstrated. For each white matter tract, Matlab (v. 7.0) was used to calculate a mean FA (Basser and Pierpaoli 1996) value along the selected tract.

8.3.3 Genetics

The BDNF val66met polymorphism (rs6265) was genotyped in each subject. This polymorphism lies in the 5’ region of the BDNF gene and affects intracellular packaging and secretion of BDNF(Egan, Kojima et al. 2003). Genotyping of this polymorphism was performed using a standard ABI (Applied Biosystems Inc.) 5’ nuclease Taqman assay-on-demand protocol in a total volume of 10 µL. Postamplification products were analyzed on the ABI 7500 Sequence Detection System (ABI, Foster City, California, USA) and genotype calls were performed manually. Results were verified independently by two laboratory personnel blind to demographic and phenotypic information. Quality control analysis was performed on 10 percent of the sample. All participants also underwent genotyping at the Apolipoprotein E (APOE) gene to determine APOE4 allele status.

8.3.4 Cognitive Measures

Sixty-five of the study participants completed cognitive testing that included the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Verbal episodic memory performance and visuospatial episodic memory performance were measured using the list recall and figure recall tests of the RBANS respectively.
8.3.5 Statistical Analysis

Three separate analyses were performed according to the general linear model in order to examine the effects of the BDNF gene and age on (1) cortical thickness, (2) white matter tract integrity and (3) cognitive performance. Two genotypic groups were created: met allele carriers, and val/val individuals. Genotypic group served as the between group factor in each model. (1) The first model examined an ANCOVA relating BDNF genotype and age to cortical thickness. Statistical thresholds were determined by application of a 5 percent false discovery rate (FDR) correction, where \( q < 0.05 \) was considered significant (Genovese, Lazar et al. 2002).

(2) The second model used a repeated measures ANCOVA with BDNF genotype as the between-group factor, and age as the covariate, to examine white matter tract FA (all tract FA values were within-group measures) of association fiber tracts and of the genu of the corpus callosum.

(3) For episodic memory performance, a repeated measures ANCOVA was conducted with BDNF genotype as the between-group factor and age as the covariate. Scores on the list recall and figure recall tests of the RBANS were the two within-group measures.

8.4 Results

The two genotypic groups did not differ in terms of age, gender, IQ, years of education, ethnicity, socioeconomic status, systolic blood pressure, diastolic blood pressure, or body mass index (all \( p > 0.10 \) -- Table 1). Of the 69 healthy volunteers there were 28 met allele carriers (including 5 met homozygotes), and 41 individuals who were val/val homozygotes. A one hundred percent genotyping success rate was achieved. The sample did not deviate from Hardy-Weinberg equilibrium (\( X^2 = 0.487, 1 \) degree of freedom, 2 tailed \( p = 0.485 \)). No individual had two APOE4 alleles, and 12 individuals were carriers of one APOE4 allele.
A BDNF genotype by age interaction predicted cortical thickness at several regions in the temporal lobe, prominently at entorhinal cortex \((F_{1,65}) = 12.5, q = 0.03\), and inferior temporal gyrus \((F_{1,65}) = 13.9, q = 0.016\), following false discovery rate correction (Figure 3a). Cortical thickness at middle temporal gyrus and superior temporal gyrus, along with parieto-occipital sulcus also met the FDR threshold for the BDNF genotype by age interaction (Supplementary Table). No main effects of BDNF genotype, but significant effects of age were present (all \(q <0.05\), except for right inferior temporal gyrus).

For white matter tract integrity a significant BDNF genotype by age interaction was present \((F_{1,65}) = 14.0, p < 0.001\). Main effects of genotype and age were \((F_{1,65}) = 9.0, p = 0.004\) and \(F(1,65) = 53.7, p < 0.001\). Since the overall model for white matter integrity was statistically significant, follow-up univariate ANCOVAs were used with a Bonferroni corrected threshold \(p\) value for 11 comparisons, at \(p = 0.0045\). The interaction was most notable at the left cingulum bundle \(F(1,65) = 10.8, p = 0.002\) (Figure 3b) and left inferior longitudinal fasciculus \(F(1,65) = 10.2, p = 0.002\), white matter tracts connecting to the medial temporal lobe, and the left arcuate fasciculus, \(F(1,65) = 10.0, p = 0.002\), a white matter tract with tempo-parietal and tempo-frontal fibers. There was no significant interaction between BDNF genotype and the integrity of any of the other white matter tracts studied. In particular, there was no interaction for the genu of the corpus callosum, the white matter tract typically most vulnerable in healthy aging studies \((F_{1,65} = 4.0, p = 0.05)\) (Supplementary Table).

Finally, for episodic memory performance, a BDNF genotype by age interaction was also present \((F_{1,61}) = 6.2, p = 0.016\) (Figure 3c). Main effects of BDNF genotype and age were \((F_{1,61}) = 4.5, p = 0.039\), and \(F(1,61) = 18.6, p < 0.001\). Since the overall model for episodic memory was statistically significant, follow-up univariate ANCOVAs were used with a Bonferroni corrected \(p\)
value for 2 comparisons, at threshold \( p = 0.025 \), to investigate each episodic memory task separately. For visuospatial episodic memory performance, BDNF genotype by age interaction revealed \( F(1,61) = 4.7, p = 0.034 \), and for verbal episodic memory performance, \( F(1,61) = 3.2, p = 0.08 \).

Cortical thickness, white matter tract integrity and episodic memory performance results remained significant following re-analysis of the data without the two participants of Asian ethnicity, or of the 12 APOE4 carriers.

**8.5 Discussion**

We found that the BDNF val66met polymorphism interacts with age in a biologically convergent manner to predict variation in at-risk neural structures and cognitive functions of AD in healthy humans. Our findings support BDNF as a genetic susceptibility mechanism in an intermediate phenotype related to AD via its impact on: thickness of temporal lobe structures, including entorhinal cortex(Gomez-Isla, Price et al. 1996; Devanand, Pradhaban et al. 2007; Desikan, Cabral et al. 2009), white matter integrity of association fiber tracts connecting to the medial temporal lobe(Zhang, Schuff et al. 2007; Damoiseaux, Smith et al. 2009; Mielke, Kozauer et al. 2009), and episodic memory formation(deToledo-Morrell, Stoub et al. 2004; Stoub, deToledo-Morrell et al. 2006).

Multiple lines of evidence implicate BDNF in the AD process: BDNF expression is reduced in hippocampus and in entorhinal cortex in AD(Narisawa-Saito, Wakabayashi et al. 1996); neurons containing neurofibrillary tangles, the hallmark pathology of AD, do not have detectable levels of BDNF immunoreactive material, whereas neurons more intensely labeled with BDNF-specific antibodies are free of tangles(Murer, Boissiere et al. 1999); altered levels of BDNF in both serum
and CSF have been found in AD \textit{in vivo} (Peng, Wuu et al. 2005; Laske, Stransky et al. 2006), and have been associated with disease severity and episodic memory performance. Furthermore, recent data suggest potentially dramatic effects of BDNF as a therapeutic agent: hippocampal neural stem cell transplantation rescued spatial learning and memory deficits in aged triple transgenic mice, expressing pathogenic forms of amyloid precursor protein, presenilin, and tau, without altering Aβ or tau pathology (Blurton-Jones, Kitazawa et al. 2009), but rather, mediated via BDNF. In another study (Nagahara, Merrill et al. 2009), BDNF gene delivery to entorhinal cortex in amyloid transgenic mice reversed synaptic loss, improved cell signaling, and restored learning and memory without altering amyloid plaque load. Therefore, BDNF can exert substantial protective effects on crucial neuronal circuitry in AD by acting through amyloid-independent mechanisms.

Despite the wealth of evidence implicating BDNF in AD, the data examining association of the BDNF gene with AD have been mixed. Early genetic studies of the BDNF val66met demonstrated that the val/val genotype was associated with AD (Ventriglia, Bocchio Chiavetto et al. 2002). However, this finding has not been consistently replicated (Li, Rowland et al. 2005). Prospective data from the large Lothian birth cohort demonstrated that BDNF val/val individuals in late life experience a greater age-related decline in reasoning skills than met carriers (Harris, Fox et al. 2006). However, in a follow up study this finding was not replicated (Houlihan, Harris et al. 2009). Such difficulties in genetic association studies of complex disorders have been well-characterized, and a number of explanations have been put forward (de Bakker, Yelensky et al. 2005; Cardon 2006). One challenge may be that the rate-limiting step in gene identification in complex behavioural disorders can be the effect size of the risk allele on phenotypic variance (Meyer-Lindenberg and Weinberger 2006).
Imaging genetics offers an alternative strategy to conventional genetic association studies by delineating neural systems that are affected by genetic variation via the intermediate phenotype strategy (Meyer-Lindenberg and Weinberger 2006). Genotype to brain phenotype associations can be shown in carriers of risk alleles even if the carriers do not exhibit the clinical phenotype. Our findings are most robust at the level of brain structure, and least robust at the level of observable behavior (i.e. cognition), consistent with the intermediate phenotype concept. Importantly, BDNF variation is not related in our study to structures prominently affected in healthy aging, namely frontal gray matter (Head, Snyder et al. 2005) or white matter tracts that are frontally based, such as the genu of the corpus callosum (Voineskos, Rajji et al. 2010). Rather, the structures impacted by BDNF in our healthy subjects are the structures affected in the preclinical and earliest clinical stages of AD. In gray matter, medial and then lateral temporal areas are affected first, before extending to cingulate cortex and temporoparietal regions (Whitwell, Przybelski et al. 2007); in white matter, cortico-cortical association pathways, (e.g. cingulum bundle, inferior longitudinal fasciculus, arcuate fasciculus) the latest-myelinating fiber pathways in the brain, are affected earliest in AD (Stricker, Schweinsburg et al. 2009); cognitively, episodic memory performance is also affected in preclinical and in the earliest clinical stages of AD. Intermediate phenotypes in other neuropsychiatric disorders, such as schizophrenia (Meyer-Lindenberg, Nichols et al. 2006) and depression (Pezawas, Meyer-Lindenberg et al. 2005), have been previously characterized using similar approaches.

Unlike the APOE gene, where APOE4 allele carriers are at a disadvantage, even in early adult life (Filippini, MacIntosh et al. 2009), the direction of the effects of the BDNF val66met on brain structure and cognitive function found in our study differ in an age-dependent manner. Previous investigations in young healthy individuals suggest that BDNF met allele carriers demonstrate
reduced hippocampal/parahippocampal complex volumes, function, and episodic memory performance (Hariri, Goldberg et al. 2003; Pezawas, Verchinski et al. 2004). One explanation for met-allele vulnerability in early adult life is based on findings that the met-allele may fail to localize BDNF to secretory granules or synapses (Egan, Kojima et al. 2003), in the process altering activity-dependent processes of cortical development and plasticity. Our results support these findings, and add new evidence based on cortical thickness and tractography measures: young BDNF-met allele carriers are more likely to have reduced cortical thickness of medial and lateral temporal lobe structures, and reduced microstructural integrity of white matter tracts connecting to medial and lateral temporal lobe regions.

In contrast to our findings in early adult life, val/val individuals in late adult life had diminished entorhinal cortex thickness, white matter tract integrity and episodic memory performance. Here, our findings also align with previous findings, where others have shown that val/val individuals are at increased risk in later-life for poor cognitive performance (Harris, Fox et al. 2006) and AD (Ventriglia, Bocchio Chiavetto et al. 2002). The substantial literature implicating BDNF in the pathophysiology of mood disorders provides an intriguing genetic mechanism for an overlapping clinical picture with AD. First, a history of depressive mood episodes is a risk factor for subsequent AD (Ownby, Crocco et al. 2006). Second the BDNF val/66met has been associated with risk for mood disorders (Neves-Pereira, Mundo et al. 2002; Sklar, Gabriel et al. 2002), and for neuroticism (Sen, Nesse et al. 2003; Lang, Hellweg et al. 2005), though the manner in which risk is determined (i.e. met-carrier, vs. val/val) is under debate. A recent investigation highlighted the complexity of risk determined by the BDNF val66met on physiological measures of depression and anxiety (Gatt, Nemeroff et al. 2009). It is possible, therefore, that there is a lifetime ‘burden’ with a val/val genotype, whereby effects of mood vulnerability, highly
sensitive plasticity (e.g. high stress sensitivity), or reduced resilience contribute to the intermediate phenotype found in our study.

One limitation of our study is its cross-sectional design. Specifically, we are only able to conclude that val/val individuals in late life, and met allele carriers in early adult life may be at a disadvantage given the phenotypic measures employed. A longitudinal study would have allowed us to examine progression across adult life of our phenotypic measures according to BDNF genotype. However, such a study design carries its own set of challenges including technical limitations of repeated imaging measures, attrition, and cost. Despite the cross-sectional nature of our sample, our finding is unlikely to be due to a sampling bias or cohort effect, since our elderly individuals were not different from our younger individuals for IQ or levels of education. Another limitation is that we did not include the fornix of the hippocampus, a commissural white matter tract, for study in our sample, due to challenges in achieving high reliability (Wakana, Caprihan et al. 2007; Voineskos, O'Donnell et al. 2009) using streamline tractography for the fornix. Investigation of this fiber tract in relation to BDNF genotype would be useful, since the fornix is an important part of the hippocampal system, may be involved in learning and memory, and may be disrupted in AD (Mielke, Kozauer et al. 2009).

While others have investigated the effects of the BDNF gene on brain structure in healthy individuals across the adult lifespan (Sublette, Baca-Garcia et al. 2008; Kennedy, Rodrigue et al. 2009), none, to our knowledge, has investigated effects of BDNF on cortical thickness or on association fiber tracts as intermediate phenotype measures. The convergent pattern of our findings across gray matter, white matter, and cognitive performance provide a more convincing picture of the impact of the BDNF val66met on an intermediate phenotype related to AD than any one of these findings alone. Our findings suggest that the BDNF gene may be a
susceptibility mechanism for healthy vs. pathologic aging (AD), and highlight a critical alternative pathway in this neurodegenerative disorder.

### Table 8-1. Participants’ Characteristics

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<td>52 ± 9</td>
<td>48 ± 10</td>
<td>t = 1.4, p = 0.15</td>
</tr>
<tr>
<td>IQ (WTAR)</td>
<td>118 ± 9</td>
<td>119 ± 6</td>
<td>t = -0.6, p = 0.54</td>
</tr>
<tr>
<td>MMSE</td>
<td>29 ± 1</td>
<td>29 ± 1</td>
<td>t = 1.1 p = 0.30</td>
</tr>
<tr>
<td>BMI</td>
<td>25 ± 3</td>
<td>26 ± 5</td>
<td>t = -1.3, p =0.18</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>124 ± 14</td>
<td>123 ± 13</td>
<td>t = 0.2, p = 0.85</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>77 ± 6</td>
<td>74 ± 9</td>
<td>t = 1.7, p = 0.10</td>
</tr>
<tr>
<td>CIRS-G (ratio score)</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>t = 0.3, p =0.78</td>
</tr>
<tr>
<td>Number</td>
<td>9 F, 19 M</td>
<td>16 F, 25 M</td>
<td>X² = 0.34, p = 0.56</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
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</tr>
</tbody>
</table>
df - degrees of freedom

WTAR – Wechsler Test of Adult Reading

MMSE – Mini-mental state examination

BMI – Body Mass Index

BP – Blood Pressure

CIRS-G – Cumulative Illness Rating Scale – Geriatrics

*Four factors are education, occupation, sex, and marital status
Figure 8-1. Processing pipeline for extraction of cortical thickness measures used to derive anatomical information from T1-weighted MRIs.

Each image is aligned to stereotaxic space, corrected for non-uniformity artefacts, tissue classified, masked, and inner and outer cortical surfaces are extracted.
Figure 8-2. Models of white matter tracts measured.

From left to right (top panel): left cingulum bundle, left inferior longitudinal fasciculus, left arcuate fasciculus; left to right (bottom panel): left uncinate fasciculus, left inferior longitudinal fasciculus, genu of corpus callosum (in red)
Figure 8-3. Significant interaction of BDNFval66met variant with age in a similar pattern to predict cortical thickness, microstructural integrity of white matter tracts, and episodic memory performance.
Table 8-2. Supplementary Data.

<table>
<thead>
<tr>
<th>Cortex</th>
<th>BDNF by Age Interaction&lt;sup&gt;a&lt;/sup&gt;</th>
<th>x&lt;sup&gt;b&lt;/sup&gt;</th>
<th>y</th>
<th>z</th>
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</thead>
<tbody>
<tr>
<td>L EC</td>
<td>$F = 11.2, q = 0.030$</td>
<td>-26</td>
<td>-22</td>
<td>-13</td>
</tr>
<tr>
<td>L ITG</td>
<td>$F = 14.0, q = 0.016$</td>
<td>-50</td>
<td>-32</td>
<td>-27</td>
</tr>
<tr>
<td>R ITG</td>
<td>$F = 9.7, q = 0.045$</td>
<td>32</td>
<td>-5</td>
<td>-43</td>
</tr>
<tr>
<td>L MTG</td>
<td>$F = 13.2, q = 0.018$</td>
<td>-65</td>
<td>-43</td>
<td>-4</td>
</tr>
<tr>
<td>L STG</td>
<td>$F = 10.3, q = 0.038$</td>
<td>-59</td>
<td>-38</td>
<td>19</td>
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<tr>
<td>L OPS</td>
<td>$F = 10.3, q = 0.038$</td>
<td>-15</td>
<td>-60</td>
<td>11</td>
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<tr>
<td>R OPS</td>
<td>$F = 13.0, q = 0.019$</td>
<td>20</td>
<td>-59</td>
<td>-17</td>
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</tbody>
</table>

<sup>a</sup> $q$ values less than 0.05 survived 5% false discovery rate correction; <sup>b</sup>MNI space coordinates

L = left, R = right, EC = entorhinal cortex, ITG = inferior temporal gyrus, MTG = middle temporal gyrus, STG = superior temporal gyrus, OPS = occipitoparietal sulcus

<table>
<thead>
<tr>
<th>Tract&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BDNF by Age Interaction</th>
<th>Main Effect BDNF</th>
<th>Main Effect Age</th>
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<tr>
<td></td>
<td>$F(1,65)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L CB</td>
<td>$F = 10.8, p = 0.002$</td>
<td>$F = 8.6, p = 0.005$</td>
<td>$F = 28.4, p &lt; 0.001$</td>
</tr>
<tr>
<td>R CB</td>
<td>$F = 5.4, p = 0.02$</td>
<td>$F = 4.7, p = 0.03$</td>
<td>$F = 6.2, p = 0.02$</td>
</tr>
<tr>
<td>L ILF</td>
<td>$F = 10.2, p = 0.002$</td>
<td>$F = 6.0, p = 0.02$</td>
<td>$F = 30.2, p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>BDNF by Age Interaction F (1,61)</td>
<td>Main Effect BDNF</td>
<td>Main Effect Age</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Visuo. Mem.</td>
<td>F = 4.7, p = 0.03</td>
<td>F = 3.5, p = 0.07</td>
<td>F = 14.9, p &lt; 0.001</td>
</tr>
<tr>
<td>Verb. Mem.</td>
<td>F = 3.2, p = 0.08</td>
<td>F = 2.05, p = 0.16</td>
<td>F = 8.3, p = 0.005</td>
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</tbody>
</table>


a Bonferroni corrected p value significance threshold = 0.0045

b Bonferroni corrected p value significance threshold = 0.025
Chapter 9

9 Oligodendrocyte Genes, White Matter Tract Integrity, and Cognition in Schizophrenia: An Imaging-Genetics Study

9.1 Abstract

Introduction: Oligodendrocyte and myelin related (OMR) genes have been implicated in schizophrenia. At the same time, diffusion tensor imaging (DTI) studies have implicated white matter tract disruption in schizophrenia. No study has yet related these molecular genetic and neuroimaging findings. The objective was to determine whether (i) white matter tract integrity is related to cognitive performance, (ii) OMR gene variants are related to white matter tract integrity and (iii) whether OMR gene variants are related to cognitive performance.

Methods: 48 schizophrenia patients were matched to 48 controls on measures of age, gender, handedness, and ethnicity. All participants completed DTI, genetics, and cognitive protocols. Participants were recruited at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada, via referrals, study registries, and advertisements. Using the multivariate partial least squares (PLS) approach, singular value decomposition (SVD) was performed to discover latent variables that might relate DTI measures to cognitive performance, OMR gene variants to DTI measures, and OMR gene variants to cognitive performance.

Results: Significant latent variables (LVs) were found across several comparisons using PLS: (i) white matter tract integrity predicted cognitive performance -- schizophrenia patients (SV=2.0, cross-block covariance (CC) = 63.4% p < 0.001) and controls (SV=1.5, CC = 45.6% p = 0.01), (ii) OMR gene variants influenced DTI white matter measures – schizophrenia patients...
OMR gene variants influenced cognitive performance -- schizophrenia patients (SV=1.2, CC = 33.8 p = 0.03), and controls (SV=1.1, CC = 37.4 p = 0.09).

Conclusions: Overall, our findings suggest that disruption of the oligodendrocyte/white matter pathway may contribute to impaired cognitive performance in schizophrenia, and provide support for the imaging-genetics approach in disease mechanism discovery in neuropsychiatric disorders.

9.2 Introduction

Disruption of the oligodendrocyte/myelin/white matter pathway has emerged as a key theory of schizophrenia pathophysiology over the past decade (Dwork, Mancevski et al. 2007; Haroutunian and Davis 2007; Voineskos 2009). Converging evidence supporting this theory has been generated from several lines of neuroscientific enquiry, including postmortem, genetic association (Hakak, Walker et al. 2001; Tkachev, Mimmack et al. 2003; Peirce, Bray et al. 2006; Voineskos, de Luca et al. 2008), animal models (Lappe-Siefke, Goebbels et al. 2003; Segal, Koschnick et al. 2007), and electron microscopy (Uranova, Orlovskaya et al. 2001) studies. These findings are neuroanatomically congruent with diffusion tensor imaging (DTI) studies of schizophrenia that implicate white matter tracts (Friedman, Tang et al. 2008; McIntosh, Maniega et al. 2008; Voineskos, Lobaugh et al. 2010) connecting the same cortical regions where oligodendrocyte and myelin-related (OMR) genes are downregulated, and oligodendrocyte number is reduced (Haroutunian and Davis 2007). Despite these compelling findings, no
published report, to our knowledge, has examined the relationship of OMR gene variants with DTI measures of white matter in the same individuals.

DTI is based on water diffusion where decreased diffusion anisotropy, within white matter tracts, may be due to fewer axons, smaller diameter of axons, loss of coherence of axonal membranes, or changes in myelination (Beaulieu 2002). DTI tractography studies demonstrate disruption of fronto-temporal and interhemispheric white matter tracts in schizophrenia (Voineskos, Lobaugh et al. 2010), indexed via fractional anisotropy (FA), a metric reflecting axonal membrane and myelin integrity (Beaulieu 2002). Such disruption may be clinically important in its relation to impaired cognitive performance in schizophrenia. Emerging evidence, primarily from DTI studies of healthy (Catani, Allin et al. 2007), or healthy aging (Voineskos, Rajji et al. 2010) individuals, suggests that DTI metrics, particularly FA, predict cognitive performance. Although some correlations between white matter integrity and cognition have been reported in schizophrenia, the relationship of white matter tract integrity to cognitive performance is not well understood.

OMR genes influence the microstructural components of white matter tissue that form the main barriers to water diffusion in the brain as measured with DTI. Therefore, it stands to reason that OMR gene risk variants may predict reduced white matter integrity, and subsequently, impaired cognitive performance, processes highly relevant to schizophrenia. OMR genes are predominantly expressed in oligodendrocytes, and are directly involved in myelination (initiation, deposition, compaction, and maintenance) (Davis, Stewart et al. 2003), axonal support (McTigue and Tripathi 2008), mediate axo-glial interactions (Pernet, Joly et al. 2008), and provide trophic support for oligodendrocytes (Segal, Koschnick et al. 2007). OMR gene variation could lead to altered myelin protein structure or function, or disrupt adhesion of myelin.
to axons, and in the process altering white matter tract integrity, which in turn would disrupt transmission of such neural signals and impair cognitive performance.

In this study, we examined relationships among gene variants, white matter tract integrity, and cognitive performance in a sample of schizophrenia patients and matched controls. We examined OMR genes that have strong a priori evidence for a role in schizophrenia from postmortem, genetic association, and animal model data: oligodendrocyte transcription factor-2 (OLIG2); 2’,3’ cyclic nucleotide 3’-phosphodiesterase (CNP), quaking (QKI), and myelin associated glycoprotein (MAG). We also included gene variants from the Neuregulin1-ErbB4 gene system, that have been previously associated with white matter integrity(McIntosh, Moorhead et al. 2008; Konrad, Vucurevic et al. 2009). The Neuregulin1-ErbB4 system has strong evidence for a role in schizophrenia(Harrison and Law 2006; Law, Lipska et al. 2006; Law, Kleinman et al. 2007), and disruption of these genes can alter myelination, oligodendrocyte morphology and function(Roy, Murtie et al. 2007). In order to assess relationships among gene variants, white matter tract integrity, and cognitive performance we used the partial least squares (PLS) multivariate approach that is well suited to combining such data, and holds specific advantages over conventional univariate approaches (McIntosh and Lobaugh 2004). Our main hypotheses were: (i) white matter tract integrity would demonstrate a significant relationship with cognitive performance, and such a relationship would be particularly strong in schizophrenia patients; (ii) OMR and NRG1-ErbB4 gene risk variants would influence white matter tract integrity -- again, this relationship would be particularly strong in schizophrenia patients; and (iii) gene variants that influence white matter tract integrity would also influence cognitive performance. Consistent with other imaging-genetics studies, we anticipated that gene effects would be most prominent in relation to neuroimaging phenotypes, rather than in relation to behavioral measures.
9.3 Methods

9.3.1 Study Participants

Participants were recruited at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada, via referrals, study registries, and advertisements. All participants were between the age of 20-62 years, were administered the Structured Clinical Interview for DSM-IV Disorders (First MB 1995), and were interviewed by a psychiatrist to enhance diagnostic accuracy. The Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein et al. 1987) was administered to further characterize illness symptoms. Comorbid illness burden was measured by administration of the Clinical Information Rating Scale for Geriatrics (CIRS-G) (Miller, Paradis et al. 1992). The Mini Mental Status Exam was also performed to screen for dementia (Folstein, Folstein et al. 1975). Medication histories were recorded via self-report, and verified when necessary from the patient’s treating psychiatrist and chart review. Patients or controls with current substance abuse or any history of substance dependence were excluded, and urine toxicology screens were performed on all subjects. Individuals with previous head trauma with loss of consciousness, or neurological disorders were also excluded. A history of a primary psychotic disorder in first-degree relatives was also an exclusion criterion for controls. Criteria used to match controls with patients were: age within 5 years, gender, handedness (Edinburgh Handedness Inventory) (Oldfield 1971), and ethnicity. After complete description of the study to the subjects, written, informed consent was obtained. The study was approved by the Centre for Addiction and Mental Health Research Ethics Board.
9.3.2 Cognitive Testing

Participants underwent a battery of well-established cognitive tests that was administered over approximately one and one-half hours. This battery included tasks that measure a wide range of cognitive functions, all of which have shown impairment in schizophrenia (Snitz, Macdonald et al. 2006): executive function, working memory, attention, verbal fluency, verbal memory, visual memory, set-shifting, response inhibition, mental flexibility, spatial ability, and sensorimotor function.

9.3.3 Neuroimaging

9.3.3.1 DTI Acquisition

Images were acquired using an eight-channel head coil on a 1.5 Tesla GE Echospeed system (General Electric Medical Systems, Milwaukee, WI), which permits maximum gradient amplitudes of 40 mT/m. A single shot spin echo planar sequence was used with diffusion gradients applied in 23 non-collinear directions and b=1000 s/mm². Two b=0 images were obtained. Fifty-seven slices were acquired for whole brain coverage oblique to the axial plane. Slice thickness was 2.6 mm, and voxels were isotropic. The field of view was 330 mm and the size of the acquisition matrix was 128 x 128 mm, with TE = 85.5 ms, TR = 15,000 ms. The entire sequence was repeated three times to improve signal to noise ratio.

9.3.3.2 Image Analysis and Tractography

The three repetitions were co-registered to the first b=0 image in the first repetition using FSL (v. 4.0) [www.fmrib.ox.ac.uk](http://www.fmrib.ox.ac.uk) to produce a new averaged image, with gradients re-oriented according to the registration transformation. A final diffusion tensor was then estimated based on all 75 aligned volumes using a weighted least squares approach. Registration corrects eddy current distortions and subject motion, important artifacts that can affect the data, and averaging
improves the signal to noise ratio. A brain ‘mask’ was then generated. Points were seeded throughout each voxel of the brain. Whole-brain tractography was performed with a deterministic (streamline) approach (Runge-Kutta order two tractography with a fixed step size of 0.5 mm). More detailed descriptions of our tractography approach and our clustering segmentation algorithm have been recently published(O'Donnell, Kubicki et al. 2006; Voineskos, O'Donnell et al. 2009), and thus are summarized here. Threshold parameters for tractography were based on the linear anisotropy measure $C_L$, where $C_L = (\lambda_1 - \lambda_2)/\lambda_1$ and $\lambda_1$ and $\lambda_2$, the two largest eigenvalues of the diffusion tensor sorted in descending order. Thresholds were based on the $C_L$ rather than on FA, because FA can be relatively high in regions of planar anisotropy which may indicate tract crossings or branching (Ennis and Kindlmann 2006). Tractography and creation of white matter fiber tracts were performed using 3D Slicer (www.slicer.org) and Matlab 7.0 (www.mathworks.com).

A pairwise fiber trajectory similarity was quantified by first computing a pairwise fiber distance, and a mean closest point distance was then employed. The directed distances between fibers ‘A’ and ‘B’ were converted to a symmetric pairwise fiber distance. Each distance was then converted to an affinity measure suitable for spectral clustering via a Gaussian kernel $(W_{ij}) = e^{-d_{ij}^2/\sigma^2}$(Shi and Malik 2000). The role of $\sigma$ ($\sigma=60$ mm) is to define the size scale of the problem by setting the distance over which fibers can be considered similar. A spectral embedding of fibers was then created based on the eigenvectors of the fiber affinity matrix. We used the top 15 eigenvectors of the fiber similarity matrix to calculate the most important shape similarity information for each fiber, using a k-way normalized cuts clustering algorithm(O'Donnell, Kubicki et al. 2006).
Once the whole brain cluster model was produced, a trained operator (ANV) combined the clusters that correspond to a given fiber tract. Left and right uncinate fasciculus, inferior occipitofrontal fasciculus, cingulum bundle, inferior longitudinal fasciculus, arcuate fasciculus, and genu and splenium (parietal, temporal, occipital fibers) of the corpus callosum were selected (see illustration in (Voineskos, Lobaugh et al. 2010)). As reported elsewhere (Voineskos, O'Donnell et al. 2009), two individuals, blind to participant information, separately performed the entire clustering procedure on ten individuals with schizophrenia, and ten healthy controls and achieved excellent spatial and quantitative reliability using this clustering method (i.e., both voxel overlap and scalar measures of the tensor showed high agreement). Matlab (v. 7.0) was then used to calculate FA (Basser and Pierpaoli 1996). Presented data represents the mean values along the selected tracts.

9.3.4 Genetics

9.3.4.1 SNP Selection

OMR gene variants with strong a priori rationale were selected for study. For OMR genes, this rationale included replicated genetic associations in independent samples with schizophrenia, and replicated evidence from independent samples indicating significant downregulation of these genes in schizophrenia postmortem brain. Four genes met these selection criteria: QKI, MAG, CNP, and OLIG2. SNPs from these genes with a priori rationale were then selected, that had either demonstrated or theoretical rationale for effects on gene expression in this pathway, such as the exonic CNP SNP, rs2070106 and the 3’UTR OLIG2 SNP rs1059004, which have both been associated with schizophrenia, and associated with reduced expression of the respective gene in schizophrenia postmortem brain (Peirce, Bray et al. 2006). The expression of several OMR genes is tightly coordinated primarily by QKI (Aberg, Saetre et al. 2006). A mutation in
the 5’ untranslated region (UTR) of QKI directs its own alternative splicing, leading to the QKI-7b splice variant, which directs regulation of several OMR genes by binding to their 3’ UTR, thus providing a plausible mechanistic explanation of OMR gene downregulation in schizophrenia (Aberg, Saetre et al. 2006). QKI has been demonstrated to regulate both MAG and CNP expression. In addition, the MAG SNP rs756796 lies within or very near the putative QKI binding site, which directs alternative splicing of the MAG gene (Aberg, Saetre et al. 2006). Therefore, the SNPs selected include: the 5’ UTR SNP of QKI (rs2784865), the MAG SNP rs756596 just downstream of the MAG 3’UTR, the previously identified MAG schizophrenia risk SNPs rs720308, rs720309 and rs2301600, CNP rs2070106, and OLIG2 rs1059004 and rs9653711. In addition, SNPs from the NRG1-ErbB4 system were genotyped which have each recently shown association with white matter integrity using DTI. These include: (i) SNP8NRG243177, which leads to differential expression of a neuregulin transcript, particularly in schizophrenia patients (Law, Lipska et al. 2006) (ii) SNP8NRG221533, which gave the strongest association with schizophrenia in original reports (Stefansson, Sigurdsson et al. 2002), and (iii) ErbB4 rs839523, which was associated with elevated expression of ErbB4 splice variants in schizophrenia (Law, Kleinman et al. 2007).

9.3.4.2 Genotyping Protocol

Genotyping of SNPs was performed using a standard ABI 5’ nuclease Taqman® assay-on-demand protocol in a total volume of 10 µl. Post-amplification products were analyzed on the AB 7500 Sequence Detection System (Applied Biosystems, Foster City, USA) and genotype calls were performed manually. Results were verified independently by two laboratory personnel blind to demographic or diagnostic information. Ten percent of the sample was genotyped at each SNP for a second time for quality control analysis.
9.3.5 Statistical Analysis

9.3.5.1 Demographic and Group Differences

All SNPs were tested for Hardy-Weinberg Equilibrium. Demographic measures, such as age, IQ, and education were compared between the schizophrenia and control groups using two-tailed independent samples t-tests.

Although not a focus of this study, potential associations between genotypes and diagnostic group were tested using chi-square. For potential white matter tract differences and cognitive differences between schizophrenia patients and healthy controls, univariate ANCOVAs, with age as a covariate were used. Bonferroni correction for multiple comparisons was applied in each of these series of tests.

9.3.5.2 Multivariate Data Analysis

Multivariate analysis was performed using partial least squares (PLS), an approach that can assess a large covariance matrix in multivariate neuroimaging (McIntosh, Bookstein et al. 1996; McIntosh and Lobaugh 2004) and genetics data (Raadsma, Moser et al. 2008; Opiyo and Moriyama 2009). PLS has several advantages over conventional univariate approaches (McIntosh and Lobaugh 2004), including: (i) greater power, (ii) the capability to deal with data sets where the dependent measures within a block are highly correlated, and (iii) the capability to evaluate the reliability of the findings over and above tests of significance. The use of resampling algorithms to evaluate reliability enables a degree of certainty in the analysis that conventional parametric statistics cannot provide. Therefore, we used PLS (McIntosh, Bookstein et al. 1996; McIntosh and Lobaugh 2004) to examine the relationship among gene variants, white matter tract integrity, and cognitive performance in healthy individuals and schizophrenia patients. For analyses with genetic data, risk allele carriers were grouped together and compared to non-risk
allele carriers (based on previously published reports), except for the ErbB4 rs839523 variant where G allele homozygotes have been previously identified as the risk genotype.

The first PLS analysis examined the correlations between white matter tract integrity (for all 12 white matter tracts) and cognitive performance (for all 20 cognitive tests). A second PLS analysis examined correlations between gene variants (reduced to nine gene variants rather than 11, since the two OLIG2 variants were in near-complete linkage disequilibrium (LD), and two of the MAG variants were in complete LD) and white matter tract integrity (for all 12 white matter tracts). A third analysis was performed on correlations between gene variants and cognitive performance. Finally, relationships among gene variants, white matter tract integrity and cognitive performance were examined simultaneously. For each of these analyses, the two diagnostic groups were analyzed together to characterize similarities and differences in correlation patterns, and then each group was examined separately to further characterize any differences. In each analysis, age and medication exposure were residualized from brain measures and cognitive performance scores.

For each PLS analysis, correlation matrices were constructed by stacking the between-subject correlations of two blocks of data (e.g., allelic variants with DTI measures, DTI measures with cognitive performance, etc). The correlation matrices were constructed separately for each diagnostic category. The matrices were analyzed with singular value decomposition (SVD) to produce mutually orthogonal latent variables (LVs), each comprised of a singular ‘independent variable image’ (e.g. in the first analysis consisting of DTI measures that reliably contributed to the latent variable) and a singular dependent variable image (e.g. in the first analysis a composite of cognitive test scores). Each LV also has a singular value (SV), which is the covariance between the independent and dependent variable image. The cross-block covariance (CC) is the
of percent of total covariance explained by the LV between the two data matrices. In the first analysis, the weights within the ‘independent variable image’ are the linear combinations of FA of those white matter tracts that covaried with the dependent cognitive performance measures. In the second analysis, the weights within the ‘independent variable image’ are the linear combinations of those allelic variants that covary with the DTI dependent variable measures. For independent measures, e.g. in the case of allelic variation, the weightings reflect the contribution of individual SNPs to the latent variable. In the third analysis, weighted linear combinations of allelic variation that covary with cognitive performance were shown. Finally, weighted combinations of allelic variation that covaried with white matter tract integrity and cognitive performance were shown.

The significance of the singular value (i.e. whether the LV accounts for an amount of covariance that is unlikely to have arisen by chance) is determined by permutation sampling that involves randomly reassigning participants across groups. Here we used 1000 permutations. The stability of these results is then determined by the bootstrap resampling (done 500 times), which involves sampling the dataset with replacements to derive estimates of standard errors of the LV. For instance, in the second analysis, reliability of contribution of gene variants at ratio of weight to standard error (SE) greater than 2.0, corresponds to approximately 95% confidence limits, and gene variants with a ratio of weight to SE greater than 3.0 corresponds to approximately 99% confidence limits. Thus, when gene variants were examined in relation to DTI phenotypes and cognitive scores, the bootstrap ratio reflects the consistency with which a gene variant is manifested across subjects. When DTI measures were examined in relation to cognitive scores, the bootstrap ratio reflects the consistency with which the integrity of a white matter tract is manifested across subjects.
9.3.6 Bioinformatic Analysis – in silico SNP function prediction

In order to enhance the understanding of the biological meaningfulness of the genetic associations, we used in silico methods to predict potential function of the SNPs investigated in this study. Depending on their location, SNPs were assessed for alteration in transcription factor binding using MatInspector (Genomatix; promoter and intron 1). Presence of splicing enhancers, repressors or intronic regulatory elements (intronic and exonic, synonymous and nonsynonymous SNPs) were determined using F-SNP (http://compbio.cs/queensu.ca/F-SNP) and Human Splicing Finder (http://www.umd.be/HSF). The F-SNP also includes prediction for the possible damaging effect of the amino acid change using PolyPhen/ SIFT etc. We also assessed if exonic SNPs leading to synonymous amino acid substitution causes codon usage bias, i.e. the codon changes from a frequently used to a rarely used codon. In addition, exonic and 3’UTR SNPs were examined with Centroidfold predictive software to determine their effects on the structure of RNA (Hamada, Kiryu et al. 2009), and 3’UTR SNPs were also assessed for alteration in microRNA binding sites (http://www.mirbase.org/search.shtml).

9.4 Results

All SNPs were genotyped at a 100 percent success rate and were in Hardy Weinberg equilibrium (Supplementary Data). Demographic measures, along with clinical characterization of the study participants are shown in Table 1. No SNP was significantly associated with schizophrenia following multiple comparison correction. For DTI measures, schizophrenia patients had lower FA at the left uncinate fasciculus.
(F₁,₉₃ = 10.6, p = 0.002). No other white matter tracts were different between groups at the Bonferroni corrected threshold of 12 comparisons (since 12 tracts were measured) of p = 0.0042. Nearly all the cognitive tests demonstrated significant differences between schizophrenia patients and healthy controls (Supplementary Data).

For DTI-cognition relationships, when all individuals were examined together we found a robust pattern demonstrating that white matter tract FA influences cognitive performance as demonstrated by a significant LV (SV = 2.4, CC = 51.1 %, p <0.001) (Figure 1A). Upon examination by diagnostic group, in healthy controls, left and right IFOF, along with right UF and ILF, and CC5 FA predicted cognitive performance in tasks of working memory, visuospatial ability, visual attention, and motor speed (SV = 1.5, CC = 45.6%, p = 0.01) (Figure 1B). In schizophrenia patients, we found a robust pattern relating white matter tract integrity to cognition (SV = 2.0, CC = 63.4%, p < 0.001) where a distributed network of white matter tracts collectively influenced cognitive tests that measured visual attention, processing speed, visuospatial ability, and language/executive function (Figure 1B).

PLS analysis for gene variants with DTI measures yielded a significant LV (SV = 2.1, CC = 57.5 %, p < 0.001) (Figure 2A). Gene variants reliably associated with this LV were MAG rs756796, CNP rs2070106, OLIG2 rs1059004, and SNP8NRG243177. White matter integrity of all tracts was associated with these gene variants, except for the left and right uncinate fasciculus in healthy controls. When this analysis was performed separately for each diagnostic group, significant latent variables were found for the control group (SV = 1.5, CC= 67.7%, p = 0.01) (Figure 2B), and for the schizophrenia group (SV = 1.8, CC = 74.1%, p < 0.001) (Figure 2B). For controls, MAG rs720309 and rs756796, and OLIG2 rs1059004, reliably contributed to this LV. In schizophrenia patients, MAG rs756796 and SNP8NRG243177 reliably contributed to this
In schizophrenia patients, these SNPs were correlated with white matter integrity across all white matter tracts, particularly at the left uncinate fasciculus.

PLS analysis relating gene variants to cognitive performance for all individuals examined together demonstrated a significant LV (SV = 1.5, CC = 28.1%, p = 0.01) (Figure 3A). MAG rs2301600 and rs720309, and SNP8NRG221533 and SNP8NRG243177 gene variants were reliably associated with visuomotor speed and attention. When the groups were examined separately, a significant LV was found in schizophrenia patients only, although a trend significant LV was found in healthy controls. In healthy controls, the relationship between gene variants and cognition (SV = 1.1, CC=37.4%, p = 0.09) (Figure 3B) was reliably predicted by OLIG2 rs1059004 and ErbB4 rs839523. This LV revealed that in healthy individuals, these gene variants were possibly related to most of the same cognitive tasks that were related to white matter integrity in the DTI-cognition analysis in healthy individuals. These included working memory, visuomotor speed and attention, and visuospatial function. In schizophrenia patients, a significant LV (SV = 1.2, CC = 33.8 %, p = 0.03) (Figure 3B) related MAG rs2301600 and rs720309, and SNP8NRG221533 and SNP8NRG243177 gene variants to cognitive performance on visuomotor dexterity, visuospatial function, processing speed, and language/executive function. These tasks were similar to those predicted by white matter integrity in the DTI-cognition analysis.

Finally, the combined PLS analysis among gene variants, white matter tract integrity and cognitive performance, showed that MAG rs720309 and rs756796, CNP rs2070106, OLIG2 rs1059004, and SNP8NRG243177 were correlated primarily with several white matter tract integrity measures in healthy controls and schizophrenia patients (SV = 2.3, CC = 34.2%, p < 0.001) (Figure 4A). When analyzed separately, MAG rs2301600, rs720309, and rs756796, and
OLIG2 rs1059004 predicted correlations with a wide range of integrity of white matter tracts along with tests of visuospatial ability, sensorimotor function, and visuomotor attention (SV = 1.8, CC = 46.9%, p < 0.001) (Figure 4B). In schizophrenia patients, MAG rs756796, OLIG2 rs1059004, SNP8NRG243177, and SNP8NRG221533 predicted correlations with integrity of a wide range of white matter tracts along with visuospatial ability (SV = 1.9, CC = 42.1%, p = 0.01) (Figure 4B).

Where a first latent variable was both significant and produced reliable independent and dependent variable images, second latent variables were also generated. However, for each analysis only one LV was significant.

The in silico predictions provided functional information on several SNPs studied. Functional information was found for both NRG1 SNPs and the ErbB4 SNP that agreed with previous descriptions and analyses (Law, Lipska et al. 2006; Law, Kleinman et al. 2007) (Supplementary Data). Other key predictions included alteration in exon splicing enhancer predicted at MAG rs2301600 (http://www.umd.be/HSF/). For the OLIG2 rs1059004, in the 3’-untranslated region, presence of the C-allele, but not the A-allele, predicted transcription factor binding for two factors, Zinc binding protein factors (ZNF219) and EGR/nerve growth factor induced protein C and related factors (WT1). The presence of the A- allele predicted a binding site for microRNA hsa-miR-323-5p and hsa-miR-608. For the synonymous exonic CNP SNP rs2070106 is associated with the alteration in free energy of the predicted local mRNA structure (100bp oligonucleotide, CentroidFold). The free energy of the A-allele carrying mRNA was -16.70 Kcal/mol compared to the G-allele carrying mRNA -26.40 Kcal/mol (Supplementary Figure).
9.5 Discussion

Our findings provide convergent evidence from genetics, neuroimaging, and cognitive data that implicate involvement of the oligodendrocyte/myelin/white matter pathway in schizophrenia.

First, we demonstrated a significant relationship between white matter tract integrity and cognitive performance in healthy individuals and in schizophrenia patients. Consistent with our hypothesis, this relationship was particularly strong in schizophrenia patients. Second we combined genetics and neuroimaging to demonstrate that OMR and NRG1-ErbB4 gene variants influence white matter tract integrity. Third, we found that cognitive measures predicted by white matter integrity were influenced by gene variants in the OMR and NRG1-ErbB4 pathway. Certain gene variants that influenced white matter integrity also influenced cognitive performance. As expected, gene effects were more evident on brain measures than on cognitive performance, highlighting the importance of the imaging-genetics approach. However, these relationships differed somewhat in schizophrenia patients compared to healthy controls, with NRG1 variants playing a role in determining white matter integrity and cognitive performance in schizophrenia patients only. Finally, *in silico* predictions provided insight into potential functional roles for certain gene variants that influenced white matter integrity and/or cognitive function, thus providing some preliminary mechanistic explanation for these associations.

By examining potentially vulnerable fronto-temporal and interhemispheric white matter tract integrity in conjunction with a cognitive battery, we found a significant latent variable that explained the majority of the cross-block covariance between white matter tract integrity and vulnerable cognitive domains in schizophrenia. This relationship was characterized by white matter tracts including the left UF, bilateral CB, bilateral IFOF, and corpus callosum (Kubicki, McCarley et al. 2007; Friedman, Tang et al. 2008), predicting cognitive performance on tasks
where schizophrenia patients are impaired, namely executive function, visual attention, visuospatial ability, language, and processing speed (Bokat and Goldberg 2003; Gur, Nimgaonkar et al. 2007). These findings provide support for the theory that white matter tract integrity is a key neurobiological substrate of cognitive performance in schizophrenia. That these findings were especially strong in schizophrenia patients highlights the possibility that a subtle perturbation in already vulnerable white matter tracts may lead to a particularly significant impact on cognitive performance. This finding may, therefore, lend weight to the theory of decreased cognitive reserve in schizophrenia patients (Dwork, Mancevski et al. 2007), also supported by our finding that in schizophrenia patients a more distributed pattern of integrity of white matter tracts was correlated with cognitive task performance compared to healthy controls.

We then demonstrated that gene variants from both the OMR and NRG1-ErbB4 gene systems were reliably correlated with white matter tract integrity. The rationale for combining genetics and imaging measures was strong since these genes influence the same microstructural components of white matter that DTI indices reflect. Of the OMR genes, MAG, CNP, and OLIG2 were associated with white matter tract integrity. These genes are downregulated in frontal and temporal cortical regions (Haroutunian and Davis 2007), connected by the white matter tracts examined in the present study, and MAG and CNP are also downregulated in white matter in postmortem schizophrenia brain (McCullumsmith, Gupta et al. 2007). The MAG gene is critical for axo-glial interactions, and is a key component of the myelin-mediated complex that inhibits axonal growth (Budel, Padukkavidana et al. 2008). The OLIG2 gene is an important transcription factor for oligodendrocytes, for their genesis, myelination (Georgieva, Moskvina et al. 2006), specification, and maturation (Nicolay, Doucette et al. 2007). Both OLIG2 rs1059004 and CNP rs2070106 have replicated associations with schizophrenia, and these variants are
associated with OLIG2 and CNP expression respectively in postmortem schizophrenia brain (Georgieva, Moskvina et al. 2006; Huang, Tang et al. 2008). Our bioinformatic predictions support functional roles for CNP rs2070106, and OLIG2 rs1059004 via effects on mRNA secondary structure, and binding to microRNA (which can lead to mRNA degradation) respectively. Thus each of these SNPs may influence mRNA (and possibly protein) quantitatively, providing a possible explanation for their effects on white matter integrity.

The relationship of gene variants with brain phenotypes was considerably stronger than on behaviour (cognitive performance). This has been a key premise of the intermediate phenotype approach (Meyer-Lindenberg and Weinberger 2006). However, our approach differed from the conventional intermediate phenotype approach, since we did not limit ourselves to the study of healthy individuals. By also examining schizophrenia patients, we demonstrated certain differences between groups in relationships for all three major results (white matter – cognition, gene variants – white matter, gene variants – cognition). The value of our decision to include schizophrenia patients was demonstrated by the relationship between NRG1 gene variants, white matter integrity, and cognitive performance in schizophrenia patients, but not in controls. These findings are supported by postmortem work demonstrating that SNP8NRG243177 influences expression of neuregulin isoforms in a more pronounced manner in schizophrenia brain compared to healthy controls (Law, Lipska et al. 2006). Our findings are also supported by reliability measures via bootstrapping in each analysis, not provided in previous imaging genetics studies of NRG1 (McIntosh, Moorhead et al. 2008; Winterer, Konrad et al. 2008) or ErbB4 (Konrad, Vucurevic et al. 2009).

An important limitation of our study is the likelihood that other gene systems not investigated in the present study, influence the oligodendrocyte/white matter pathway in schizophrenia. Genes
in the glutamatergic, oxidative stress, and autoimmune/cytokine system may also influence white matter integrity in schizophrenia. Specifically, NMDA receptors are present on oligodendrocyte processes (Salter and Fern 2005), and therefore, NMDA receptor dysfunction may lead to subtle disruption or damage of the myelin sheath in schizophrenia. Oxidative stress is particularly relevant to oligodendrocytes (French, Reid et al. 2009), since oligodendrocytes operate at the highest metabolic rate of any cell in the brain, and have high iron requirements, necessary for myelin production, and in the process evoking free radical formation and lipid peroxidation (McTigue and Tripathi 2008). Finally, oligodendrocyte disruption may also be related to autoimmune/cytokine abnormalities in schizophrenia that may occur during development or in utero (Harry, Lawler et al. 2006). Future investigations should examine the collective role of variants from gene systems related to cytokines, glutamate, and oxidative stress, and their relationship with white matter integrity in schizophrenia. In the present study, however, we conducted a more focused strategy by carefully selecting each variant based on strong a priori evidence where it was anticipated that these variants might be related to microstructural integrity of white matter. Despite careful matching, our study sample was also limited by a rather wide age-range, and some degree of ethnic heterogeneity. Although we residualized mean chlorpromazine doses from dependent variable measures, and recent data suggests relationships between white matter integrity and cognitive performance in schizophrenia patients are not influenced by medication (Perez-Iglesias, Tordesillas-Gutierrez et al.), the potentially confounding effects of medication on white matter and cognitive data in our schizophrenia group cannot be conclusively ruled out. Finally, despite growing evidence that diffusion indices are biologically relevant, these indices are ultimately based on diffusion of water and only indirectly measure properties of white matter tissue microstructure.
In summary, we have shown that white matter integrity is an important predictor of cognitive performance in schizophrenia patients, on a number of key cognitive domains. OMR and NRG1 gene variants influence measures of white matter integrity and myelination, and influence many of the same cognitive tasks predicted by white matter tract integrity. The relationship of OMR gene variants with white matter integrity provides novel data that emphasizes the biological relevance of DTI metrics (Mori and Zhang 2006). Such relevance is further emphasized by the significant relationships demonstrated between white matter tract integrity and cognitive performance. Taken together, our findings provide further evidence for the oligodendrocyte/myelin/white matter hypothesis in schizophrenia by bridging findings from genetics and neuroimaging, and lend insight into this pathway as a potential neurobiologic substrate of impaired cognitive performance in schizophrenia.
Table 9-1. Demographic and Clinical Characterization of Subjects

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Schizophrenia Patients (n=48)</th>
<th>Healthy Controls (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>aEducation</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>WTAR (IQ)</td>
<td>111</td>
<td>118</td>
</tr>
<tr>
<td>MMSE</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>bCIRS-G</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>23</td>
<td>NA</td>
</tr>
<tr>
<td>Chlorpr. equiv. (mg)</td>
<td>249</td>
<td>178</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>NA</td>
</tr>
<tr>
<td>General</td>
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<td>NA</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>41 SZ, 7 SA</td>
<td>NA</td>
</tr>
<tr>
<td>Gender</td>
<td>32M, 16F</td>
<td>32M, 16F</td>
</tr>
<tr>
<td>Handedness</td>
<td>47 R, 1 L</td>
<td>47 R, 1 L</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>42 C, 6 As</td>
<td>42 C, 6 As</td>
</tr>
<tr>
<td>Antipsychotic treatment</td>
<td>4 1º, 44 2º</td>
<td>NA</td>
</tr>
</tbody>
</table>

SZ = Schizophrenia, SA = Schizoaffective, NA = Not Applicable, N = Number

1º = first generation antipsychotic, 2º = second generation antipsychotic

M = Male, F = Female

WTAR – Wechsler Test for Adult Reading
MMSE – Minimental State Examination

CIRS-G – Clinical Information Rating Scale, Geriatrics

PANSS – Positive and Negative Syndrome Scale

Chlorpr. Equiv. – Chlorpromazine Equivalent

C = Caucasian, As = Asian (based on self-report)

\[ ^{a,b} \text{Schizophrenia Patients were compared to Healthy Controls using two-tailed independent samples t tests on Age, Education, WTAR, MMSE, and CIRS-G. Significant differences (i.e. } p<.05) \text{ were present on Education } (t_{94}=4.9, p<0.001), \text{ IQ } (t_{94}=2.7, p=0.008) \text{ and CIRS-G } (t_{94}=9.6, p<.001), \text{ and } (t_{48}=4.0, p<.001) \]
Figure 9-1. Latent Variable Demonstrating White Matter Tract Integrity Covarying with Cognitive Performance.

Left (L) and right (R) uncinate fasciculus (UF), inferior occipitofrontal fasciculus (IFOF), arcuate fasciculus (AF), inferior longitudinal fasciculus (ILF), cingulum bundle (CB), and genu (CC1) and splenium (CC5) of corpus callosum.
A. Schizophrenia patients and healthy controls analyzed together. Predictors are those white matter tracts whose integrity reliably predicts cognitive performance (i.e. standard error to salience ratio of greater than 2.0). Cognitive measures reliably correlated (95% confidence interval) with predictors are strongly coloured with asterisk. This latent variable was driven by the relationship of white matter tract integrity (all 12 tracts were reliable predictors) with letter fluency, category fluency, line orientation, and letter cancellation task performance in schizophrenia patients.
B. Schizophrenia patients and healthy controls are analyzed separately. Predictors are those white matter tracts whose integrity reliably predicts cognitive performance (i.e. standard error to salience ratio of greater than 2.0). Cognitive measures reliably correlated (95% confidence interval) with predictors are strongly coloured.
A. Schizophrenia patients and healthy controls analyzed together. Predictors are those gene variants that reliably predict white matter tract integrity (i.e. standard error to salience ratio of greater than 2.0). White matter tract integrity reliably correlated as part of this LV (95% confidence interval) are strongly coloured with asterisk.
B. Schizophrenia patients and healthy controls analyzed separately. Predictors are those gene variants that reliably predict white matter tract integrity (i.e. standard error to salience ratio of greater than 2.0). White matter tract integrity reliably correlated (95% confidence interval) with genetic predictors are strongly coloured with asterisk.
A. Schizophrenia patients and healthy controls analyzed together. Predictors are those gene variants that reliably predict cognitive task performance (i.e. standard error to salience ratio of greater than 2.0). Cognitive tasks reliably correlated (95% confidence interval) with genetic predictors are strongly coloured with asterisk.
B. Schizophrenia patients and healthy controls analyzed separately. Predictors are those gene variants that reliably predict cognitive task performance (i.e. standard error to salience ratio of greater than 2.0). Cognitive tasks reliably correlated (95% confidence interval) with genetic predictors are strongly coloured with asterisk.
A. Schizophrenia patients and healthy controls analyzed together. Predictors are those gene variants that reliably predict white matter tract integrity and cognitive task performance (i.e. standard error to salience ratio of greater than 2.0). White matter tract integrity measures and cognitive tasks reliably correlated (95% confidence interval) with genetic
predictors are strongly coloured with asterisk. This LV is driven by the relationship of gene variants with white matter tract integrity.

B. Schizophrenia patients and healthy controls analyzed separately. Predictors are those gene variants that reliably predict white matter tract integrity and cognitive task performance (i.e. standard error to salience ratio of greater than 2.0). White matter tract integrity measures and cognitive tasks reliably correlated (95% confidence interval) with genetic predictors are strongly coloured with asterisk.
Table 9-2. Supplementary Data. Genetics

Frequency table with Hardy-Weinberg equilibrium (HWE) p-values

<table>
<thead>
<tr>
<th>Chr./Mb</th>
<th>Locus</th>
<th>SNP</th>
<th>Alleles (maj./min.)</th>
<th>Allele Freq.</th>
<th>Genotype Frequency (N)</th>
<th>HWE p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>major</td>
<td>minor</td>
<td>homozygous major</td>
<td>heterozygous</td>
</tr>
<tr>
<td>17/37.38</td>
<td>CNP</td>
<td>rs2070106</td>
<td>G/A</td>
<td>.646</td>
<td>.354</td>
<td>40</td>
</tr>
<tr>
<td>2/212.25</td>
<td>ErbB4</td>
<td>rs839523</td>
<td>G/A</td>
<td>.699</td>
<td>.301</td>
<td>47</td>
</tr>
<tr>
<td>19/40.49</td>
<td>MAG</td>
<td>rs720308</td>
<td>A/G</td>
<td>.839</td>
<td>.161</td>
<td>68</td>
</tr>
<tr>
<td>19/40.49</td>
<td>MAG</td>
<td>rs720309</td>
<td>T/A</td>
<td>.839</td>
<td>.161</td>
<td>68</td>
</tr>
<tr>
<td>19/40.48</td>
<td>MAG</td>
<td>rs2301600</td>
<td>C/T</td>
<td>.765</td>
<td>.235</td>
<td>57</td>
</tr>
<tr>
<td>19/40.50</td>
<td>MAG</td>
<td>rs756796</td>
<td>G/A</td>
<td>.766</td>
<td>.234</td>
<td>58</td>
</tr>
<tr>
<td>8/31.59</td>
<td>NRG1</td>
<td>rs35753505</td>
<td>T/C</td>
<td>.677</td>
<td>.323</td>
<td>44</td>
</tr>
<tr>
<td>8/31.62</td>
<td>NRG1</td>
<td>rs6994992</td>
<td>C/T</td>
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<td>.386</td>
<td>39</td>
</tr>
<tr>
<td>21/33.32</td>
<td>OLIG2</td>
<td>rs1059004</td>
<td>C/A</td>
<td>.527</td>
<td>.473</td>
<td>30</td>
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<tr>
<td>21/33.32</td>
<td>OLIG2</td>
<td>rs9653711</td>
<td>G/C</td>
<td>.532</td>
<td>.468</td>
<td>30</td>
</tr>
<tr>
<td>6/163.75</td>
<td>QKI</td>
<td>rs2784865</td>
<td>T/A</td>
<td>.777</td>
<td>.223</td>
<td>57</td>
</tr>
</tbody>
</table>
Linkage Disequilibrium plot for MAG SNPs

Blocks defined by confidence intervals according to Gabriel et al.

$r^2$ values shown in plot.

<table>
<thead>
<tr>
<th>SNP comparison (rs#)</th>
<th>$r^2$</th>
<th>$D'$</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAG 2301600-720308</td>
<td>0.629</td>
<td>1</td>
<td>17.52</td>
</tr>
<tr>
<td>2301600-720309</td>
<td>0.629</td>
<td>1</td>
<td>17.52</td>
</tr>
<tr>
<td>720308-720309</td>
<td>1</td>
<td>1</td>
<td>29.34</td>
</tr>
<tr>
<td>2301600-756796</td>
<td>0.094</td>
<td>1</td>
<td>3.67</td>
</tr>
<tr>
<td>720309-756796</td>
<td>0.059</td>
<td>1</td>
<td>2.47</td>
</tr>
<tr>
<td>720308-756796</td>
<td>0.059</td>
<td>1</td>
<td>2.47</td>
</tr>
<tr>
<td>NRG1 35753505-6994992</td>
<td>0.483</td>
<td>0.797</td>
<td>13.88</td>
</tr>
</tbody>
</table>

Comparison of cognitive scores between schizophrenia patients and healthy controls

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Schizophrenia Patients)</td>
<td>(Healthy Controls)</td>
<td></td>
</tr>
<tr>
<td>EXIT*</td>
<td>-4.2 ± 2.6</td>
<td>-2.4 ± 1.9</td>
<td>F = 11.7, p = 0.001</td>
</tr>
<tr>
<td>Letter Number Span</td>
<td>12.7 ± 3.8</td>
<td>16.2 ± 3.3</td>
<td>F = 20.8, p &lt; 0.001</td>
</tr>
<tr>
<td>Stroop Ratio *</td>
<td>-2.4 ± 0.7</td>
<td>-2.1 ± 0.6</td>
<td>F = 6.6, p = 0.01</td>
</tr>
<tr>
<td>Letter Cancellation*</td>
<td>-78.0 ± 29.6</td>
<td>-57.1 ± 13.7</td>
<td>F = 17.5, p &lt; 0.001</td>
</tr>
<tr>
<td>Finger Taps (DH)</td>
<td>39.7 ± 11.1</td>
<td>47.4 ± 9.5</td>
<td>F = 10.3, p = 0.002</td>
</tr>
<tr>
<td>Finger Taps (NDH)</td>
<td>37.3 ± 9.4</td>
<td>42.2 ± 8.8</td>
<td>F = 4.2, p = 0.04</td>
</tr>
<tr>
<td>Grooved Pegboard (DH)*</td>
<td>-92.0 ± 27.3</td>
<td>-69.3 ± 14.6</td>
<td>F = 22.2, p &lt; 0.001</td>
</tr>
<tr>
<td>Grooved Pegboard (NDH)*</td>
<td>-107.2 ± 36.6</td>
<td>-79.4 ± 19.8</td>
<td>F = 17.7, p &lt; 0.001</td>
</tr>
<tr>
<td>RBANS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>List Learning</td>
<td>26.2 ± 5.3</td>
<td>30.3 ± 4.3</td>
<td>F = 14.4, p &lt; 0.001</td>
</tr>
<tr>
<td>Figure Copy</td>
<td>17.1 ± 3.3</td>
<td>17.6 ± 2.6</td>
<td>F = 0.2, p = 0.67</td>
</tr>
<tr>
<td>Line Orientation</td>
<td>15.3 ± 4.4</td>
<td>18.0 ± 2.0</td>
<td>F = 12.7, p = 0.001</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>18.1 ± 5.6</td>
<td>24.1 ± 5.3</td>
<td>F = 25.0, p &lt; 0.001</td>
</tr>
<tr>
<td>F + A + S</td>
<td>35.3 ±11.5</td>
<td>49.9 ± 12.8</td>
<td>F = 30.1, p &lt; 0.001</td>
</tr>
<tr>
<td>Test</td>
<td>DH Mean ± SEM</td>
<td>NDH Mean ± SEM</td>
<td>F-value</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Digit Span</td>
<td>12.3 ± 2.3</td>
<td>10.3 ± 2.4</td>
<td>14.7</td>
</tr>
<tr>
<td>Coding</td>
<td>40.8 ± 11.9</td>
<td>52.3 ± 12.4</td>
<td>18.6</td>
</tr>
<tr>
<td>List Recall</td>
<td>4.9 ± 2.6</td>
<td>7.1 ± 2.0</td>
<td>18.2</td>
</tr>
<tr>
<td>Story Memory</td>
<td>15.7 ± 4.3</td>
<td>19.5 ± 3.3</td>
<td>22.0</td>
</tr>
<tr>
<td>Story Recall</td>
<td>8.3 ± 2.8</td>
<td>10.4 ± 1.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Figure Recall</td>
<td>11.5 ± 4.5</td>
<td>13.0 ± 4.6</td>
<td>0.9</td>
</tr>
<tr>
<td>TrailsB/TrailsA *</td>
<td>-2.5 ± 1.4</td>
<td>-2.3 ± 0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Tests for which higher scores are indicative of poorer performance

DH= Dominant Hand

NDH = Nondominant Hand

F + A + S – total words starting with F, A, and S

EXIT – Executive Interview

RBANS – Repeatable Battery for the Assessment of Neuropsychological Status
Table 9-4. Supplementary Data. Bioinformatics.

Predicted/ reported functional effect of SNPs tested in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Location</th>
<th>Prediction Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAG</td>
<td>rs720308</td>
<td>Intron 7</td>
<td>No microRNA binding sites</td>
</tr>
<tr>
<td></td>
<td>rs720309</td>
<td>Intron 7</td>
<td>No microRNA binding sites</td>
</tr>
<tr>
<td></td>
<td>rs2301600</td>
<td>Synonymous</td>
<td>AGC (19.5) to AGT (12.1) triplet frequency per thousand; Exon splicing enhancer sites the C-allele –SRp40; T-allele –SC-35</td>
</tr>
<tr>
<td></td>
<td>rs756796</td>
<td>Intergenic</td>
<td>LINE-L2 element</td>
</tr>
<tr>
<td>OLIG2</td>
<td>rs1059004</td>
<td>3’UTR</td>
<td>Presence of C-allele creates a binding site for Zinc binding protein factors (ZNF219) and EGR/nerve growth factor induced protein C &amp; related factors (WT1).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Presence of the A- allele creates a binding site for microRNA hsa-miR-323-5p and hsa-miR-608</td>
</tr>
<tr>
<td></td>
<td>rs9653711</td>
<td>Intergenic</td>
<td>--</td>
</tr>
<tr>
<td>CNP</td>
<td>rs2070106</td>
<td>Synonymous</td>
<td>GGG to GGA (Glycine) No codon usage bias was seen for these codons i.e. Are used at the same frequency (16.5/1000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>The RNA structure predicted using centroidFold. The free energy of these structures were: A-allele</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP</td>
<td>Location</td>
<td>Information</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>QKI</td>
<td>rs2784865</td>
<td>Promoter</td>
<td>SINE element (mammalian-wide interspersed repeat, MIRb).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Present in the 5’UTR of a LOC100128405</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Presence of the T allele creates binding sites for Selenocysteine tRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>activating factor and Iroquois homebox transcription factors</td>
</tr>
<tr>
<td>NRG1</td>
<td>rs6994992</td>
<td>Promoter</td>
<td>C-allele presence creates a binding site to MYT1 (MyT1 zinc finger</td>
</tr>
<tr>
<td></td>
<td>(SNP8NRG24</td>
<td></td>
<td>transcription factor involved in primary neurogenesis) and Serum</td>
</tr>
<tr>
<td></td>
<td>3177)</td>
<td></td>
<td>response factor (SRF)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T-allele presence creates a binding site to HMGA family of architectural</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>transcription factors (HMGA1, HMGA2) and Homeobox transcription factor</td>
</tr>
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<td></td>
<td></td>
<td>Nanog (NANOG)</td>
</tr>
<tr>
<td></td>
<td>rs35753505</td>
<td>5’ upstream region</td>
<td>C-allele presence creates a binding site to CCAAT/enhancer binding protein</td>
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<td>(SNP8NRG22</td>
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<td>beta (CEBPB) and Muscle-specific Mt binding site (MTBF)</td>
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<td></td>
<td>1533)</td>
<td></td>
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<tr>
<td>ErbB4</td>
<td>rs839523</td>
<td>Intron 2</td>
<td>Repeat element, LTR54-LTR-ERV1; Law et al., 2007</td>
</tr>
</tbody>
</table>
Figure 9-5. Supplementary.

mRNA secondary structure prediction for CNP rs2070106 'G' allele

Gibbs free energy = -26.4 kcal/mol

mRNA secondary structure prediction for CNP rs2070106 'A' allele

Gibbs free energy = -16.7 kcal/mol

Supplementary Figure. mRNA Secondary Structure Prediction from RNA sequence using Centroidfold predictive software
Chapter 10

10 Discussion

10.1 Summary of Results

The results described in the previous chapters demonstrated several key findings: first, preliminary associations of myelin genes with schizophrenia were found; second, association of the MAG gene with brain volumetric measures was shown; third, reliability of a novel clustering segmentation approach using diffusion tensor tractography in healthy individuals and schizophrenia patients was demonstrated; fourth, using diffusion tensor tractography, white matter integrity was found to be disrupted in schizophrenia in fronto-temporal white matter tracts in younger chronic schizophrenia patients, but not in elderly community-dwelling patients compared to age-matched controls; fifth, using DTI tractography in healthy individuals across the adult lifespan, relationships among age, white matter integrity, and cognitive performance were shown using structural equation modeling. These studies suggested that in both healthy aging and schizophrenia populations, considerable heterogeneity was present. Imaging-genetics approaches can help parse out such heterogeneity. Furthermore, the effects of gene variants may be more observable at the level of brain phenotypes, rather than at the level of disease phenotypes. In the sixth study, the intermediate phenotype approach was used in a healthy aging population to demonstrate that a BDNF gene variant was associated with patterns of healthy vs. pathologic aging in the neural structures and cognitive functions characteristically impaired in Alzheimer’s disease. Finally, in the seventh study, the intermediate phenotype approach was extended to examine multiple levels of genetic-phenotypic relationships. In schizophrenia patients and healthy controls, the multivariate statistical approach, PLS, was used to successfully relate a system of gene variants that influence oligodendrocyte development, survival and
viability to a network of white matter structures and cognitive tasks. Characterization of the mechanisms of these gene variant-brain relationships \textit{in vivo} are currently being followed up by our group and collaborators \textit{in vitro} to demonstrate functional effects of these variants on protein expression. These additional investigations may help reveal mechanisms of how these gene variants influence brain structure and cognitive performance in schizophrenia.

10.2 DTI as a Tool to Measure White Matter and the Functional Ramifications of DTI Metrics

DTI metrics, such as fractional anisotropy, directly index water diffusion, and are thought to index properties of white matter tissue indirectly. The consistently demonstrated declines in FA of white matter with age suggest reductions in microstructural integrity of white matter in elderly individuals. Those white matter tracts that experience considerable age-related decline of white matter integrity connect the higher order cortical regions responsible for higher-order cognitive functions. In addition, \textit{in vivo} measurement of white matter using DTI supports post-mortem findings in healthy aging of alterations in oligodendrocyte morphology, decline in number and length of myelinated fibers (Marner, Nyengaard et al. 2003), and breakdown in the myelin sheaths (Bartzokis 2004; Bartzokis, Sultzer et al. 2004; Sullivan and Pfefferbaum 2006).

In schizophrenia, postmortem findings also align with the \textit{in vivo} findings from DTI. In the study described in Chapter 6, younger chronic schizophrenia patients had reduced FA in left uncinate fasciculus and right cingulum bundle. Glial cell loss has been demonstrated in cingulate cortex in schizophrenia, and oligodendrocyte related genes are downregulated in three regions connected by the cingulum bundle: the cingulate, frontal, and temporal cortex (Davis, Stewart et al. 2003). Downregulation of oligodendrocyte genes has also been shown in cingulate white matter (McCullumsmith, Gupta et al. 2007). Reduced oligodendrocyte number and gene expression in
frontal and temporal cortex in schizophrenia have been reported (Hakak, Walker et al. 2001; Uranova, Orlovskaya et al. 2001; Katsel, Davis et al. 2005), that align with the finding of reduced FA in the left uncinate fasciculus.

White matter integrity is also closely tied to cognitive performance in schizophrenia patients. In the schizophrenia imaging-genetics study (Chapter 9), the relationship between white matter tract integrity and cognitive performance was examined directly. Although white matter integrity was significantly correlated with cognitive performance in both groups, in schizophrenia patients the relationship was particularly impressive. The first LV explained approximately 63.4% of the cross-block covariance between white matter integrity and cognitive performance (compared to 45.6% of the cross covariance between the same variables in healthy controls). Such a finding suggests that white matter integrity is a key neurobiological correlate of cognitive performance in schizophrenia, a finding that adds to, and supports the work of previous investigations (Karlsgodt, van Erp et al. 2008; Nestor, Kubicki et al. 2008). Since the schizophrenia patients have diminished cognitive performance compared to healthy controls on a number of cognitive domains, a subtle perturbation of white matter integrity in schizophrenia may impact cognitive performance in a manner over and above the effect in healthy controls. The data also provide novel evidence that schizophrenia patients may rely on a more distributed network of white matter tracts in order to perform certain cognitive tasks, compared to healthy controls. Finally, the biological relevance of DTI metrics is further advanced by the newly discovered relationships between certain gene variants and white matter integrity. The genes examined in this study influence the same microstructural components of white matter that DTI indices have been shown to measure. Microscopic factors (i.e. axons and myelin), are major determinants of FA, as measured by DTI (Mori and Zhang 2006). The OMR genes studied play key roles in
oligodendrocyte development, maintaining the structural integrity of the myelin sheath, maintaining interactions between oligodendrocytes and axons, and enhancing oligodendrocyte survival (Davis, Stewart et al. 2003; Haroutunian and Davis 2007; Haroutunian, Katsel et al. 2007). Although it was expected that these genes might influence white matter integrity, our data indeed demonstrate this to be the case for the first time. Associations of Neuregulin1 SNPs with white matter integrity were also replicated, but are novel in that Neuregulin1 variants are associated with integrity of a wide range of white matter tracts, and are associated with cognitive performance in schizophrenia patients, but not in healthy controls. Overall, our findings provide a critical link between molecular genetics and neuroimaging work implicating oligodendrocyte genes and white matter in schizophrenia, and suggest that disruption of this pathway may be related to cognitive deficits in schizophrenia. Furthermore, our results illustrate the value of examining the role of gene variants in conjunction with at-risk neural circuitry, using the imaging-genetics approach, rather than simply examining association with the disease phenotype.

10.3 Schizophrenia: A Syndrome of Accelerated Aging?

The results of the study in Chapter 6 indicate that older controls, younger schizophrenia patients, and older schizophrenia patients had similar white matter tract integrity values. Differences that appear to be present between the younger groups are not found between the older groups. One explanation considered was that perhaps, due to selection of community dwelling elderly patients, the elderly individuals with schizophrenia studied may represent a subset of schizophrenia patients who are resilient to white matter disruption. Furthermore, many of these individuals have exceeded the expected lifespan for schizophrenia patients (Tiihonen, Lonqvist et al. 2009), thus providing another indication that this group may be particularly resilient.
It has been proposed that schizophrenia may be a syndrome of accelerated aging (Kirkpatrick, Messias et al. 2008). The term chosen by Kraepelin to describe this disorder, ‘dementia praecox’ invokes this concept, and was specifically chosen because Kraepelin viewed schizophrenia as a disorder of progressive cognitive decline. Various changes occur in schizophrenia that are similar to age-related changes. While various comparisons have been made, even at the level of physiologic changes, such as greater pulse pressure (Kirkpatrick, Messias et al. 2008), the most notable similarities are that some of the cognitive changes seen in schizophrenia, even at the time of the first episode, overlap with those cognitive abilities that are vulnerable in normal aging.

Some of the more substantial cognitive deficits in schizophrenia (processing speed, episodic memory, learning, and high-load information processing) align with some of the more substantial changes in healthy individuals in late-life. That white matter deficits are present in both schizophrenia and healthy aging may be of more than coincidental importance. One overlapping common pathway between schizophrenia and healthy aging may be disruption of oligodendrocytes and myelin as shown in postmortem tissue, and white matter as shown by DTI studies. It will be important for future work in this area to disentangle how these mechanisms may contribute to the overlapping cognitive changes in schizophrenia and healthy aging.

10.4 From Molecular Genetics to Neuroimaging: How Big is the Leap?

Genes code for proteins, and contribute to brain structure and function. However, important intermediate steps occur from DNA to protein, that can contribute to considerable phenotypic variation. For instance, epigenetic modifications, such as cytosine methylation and histone acetylation, can influence gene expression (Petronis 2004). Other processes that can influence gene expression might be related to noncoding RNAs. While genetic variation in DNA coding
regions can contribute to changes in protein conformation and function via amino acid changes, evidence increasingly points to the importance of noncoding RNA molecules in phenotypic variation, particularly in complex diseases, non-Mendelian disorders that range from cancer to neurodegenerative disorders (Mattick 2009). For instance gene variants in a gene’s untranslated regions may lead to differences in transcription factor binding or microRNA binding, that may in turn create differences in mRNA production. In turn, this process would lead to differences in amount of protein, a quantitative difference that can have important phenotypic effects (Mattick 2009). Another type of variation with important functional effects can occur at synonymous exonic variants, previously thought to simply be redundant components of the genetic code (since no amino acid change occurs). Genetic variation at such sites may lead to modifications of mRNA secondary structure, that then lead to differences in translation. Sophisticated software is now available for in silico analysis of a given nucleotide sequence, in order to help predict mRNA secondary structure based on nucleotide changes (Hamada, Kiryu et al. 2009). One such example was shown by other investigators at a synonymous exonic variant of the COMT gene, where bioinformatics tools were utilized to predict different mRNA secondary structure due to that nucleotide change (Nackley, Shabalina et al. 2006). Then, in vitro work demonstrated effects of that variant both on expression of the COMT protein, and COMT activity. Such a successful combined in silico and in vitro approach can provide a valuable mechanistic explanation of how a gene variant can exert effects on protein structure and/or function.

In order to provide added confidence in imaging-genetics findings, which are statistical associations, mechanistic investigations, as described above, are particularly useful, and provide an understanding of the pathway from DNA to RNA to protein. In the final study of this thesis, association of several gene variants was made with white matter tract integrity. In particular, the
CNP rs2070106 SNP and the OLIG2 rs1059004 SNP had been previously associated with schizophrenia, and had been associated with expression of each of those genes (respectively) in postmortem schizophrenia brain. Although there is good \textit{a priori} rationale for involvement of these genes in white matter, as described earlier in this thesis, the mechanism by which these variants might differentially influence white matter is not known. As described in Chapter 9, \textit{in silico} prediction software (using Centroidfold) demonstrated a considerable free energy difference for mRNA secondary structure for the CNP rs2070106 variant where a greater Gibbs free energy was predicted for the ‘G’ allele (more stable) compared to the ‘A’ allele at that site. Therefore, those differences in mRNA secondary structure may possibly contribute to the observed association between the rs2070106 variant and white matter tract integrity. With further work with collaborators, this software prediction will also be tested \textit{in vitro}, in order to conclusively demonstrate whether this predicted mRNA difference will translate into altered protein expression.

10.5 Limitations

Certain limitations with the approaches and findings described should be considered. First of all, positive findings require replication. This is particularly true of genetic association studies, where the “winner’s curse” has been documented (Zollner and Pritchard 2007), and positive studies can be notoriously difficult to replicate (i.e. reproducing a result at the same SNP with the same allele), an issue that may also extend to imaging-genetics studies. However, the value of imaging-genetics studies is demonstrated by the opportunity for association at a particular neuroanatomic location or structure, brain function, and/or cognitive task. As such, considerably more biological meaning is provided compared to a conventional genetic association study. Nevertheless, when a gene variant without a clearly known function is examined, confidence in
genetic associations with imaging measures can be obtained by utilizing additional neuroscientific lines of enquiry that can provide convergent evidence to support a hypothesis. Therefore, the approach employed in the final study of the thesis, using in silico bioinformatics testing, along with the in vitro work currently in progress is particularly important to add confidence to the imaging-genetics findings.

Another important limitation in our work is that all SNPs at each gene of interest were not examined. In the final study, we chose SNPs from two gene systems (myelin protein genes, and the NRG1-ErbB4 gene system) based on the literature that had good a priori rationale for involvement in schizophrenia, and had been associated with expression of each gene. However, an optimal manner to examine association of gene variants with imaging measures is to systematically investigate each variant in each gene. This can be done by starting with all the tagged SNPs from the HapMap database and measuring association of each SNP with the brain measure of interest. However, this method is now being replaced by newer approaches using next generation sequencing that allow for the entire gene to be sequenced. This newer method not only allows for examination of all known SNPs, but may also lead to discovery of novel variants including duplications and deletions, in each gene. The discovery of novel variants offers opportunity for study in conjunction with neuroimaging phenotypes of interest, although achieving adequate power may be a challenge due to the fact that many of the novel variants discovered may occur with rare frequency.

Several studies in this thesis examined age-related questions. A limitation in this regard is our cross-sectional approach. Conclusions cannot be drawn regarding progression of brain changes or cognitive changes specifically in a cross-sectional study. Rather, patterns can be observed during earlier phases of adult life compared to later phases of adult life. A longitudinal study
would be valuable in answering questions regarding (i) progression of decline of white matter integrity and cognitive performance in the DTI healthy aging and cognition study, (ii) progression of white matter deficits in schizophrenia that can be assessed in a tract specific manner across the adult lifespan, and (iii) effects of gene variants on progression of white matter or other brain measures, or cognitive performance across adult life. Longitudinal studies have their own challenges however. These include cost, participant attrition, and methodologic challenges regarding repeated cognitive and imaging measures.

Other limitations, such as medication effects on white matter integrity (see discussion in Chapter 6), are discussed in more detail within each paper. A final, important limitation, is that of DTI deterministic tractography, as it pertains to the crossing fiber problem (see discussion in Chapter 5), that prevents optimal segmentation of limbic system and other tracts that may play important roles in schizophrenia and other neuropsychiatric disorders. Newer DTI methods may help resolve this problem, and are discussed in the next section.

10.6 Future Directions

10.6.1 Newer Diffusion Imaging Methods

Major strides in our understanding of white matter neuroanatomy and in our understanding of disease related white matter change have been achieved with current DTI methods. However, certain challenges remain. One challenge particularly relevant to further understanding of neuropsychiatric disorders is the need to accurately and reliably segment crossing fibers. This challenge is important because most deterministic tractography algorithms cannot optimally segment various limbic system white matter tracts. The limbic system is critically important in schizophrenia and in other major mental illnesses. The limbic system contains white matter tracts
that cross near each other, and thus are difficult to segment reliably, e.g. the fornix of the hippocampus, and the posterior projection of the cingulum bundle. One way to improve resolution and separation of crossing fibers is through high angular resolution diffusion imaging (HARDI) (Frank 2002). This technique uses a large number of encoding directions (up to several hundred) at a fixed level of diffusion weighting. Spherical harmonic decomposition methods are used to estimate the complexity of the diffusion profiles (Alexander and Lobaugh 2007).

HARDI, however, has its own limitations for crossing white matter tracts, and may not be able to consistently resolve this problem due to the manner in which it models the ADC profile. Q-ball imaging, on the other hand, interpolates diffusivities over the entire surface of a sphere using an integration function (Tuch 2004). As a result, the peaks in the orientational distribution function (ODF) profiles correspond to the specific white matter tract direction (Alexander and Lobaugh 2007). A very recent study recommended Q-ball imaging for quantification at higher b-values and especially at regions of heterogeneous fiber orientation (Fritzsche, Laun et al.).

At higher b values (or diffusion weighting), which can provide improved detection of lesions with mild diffusion changes, the diffusion tensor model may not be consistently accurate in describing signal behaviour. First, apparent fast and slow diffusing components can cause the signal decay with diffusion-weighting to appear biexponential. Second, partial volume averaging can cause ambiguities in the interpretation of diffusion tensor measurements, e.g. due to crossing white matter tracts, or due to averaging between white matter and gray matter tissues (Alexander and Lobaugh 2007). A voxel that contains crossing white matter tracts may have low diffusion anisotropy, not due to disruption of the white matter tissue, but rather due to the fact that water diffusion does not follow a principal direction due to the multiple directions of various fibers present in that voxel. It has been estimated that over one third of voxels in the brain exhibit
marked departures from the Gaussian diffusion behaviour characterized by the tensor model (Behrens, Berg et al. 2007). Newer modalities that have attempted to resolve these issues have yet to gain traction in disease-related studies, perhaps due to the difficulty in knowing how to handle the new information that is provided (Jones 2008).

10.6.2 Other Imaging Approaches Potentially More Specific to Myelin

As described in previous Chapters, although myelin is one barrier to diffusion in the brain, it may not be the main barrier that influences fractional anisotropy, the most commonly used metric in DTI studies to date. Radial diffusivity (or transverse diffusivity), may be more specific to myelin (Song, Sun et al. 2002), however our understanding of the biological meaning of this metric is still developing. One MRI based approach, known as T2 relaxation, may be useful for measuring myelin since it can take advantage of the T2 distribution in human brain. This type of imaging is sensitive to water in the brain. A specific T2 component is sensitive to myelin water (i.e. the water trapped between the bilayers of the myelin sheath) (MacKay, Laule et al. 2006). Although one study demonstrated significantly reduced myelin water fraction in the schizophrenia patients compared to healthy controls in the frontal lobes (Flynn, Lang et al. 2003), there is a paucity of published data, in this regard. Therefore, future T2 relaxometry studies in schizophrenia (and in aging) are certainly warranted.

Magnetization transfer imaging (MTI) is another type of MRI technique that may be useful for measuring myelin. MTI, via a measure known as the magnetization transfer ration (MTR) measures the exchange of magnetization between bound protons and free water after the application of a specific pulse sequence (MacKay, Laule et al. 2006). MTR is a reproducible measure (Barker, Tofts et al. 1996), and reductions have been reported in myelin-based disorders, such as multiple sclerosis (Gass, Barker et al. 1994). A small number of studies have examined
MTR in schizophrenia, generally demonstrating some reduction in this measure in certain regions, e.g. temporal white matter (Foong, Maier et al. 2000), corpus callosum, and fornix (Kubicki, Park et al. 2005). The combination of DTI and MTI may be particularly useful, as in (Kubicki, Park et al. 2005). That is, white matter structures demonstrating reductions in anisotropy and MTR, may be specifically due to changes in myelin integrity. Further investigations combining DTI and MTI may help provide more information regarding white matter tissue disruption than either technique alone.

### 10.6.3 Novel Sequencing Methods in Genetics

Newer sequencing technologies that are rapidly becoming affordable permit the sequencing of parts of genes, or of entire genes of each individual. Such sequencing will lead to the discovery of novel polymorphisms, insertions/deletions, microdeletions and microduplications in genes of interest. For instance a fairly recent study sequenced the NOGO-66 receptor gene, that codes for the receptor (Nogo-66) for a myelin mediated complex (including MAG, MOG, and ErbB3) that inhibits axonal growth, in schizophrenia patients and healthy controls (Budel, Padukkavidana et al. 2008). The authors found novel sequence variants in the schizophrenia patients but not in the healthy controls. Functional analysis of these variants revealed potential effects on binding of myelin ligands (e.g. MAG). Comparisons of the frequency of these novel variants in schizophrenia cases vs. matched controls could then be studied to determine whether these novel variants are associated with disease. Then, these novel variants or genomic changes can be tested in conjunction with white matter phenotypes or cognitive performance (assuming they occur frequently enough to have adequate power to compare groups) to study the potential pathophysiologic link between such a genetic change and the phenotype of interest.

Alternatively for rare, n of 1 cases, where a new genetic lesion is discovered via sequencing,
reverse phenotyping could be employed (a bottom-up approach). The patient who is a carrier of a novel genetic lesion that is determined to be potentially causative for disease, could be characterized at the level of brain imaging, cognitive function, and symptom profile, and thus provide phenotypic characterization of molecular subtypes of schizophrenia.

10.6.4 Other Gene Systems and White Matter Integrity

Other genes or groups of genes may influence white matter integrity that have not yet been examined using DTI-genetics approaches. Some of these include the DISC1 system, the oxidative stress system, and the recently discovered genome wide significant variant at the ZNF804A gene. The DISC1 binding partner FEZ1 is key for axonal development. A mouse model of DISC1 demonstrated reduction in cerebral cortical layers containing pyramidal neurons necessary for interhemispheric communication, impaired neurite outgrowth, and subtle alterations of the CC, such as thinning and impaired midline crossing, consistent with changes noted in schizophrenia (Shen 2008). Furthermore, DISC1 has been related to transcription of oligodendrocytes via the OLIG2 gene in zebrafish (Wood, Bonath et al. 2009). The DISC1 Ser704Cys functional polymorphism has been associated with prefrontal cortical function (Prata, Mechelli et al. 2008) and hippocampal structure and activation during a working memory task (Callicott, Straub et al. 2005). Therefore, DISC1 variants point to intermediate phenotypes for schizophrenia via imaging-genetics approaches, and there is good rationale to examine these DISC1 variants in conjunction with white matter integrity measures.

Of all cells in the brain, oligodendrocytes are particularly vulnerable to oxidative damage due to a combination of high metabolism, numerous peroxisomes, lipid byproducts, and iron (McTigue and Tripathi 2008). Therefore, genes involved in oxidative stress processes may be related to white matter integrity. Iron is necessary for myelin production, that may explain previous
association of the transferrin gene with schizophrenia (Qu, Yue et al. 2008). When
oligodendrocyte precursor cells are exposed to oxidative stress, expression of genes important in
promoting oligodendrocyte differentiation (e.g. OLIG2, SOX10) is suppressed, and expression of
genes that inhibit oligodendrocyte differentiation is increased. Such disruption of
oligodendrocyte differentiation leads to the loss of mature myelinating oligodendrocytes (French,
Reid et al. 2009). Several studies support the concept that oligodendrocytes display maturation
dependent vulnerability to oxidative damage caused by a developmental delay in the expression
of antioxidant enzymes (superoxide dismutases-1 and –2, catalase, glutathione peroxidase),
glutathione depletion, or exogenous reactive oxygen species (Back, Gan et al. 1998; Baud,
Greene et al. 2004).

Recent genome-wide studies have been conducted in schizophrenia (O'Donovan, Craddock et al.
replicated finding for genome-wide significance occurs at the ZNF804A gene. Examination of
the ZNF804A genome-wide significant variant in a healthy sample of individuals demonstrates a
schizophrenia-related intermediate phenotype via effects on functional coupling of the
hippocampal-DLPFC circuitry (Esslinger, Walter et al. 2009). Therefore, this variant may
increase risk for schizophrenia via effects on fronto-temporal connectivity. Given that this
variant may serve as a binding site for a myelin related transcription factor (Williams, Norton et
al.), examination in conjunction with fronto-temporal white matter phenotypes is a priority to
further the field’s understanding of how this variant might confer risk for schizophrenia.
10.6.5 White Matter Integrity as a Quantitative Phenotype in Gene Discovery

Neuroimaging measures that directly index the brain should be more optimal than behavioural indices in studies pursuing the biological and genetic components of psychiatric disorders. In other words, a strategy of studying quantitative brain traits via neuroimaging should be more effective in revealing susceptibility genes in complex neuropsychiatric disorders, such as schizophrenia, where the brain is the target organ of study (Turner, Smyth et al. 2006). Both white matter integrity and cognitive performance can be considered as quantitative traits and are heritable (Bertisch, Li et al.). While there is very strong rationale for gene systems examined in this thesis in relation to white matter phenotypes, it is possible that other genes across the genome also may influence white matter phenotypes. DNA microarray technology can permit examination of tagged SNPs (now up to 1,800,000 SNPs) across the entire genome providing broad coverage for gene discovery by utilizing white matter integrity as a quantitative phenotype. This exploratory approach can be used to search for genetic signals that may point to genomic regions (either at, or near these SNPs) associated with white matter integrity in schizophrenia. This approach has recently been utilized with success in identifying genetic correlates of fMRI activation in the DLPFC in schizophrenia (Potkin, Turner et al. 2009), with similar sample sizes to the ones used in this thesis. PLS can be used to combine the large amount of SNP data with imaging data, and in this way provides a major advantage over other whole genome approaches. There has been great debate over the type and stringency of multiple comparison correction due to the concern regarding false positives in imaging-genetics (Meyer-Lindenberg, Nicodemus et al. 2008). However, with PLS, these large amounts of data can be combined into a single analysis, and correction for multiple comparisons is not required. And, as previously described, PLS has the additional advantage of providing reliability measures for the data. Thus an exciting
next step would be to perform a whole genome analysis for the quantitative phenotype of white matter integrity using PLS.

10.6.6 Combining *in vivo* Biomarkers

Methods now exist to examine gene expression *in vivo*. Correlations of expression of genes in blood with expression of those same genes in brain is only now being investigated (Rollins, Martin et al.). However, a recent study in bipolar disorder, where postmortem studies show downregulation of myelin genes, demonstrated that expression of myelin genes *in vivo* varied with mood state. The investigators used a microarray approach, and thus thousands of transcripts were examined (Le-Niculescu, Kurian et al. 2008). The same pattern, characterized by downregulation of myelin genes in postmortem brain, and altered regulation in blood may also be present in schizophrenia. A recent study examined two myelin protein genes in schizophrenia *in vivo* using this approach and did not find any significant differences. However, the investigators did not investigate the myelin gene system as a whole, nor did they examine correlation of expression of these genes with any potentially more informative disease state measures, such as negative symptoms, or cognitive deficits (Gutierrez-Fernandez, Gonzalez-Pinto et al.). The study of correlation of these transcripts with white matter integrity would be a novel approach for biomarker discovery in schizophrenia, that may be specifically related to severity of cognitive deficits or other symptom domains.

Imaging and genetics represent two of the most important areas of study in clinical neuroscience. Schizophrenia is primarily a genetic disorder, and neuroimaging permits *in vivo* measurement of structure and function of the brain, the target organ of this disease. By obtaining clinical and cognitive data, as well as imaging and genetics data and gene expression measures (in the future) on each patient, there is now the potential to provide more powerful answers than possible in any
one field alone. Such an integrated approach may greatly illuminate our understanding of schizophrenia, discover prognostic markers, and novel treatment targets in this devastating disorder.
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