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Investigating the Effects of Schizophrenia Genome-wide Associated Variants on White Matter Microstructure and Brain Morphology using Diffusion Tensor Imaging and Quantitative MRI

Sinead Kelly

Ph.D. 2014
Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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[Signature]

Sinéad Kelly
Note

MRI scanning for this project commenced in 2007 in the Trinity College Institute of Neuroscience. MRI scans were initially collected by Dr. Sarah Jacobson, Dr. Ciara Greene and Dr. Emma Rose of the Dept. of Psychiatry. Throughout the duration of the present thesis which commenced in 2011, the continuation of MRI data collection was carried out by the candidate, Ms. Sinéad Kelly, and her colleague in the Dept. of Psychiatry, Mr. Omar Mothersill.
Summary

This thesis describes four studies that aim to elucidate the effects of schizophrenia genome-wide associated variants on measures of white matter microstructure, cortical thickness, surface area and subcortical volume. Genome-wide association studies are considered a major advance in the identification of risk genes involved in schizophrenia pathology. However, the underlying mechanisms of how these genes act to confer risk for disease is unknown. As variation in white matter connectivity and brain structure are considered suitable endophenotypes for the disorder, this thesis examined the effects of six genome-wide associated variants on these brain measures in a sample of healthy control participants.

The first study described in this thesis comprised a review of the literature and meta-analysis investigating the effect sizes of psychosis risk variants on structural and functional connectivity. This study showed that, on average, the effects of common schizophrenia risk variants on measures of structural and functional brain connectivity are large, with a wider range of effect sizes being associated with structural connectivity studies utilising diffusion tensor imaging.

The second study described here examined the effect of a novel genome-wide associated variant at CNNM2 on measures of white matter microstructure using diffusion tensor imaging. Region of interest deterministic tractography analysis revealed that healthy controls carrying at least one copy of the risk G allele showed significantly decreased mean and radial diffusivity in comparison to non-risk allele carrier in the right inferior longitudinal fasciculus, the right uncinate fasciculus, the left inferior fronto-occipital fasciculus, as well as increased fractional anisotropy in the right anterior thalamic radiation. Whole-brain voxel-wise tract-based spatial statistics analysis revealed genotype effects on fractional anisotropy of the left
inferior fronto-occipital fasciculus, the right inferior longitudinal fasciculus, the anterior thalamic radiation, as well as the forceps minor, splenium and corticospinal tract. Analysis of the mean and radial diffusivity data also revealed increases in diffusivity in the forceps major and minor.

The third study sought to characterize the effects of the additional genome-wide associated schizophrenia variants on WM microstructure in healthy controls, using the same methodology as Chapter 3, namely ZNF804A, MIR137, TCF4, CSMD1 and NOS1. However, no significant effects of these variants on WM microstructure were observed. Voxelwise tract-based spatial statistics of the fractional anisotropy data was performed to determine if the genotype effects are specific to the tracts selected or if they are more generalized. Similar to the tractography results, no significant effects of variants at CSMD1, MIR137, TCF4 or ZNF8041 were observed at a whole-brain level.

The fourth study examined the effects of the same genome-wide associated schizophrenia risk variants at CNNM2, CSMD1, MIR137, NOS1, TCF4 and ZNF804A on measures of cortical thickness and surface area, as well as subcortical volume, in healthy controls. No significant effects of genotype were observed for measures of cortical surface area or subcortical volume. For cortical thickness measures, however, both risk variants at TCF4 were associated with significant differences in post-hoc t-tests. For rs4309482 at TCF4, a significant difference between homozygous A carriers and homozygous G carriers was observed in the superior frontal lobe for both the left hemisphere and the right hemisphere without a significant f-test difference. In both cases, risk AA carriers had increased cortical thickness in comparison to non-risk GG carriers. For the second variant, rs9636107 at TCF4, a significant difference between homozygous A allele carriers and homozygous G carriers was found in the rostral middle frontal
region with increased cortical thickness for risk G homozygotes in comparison to non-risk A homozygotes.

Overall, this thesis demonstrated the utility of quantitative MRI and diffusion tensor imaging studies in elucidating the neural effects of GWAS identified variants in schizophrenia.
Acknowledgements

I would like to gratefully acknowledge the support and guidance of my supervisor, Professor Gary Donohoe. Your enduring optimism and continued encouragement of my work has allowed me to grow as a research scientist. Thank you for giving me this wonderful opportunity.

My grateful thanks also to the following people for assistance at various stages of the project:
Dr. Emma Jane Rose for your invaluable advice and vast knowledge regarding the design and analysis of the neuroimaging data, as well as your constructive criticism and feedback on manuscript preparation. My continuing development of critical thinking I owe to you. Thank you for your support and encouragement over the last three years.
Dr. Erik O'Hanlon for your practical support on all things DTI. Thank you guiding me through my analysis, for always replying to my pestering emails and for your insightful feedback on my work.
Dr. Cathy Scanlon for your vital FreeSurfer support. I couldn't have got through this analysis without your help. Thank you for taking the time to look at my data as well as my write-up.
Dr. Derek Morris and everyone at the Neuropsychiatric Genetics Research Group in St. James' Hospital for teaching me all I know about psychiatric genetics,
Dr. Jane McGrath for assisting me with my DTI analysis. Thank you for being so helpful and patient.
Dr. Arun Bokde and Dr. Liz Kehoe for your practical help and guidance throughout my PhD.
Prof Michael Gill, Prof Aidan Corvin and Prof Louise Gallagher for your continued support and encouragement throughout my time in the Department of Psychiatry. It has been a privilege to work with you.

Prof Paul Thompson, Dr. Jessica Turner, Dr. Neda Jahanshad and members of the ENIGMA consortium and Imaging Genetics Centre, USC, for their collaboration and expertise on large-scale imaging genetics projects. It was a joy to work with you and contribute to the ENIGMA Schizophrenia working group.

Denise Hogan and Christina Mooney for the recruitment of patients for the study as well as your cherished friendship over the past three years. Your camaraderie made the whole experience even more enjoyable. I will miss our chats and coffee breaks.

Dr. Eric Kelleher and Catherine Delaney for the recruitment of patients for our study.

Omar Mothersill for all your help with scanning, recruitment, analysis and for teaching me so much about functional analysis and connectivity.

Dr. Ruben Keane, Rachael Dillon and Dr. April Hargreaves, it has been a pleasure to work with you on the CRT project. I have learned so much about CRT from your collaboration and I wish you the very best with this exciting and ambitious study.

Dr. Ken O'Reilly for your engaging conversations, vast knowledge and passion for psychology. You have instilled in me a fervour for the subject.

Dr. Therese O'Donoghue for your assistance with EEG analysis and teaching me everything I know about BESA.

Professor Richard Reilly and Hanni Kiiski for EEG data collection training and for including me in your stimulating lab meetings. I have learned so much about neural engineering from you and your group – thank you for being so supportive and inclusive.

Sojo Joseph for scanning our participants in TCIN and for your help with any radiography-related queries I have had about our data. Thank you for sharing your knowledge with us.

Keith McGrath, Dr. Jason McMorrow, Dr. Andrew Fagan and everyone at CAMI for assisting us in the start-up and collection of a new-wave of MRI data.
Geraldine Quinn for your administerial assistance throughout the past three years. It has been a pleasure to work with you.

Members and staff at the Trinity Centre for High Performance Computing (TCHPC) for your provision of a high performance server for analyses and storage of data. Thank you for always being so helpful in your assistance of any queries I have had.

Finally, I would particularly like to acknowledge the encouragement of my family and friends over the last few years, without you this thesis would not have been possible. I would especially like to thank my mam, Annora, for being there for me throughout the highs and lows of this journey and for supporting me in every way throughout my life.

Last but by no means least I would like to thank my fiancée and best friend, Chris. Thank you for your help with my analyses, for proof reading my manuscripts, for putting up with my tears, for your incessant belief in my abilities, for sending flowers to my office, and for encouraging me to strive towards my goal. I am forever indebted to you.

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<th>Description</th>
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<tr>
<td>5-HTTLPR</td>
<td>serotonin-transporter-linked polymorphic region</td>
</tr>
<tr>
<td>AD</td>
<td>axial diffusivity</td>
</tr>
<tr>
<td>ATR</td>
<td>anterior thalamic radiation</td>
</tr>
<tr>
<td>C10orf26</td>
<td>chromosome 10 open reading frame 26</td>
</tr>
<tr>
<td>CACNA1c</td>
<td>calcium channel, voltage-dependent, L type, alpha1C subunit</td>
</tr>
<tr>
<td>CAM</td>
<td>Cell adhesion molecule</td>
</tr>
<tr>
<td>CB</td>
<td>cingulum bundle</td>
</tr>
<tr>
<td>CC</td>
<td>corpus callosum</td>
</tr>
<tr>
<td>CDCA</td>
<td>Common disease common allele</td>
</tr>
<tr>
<td>CDRA</td>
<td>Common disease rare allele</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CNNM2</td>
<td>cyclin M2</td>
</tr>
<tr>
<td>CogG</td>
<td>centre of gravity</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CSMD1</td>
<td>CUB and sushi multiple domains 1</td>
</tr>
<tr>
<td>CWP</td>
<td>cluster-wise probability</td>
</tr>
<tr>
<td>DISC1</td>
<td>disruptef in schizophrenia 1</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
</tr>
<tr>
<td>ENIGMA</td>
<td>enhancng neuroimaging genetics through meta-analysis</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FS</td>
<td>freesurfer</td>
</tr>
<tr>
<td>FWE</td>
<td>family-wise error</td>
</tr>
<tr>
<td>GABRA1</td>
<td>gamma-aminobutyric acid (GABA) A receptor, alpha 1</td>
</tr>
<tr>
<td>GRIN2A</td>
<td>glutamate [NMDA] receptor subunit epsilon-1</td>
</tr>
</tbody>
</table>
GRM5  glutamate receptor, metabotropic 5
GSK3B  glycogen synthase kinase-3 beta
GWAS  genome-wide association study
HTR2C  5-hydroxytryptamine (serotonin) receptor 2C
IFOF  inferior fronto occipital fasciculus
ILF   inferior longitudinal fasciculus
MANCOVA multiivariate analysis of covariance
MD    mean diffusivity
MIR137 microRNA 137
MiRNA microRNA
MNI   montreal neurological institute
MTHFR methylenetetrahydrofolate reductase
NOS1  nitric oxide synthase 1
NRG1  Neuregulin1
PDF   probability distribution function
PFC   prefrontal cortex
PGC   psychiatric GWAS consortium
PPP1R1B Protein phosphatase 1 regulatory subunit 1B
PRODH proline oxidase dehydrogenase
RD    radial diffusivity
ROI   region of interest
SD    standard deviation
SE    standard error
SNP   single nucleotide polymorphism
STG   superior temporal gyrus
SZ    schizophrenia
TBSS  tract-based spatial statistics
TCF4  transcription factor 4
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TE</td>
<td>time to echo</td>
</tr>
<tr>
<td>TFE</td>
<td>turbo field echo</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TR</td>
<td>time to repetition</td>
</tr>
<tr>
<td>UF</td>
<td>uncinate fasciculus</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel-based morphometry</td>
</tr>
<tr>
<td>VWT</td>
<td>vertex-wise threshold</td>
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<tr>
<td>WM</td>
<td>white matter</td>
</tr>
<tr>
<td>ZNF804a</td>
<td>zinc finger protein 804a</td>
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1. Introduction
1.1 Background

Schizophrenia (SZ) is a complex neuropsychiatric disease affecting roughly 1% of the world's population (see (Lewis & Lieberman, 2000) for a review). It is characterized by positive (i.e. hallucinations, delusions), negative (i.e. apathy, lack of emotion, poor social functioning), cognitive (i.e. disorganized thoughts, difficulty concentrating, memory problems) and affective symptoms, and imposes a heavy cost on society. For example, the total cost of psychotic disorders in Europe in 2010 was recently calculated as €93.9 billion; (Gustavsson et al., 2011). It is also a disease of considerable heritability with up to 65% concordance rates in monozygotic twins and up to 28% in dizygotic twins (Cardno & Gottesman, 2001). While there is no consensus about its exact causes, the heritability of SZ is estimated to be about 80% (Sullivan et al., 2003).

Advances in neuroimaging techniques have allowed for the identification of various brain networks that are affected in patients with SZ and it is hoped that these innovations will foster diagnostic and therapeutic improvements for the illness. However, evidence suggests that the disease process of SZ occurs long before the disease manifests (Weinberger, 1987) and that brain abnormalities evident in patients may be part of an advanced condition that is too late to guide treatment or prevention (Meyer-Lindenberg, 2010b). The complex genetics of SZ poses significant problems for understanding the mechanisms by which genetic variants confer risk for this disease. Genome wide association studies (GWAS) that use high throughput technologies to examine genetic variation in large samples of SZ patients have shed light on the identification of possible risk genes (O'Donovan et al., 2008). Understanding the function of these variants at the level of the brain could, therefore, lead to a greater understanding of disease pathogenesis, which could direct new treatments.

This chapter describes the structural brain phenotypes investigated in SZ, including measures of white matter (WM) integrity using diffusion tensor imaging (DTI) as well as
quantitative MRI measuring cortical thickness, surface area and subcortical volume. The role of common genetic variants for SZ in brain structure is also reviewed and the aims and hypotheses of the current research are outlined.

1.2 Endophenotype approach

As already mentioned above, SZ is considered highly heritable. Heritability \( \left( h^2 \right) \) is the estimation of the proportion of phenotypic variation in a population at a given time that is due to genetic variation. It is estimated from similarities observed in subjects varying in their level of genetic similarity. In an attempt to address the complexity of gene function, researchers have focused on so-called ‘intermediate phenotypes’, which are measurable variations that occur on the pathway between genes and disease, and as such may be closer to the underlying genetic architecture than clinical symptoms (Gottesman & Gould, 2003). The application of endophenotypes can help identify mechanisms that link genes to behaviour and psychiatric disease. The endophenotype approach centers on co-segregating, state-independent and heritable phenotypes associated with a disorder with the ultimate aim of deconstructing an illness into less complex components involving fewer susceptibility variants compared to the number of variants involved in producing the disorder itself. This approach may facilitate identification of genes involved in illness, but also, where specific genes for a disorder have been identified, endophenotypes may reveal biological pathways by which individual genes confer increased risk. Endophenotypes should be: a) associated with illness in the population b) genetically mediated c) state-independent d) present in non-affected family members d) and be reliably measured (Gottesman & Gould, 2003).

An endophenotype that has proven to be very successful in characterizing genetic influences on brain structure and function is neuroimaging (Hariri, 2003) spurring a new field referred to as imaging genetics. Neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have been employed to examine the physiological
effects that genetic influences may have on patterns of brain activation of human subjects who have been genotyped for particular genetic polymorphisms (Greene et al., 2008).

1.3 Connectivity as an endophenotype

Other possible imaging genetic phenotypes for SZ include changes in structural and functional brain connectivity (Meyer-Lindenberg, 2010b). In the early 20th century, German neurologist Carl Wernicke proposed that SZ arises from altered neural connectivity (or dysconnectivity) rather than from abnormalities in specific parts of the brain (Stephan et al., 2009). One hundred years later, advances in neuroimaging technology have enabled scientists to empirically consider dysconnectivity as a key component of SZ pathogenesis. If the activity of two or more brain regions is correlated over time, they are said to be functionally connected (Friston et al., 1993). The “disconnection” hypothesis was first proposed by Karl Friston and colleagues in the 1990s (Friston, 1998). This hypothesis postulates that SZ is primarily caused by abnormal N-methyl-D-aspartate (NMDA)-receptor mediated synaptic plasticity, which in turn, is caused by dysregulation of these receptors by neurotransmitters such as dopamine. While some dysconnectivity theories emphasize the role of synaptic plasticity in SZ etiology (Stephan et al., 2009), others acknowledge the role of compromised fiber connections between neurons - i.e. white matter (WM). WM contains myelinated nerve cells that connect various grey matter areas of the brain to each other, and carry nerve impulses between neurons. Compromised WM integrity is evident in SZ (Ellison-Wright & Bullmore, 2009; Kubicki et al., 2007). Moreover, WM abnormalities are apparent in individuals at high risk of SZ and also in patients during the early stages of illness, suggesting that these abnormalities may be a stable characteristic of the disease (Pérez-Iglesias et al., 2010; Witthaus et al., 2008;). The macro-circuit theory proposes that specific WM tracts are compromised, which may be a cause or consequence of abnormalities in the grey matter regions these tracts connect (Konrad & Winterer, 2008).
Although there are robust links between functional and structural connectivity in the brain (Hermundstad et al., 2013; Sharp et al., 2011), a review by Park & Friston (2013) acknowledges that for any one structural connectivity pattern, there are many possible patterns of functional connectivity. They claim that function may deviate from structure to exhibit dynamic and contextualized behavior and, with advances in graph and network theory, a range of functional networks will develop from the (hidden) structural architecture that allows for efficient global integration (long-range connections subserving higher cognition) of local integrations (short-range connections).

1.3.1 Pathophysiological mechanisms of compromised WM

As the integrity of axons is dependent on myelination and factors influencing myelination, it is possible that myelin and oligodendroglia function also plays a role in the pathophysiology of SZ (Davis et al., 2003). Since myelination also impacts synaptic plasticity, oligodendrocyte abnormality and subsequent myelin dysfunction may contribute to the development of SZ by altering synaptic function and information processing (Fields, 2008). Conduction velocity along axons is also thought to be essential for learning processes (Fields, 2008) and disruption of this has the potential to lead to the range of cognitive impairments observed in SZ (Tanaka et al., 2009). Furthermore, oligodendrocyte and myelin impairment also impacts neuronal activity that is relevant to SZ, such as glutamate and dopamine signalling. Evidence from psychotic episodes of multiple sclerosis (MS) patients and experimentally induced demyelination suggests that altered myelin function leads to changes in dopamine signalling (Takahashi et al., 2011). Similar analyses have also revealed increased levels of glutamate in brains of multiple sclerosis (MS) patients as well as increased expression of glutamate receptors on oligodendrocytes (Takahashi et al., 2011). Glutamate transporters are also present on oligodendroglia and are thought to regulate glutamate concentrations to prevent glutamate-induced excitotoxicity (Pitt et al., 2003).
Furthermore, over activation of oligodendroglial glutamate receptors is excitotoxic and can result in oligodendrocyte death (Davis et al., 2003).

Recently, attention has turned toward the consideration of genes that influence oligodendrocyte architecture and how these genes may also be associated with SZ risk. Hakak et al. (2001) examined the expression of 6500 genes derived from postmortem cortical tissue of SZ patients and controls. The expression levels of six myelin-related genes were significantly down regulated for SZ patients in comparison to control subjects. These genes included: myelin-associated glycoprotein (MAG), CNP, myelin and lymphocyte protein (MAL), gelosn (GSN), ErbB3, and transferring. Down regulation of these genes supports the view that oligodendrocytes, the cell type from which all these genes derive in the brain, contribute to the pathophysiology of SZ.

1.4 Utility of DTI as an endophenotype

Conventional MRI methods utilising T1 and T2 contrasts to differentiate WM, grey matter and cerebral spinal fluid can be analysed using region of interest (ROI) and voxel-based methods to characterise WM differences in SZ (Konrad & Winterer, 2008). However, a more advanced and more widely used MRI method called Diffusion Tensor Imaging (DTI) has the ability to assess integrity of WM and subcortical WM tracts by characterizing the diffusion of water molecules in brain WM (Jones, 2008; Tournier et al., 2011). A 3D diffusion model (the tensor) is estimated for DTI and eigenvalues are calculated for the tensor. Various scalars to characterize diffusion can then be extracted from the tensor eigenvalues. Healthy brain WM has a complex axonal structure and, therefore, water diffusion will be restricted along the direction of the axons. This is known as anisotropic diffusion. However, if brain WM is impaired, water diffusion can become less restricted (i.e. isotropic). A common measure derived from DTI to describe the degree of anisotropy during diffusion is fractional anisotropy (FA). However, other measures of diffusion such as mean (average of the eigenvalues) radial (average of the
second and third eigenvalues) and axial (measure of the first eigenvalue) diffusivity can also be obtained. Based on the voxel-wise information provided by DTI, fibre tracking algorithms can also be implemented in regions of interest to reconstruct the underlying three-dimensional WM pathways. While caution must be exercised when interpreting measures of anisotropy (Jones, 2008; Tournier et al., 2011) such measures are thought to index structural integrity of WM tracts and, thus, may be reasonably considered to be implicit indices of brain structural connectivity.

![3D diffusion model](image)

**Figure 1** 3D diffusion model (the tensor) and its associated eigenvariates ($\lambda_1$, $\lambda_2$ and $\lambda_3$).

A review by Kubicki and colleagues (2007) noted that the most frequent positive findings of DTI studies in SZ were decreased FA within the prefrontal and temporal lobes, as well as abnormalities within the fibre bundles connecting these regions. Ellison-Wright and Bullmore (2010) conducted a meta-analysis of 15 DTI studies, which included a total of 407 patients with SZ and 383 comparison subjects. Results identified two regions of FA decreases in SZ subjects in comparison to controls. The first region was in the left frontal deep WM, which is traversed by WM tracts interconnecting the frontal lobe, thalamus and cingulate gyrus. The tracts included: a) the anterior thalamic radiation; b) corticobulbar tracts; c) inter-hemispheric fibres running through the genu of the corpus callosum; d) the inferior fronto-occipital fasciculus and e) the cingulum bundle. The second region was in the left temporal deep WM that is traversed by WM tracts interconnecting the frontal lobe, insula, hippocampus–amygdala, temporal and occipital
lobes. These tracts include: a) inter-hemispheric fibres running through the splenium of the corpus callosum; b) the inferior fronto-occipital fasciculus; c) the inferior longitudinal fasciculus; d) the fornix/striaternalis. WM abnormalities are also present in first-episode subjects (Pérez-Iglesias et al., 2010) suggesting that these variations are stable characteristics of the disease. These two reviews of the current DTI/SZ literature suggest that specific networks of WM are disrupted in SZ providing support for the macro-circuit theory of WM disruption in the disease.

Disruption of these WM networks may also contribute to cognitive deficits and symptoms in SZ. For example, memory impairment has been associated with reduced FA in the fornix (Nestor et al., 2007) while executive function episodic memory deficits have been associated with reduced FA in the cingulum bundle and uncinate fasciculus respectively (Nestor et al., 2008). It has been postulated that inferior longitudinal fasciculus disruption may contribute to impaired social cognition in SZ (Ashtari et al., 2007) and auditory hallucinations in SZ have been associated with FA changes in multiple tracts including the cingulum bundle, corpus callosum and left inferior longitudinal fasciculus (Shergill et al., 2007). Kubicki et al. (2009) concluded that disruption in anatomical connectivity between areas involved in attentional control might be responsible for attention and memory deficits in SZ as the integrity of the cingulum bundle was associated with executive function in SZ patients.

Evidence from family and twin studies using DTI measures indicates that the microstructure of cerebral white matter is under strong genetic control (Kochunov et al., 2010). In one study of 467 healthy individuals recruited from randomly ascertained pedigrees of extended families, significant heritability was observed for FA and radial diffusivity (Kochunov et al., 2010). Genetic correlation analysis indicated that FA and radial diffusivity shared 46% of the genetic variance (Kochunov et al., 2010). A twin study of 92 identical and fraternal twins also demonstrated that FA is under strong
genetic control and is highly heritable (Chiang et al., 2009). FA data from SZ patients, non-affected first-degree relatives and controls also show a significant linear relationship to genetic liability for SZ (Clark et al., 2011). Furthermore, candidate common genetic variants associated with genetic susceptibility for SZ including neuregulin 1 (NRG1) and its receptor ErbB-4. Both have previously been associated with compromised WM integrity (McIntosh et al., 2006; Zuliani et al., 2011). Chapter 2 details these findings further, providing a meta-analysis of effects on white matter associated with SZ implicated genes to date.

From the evidence outlined, WM connectivity qualifies as an endophenotype for SZ. The connectivity hypothesis is now a well-established hypothesis of the disease, however, the underlying molecular pathways and genetic underpinnings of dysconnectivity remain to be delineated. Investigating the impact of SZ risk genes on structural connectivity would provide a greater understanding of disease etiology and may have the potential to direct new evidence-based treatments.

1.5 Schizophrenia and brain structure

As well as WM microstructure and connectivity, brain structural alterations evident in SZ using quantitative MRI may also be considered suitable endophenotypes for the disorder. Systematic reviews and meta-analyses of studies of structural MRI modalities in SZ indicate that the whole-brain volume is reduced, while the ventricular volume is increased (Steen et al., 2006; Ward et al., 1996; Wright et al., 2000). Volume reductions have been found in the temporal lobe, including the hippocampus, amygdala, and superior temporal gyrus (STG) (Lawrie & Abukmeil, 1998; Nelson et al., 1998). Meta-analyses of individual brain regions have also revealed reductions in thalamic volume (Konick & Friedman, 2001), corpus callosum area (Woodruff et al., 1995), and hippocampal volume (Adriano et al., 2012). Whole-brain and hippocampal reduction as well as ventricular enlargement are also apparent in first-episode patients (Steen et al.,
In a meta-review conducted by Shepherd et al. (2010) integrating volumetric and voxel-based estimates have found grey matter reductions of anterior cingulate, frontal (particularly medial and inferior) and temporal lobes, hippocampus/amygdala, thalamus, and insula that may be magnified over time.

In a voxel-based morphometry (VBM) meta-analysis of high-risk, first-episode and chronic SZ, Chan et al. (2010) found decreased grey matter in the high-risk group relative to controls in anterior cingulate regions, left amygdala and right insula. Decreased grey matter volumes in first-episode in comparison to controls were also found in the anterior cingulate and right insula but not the amygdala. The chronic group had more extensive grey matter reduction, incorporating similar regions to those found in first-episode and high-risk groups but extending to superior temporal gyri, thalamus, posterior cingulate, and parahippocampal gryus. The authors concluded that as the grey matter abnormalities become more extensive through first-episode and chronic illness, SZ might be a progressive cortico-striato-thalamic loop disorder.

1.5.1 Brain structure as an endophenotype for schizophrenia

A review analyzing the suitability of brain structural variations as endophenotypes for SZ conducted by Prasad & Keshavan (2008) found that MRI morphometric measures meet many but not all of the criteria in order to be considered endophenotypes. They found that the brain structural alterations (1) are robustly associated with SZ subjects, (2) are relatively specific to SZ, (3) are present in unaffected relatives, (4) co-segregate with the disorder, (5) appear to co-vary with the presence of broader spectrum psychopathology related to schizophrenia and (6) are moderately to highly heritable. They state that overall, morphometric measures appear to be as highly heritable as the disease phenotype. They acknowledge that global cerebral measures might have higher heritability but the relative contributions of gene and environmental factors may vary across the different brain structures. The authors also acknowledge that the measures
(7) are reliably quantifiable and (8) have some regional variations that probabilistically predict the disease. However, the data do not definitively support the view that brain structural alterations are state independent. Overall, brain structural alterations have been reported at every stage of the illness, however, there is some debate about whether they change with illness chronicity (Prasad & Keshavan, 2008).

Finally, these structural differences have also been associated with SZ risk genes. Prasad et al. (Prasad et al., 2004) list several examples, including an association between reduced prefrontal grey matter volume and polymorphisms of the gene encoding the regulator of G-protein signaling, subtype 4 (RGS4) (Buckholtz et al., 2007; Prasad et al., 2004), as well as an association between polymorphisms in the brain-derived neurotrophic factor (BDNF) gene with differences in frontal and medial temporal lobe (MTL) volumes (Pezawas et al., 2004; Szeszko et al., 2005). The DISC1 gene has also been implicated in hippocampal structure variation (Callicott et al., 2005; Roberts, 2006) and COMT variants have been associated with increased grey matter density in the superior temporal gyrus and increased WM density in the left inferior frontal pole (Zinkstok et al., 2007). Furthermore, homozygote Val allele carriers of COMT with chronic SZ had decreased volume of left anterior cingulate cortex, thalamus, amygdala uncus, and left middle temporal gyrus (McIntosh et al., 2007a).

This broad body of evidence suggests that brain structural measures are suitable endophenotypes for SZ and the use of such endophenotypes may assist genetic studies in delineating the pathophysiology of major psychiatric disorders such as SZ.

1.6 Genomic methods – identification of illness associated genes
A prevailing hypothesis regarding the underlying genetic architecture of SZ susceptibility is the common disease–common allele (CDCA) hypothesis (Rodriguez-Murillo et al.,
The CDCA hypothesizes that multiple, relatively common variants, each conferring weak or modest risk, work in combination to increase disease risk (Rodriguez-Murillo et al., 2012). This model explains the high prevalence of SZ by suggesting that small-effect alleles are less susceptible to negative selection, and assume that these alleles occasionally recombine in high-risk combinations in sporadic cases (Rodriguez-Murillo et al., 2012).

1.6.1 Genome-wide association studies

In genetics, a genome-wide association study (GWA study, or GWAS) is an examination of many common genetic variants in different individuals to see if any variant is associated with a trait or disease. GWAS usually focus on associations with single nucleotide polymorphisms (SNPs). A SNP is a change in DNA sequence occurring when nucleotides (A, T, C or G) in the genome differ between base-pair positions on a chromosome. These polymorphisms are the most common type of genetic variation among individuals. GWA studies usually compare the DNA of two groups of participants: people with a disease (cases) and unrelated, albeit ethnically similar, healthy individuals (controls). Each person gives a sample of DNA, from which millions of genetic variants are read using SNP arrays. If one allele of a polymorphism is significantly more frequent in people with the disease, the SNP is said to be "associated" with the disease. The associated SNPs are then considered to mark a region of the human genome that influences the risk of disease. A large majority of SNPs have very little effect on biology, however, some can have functional significance with effects on amino acid production, transcription factor binding and mRNA transcription (Bush & Moore, 2012). As GWAS investigates the entire genome, the approach is considered non-candidate-driven and hypothesis-free.

Technological advances for high-throughput genotyping (microarrays), and the development of statistical methods to analyze the large amount of data obtained in
these studies have made GWAS possible. However, to identify small effects, very large sample sizes are required for GWAS due to the significant multiple testing burden (Corvin, 2011). This requirement has encouraged large-scale collaborations such as the Psychiatric GWAS consortium (PGC) that has performed meta-analyses of GWAS data for psychiatric disorders including SZ, bipolar and major depression. The consortium has recently published one of the largest SZ GWAS conducted to date (Ripke et al., 2011).

1.6.2 Schizophrenia GWAS

By 2011, ten SZ common risk variants had been identified (Corvin, 2011) with five new SZ loci identified in the PGC GWAS by Ripke et al. (2011) along with the association of two identified SZ risk variants at neurogranin and transcription factor 4 (TCF4). Even more recently, thirteen new loci reached genome-wide significance for SZ (Ripke et al., 2013) with the authors estimating that over 8,000 independent, mostly common SNPs contribute to risk for SZ and collectively account for 32% of the variance in liability (Ripke et al., 2013). Moreover, the PGC are currently preparing a manuscript for publication of the largest SZ GWAS conducted to date with over sixty variants reaching genome-wide significance. The statistical association of these variants with SZ is only the first step in elucidating the role that common variants have on the pathophysiology of the disorder. Furthermore, from what is known about the function of these genes, it is not immediately apparent how they act to confer risk for SZ. For example, the functions of some of these variants include complement system regulation, magnesium transport and tumor growth suppression (See chapters 3 and 4). Therefore, endophenotype analyses are required to fully elucidate the biological role of these variants on SZ pathophysiology. In the case of GWAS variants, a definitive a-priori hypothesis may be lacking due to the ambiguity surrounding the relationship between their function and SZ biology, as well as a scarcity or previous studies. However, such investigations are validated on the basis that genes, whose functions are predominantly unknown, yet are statistically associated with an illness which features compromised WM integrity and
cerebral morphometry in its phenotype, may have a role to play in brain structure and connectivity. Table 1 below outlines the GWAS variants selected for this thesis.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Variant</th>
<th>P-value</th>
<th>Odds Ratio</th>
<th>95% Cl</th>
<th>Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p21.3</td>
<td>rs1625579</td>
<td>1.5 × 10^-11</td>
<td>1.12</td>
<td>1.09-1.16</td>
<td>MIR 137</td>
<td>(Ripke et al., 2011)</td>
</tr>
<tr>
<td>2q32.1</td>
<td>rs1344706</td>
<td>2.5 × 10^-11</td>
<td>1.1</td>
<td>1.07-1.14</td>
<td>ZNF804A</td>
<td>(O'Donovan et al., 2008)</td>
</tr>
<tr>
<td>8p23.2</td>
<td>rs10503253</td>
<td>1.45 × 10^-8</td>
<td>1.16</td>
<td>1.11-1.21</td>
<td>CSMD1</td>
<td>(Ripke et al., 2011)</td>
</tr>
<tr>
<td>10q24.32*</td>
<td>rs7914558</td>
<td>2.23 × 10^-8</td>
<td>1.22</td>
<td>1.15-1.29</td>
<td>CNNM2</td>
<td>(Ripke et al., 2011)</td>
</tr>
<tr>
<td>18q21.2*</td>
<td>rs4309482</td>
<td>7.8 × 10^-9</td>
<td>1.09</td>
<td>1.06-1.12</td>
<td>TCF4</td>
<td>(Steinberg et al., 2011)</td>
</tr>
<tr>
<td>18q21.2*</td>
<td>rs9636107</td>
<td>4.74 × 10^-9</td>
<td>0.928</td>
<td></td>
<td>TCF4</td>
<td>PGC (Manuscript in preparation)</td>
</tr>
<tr>
<td>12</td>
<td>rs1607817</td>
<td>2.72 × 10^-9</td>
<td>0.923</td>
<td></td>
<td>NOS1</td>
<td>PGC (Manuscript in preparation)</td>
</tr>
</tbody>
</table>

Table 1 SZ GWAS variants selected for this thesis and their locations and effect sizes. The Odds Ratio (OR) is a measure of effect size. It is the ratio of the odds of the variant occurring in the group of people with disease versus the ratio in the control group. The 95% confidence interval (CI) gives the range within which the true OR lies with a 95% probability. An asterisk indicates that more than one variant has been implicated at this locus (see Corvin et al., 2011).

1.7 Conclusion

Variations in WM integrity and cortical/subcortical brain structure are characteristics of the highly heritable disorder that is SZ. These differences are evident across all stages of the illness as well as in first-degree relatives and are considered suitable endophenotypes for SZ. Therefore, understanding the molecular mechanisms of variation in brain structure may be crucial to understanding the pathophysiology of SZ. Advances in genomic research including the application of GWAS can provide the opportunity to further explore these underlying molecular mechanisms and potentially
enable the development of more effective and advanced treatments for this debilitating disorder. However, due to the rapid progression and advancement in genomic research, many questions regarding the role of common variants in SZ remain unanswered.

The work described in the following chapters attempts to characterize the effects of common, GWAS-significant SZ variants on the brain by employing MRI based measures of WM integrity and cortical/subcortical brain structure. Specifically, we will attempt to address (i) the role these variants play in measures of WM integrity using DTI, (ii) the role of these variants in cortical and subcortical brain structure and (iii) the extent, if any, of which these novel SZ associated loci impact overall brain structure and microstructure.

1.8. Aims of the thesis

The overall aim of the project was to investigate the effects of common genetic variants on measures of structural brain connectivity as well as cortical and subcortical brain structure. Genes selected were based on recent findings from SZ GWAS studies. These studies investigated the effects of risk alleles by comparing carriers with non-carriers of the allele or investigating the impact of allele dosage if permitted by the minor allele frequencies.

The first study described in this thesis (discussed in detail in Chapter 2) will investigate effect size differences between the impact of common risk variants on measures of structural and functional brain connectivity by conducting a review of the literature and a subsequent meta-analysis. The examination of effect sizes may illustrate the sensitivity of functional and structural measures of connectivity to effects of common SZ risk variants.
The second study outlined in the present thesis is an empirical investigation of a SZ GWAS variant at the CNNM2 gene, identified in the Ripke et al. (2011) analysis, on measures of WM connectivity. This study is discussed in Chapter 3.

The third study, discussed in Chapter 4, will assess the effects of five other SZ GWAS variants on measures of brain structural connectivity. Two of these variants (MIR137, and CSMD1) were also identified in Ripke et al. (2011). One of the TCF4 variants (rs4309482) was originally identified by Steinberg et al. (2011). The common variant at ZNF804A was the first SZ GWAS variant identified in O'Donovan et al. (2008), while the fifth and sixth variants, an indel at NOS1 and another SNP within the TCF4 gene (rs9636107), were recently identified in the largest Psychiatric Genetics Consortium GWAS (Manuscript in preparation).

Finally, the fourth study, described in detail in Chapter 5, will examine the effects of these six SZ GWAS variants on subcortical volume in structures implicated in SZ, as well as measures of cortical thickness and surface area, considered more sensitive endophenotypes in comparison to volume (Winkler et al., 2010).
2. The Effects of Psychosis Risk Variants on Brain Connectivity:

A Review and Meta-analysis
2.1 Introduction

While changes in functional and structural connectivity may be a critical aspect of the SZ disease profile, there has been little systematic evaluation of the relative sensitivity of these different indices to genetic risk for SZ. In this chapter, empirical investigations that have utilized functional magnetic resonance imaging (fMRI) or diffusion tensor imaging (DTI) to investigate the effects of SZ risk variants on functional and structural brain connectivity are outlined. The relative magnitude of these effects is also considered in order to determine the extent of the genetic impact on brain connectivity.

Previous meta-analyses have considered the magnitude of the impact of gene variants on brain function, each reporting large effect sizes (Mier et al., 2010; Munafo et al., 2008). Munafo et al. (2008) examined the effect sizes of the 5-HTTLPR polymorphism and amygdala activation, while Mier et al. (2010) examined the magnitude of effect of the catechol-O-methyltransferase (COMT) Val158Met polymorphism on brain function, reporting association between this variant and activation of the prefrontal cortex (PFC). Rose & Donohoe (2012) also investigated if SZ risk variants are more penetrant at the level of the brain structure and function in comparison to the level of behaviour. They found medium and large effects for imaging studies and small effects for cognitive investigations, concluding that SZ risk variants demonstrate greater penetrance at the level of the brain. However, to our knowledge no studies have specifically considered the effect size of gene variants in studies of functional and structural connectivity, or compared effect sizes between these phenotypes. Consideration of the relative impact of these two measures of brain connectivity will help delineate whether or not one phenotype is more proximal to the underlying genetics, and thus preferential as an intermediate phenotype for studies of SZ. This could not only aid our theoretical understanding of the SZ disease trajectory but may also have significant practical implications for future investigations.
2.2 Methods

Relevant papers were retrieved based on the criteria of studies that included genes implicated in SZ risk and measures of either DTI or functional connectivity. PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used to search for relevant functional and structural connectivity papers published until June 2011. The following search terms were included in this search: [schizophrenia OR schiz*] AND [genetic or gene*] AND [MRI OR DTI] AND [connectivity] AND [structural OR functional]. This literature search was supplemented with a review of the references from each of the papers identified. In total 24 studies meeting these search criteria were retrieved, including 12 DTI studies and 12 functional connectivity studies. Individual studies differed slightly in terms of MRI acquisition and analysis parameters (e.g., voxel size, size of Gaussian function used for smoothing). However, all studies were included regardless of these differences, due to the small number of studies available. Where the data presented were insufficient for effect size calculations, a request for supplementary data was sent to the corresponding author. This led to data being available for 19 out of the 24 studies identified (10 DTI studies and 9 functional connectivity studies).

Effect size calculations were performed using two online effect size calculators http://www.uccs.edu/~faculty/lbecker/ and www.lyonsmorris.com/ma1/index.cfm. Estimates of effect size were calculated based upon either descriptive data (i.e., mean, SD, and N), or statistical data (i.e., t, F). The purpose of this paper was to estimate differences in effect size rather than differences in direction of effect. That is, we were interested in delineating the relative sensitivity of these two indices of brain connectivity to genetic variability, rather than accounting for the overall impact of a specific variant or group of variants. Therefore, direction of effect was not included in the analysis and all effect sizes were considered positive.
A random effects meta-analysis considering the relative difference in the impact of SZ risk genes on functional and structural connectivity was carried out using the comprehensive meta analysis software (CMA; software package v2; www.meta-analysis.com). For the purposes of this analysis, Hedge's $g$ and its associated variance were calculated for the outcome of each significant effect in each study. As with prior estimates of Cohen's $d$, $g$ was calculated using a variety of input variables including descriptive and inferential statistics. In the first analysis, the largest effect for each study was chosen so to reflect the maximal sensitivity to gene effects within each investigation. In a secondary analysis, all of the effects for each significant result in each paper were taken into account. This strategy allowed us to account for both variability in the number and range of significant effects reported across methodologies.

2.3 Results

2.3.1 Summary

Overall, 8 fMRI and 10 DTI studies were included in the meta-analysis. Summary information from all of these studies is presented in Tables 2 and 3.

<table>
<thead>
<tr>
<th>First author and date</th>
<th>Gene of interest</th>
<th>Connectivity</th>
<th>Method</th>
<th>Statistic</th>
<th>n</th>
<th>Cohen's d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Meyer-Lindenberg et al., 2007)</td>
<td>PPP1R1B</td>
<td>L. PFC - striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
<td>SC</td>
<td>4.41†</td>
<td>126</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. PFC - striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
<td>SC</td>
<td>4.57†</td>
<td>126</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. PFC - striatum, frequent</td>
<td>SC</td>
<td>4.31†</td>
<td>142</td>
<td>0.73</td>
</tr>
<tr>
<td>Study</td>
<td>Gene</td>
<td>Region</td>
<td>Haplotype Carriers</td>
<td>Statistic</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
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<td>-----------------------</td>
<td>------------------------------------</td>
<td>-----------</td>
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<td></td>
</tr>
<tr>
<td>(Kempf et al., 2008)</td>
<td>PRODH</td>
<td>dlPFC - striatum</td>
<td>&gt; non-frequent</td>
<td>SC</td>
<td>3.91*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>reference haplotype</td>
<td>carriers &gt; protective</td>
<td></td>
<td>103/108</td>
<td></td>
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<td>PPI</td>
<td>3.58*</td>
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<td>R. dlPFC, Ser/Ser&gt;Cys</td>
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21
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<tr>
<th>(Walter et al., 2011) ZNF804A</th>
<th>L. TPJ - L. inferior frontal gyrus, AA &gt; CA &gt; CC</th>
<th>SC</th>
<th>3.77*</th>
<th>109</th>
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<td>R. dIPFC - L. MTG, CC &gt; CA &gt; AA</td>
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<td>R. dIPFC - L. LG, CC &gt; CA &gt; AA</td>
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<td>3.83*</td>
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<td>(Paulus et al., 2011) ZNF804A</td>
<td>R. dIPFC - L. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.3*</td>
<td>94</td>
<td>0.48</td>
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<tr>
<td>R. dIPFC - L. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.22*</td>
<td>94</td>
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<td>R. dIPFC - R. HF, AA &gt; CA &gt; CC</td>
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<td>0.46</td>
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<tr>
<td>R. dIPFC - L. dIPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.42*</td>
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<td>R. dIPFC - L. dIPFC, AA &gt; CA &gt; CC</td>
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<td>94</td>
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<td>R. dIPFC - R. dIPFC, AA &gt; CA &gt; CC</td>
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<td>R. dIPFC - R. dIPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
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<td>94</td>
<td>0.49</td>
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<tr>
<td>R. dIPFC - R. dIPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.33*</td>
<td>94</td>
<td>0.49</td>
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</tr>
<tr>
<td>ZNF804A</td>
<td>R.dIPFC- R.dIPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>2.43*</td>
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<td>0.51</td>
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<tr>
<td>Controls:</td>
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<td>2.72*</td>
<td>96</td>
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<tr>
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<td>3.21*</td>
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<td>3.74§</td>
<td>96</td>
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<tr>
<td>Controls:</td>
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<td>PPI</td>
<td>2.89†</td>
<td>96</td>
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<td>Siblings:</td>
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<td>2.53*</td>
<td>83</td>
<td>0.57</td>
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<tr>
<td>Siblings:</td>
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<td>Siblings:</td>
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<td>Patients:</td>
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<td>4.58§</td>
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<tr>
<td>Patients:</td>
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<td>PPI</td>
<td>3.56§</td>
<td>33</td>
<td>1.28</td>
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Table 2 Details of the functional connectivity studies included in this meta-analysis

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<th>First author and date</th>
<th>Gene of interest</th>
<th>Connectivity</th>
<th>Statistic (t or F)</th>
<th>N</th>
<th>Cohen’s d</th>
</tr>
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<tbody>
<tr>
<td>(McIntosh, et al., 2007b)</td>
<td>NRG1 SNP8NRG243177</td>
<td>Reduced FA in ALIC</td>
<td>t = 2.65**</td>
<td>43</td>
<td>0.83</td>
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<tr>
<td>(Winterer et al., 2008)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in MF subcortical WM</td>
<td>t = 4.67***</td>
<td>50</td>
<td>1.35</td>
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<tr>
<td>(Sprooten et al., 2009)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in left ATR</td>
<td>t = 5.52***</td>
<td>28</td>
<td>1.95</td>
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<tr>
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<td>NRG1 SNP8NRG243177</td>
<td>Reduced FA in left ATR</td>
<td>t = 4.69***</td>
<td>28</td>
<td>1.66</td>
</tr>
<tr>
<td>(Wang et al., 2009)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in anterior cingulum</td>
<td>F = 5.27*</td>
<td>31</td>
<td>0.86</td>
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<tr>
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<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in anterior cingulum</td>
<td>F = 18***</td>
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<td>(Konrad et al., 2009)</td>
<td>ErbB4 rs707284</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 4.24***</td>
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<td>ErbB4 rs758440</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 2.81***</td>
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<td>Reduced FA in temporal lobe WM</td>
<td>t = 4.31***</td>
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<td>ErbB4 rs839523</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 4.73***</td>
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<td>1.37</td>
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<tr>
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<td>G-T-G-T v lower risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 3.85***</td>
<td>32</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Note: n, sample size; SC, seeded connectivity; PPI, psychophysiological interaction; dIPFC, dorsolateral prefrontal cortex; MFG, middle frontal gyrus; SFG, superior frontal gyrus; TPJ, temporo-parietal junction; MTG, middle temporal gyrus; LG, lingual gyrus; IFG, inferior frontal gyrus; * p-value is uncorrected for multiple comparisons; † false discovery rate corrected within region of interest; ‡ false discovery rate corrected for whole brain; § family wise error corrected within region of interest
<table>
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<tr>
<th></th>
<th>Reduced FA in</th>
<th>t</th>
<th>n</th>
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<tr>
<td></td>
<td>temporal lobe WM</td>
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<tr>
<td>G-T-G-T v all</td>
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<td>All other v nonrisk</td>
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<td>Reduced FA in</td>
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<td>left ALIC</td>
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<td>ErbB4 rs4673629</td>
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<td>3.98**+ 36</td>
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<td>(Thomason et al., 2010)</td>
<td>COMT val158met</td>
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<td>Main effect of genotype on FA, AD, RD in GCC</td>
<td>3.04* 40</td>
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<td>Main effect of genotype on FA, AD, RD in ATR</td>
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<td>Main effect of genotype on FA, AD, RD in UF</td>
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<td>(Liu et al., 2010)</td>
<td>COMT val158met</td>
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<td>Decreased FA in right CST for Val/Val carriers</td>
<td>5.197* 79</td>
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<tr>
<td></td>
<td>Association with mean FA in left PF lobe</td>
<td>2.79* 68</td>
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<td>Association with mean FA in right PF lobe</td>
<td>3.58* 68</td>
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<td>Association with mean FA in right UF</td>
<td>3.507* 68</td>
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<td>(Roffman et al., 2011)</td>
<td>MTHFR 677T</td>
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<td>Reduced FA in bilateral DACC</td>
<td>6.59* 18</td>
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<td>(Pacheco et al., 2009)</td>
<td>5-HTTLPR</td>
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<td></td>
<td>Increasing number of low expressing alleles - decreasing FA in left FUF</td>
<td>-3.03* 37</td>
<td>-0.92</td>
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Table 3 Details of the structural connectivity studies using DTI included in this meta-analysis Note: n, sample size; FA, fractional anisotropy; MF, medial frontal; WM, white matter; ALIC, anterior limb of internal capsule; AD, axial diffusivity; RD, radial diffusivity; ATR, anterior thalamic radiation; UF, uncinate fasciculus; GCC, genu of corpus callosum; CST, corticospinal tract; PF, prefrontal; DACC, dorsal anterior cingulate cortex; FUF, frontal uncinate fasciculus; * p < 0.05; ** p < 0.001; *+ p < 0.05 family-wise error corrected
2.3.2 Functional Connectivity

A total of 44 effect sizes were calculated from the eight functional connectivity studies. Effect sizes (i.e., Cohen's $d$) ranged from medium to large ($d = 0.46-1.65$) with an average effect size of $0.76$ (SD $\pm$ 0.23). The largest effect size ($d = 1.65$) was reported for the impact of a single nucleotide polymorphism (SNP) in ZNF804A on functional connectivity within the right PFC in SZ patients (Rasetti et al., 2011). While large effect sizes ($d > 0.7$) were also calculated in other studies examining the effects of this SNP on functional connectivity (Esslinger et al., 2009; Rasetti et al., 2011) these results were not consistent: the smallest effect size was also reported for this SNP ($d = 0.46$; (Paulus et al., 2011).

2.3.3 Structural Connectivity

A total of 24 effect sizes were calculated for ten structural connectivity investigations. Effect sizes ranged from small to large ($d = 0.38-1.95$) with an average effect size of 1.04 (SD = 0.42). The largest effect size was revealed for the impact of NRG1 SNP on WM integrity in the left anterior thalamic radiation (ATR) (Sprooten et al., 2009). Large effect sizes were also observed for all the other studies examining the impact of NRG1 on WM integrity (all $d > 0.80$). Similar effect sizes were revealed for studies investigating the ErbB4 gene, with Cohen's $d$ for these studies ranging from 0.81 to 1.41. Both the MTHFR gene and the 5-HTT gene had large effect sizes of 1.29 and 0.92 respectively. The smallest effect size of 0.38 was computed for the effect of a COMT haplotype on left prefrontal WM integrity. Cohen's $d$ for the COMT papers ranged from 0.38 to 0.76.

2.3.4 Meta-Analysis

In the first meta-analysis, only the largest effect sizes in each study were considered (Figure 2; Table 4). This analysis revealed no significant difference in outcome variability between the effect sizes for functional and structural studies ($Q = 2.171$, $p = 0.141$). The
second analysis examined all of the effects for each result in each paper (Figure 3; Table 4). This analysis revealed a significant difference between effects sizes in functional and structural studies ($Q = 6.928, p = 0.008$).

![Figure 2 Forest plot reporting Hedges' $g$ and 95% CI for the analysis showing the largest effect size in each paper. CI, confidence interval; $g$, Hedges' $g$; SE, standard error.](image-url)
Figure 3 Forest plot reporting Hedges’ g and 95% CI for each functional and structural connectivity analysis. CI, confidence interval; g, Hedges’ g; SE, standard error.
Table 4. Results of random-effects meta-analysis comparing the relative difference in the impact of variants on functional and structural connectivity.

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<th>Structural</th>
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2.4 Discussion

The aim of this review was to consider the nature and magnitude of effect of SZ risk variants on functional and structural connectivity. Our focus was the overall magnitude of such effects, rather than delineating the direction of effect of a specific variant.
Therefore, we focused on the size, rather than the direction of individual results. Examining the effect of risk variants on connectivity in fMRI and DTI studies, we found that variation in genes implicated in neurotransmission, plasticity, development and myelin function are associated with altered neural connectivity. Meta-analyses of effect size data revealed that there was no significant difference between the effect sizes of functional and structural studies when the largest effect size of each study was analysed. However, when all effects were taken into consideration, the effect sizes for the structural studies were larger than in the functional connectivity studies, and there was a significant lack of homogeneity across the modalities. Most likely, given the absence of difference when only the largest effects from each set of studies are considered, this difference reflects the greater variation in effect sizes in structural studies compared to fMRI studies.

2.4.2 Structural Connectivity Studies

A number of candidate SZ risk genes have been investigated in terms of their effects on structural connectivity. This includes genes that are involved in myelination (NRG1, ErbB4) neurotransmission (COMT, MTHFR, 5-HTTLPR) and neurodevelopment (BDNF and DISC1). Almost all variants considered here were associated with significant variation in FA scores using DTI.

The average effect size for these studies was large, with the largest effect size computed for the impact of neuregulin1 (NRG1) on FA in the left anterior thalamic radiation (ATR) (Sprooten et al., 2009). The NRG1 gene codes for the NRG1 protein, which is involved in growth and differentiation of neuronal and glial cells and is necessary for the normal development of the nervous system. ErbB4 is a receptor for the NRG1 protein. It is thought that NRG1 may mediate its effects on SZ susceptibility through functional interaction with ErbB4 (Norton et al., 2006). Interestingly, ErbB4 was also observed here to show effects on WM integrity that would be considered to be of
large magnitude. The role of these genes in myelin function suggests a mechanism by which they confer risk for SZ. The relatively large impact of these genes on structural connectivity noted here, suggests that genetically mediated “dysconnectivity” in SZ results from macro-circuit WM abnormalities in addition to micro-circuit synaptic plasticity. However, since oligodendrocyte dysfunction may also impact synaptic function and information processing via a myelin-dependent impact on synaptic plasticity (Fields, 2008), it remains to be established if the influence of these variants is specific to structural connectivity or if they also impact upon functional connectivity.

Other gene variants associated with variation in WM connectivity included COMT, MTHFR, 5-HTTLPR, BDNF and DISC1. Results for both the MTHFR gene and the 5-HTT gene had large effect sizes. The MTHFR gene codes for an enzyme that plays a role in the regulation of intracellular methylation reactions and may influence dopamine signaling (Roffman et al., 2011). The hypofunctional 677T variant of this gene has been associated with increased SZ risk (Gilbody et al., 2007). The serotonin transporter (5-HTT) regulates the reuptake of serotonin to the presynaptic neuron for recycling or degradation. Although the 5-HTTLPR polymorphism has been found to be associated with SZ in a South India population (Vijayan et al., 2009), other genetic association studies have given conflicting results (Rao et al., 1998). The smallest effect size was computed for the effect of a COMT haplotype on left prefrontal WM integrity (Liu et al., 2010). The COMT gene codes for an enzyme that is involved in the degradation of dopamine. Therefore, the evidence from DTI studies investigating the impact of the COMT gene on WM indicates that neurosignalling processes involved in SZ may also impact structural connectivity. Finally, BDNF (Chiang et al., 2011) and DISC1 (Sprooten et al., 2011), genes that are crucial for neurodevelopment, were also associated with WM connectivity in SZ. However, as insufficient data was available, effect sizes for these studies were not calculated.
2.4.3 Meta-Analysis

The papers included in this review most commonly report a large effect of gene variants on functional and structural connectivity. This result is similar to previous meta-analyses in imaging genetics, which reported large effect sizes of gene variants (i.e., 5-HTTLPR polymorphism and the COMT Val158Met polymorphism) on brain function (Mier et al., 2010; Munafò et al., 2008). This result is also consistent with the intermediate phenotype hypothesis that common SZ risk variants will show small effects on behaviour and disease risk, but large effects at the level of the brain (Tost et al., 2012).

When the maximum effect size value for each paper in our meta-analysis was compared between fMRI and DTI studies, no significant difference was found between these measures. As only a small number of studies were obtained, there may be a lack of power to detect such differences. However, examination of effect sizes for all significant effects indicates that structural connectivity studies were associated with overall larger and more variable effect sizes. This suggests that measures of structural connectivity, such as DTI, may be sensitive to a wider range of effects compared to functional connectivity measures, which may only be able to accurately detect large effects. This result may also indicate that structural connectivity is closer to the level of genes than functional connectivity.

2.4.4 Limitations

A number of limitations need to be considered in evaluating the findings of the present study. Firstly, many of the studies included in the meta-analysis have examined the effects of polymorphisms that do not have consistent association with SZ phenotypes. This makes it difficult to determine the relevance of these genes for our understanding of SZ pathogenesis (for a review, see (Meyer-Lindenberg, 2010a). Secondly, it should also be noted that the sample sizes included in these studies are relatively small and thus,
are underpowered to detect differences in brain connectivity conferred by individual variants. Due to the interplay between sample size, power, and effect size, smaller studies generally show larger effects in meta-analyses (Sterne et al., 2000) and may lack sufficient power to detect smaller effects. Related to the general issue of sample size, it is important to note that the average sample size of the studies utilizing DTI was smaller than that for the functional studies. As a result, the effect sizes for the structural papers may be over-inflated. However, the results of our meta-analysis suggest that despite smaller samples, the structural imaging studies were associated with a wider range of effects, suggesting that sample size is not the only factor at play here.

Due to the under-representation of publications with negative results, the studies included in this review may not be representative of connectivity research in its entirety, but rather a bias toward only published papers showing statistically significant results. Therefore, while our effect size findings are calculated on the basis of published effect sizes, it is possible that the true effect sizes are smaller, and to an extent that is unknown. Similarly, it is also unclear to what extent differences in scanning parameters between the studies included in this meta-analysis influenced results. More systematic investigation of these differences will be possible in the future with the accumulation of more studies.

An additional limitation in the studies considered here is that each investigation examined the effects of only one particular variant. However, the true function of these genes may be affected by additive or epistatic interactions with other variants. As such, the results presented in this review may be incomplete without taking these interactions into account (Nicodemus et al., 2010).

Finally, it is probable that these results could be impacted by differences in functional and structural methodological approaches. For example, a number of analysis methods
can be employed to measure functional connectivity between brain regions. However, we are not currently aware of the relative strengths and weaknesses of these different approaches. There are also various approaches used to quantify WM connectivity using DTI, which also pose different strengths and limitations (see Jones, 2008 for a review).

2.4.5 Conclusions and Future Directions

As illustrated by this meta-analysis, imaging genetics has made important progress in delineating genetic effects on neural connectivity. In particular, it has established neural connectivity as a key intermediate phenotype for SZ, which can be used to explore the complex trajectory from genetic risk to clinical symptoms.

Despite the progress that has taken place, important advances can be made in this research field in four key areas. Firstly, future studies should examine the effects of gene variants on neural connectivity in larger sample sizes, as this can provide the extra statistical power that may be necessary to detect smaller effects of these genes. Multi-site research projects, such as the IMAGEN project in Europe, may be particularly suited for compiling imaging and genetic databases of thousands of subjects (Schumann et al., 2010). Secondly, future studies should examine additive and epistatic effects of gene variants on neural connectivity, as these variants are unlikely to be working in isolation. Thirdly, future studies should examine the effects of risk variants in healthy controls and SZ patients as the opposite effects of these genes on connectivity can be found in these different groups (e.g. Prata et al., 2009).

Finally, future studies could benefit from the novel application of recently developed analysis techniques to imaging genetics. For example, dynamic causal modelling (DCM) holds potential for constructing models of changing brain interactions that also takes into account genetic variation (Meyer-Lindenberg, 2009). Other recent advances include the
use of parallel independent component analysis (ICA) to simultaneously analyze independent components derived from fMRI and genetic data (J. Liu et al., 2009). For example, Meda et al., (2010) used this technique in a pilot study to identify simultaneous independent components of fMRI data and SNP data, derived from a sample of 35 controls and 31 SZ patients. The authors found correlations between different neural networks and a number of SNPs, including polymorphisms involved in altered dopamine transmission. While the authors only included a small number of SNPs and a small sample size, this research suggests a powerful new approach for future studies examining the effects of SZ risk variants on functional brain networks. Similarly, more advanced DTI techniques could be implemented that use high angular resolution to account for multiple crossing fibers within a single voxel. Such imaging techniques include Q-space approaches and mixture models (Tournier et al., 2011). These models provide mathematical alternatives to the tensor model for the characterization of diffusion processes. Furthermore, Jones (2008) recommends the integration of DTI with other measures of WM such as measures of axon density and myelination that can be acquired using techniques such as magnetization transfer or multicomponent relaxometry.

In conclusion, the present meta-analysis examined the nature and magnitude of effect of SZ risk variants on functional and structural connectivity. Gene variants impacting upon both synaptic plasticity and axonal connectivity have been associated with altered neural connectivity in patients and healthy controls. As such, it is likely that both mechanisms make important contributions to SZ pathogenesis. On average, risk variants exert a large effect on functional and structural connectivity. There is also more variability in the effects of variants on structural connectivity, compared to functional connectivity. While imaging genetics has made considerable progress in the field of neural connectivity in a short period of time, important advances are still to be made. It is hoped that this
research will lead to a better understanding of the biological mechanisms mediating genetic risk for SZ, which can then be used to direct novel treatments for the disorder.
3. Investigating the Impact of Schizophrenia GWAS Variant rs7914558 at CNNM2 on White Matter Microstructure: DTI study
3.1 Introduction

In Chapter 1, the potential utility of DTI as an endophenotype for SZ was outlined in detail. Compromised WM integrity is frequently associated with SZ (Ellison-Wright & Bullmore, 2009; Kubicki et al., 2007) with a meta-analysis revealing two WM affected networks firstly in the frontal lobe consisting of WM interconnecting the frontal lobe, thalamus and cingulate gyrus and, secondly, a network in the temporal WM interconnecting the frontal lobe, insula, hippocamups-amygdala and occipital lobe (Ellison-Wright & Bullmore, 2009). WM abnormalities are apparent in first-degree relatives, individuals at high risk of SZ, and in patients during the early stages of illness, suggesting that these abnormalities may represent a stable characteristic of the disease under strong genetic control (Kochunov et al., 2010; Pérez-Iglesias et al., 2010; Witthaus et al., 2008) and therefore a potential endophenotype for SZ (Mothersill et al., 2012).

The focus of this chapter is to investigate the extent to which the genome-wide significant SNP at the cyclin M2 (CNNM2) gene impacts WM microstructure. This introduction provides a brief description of CNNM2 and outlines the evidence associating this variant to WM microstructure or brain structure more generally. The tract-based and voxel-wise methods used to measure WM integrity are also described in this section.

3.1.1 Gene of interest - CNNM2/rs7914558

In one of the largest SZ GWAS conducted to date, consisting of over 51,000 patients and controls (Ripke et al., 2011), five novel loci were identified. One of these loci on chromosome 10q34.33 contains two variants 130kb apart reaching genome-wide significance, including rs7914558, located in an intron of the CNNM2 gene ($P = 1.8 \times 10^{-5}$) and rs11191580 ($P = 1.1 \times 10^{-6}$), implicating a 0.5-Mb region containing multiple...
genes. The importance of this gene to SZ risk was recently further supported by Aberg et al. (2013). CNNM2 has also shown a significant cross-disorder association for major depressive disorder as well as SZ (Smoller et al., 2013). CNNM2 is implicated in magnesium (Mg^{2+}) transport and plays an important role in Mg^{2+} homeostasis by modulating Mg^{2+} concentration. CNNM2 messenger RNA (mRNA) is also upregulated when there is a magnesium deficiency in the brain (Goytain, 2005). Furthermore, increased extracellular Mg^{2+} blocks CNNM2 mediated Mg^{2+}-sensitive sodium (Na^+) currents (Meyer et al., 2010). Hypertension risk and altered serum Mg^{2+} are associated with sequence variation in CNNM2 (Meyer et al., 2010; Stuiver et al., 2011) and mutations in CNNM2 are associated with hypomagnesaemia risk (Stuiver et al., 2011).

In a sample of 173 patients and 449 healthy controls, Ohi et al. (2013) revealed that the risk variant at CNNM2 was associated with variation of voxel-based grey matter volumes in the bilateral inferior frontal gyri. Subjects homozygous for the risk G allele rs7914558 had smaller grey matter volumes in the bilateral inferior frontal gyri than carriers of the non-risk A allele. Using bioinformatics data to establish if the rs7914558 genotype might be an expression quantitative trait loci (eQTL), in silico analysis showed that the CNNM2 gene expression of the risk G allele of the AG polymorphism was significantly lower than that of the non-risk genotype in combined lymphoblast-derived HapMap CEU and YRI samples. Ohi and colleagues (2013) reported that low expression of this gene resulted in increased Mg^{2+} levels. As increased extracellular Mg^{2+} concentrations cause a decrease in the activity of the glutamate N-methyl-D-aspartate (NMDA) receptor (Kochlamazashvili et al., 2012), the authors concluded that the risk CNNM2 allele might play an important role in the hypofunction of NMDA receptors, which is implicated in the pathophysiology of SZ.
To establish the potential effects of this gene on neurocognitive processes, Rose et al. (2014) found an association of CNNM2 with social cognition in a sample of 400 patients and 160 controls. Despite the potential role of CNNM2 in NMDA receptor hypofunction, Rose et al. (2014) found no effect of this variant on memory or general cognition. However, the risk allele was associated with reduced externalizing bias in both patients and controls. Externalizing bias is the tendency to attribute positive events to oneself and negative events to others. Decreased externalizing bias has been linked to increased depressive symptoms, however, in a post-hoc analysis, Rose et al (2014) found no association of CNNM2 with symptom severity scores (i.e. Scale for Assessment of Positive Symptoms (SAPS) and Scale for Assessment of Negative Symptoms (SANS) scores) in the sample. This suggests that variability in attributional bias associated with CNNM2 may not lead to clinical pathology. Although CNNM2 may be associated with variation in social attributions, these effects may not be deleterious. Structural imaging was also carried out on a sub-sample using voxel-based morphometry (VBM) to assess volumetric measures in areas associated with social cognition. In contrast to Ohi et al’s (2013) study, the risk CNNM2 allele was associated with increased grey matter volume in the right temporal pole and the right anterior cingulate.

These data suggest that CNNM2 is associated with variation of brain structure, social cognition and possibly NMDA receptor hypofunction. However, the exact mechanism or mechanisms by which this variant increases SZ risk or causes variability in intermediate phenotypes related to SZ remains unknown. Magnesium additionally plays a role in WM development and is required for the production of myelin sheaths (Gong et al., 2003). Consequently, Mg$^{2+}$ is considered a WM protectant (Wiseman et al., 2009) and therefore genetic variations at CNNM2 may be associated with variation in WM structure via an impact on Mg$^{2+}$ levels in the brain.
Summary

GWAS is a hypothesis-free technique to detect genetic variations in a clinical population and therefore the endeavor to elucidate the role of GWAS polymorphisms in disease is often quite exploratory in nature. However, the endophenotype approach has helped to shed some light on their function and how they may be related to pathophysiology of SZ.

3.1.2 Aims and hypotheses

The aim of the present study was to examine the effect of a GWAS variant at CNNM2 on WM microstructure using whole brain tract-based spatial statistics (TBSS) and tractography of five tracts that form part of the previously identified frontal and temporal WM networks altered in SZ (Ellison-Wright & Bullmore, 2010), namely the inferior longitudinal fasciculus (ILF), the uncinate fasciculus (UF), the inferior frontal occipital fasciculus (IFOF), the anterior thalamic radiation (ATR) the cingulum bundle (CB) and the corpus callosum (CC) (See Chapter 1).

As the exact function of these variants remains unclear, the formation of hypotheses regarding their effect on WM is problematic. However, considering the evidence outlined above and the establishment of WM integrity as a suitable endophenotype for SZ, the following hypothesis was devised:

- Given that CNNM2 is implicated in Mg2+ transport and homeostasis and as Mg2+ is required for myelination and WM protection, it is hypothesized that CNNM2 will result in variation in measures of WM microstructure and integrity as indirect measures of myelination.
3.2 Methods

3.2.1 Participants

The DTI sample consisted of 152 healthy participants recruited from the general population through local advertising. Furthermore, 59 participants with a diagnosis ofSZ were also recruited; however, after data quality checks and genotyping, the sample was not large enough for the recommended minimum sample size of 80 subjects for an imaging genetics analysis (Meyer-Lindenberg, 2010b). All participants were genetically Irish (i.e. had Irish born paternal and maternal grandparents), were between 18 and 65 years of age and were right-handed. Written, informed consent was obtained from all subjects in accordance with local ethics committee guidelines (see Supplementary section A). Exclusion criteria included a significant neurological or psychiatric history, a first-degree relative with a diagnosis of SZ or other psychosis, substance abuse in the preceding six months, pregnancy or other contraindication for MRI. Participants were not paid for participation but were offered re-imbursement for study related expenses.

3.2.2. Genotyping

Genetic analyses were based on DNA extracted from saliva samples obtained using Oragene DNA self-collection kits (DNA Genotek; Ontario, Canada). All SNPs of interest were genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium (p > 0.05). Along with these samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for each SNP for quality control purposes and were found to be concordant with available HapMap data for these SNPs.
3.2.3 DTI

Magnetic resonance images were collected using a 3-T Philips Achieva scanner. DTI images were acquired via a single-shot spin-echo echo planar imaging (EPI) with diffusion sensitizing gradients applied sequentially along 15 non-collinear directions with a b-value of 800s/mm² (TR= 12445ms, TE= 52ms, FOV=224mm x 224mm x 149mm, acquisition matrix=112 x 112, reconstruction matrix=128 x 128, 60 slices, 2.2mm slice thickness, slice gap = 0.299mm, spatial resolution = 2mm x 2mm x 2.2mm, flip angle = 90°).

3.2.4 Preprocessing

Using ExploreDTI software (Leemans et al., 2009), diffusion data were converted to ExploreDTI*.mat files with a voxel size of 2x2x2mm. Diffusion tensor estimation was weighted linear and was based on the least-squares (LS) regression model which takes into account the signal variability produced by thermal noise by including the signal variance as a weighting factor in the tensor fitting (Basser et al., 1994). A cubic interpolation and robust estimation of tensors by outlier rejection (RESTORE) (Chang et al., 2005) was used to correct for subject motion and cardiac pulsation artefacts. Data processing also routinely employed Eddy Current correction for any distortion from electric currents induced by the MRI scanner. B-matrix rotation was also used when realigning the diffusion-weighted images to correctly preserve orientational information (Leemans & Jones, 2009). A quality check of the corrected diffusion data was then performed by visually inspecting each direction of each diffusion image. Residual and outlier profiles were also examined using ExploreDTI. Finally the motion correction parameters were inspected. Movement during scanning was less than 2mm in any direction and less than 3° rotation in axial, sagittal or coronal planes for all participants. Twelve subjects were removed from analysis due to poor quality data or excess motion during scanning.
3.2.5 Atlas-based deterministic tractography

Atlas-based deterministic tractography was conducted using ExploreDTI 4.8.3. Fibre tracking or 'tractography' uses voxel-based estimates of the underlying continuous fibre orientation to infer the three-dimensions of WM tracts (Jones, 2008). Deterministic tractography assumes that the principal eigenvector is parallel to the underlying dominant fibre orientation in each voxel and forms a tangent to the space curve traced out by the WM tract (Basser et al., 2000). A single bi-directional pathway is initiated from a "seedpoint" and moves in a parallel direction with the principal eigenvector (Jones, 2008).

All data were transformed into Montreal Neurological Institute (MNI) space and a whole-brain WM tract construction was then carried out for each participant using a linear interpolation that allows for an estimation of the underlying tensor field at a sub-voxel level. This is required as it is assumed that the underlying tensor field is continuous and the step size is usually fixed (e.g., to a 10th of the voxel dimension) (Jones, 2008). This allows an estimation of the tensor field to be obtained at any point within the image, i.e., not just at the centre of an image voxel (Jones, 2008). Seed point resolution was then set at 2x2x2 mm. A seed fractional anisotropy (FA) threshold of 0.2 was specified to prevent the tract reconstruction from entering into the grey matter and/or regions where the principal fibre orientation is poorly defined. To prevent unrealistic turns in fibre orientation and to prevent the tract reconstruction from doubling back on itself, an angle threshold of 45 degrees was used.

Then, particular tracts implicated in SZ were isolated using the automated atlas based tractography segmentation tool shown to yield robust and reliable tract delineation (Verhoeven et al., 2009). These were identified as: the UF, the ILF, the IFOF, the ATR, the CB and the CC. Isolation of tracts was conducted by drawing two ROIs through which the tracts passed (using Boolean inclusion "AND" gates) and excluding areas that
the tracts didn't pass through (using exclusion "NOT" gates). The appropriate AND and NOT gates were drawn for each tract based on a randomly selected single subject. Using this information the tract of interest was automatically reconstructed for all data sets.

All ROIs were drawn on the coloured fractional anisotropy–weighted maps. For the ATR, that connects, via the anterior limb of the internal capsule, the anterior and medial thalamic nuclei and the cerebral cortex of the frontal lobe, the first ROI was placed on a coronal slice around the thalamus. The second ROI was placed on a coronal slice around the temporal pole and orbital frontal cortex. ROIs using "NOT" gates were drawn on the sagittal plane to avoid fibres from the IFOF, and fibres crossing over to the opposite hemisphere. Finally a "NOT" gate was also drawn on an inferior axial slice to avoid fibres from the cortico-spinal tract (See Figure 4 (a)).

The CB projects from the cingulate gyrus to the entorhinal cortex. Two "AND" ROIs were placed at the anterior and posterior division of the cingulate gyrus. A "NOT" operator was used to avoid fibres crossing from opposite hemispheres (See Figure 4 (b)).

The IFOF runs from the frontal lobes, through the temporal lobes and terminates in the posterior parietal cortex. The first ROI was placed in an anterior coronal slice around the orbital frontal cortex and the second ROI was placed in a posterior coronal slice in the lateral occipital cortex. A NOT gate was drawn on the sagittal plane to avoid fibres crossing over to opposite hemispheres (See Figure 4 (c)).

The ILF connects the temporal and occipital lobes. The first ROI was drawn on a coronal slice in the temporal lobe around the parahippocampal gyrus and the second ROI was
drawn on a coronal slice around the lateral occipital cortex. We used the "NOT" operator to avoid fibres crossing from opposite hemispheres (See Figure 4. (d))

For the UF, which connects the anterior temporal lobe with the orbitofrontal cortex and inferior frontal gyrus, ROIs were drawn on a coronal slice around the temporal lobe and parahippocampal gyrus. The second ROI was placed at a more anterior coronal slice and was drawn to include projections to the frontal pole and orbital frontal cortex. ROIs using "NOT" gates were drawn on the saggital plane to avoid fibres from the IFOF, and fibres crossing over to the opposite hemisphere. Finally a "NOT" gate was also drawn on an inferior axial slice to avoid fibres from the cortico-spinal tract (See Figure 4 (e)).

The CC did not require any "NOT" or "AND" gates as it was accurately delineated for each subject using the automated tractography atlas in ExploreDTI (See figure 4 (f)).

After performing the tractography, diffusion metrics including mean fractional anisotropy (FA) mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) were extracted for each tract. These diffusion metrics are defined by the intrinsic descriptive properties of the diffusion ellipsoid, which is derived from the rotationally invariant scalar parameters known as eigenvalues (L1, L2 and L3), corresponding to the three respective principle directions or eigenvectors used to characterize the size, shape and orientation of the ellipsoid. FA has a value between zero and one that indicates the degree of anisotropy of diffusion. A value of zero indicates that diffusion is isotropic, i.e. it is unrestricted (or equally restricted) in all directions. A value of one indicates that diffusion is occurring along one axis and is fully restricted along all other directions. Therefore high FA values are thought to reflect high levels of WM integrity but this should be interpreted with caution (Alba-Ferrara & de Erausquin, 2013). MD is representative of unrestricted diffusion in all directions and is the mean of the total diffusivity within a voxel. High MD values are thought to be indicative of low axonal
density and cortical atrophy (Narr et al., 2009). RD and AD measures, which are components of MD, were also extracted for each tract. Radial diffusivity (RD) or perpendicular diffusivity is the mean of the two minor eigenvectors of the tensor and is sensitive to changes in the membrane restriction of diffusion. Decreases in myelination correlate with high levels of RD (Song et al., 2005; 2002). Finally, AD or longitudinal diffusivity is the measure of diffusion along the major eigenvector and is thought to reflect levels of axonal damage and/or the extent of axonal packing (Song et al., 2002).
Figure 4 Atlas-based deterministic tract reconstruction of the ATR (A. ii and i), CB (B. I and ii), IFOF (C i and ii), IFL (D i and ii), UF (E i and ii) and CC (F) for left and right hemispheres.
3.2.6 Whole-brain TBSS

TBSS (Smith et al., 2006; 2004) combines elements of both VBM and tractography methods but solves common problems associated with these methods including alignment and smoothing as well as permitting the delineation of WM throughout the whole brain without the need to predefine tracts. TBSS of the FA and MD data were performed using FSL v4.1.6 (Smith et al., 2004) to determine if any potential genotype effects were specific to the tracts selected or if they were more generalized. If significant effects were observed for the FA and/or MD data, then further TBSS analysis of the AD and RD data would also be carried out. The main steps of TBSS are outlined below.

Step 1: Nonlinear alignment

All subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT (Andersson, Jenkinson, & Smith, 2007a; Andersson, Jenkinson, Smith, & Andersson, 2007b) that uses a b-spline representation of the registration warp field (Rueckert et al., 1999). FA images were registered to FMRIB58_FA standard-space image, an averaged FA map included with the software package. This option is recommended as it involves only one registration for each subject.

Step 2: Creating Mean FA Image and Skeleton

Once all FA images were aligned to the target, the entire data set was affine transformed into 1mm\(^3\) MNI152 space. The higher resolution was used to avoid significant interpolation blurring (i.e., increase in partial voluming) when the nonlinear warp plus standard-space affine transformation was applied to each subject's data (Smith et al., 2006). The transformed FA images were then averaged to create a mean FA image. This is a relatively smooth image as a result of averaging FA images across subjects.
The mean FA was then used to generate a tract skeleton that represents all tracts common to all subjects. Each tract of the skeleton is represented as a single line running down the centre of the tract. Away from the centre surface or line, the FA values decrease gradually, moving out of WM.

To perform skeletonisation the local surface perpendicular direction (at all voxels in the image) was estimated and then non-maximum-suppression was carried out in this direction (Smith et al., 2006). In other words, a search for voxels with maximum FA values is made along all voxels in the local “tract perpendicular direction”, and the voxel with the highest FA is identified as the centre of the tract (Smith et al., 2006).

To establish the orientation of the tract surface, it is assumed that if the voxel of interest lies away from a tract centre, FA will be higher in neighbouring voxels on one side of the voxel than on the other and the direction in which it is highest points towards the nearest tract centre (Smith et al., 2006). This is quantified by finding the centre of gravity of the local 3 x 3 x 3 voxel neighbourhood. The vector from the current voxel centre to the local centre-of-gravity (CofG, of FA values) should point towards the tract centre, in a perpendicular direction to the local tract structure (Smith et al., 2006). Therefore, as long as the centre of the current voxel does not lie close to the local CofG (within 0.1 mm), the perpendicular direction is assumed to be given by this vector (Smith et al., 2006).

However, if the centre of the current voxel is close to the local CofG and the current voxel is very close to the tract centre, a different method to estimate the perpendicular is used. In this case, the direction of maximum change is found from the local 3 x 3 x 3 voxel neighbourhood (Smith et al., 2006). The mean of each opposing pair of voxels is subtracted from the centre value, and the direction that causes the largest difference is thought to be perpendicular to the local tract (Smith et al., 2006). See Figure 5.
Then to form the tract skeleton, the centre of the tract was determined. This is achieved by comparing the FA value each with the two closest neighbours on each side, in the direction of the tract perpendicular. If the FA value is greater than the neighbouring values, then the voxel is identified as lying on the skeleton.

The FA skeleton was then thresholded in order to restrict further analysis to points that are within WM which have been successfully aligned across subjects. A threshold of 0.2 mean was used in this case to excludes grey matter or cerebro-spinal fluid (CSF) voxels. See Figure 6, for a depiction of the different skeletonisation stages.
Figure 6 Different skeletonisation stages. (A) Original mean FA image with final skeleton and the ROI used for the remaining sub-images. (B) Skeletonisation stage 1, using local FA centre-of-gravity to find tract perpendiculars. (C) Skeletonisation after stage 2, using FA image second-derivative to find remaining perpendiculars. (D) Result of smoothing the perpendicular direction vector image (adapted from Smith et al., 2006).

Step 3: Projecting individual subjects' FA onto the skeleton via perpendicular search for local tract centre

Each subject’s aligned FA image was then projected onto the mean FA skeleton. At each point in the skeleton, the maximum FA value, in a perpendicular direction, of a given subject’s FA image is assigned to the skeleton voxel (Smith et al., 2006). This achieves alignment between the skeleton and this subject’s FA image. This assignment is valid only in a perpendicular direction as the change in FA value is greatly pronounced in this direction in comparison to parallel direction (Smith et al., 2006). During the projection of individual FA maps onto the skeleton, a distance map is used to encode how far the nearest skeleton voxel is from each brain voxel to ensure that values are only taken onto the nearest part of the skeleton (See Figure 7).
For MD data, the FA images were used to achieve the nonlinear registration and skeletonisation stages, and also to estimate the projection from each individual subject onto the mean FA skeleton. The nonlinear warps and skeleton projection were then applied to MD data. If significant differences were observed for FA or MD data then RD and AD data were further analysed using the same method.

3.2.7 Statistical analysis - Tractography

The diffusivity indices for each participant were exported to PASW statistical software (Release 18; SPSS Inc., Chicago, IL, USA) and subjected to a one-way multivariate analysis of covariance with genotype group (AA, AG, GG) as fixed factors. Age and gender did not significantly differ between genotype groups and therefore these variables were included as covariates of no interest in the analysis as a matter of standard protocol (Miller & Chapman, 2001). As FA, MD, RD and AD are constructed from the three eigenvalues of the tensor, an alpha level of $p = .017$ was applied to Bonferroni correct for testing of multiple diffusion measures arising from three eigenvalues.
3.2.8 Statistical analysis – TBSS

Statistical analysis for TBSS was conducted using FSL's 'Randomise' algorithm. Randomise is a permutation program that enables modelling and inference using standard general linear model (GLM) design setup. It can output voxelwise and cluster-based tests. Randomise incorporates a cluster-based thresholding option called Threshold-Free Cluster Enhancement (TFCE) (Smith & Nichols, 2009). TFCE is a proposed method to enhance cluster-like structures in an image without having to define an initial cluster-forming threshold or carry out a large amount of data smoothing. TFCE was selected using 5000 permutations per test. Threshold-free cluster enhancement produced an output image from a raw statistic image. The voxelwise values in this output image represented the amount of cluster-like local spatial support (Smith & Nichols, 2009). Each TFCE score of each voxel was given by the sum of the scores of all supporting sections. A GLM f-test design was constructed to look for an overall difference, along with six post-hoc t-tests to compare between the three genotype groups. The statistical threshold was set at $p<0.05$, family-wise error corrected (FWE) corrected for multiple comparisons across the whole brain to find differences between genotype groups. Post-hoc t-tests were further Bonferroni-corrected for the six comparisons, as well as the three eigenvalues and an alpha level of 0.0027 (0.05/18) was implemented. Areas of significant differences were identified using the following FSL tools: the Harvard-Oxford Structural Atlas and the JHU ICBM-DTI and tractography atlases (www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#wm).

3.3 Results

3.3.1 Participant Demographics

Tables 5 below outlines participant demographics based on rs7914558 genotype.
<table>
<thead>
<tr>
<th>CNNM2 rs7914558</th>
<th>N</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>21</td>
<td>7</td>
<td>14</td>
<td>35.29 (13.0)</td>
<td>16.58 (2.92)</td>
</tr>
<tr>
<td>AG</td>
<td>45</td>
<td>21</td>
<td>24</td>
<td>31.11 (10.69)</td>
<td>17.4 (3.10)</td>
</tr>
<tr>
<td>GG</td>
<td>59</td>
<td>32</td>
<td>27</td>
<td>32.02 (11.71)</td>
<td>16.97 (2.93)</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>60</td>
<td>65</td>
<td>32.24 (11.71)</td>
<td>16.97 (2.92)</td>
</tr>
</tbody>
</table>

Table 5 Participant demographics based on rs7914558 genotype (A is minor allele; G is risk allele)

3.3.2 Tractography

Tractography was conducted on five tracts for both hemispheres including the ATR, CB, ILF, IFOF and the UF. After covarying for age and gender, an effect of genotype was observed for fibres of the right ATR ($F_{(2, 114)} = 4.51$, $p = .013$, partial $\eta^2 = .073$) with homozygous as well as heterozygous G allele carriers showing increased FA in comparison to A homozygotes, surviving Bonferroni correction (See Figure 8 (a)). An effect of genotype on FA of the left IFOF fibres was also ascertained ($F_{(2,113)} = 3.88$, $p = .023$, partial $\eta^2 = .064$) as well as the right ILF ($F_{(2,113)} = 3.97$, $p = .022$, partial $\eta^2 = .065$) with risk G homozygotes displaying increased FA in comparison to both A heterozygous and homozygous carriers. However, this did not survive Bonferroni correction.

An effect of genotype was observed for MD in the left IFOF ($F_{(2,113)} = 6.62$, $p = .002$, partial $\eta^2 = .105$), the right ILF ($F_{(2,113)} = 7.54$, $p = .001$; partial $\eta^2 = .118$) with risk homozygote and heterozygote G allele carriers displaying decreased MD in comparison to nonrisk A homozygotes, surviving Bonferroni correction (see Figure 8 (b) and (c)). The right UF also displayed a genotype effect for MD ($F_{(2,113)} = 4.82$, $p = .01$, partial $\eta^2 =$
.079) with decreased MD in homozygous G carriers compared to A carriers, also surviving Bonferroni correction (see Figure 8 (d)).

An effect of genotype on RD was noted for the left IFOF ($F_{(2, 113)} = 7.59, p = .001$, partial $\eta^2 = .118$), and the right ILF for RD ($F_{(2, 113)} = 6.92, p = .001$, partial $\eta^2 = .109$) due to decreased RD in G allele carriers in comparison to homozygous A allele carriers, all surviving Bonferroni correction (see Figure 8 (e) and (f)). An effect of genotype for fibres of the right UF was also observed ($F_{(2, 113)} = 4.76, p = .01$, partial $\eta^2 = .078$) with G homozygotes displaying decreased RD in comparison to A homozygotes, also surviving Bonferroni correction (See Figure 8 (g)).

![Figure 8 Differences between rs7914558 genotype groups in measures of a) fractional anisotropy (FA) of the right anterior thalamic radiation, b) mean diffusivity (MD) of the left inferior fronto-occipital fasciculus (IFOF), c) MD of the right inferior longitudinal fasciculus (ILF), d) MD of the right uncinate fasciculus, (UF), e) radial diffusivity (RD) of the left IFOF, f) RD of the right ILF and g) RD of the right UF). * p < .05; ** p < .01. Error bars represent +/- 2 standard error.
3.3.3 TBSS

After family-wise error (FWE) correction using threshold free cluster enhancement (TFCE, Smith & Nichols, 2009) and Bonferroni correcting for 18 comparisons, two of the six one-tailed t-tests remained significant. A allele homozygotes displayed decreased FA in comparison to homozygous G carriers in the right IFL, right IFOF, splenium of corpus callosum, forceps minor, left IFOF, left ILF and right corticospinal tract (p = 0.0027) (See Table 6 and Figure 9 (b)).

In comparison to AG carriers, A allele homozygotes had decreased FA in the left IFOF, left ILF, right corticospinal tract, right IFOF and right ATR (p < 0.0027) (See Table 7 and Figure 9 (a)).

For the MD data, homozygous A carriers had increased MD in a large cluster of the forceps minor and the ATR in comparison to G allele homozygotes (p = 0.035), however this did not remain significant after Bonferroni correction (See Table 8 and Figure 10).

Finally, two t-tests for the RD data remained significant after Bonferroni correction. Homozygous A carriers displayed increased RD in clusters of the left IFOF, (p < .0027) (See Table 9 and Figure 11 (a)). Similarly, homozygous A carriers displayed increased RD in comparison to homozygous G carriers in clusters of the forceps minor (p < .0027), remaining significant after Bonferroni correction for multiple comparisons (See Table 10 and Figure 11 (b)).
<table>
<thead>
<tr>
<th>Structure</th>
<th>p</th>
<th>t</th>
<th>Cohen's d</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior fronto-occipital fasciculus/Inferior longitudinal fasciculus/</td>
<td>0.001</td>
<td>2.39</td>
<td>0.54</td>
<td>Right</td>
<td>2874</td>
<td>38</td>
<td>-35</td>
<td>-1</td>
</tr>
<tr>
<td>Splenium of corpus callosum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Genu of corpus callosum/forceps minor                                    | 0.001| 2.73 | 0.62      | N/A        | 1371   | 6     | 27    | -1    |

| Inferior fronto-occipital fasciculus/Inferior longitudinal fasciculus    |       |      |           | Left       | 392    | -41   | -35   | -5    |
| Anterior thalamic radiation                                              |       |      |           |            |        |       |       |       |

| Corticospinal tract                                                      |       |      |           | Right      | 256    | 17    | -13   | -8    |

Table 6 Regions of increased FA for GG carriers in comparison to AA carriers (p < .0027, FWE corrected)

<table>
<thead>
<tr>
<th>Structure</th>
<th>p</th>
<th>t</th>
<th>Cohen's d</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior longitudinal fasciculus/Inferior fronto-occipital fasciculus</td>
<td>0.000</td>
<td>2.13</td>
<td>0.53</td>
<td>Left</td>
<td>11698</td>
<td>-36</td>
<td>-45</td>
<td>4</td>
</tr>
</tbody>
</table>

| Corticospinal tract                                                      | 0.001| 2.95 | 0.74      | Right      | 256    | 17    | -13   | -8    |

| Inferior fronto-occipital fasciculus/Anterior thalamic radiation         |       |      |           | Right      | 26     | 25    | 19    | 13    |

Table 7 Regions of increased FA for AG carriers in comparison to AA carriers (p < .0027, FWE corrected)
<table>
<thead>
<tr>
<th>Structure</th>
<th>p</th>
<th>t</th>
<th>Cohen's d</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forceps minor</td>
<td>0.035</td>
<td>2.26</td>
<td>0.51</td>
<td>Left</td>
<td>1790</td>
<td>-9</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td>Forceps minor</td>
<td>0.039</td>
<td>2.25</td>
<td>0.51</td>
<td>Right</td>
<td>1185</td>
<td>19</td>
<td>39</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 8 Regions of increased MD for AA carriers in comparison to GG carriers (p < .0027, FWE corrected)

<table>
<thead>
<tr>
<th>Structure</th>
<th>p</th>
<th>t</th>
<th>Cohen's d</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior fronto-occipital fasciculus</td>
<td>0.002</td>
<td>3.12</td>
<td>0.78</td>
<td>Left</td>
<td>605</td>
<td>-34</td>
<td>-40</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 9 Regions of increased RD for AA carriers in comparison to AG carriers (p < .0027, FWE corrected)

<table>
<thead>
<tr>
<th>Structure</th>
<th>p</th>
<th>t</th>
<th>Cohen's d</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forceps minor</td>
<td>0.00</td>
<td>2.63</td>
<td>0.61</td>
<td>Left</td>
<td>1977</td>
<td>-8</td>
<td>29</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 10 Regions of increased RD for AA carriers in comparison to GG carriers (p < .0027, FWE corrected)
Figure 9 a) regions showing decreased FA for AA homozygotes in comparison to AG carriers (p < .0027 FWE corrected), b) regions showing decreased FA for AA homozygotes in comparison to GG homozygotes (p < .0027 FWE corrected)

Figure 10 regions showing increased MD for AA homozygotes in comparison to GG homozygotes (p < .05 FWE corrected)
3.4 Discussion

3.4.1 Summary of results

In this study we sought to characterize the effects of the GWAS SZ risk variants at CNNM2 on WM microstructure in healthy controls. Six WM tracts were selected for analysis based on previous evidence of their association with SZ pathophysiology (Ellison-Wright & Bullmore, 2009; Kubicki et al., 2007) and SZ related clinical symptom severity and cognitive deficits (Ashtari et al. 2007; Szeszko et al. 2008; Mamah et al. 2010). Based on a number of related diffusivity metrics, CNNM2 was associated with differences in diffusivity along four of the five WM tracts investigated. Differences in WM microstructure were observed using measures of MD and RD in (1) the right ILF, (2) the right UF and (3) the left IFOF, and using measures of FA (4) in the right ATR. In each case the risk G allele carriers showed significantly increased FA or decreased MD and RD in comparison to the non-risk homozygous group. For one of these tracts, the UF, the difference was between the homozygous carriers versus non-carriers; in all other
cases differences were observed between non-carriers and both the heterozygous and homozygous carrier groups. In addition to the fact that we saw an effect in four of the six tracts investigated (and in the same direction across these tracts) the robustness of CNNM2's effects on WM connectivity was also indicated by the size of effects observed. Based on partial $\eta^2$ values, these ranged from .073-0.118, indicating that CNNM2 rs7914558 explained 7-12% of WM variation in this sample, comparable to effect sizes found in previous imaging genetic studies using DTI (Mothersill et al., 2012). Finally, the majority of these findings remained significant even after a relatively conservative Bonferroni correction for multiple testing.

Post-hoc voxelwise TBSS analyses were conducted to determine if our genotype effects were specific to the tracts selected or if they extended to additional WM fibres. Genotype effects on FA, MD and RD data were observed along the left IFOF, right ILF and ATR – supporting our tractography findings. However, these effects also extended to the corticospinal tract and the genu and forceps minor of the corpus callosum (CC). This suggests that although deterministic tractography of the entire CC initially failed to yield significant associations, prospective segmentation tractography decomposing the structure into sub-sections may reveal subtler genotype effects. Alterations in the structure of the corpus callosum (CC) have been reported in SZ (Arnone et al., 2008). With DTI studies reporting FA decreases in various sub-regions of the CC, including the genu, body and splenium, of SZ patients (Buchsbaum et al., 2006; Kim et al., 2012b; Li et al., 2014). Based on t-values derived from the post-hoc TBSS t-test data, Cohen's $d$ was calculated using an online calculator (http://www.uccs.edu/~lbecker/). Cohen's $d$ ranged from 0.51-0.87 corresponding to medium and large effect sizes comparable to the average effect size of common variants on DTI measures (Mothersill et al., 2012); See Chapter 2).
3.4.2 Interpretation of results

FA is considered a measure of WM integrity and coherence with decreases in FA usually reflecting a reduction in WM integrity (Kanaan et al., 2005), and increases in WM FA considered to be associated with myelination, coherence, and the structural integrity of fibres (Peters et al., 2008). In general, FA is globally decreased and MD is generally increased in SZ (Ellison-Wright & Bullmore, 2010; KUBICKI et al., 2007; Lee et al., 2013). In the present study, based on healthy participants carriers V non-carriers of the rs7914558 risk variant, the association fell in the opposite direction. However, the relationship between WM and SZ is complex. For example, in SZ patients, increased FA of fibres connecting temporal regions has been associated with increasing severity of hallucinations (Hubl et al., 2004; Mulert et al., 2012; Rotarska-Jagiela et al., 2009; Seok et al., 2007; Shergill et al., 2007), while increased FA within the UF and IFOF has been correlated with increased positive and negative symptoms of SZ (Lee et al., 2013; Szeszko et al., 2007).

Furthermore, findings of increased FA and decreased MD and RD in risk carriers may also represent some sort of compensatory mechanism (Alba-Ferrara & de Erausquin, 2013), with relatively preserved WM previously reported in siblings of SZ patients (Boos et al., 2007), participants at high risk ( Hoptman et al., 2008) and participants at 'ultra high-risk' of psychosis (Bloemen et al., 2010). However, this is speculative and further investigation of the functional biology of this variant is required to determine causality. Alternatively, increased FA could instead be indicative of pre-existing pathology, as it has previously been associated with decreased dendritic branching and/or compromised organisational complexity of WM (Hoeft et al., 2007).

Moreover, a number of previous studies from our group (e.g. ZNF804A (Hargreaves et al., 2011; Walters et al., 2010)) and others (e.g. DARPP-32 (Meyer-Lindenberg et al., 2007)) have previously found evidence of association between SZ risk alleles and
preserved brain structure or function. In a recent study on an overlapping sample from our group (Rose et al., 2014) the CNNM2 risk allele was also associated with variation in grey matter and social cognitive performance. In that study, risk allele carriers showed higher grey matter volumes in regions implicated in social cognition. The direction of findings in the present study would appear consistent with these previous findings.

In this chapter, the CNNM2 variant was associated with WM differences in the right hemisphere for each tract with the exception of the IFOF. This contracts with several previous studies that have found either lateralization of FA and MD differences in SZ to the left hemisphere (Kubicki et al. 2002; Wang et al. 2004; Ellisson-Wright & Bullmore 2009,) or bilateral changes (Kubicki et al., 2005). However, TBSS analysis did reveal bilateral changes for some tracts, more in line with previous findings. As the laterilization of DTI metrics are heritable (Jahanshad et al., 2010) further investigation is warranted to determine if this particular variant influences brain asymmetry. Also, the elucidation of the causal relationship between the CNNM2 allele, SZ risk and variation in brain structure and function will be required to establish whether these observed effects represent mediation of risk, or instead represent pleiotropic effects.

An important consideration in characterizing the neural effects of risk variants such as CNNM2 is their potential interactions with other SZ genetic risk factors and/or environmental risk factors for disease. The SNP studied in the present analysis, rs7914558, is located on chromosome 10q24 in a large region of high and complex linkage disequilibrium (LD). Analysis of HapMap Phase II + III data (release 28 NCBI build 36) indicates that rs7914558, positioned at 104,765,898(hg18) is in high LD (r2>0.8) with 42 other SNRs in this region (including 22 SNPs at r2=1). These SNPs span a 322kb region from rs11191438 at position 104,627,854 to rs4307650 at position 104,949,842. This region contains four RefSeq genes: C10orf32, AS3MT, CNNM2 and NT5C2. Analysis of SNP x gene expression databases does not identify a direct
functional link between rs7914558 (or proxy) and altered expression of any of the four genes in this region, and therefore all four remain candidate SZ loci.

3.4.4 Limitations

Firstly, very little is known regarding the functional effects of the CNNM2/rs7914558 SNP and therefore it is difficult to hypothesize the mechanism by which this variant acts to increase risk for SZ. As one step towards addressing this, the present study suggests a role for CNNM2 in structural connectivity in healthy controls. Confirming these effects in patients with SZ will be an important next step in establishing the relevance of these findings to SZ risk.

Similarly, repeating this analysis using high-angular resolution data will be useful for characterizing complex crossing fibers in brain WM bundles. Measures such as FA, AD and RD are have found to be sensitive to the presence of crossing fibers (Alexander et al., 2001; Wheeler-Kingshott & Cercignani, 2009). Tournier et al. (2011) advise that given the extent of crossing fiber regions in the brain, any interpretations should be made with extreme caution. Crossing fibers are particularly problematic for tractography based on the diffusion tensor (Alexander et al., 2001; Frank, 2001; 2002) as they can potentially cause the tracking algorithm to venture off course into an adjacent WM tract and this can result in both false-positive and false-negatives connections (Behrens et al., 2007). It is estimated that approximately 90% of WM voxels contain crossing fibres (Jeurissen et al., 2012), and therefore, Tournier et al. (2011) again warn that it is unlikely that any tracts will remain unaffected throughout their entire course.

Tournier et al. (2011) outlines that the orientation produced by the diffusion tensor model is likely to be close to the largest contributing fiber direction; and they explain that this is why tensor-based tractography can often follow the corticospinal tract through regions of
crossing fibers including the pons and centrum semiovale (Mori & van Zijl, 2002). The impact of this is quite significant for non-dominant tracts. Tournier et al., (2011) give examples including the crossing of the lateral projections of the corticospinal tract through regions where the superior longitudinal fasciculus is dominant, as well as the crossing of the commissural projections of the corpus callosum through the more dominant fibers of the corona radiata. Therefore it is advisable to use higher angular diffusion models to capture the DWI information more fully. An overview of more advanced models is provided in Tournier et al's (2011) paper. In addition, Sprooten et al.(2012) also point out that DTI is not sensitive to more small-scale differences in structural integrity given the scale at which FA is measured, especially close to the synapse or near the grey matter, further away from large fiber bundles. Therefore, effects of CNNM2 on WM microstructure may be even more extensive than what is observed here using DTI.

Although TBSS addresses a lot of the issues associated with VBM and it allows for a more global search approach for WM differences in the absence of a priori hypotheses about the spatial location of differences there are some disadvantages associated with the method. Edden & Jones (2011) revealed that the assumption of equal power to detect a group difference everywhere within the brain is not satisfied in TBSS. They report 'heterogeneity in the variance of data on the skeleton'. After the FA volumes have been warped to the template image with a high dimensional warp, 'core' structures (e.g. genu, splenium, internal and external capsule, and cerebral peduncles) are well normalized, but there is more anatomical variability associated with more peripheral WM structures. They conclude that it is increasingly less likely that the 'correct' voxel is projected onto the skeleton in such cases and there is an effect of increased biological variability in these peripheral regions. Furthermore, they report that the orientation of the WM skeleton in relation to the imaging matrix changes the thickness of the skeleton and this in turn changes the number of voxels that are in a local neighbourhood to contribute

66
support to the existence of a cluster of significant voxels. They refer to this as ‘orientational dependence of the statistical sensitivity’ or ‘non-stationarity’. They suggest that the number of available contiguous skeleton voxels in the neighbourhood that can lend support to a cluster should be accounted for and would potentially make the TFCE approach less orientationally biased.

3.4.5 Conclusions

The present study suggests that a novel genome-wide associated risk variant for SZ, CNNM2 (rs7914558), is associated with variation in WM microstructure in a sample of healthy controls, with the risk G allele carriers displaying decreases in MD and RD as well as increases in FA. Highlighting the utility of DTI for elucidating the biological functions of GWAS identified variants in SZ, these data suggest that the CNNM2 risk variant investigated was associated with apparently preserved WM integrity. Although requiring confirmation in other samples using the same approach, these findings were consistent with the direction of association of an earlier CNNM2 study by our group, in which the CNNM2 risk allele was associated with preserved neuropsychological function and grey matter volume (Rose et al., 2014). Further elucidation of the functional biology of CNNM2 will help elucidate if increased SZ risk is related to variation in WM microstructure or perhaps if the association with WM, as well as grey matter volume and social cognition, represents pleiotropic effects of this variant.
4. Investigating the Impact of Schizophrenia GWAS Variants at ZNF804A, CSMD1, MIR137, NOS1 and TCF4 on White Matter Microstructure: DTI study 2
4.1 Background

The potential utility of DTI as an endophenotype for schizophrenia (SZ) was outlined in detail in Chapter 2. In addition, the empirical investigation in Chapter 3 illustrated the sensitivity of DTI to the effects of a single GWAS identified SNP at CNNM2 on WM. This chapter is an investigation of the effects of other GWAS identified risk variants of interest on WM microstructure using DTI. The same analysis techniques as Chapter 2 were employed, using whole brain tract-based spatial statistics (TBSS) and tractography of six tracts that form part of the previously identified frontal and temporal WM networks altered in SZ (Ellison-Wright & Bullmore, 2009), namely the inferior longitudinal fasciculus (ILF), the uncinate fasciculus (UF), the inferior frontal occipital fasciculus (IFOF), the anterior thalamic radiation (ATR), the cingulum bundle (CB) and the corpus callosum (CC) (See Chapter 1).

As the exact function of these variants remains unclear, the formation of hypotheses regarding their effect on WM is problematic. However, considering the evidence outlined below and the establishment of WM microstructure as a suitable endophenotype for SZ, particular hypotheses for each gene of interest were devised.

4.1.2 Genes of interest

4.1.2.1 ZNF804A - rs1344706

The rs1344706 SNP of the Zinc Finger Protein 804A gene (ZNF804A) was identified in the first large SZ GWAS as the most significant association (O'Donovan et al., 2008). Independent replications have also confirmed its association with SZ as well as bipolar disorder (Riley et al., 2010; Steinberg et al., 2010; H. J. Williams et al., 2011; Zhang et al., 2010). The exact function of zinc-finger proteins remains unknown, however proteins with zinc-finger domains were considered DNA-binding molecules involved in transcription and interaction with many molecules including RNA. Transcription factor
binding sites are also thought to be present. Riley et al. (2010) found that the maintenance of binding sites for two transcription factors, myelin transcription factor 1 and octamer-binding factor 6, was predicted by the risk A allele with increased expression from the A allele in comparison to the nonrisk C allele. Both of these transcription factors are involved in oligodendrocyte differentiation and proliferation (Collarini et al., 1992.; Nielsen et al., 2004), processes that are critical to WM development. Using a mouse homologue, Chung et al. (2010) identified ZNF804A as one of the putative Hoxc8 downstream target genes. Hoxc8 encodes transcription factors required for morphogenesis suggesting that ZNF804A may play a role in embryonic neurodevelopment.

In addition, several neuroimaging and neuropsychology studies indicate that rs1344706 is associated with brain function. Walters et al. (2010) found that the ZNF804A genotype was associated with differences in episodic and working memory in patients but not in controls. Furthermore, when patients with a lower IQ were excluded, the association between ZNF804A and SZ strengthened. The authors concluded the risk variant at ZNF804A seemed to delineate a patient subgroup characterized by relatively spared cognitive ability. In a sample of 70 patients and 38 healthy participants. Donohoe et al., (2011) used voxel based morphometry (VBM) to compare homozygous risk allele carriers to heterozygous and homozygous (AC/CC) nonrisk allele carriers for both whole brain volume and specific regions implicated in earlier ZNF804A studies - the dorsolateral pre-frontal cortex, the hippocampus, and the amygdala. They found for patients, but not for controls, ‘AA’ risk carriers had relatively larger grey matter hippocampal volumes than heterozygous/homozygous non-carriers. These data are consistent with earlier behavioural data (Walters et al., 2010) and suggest that ZNF804A is delineating a SZ subtype characterized by relatively intact brain volume.
Esslinger et al. (2009) found reduced functional connectivity between the left and right dorsolateral prefrontal cortices and increased frontotemporal functional connectivity in carriers of the risk A allele during a working memory task. These findings were partly replicated in two further studies (Paulus et al., 2011; Rasetti et al., 2011). In a later study, Esslinger and colleagues (2011) also reported reduced interhemispheric prefrontal connectivity during a face-processing task as well as during resting state.

To investigate if differences in functional connectivity were mediated by changes in underlying WM connections, Lencz et al. (2010) established that individuals homozygous for the ZNF804A risk allele had reduced total WM volumes compared to carriers of the nonrisk allele. However, using DTI tractography, Voineskos et al. (2011) did not detect any effects of ZNF804A on FA in the uncinate fasciculi, arcuate fasciculi, cingulum or corpus callosum of healthy controls despite differences in cortical thickness with risk-allele homozygotes demonstrating reduced thickness in the posterior cingulate gyrus, the anterior cingulate gyrus, and the superior temporal gyrus. Furthermore, Sprooten et al. (2012) did not report any FA differences between ZNF risk and nonrisk genotype carriers at a voxel based or tract-based level in 86 high-risk subjects and 86 healthy controls. Similarly, in a group of 100 patients and 69 controls, Wei et al. (2013) found no differences between ZNF804A genotype groups. As there are conflicting reports in the literature regarding the effects of ZNF804A on WM, and as positive results have failed to be replicated, it is predicted that ZNF804A will not impact indices of WM integrity in this sample.

4.1.2.2 MIR137 - rs1625579

A common variant, rs1625579, within an intron of the MIR137 gene showed the strongest genome-wide association for SZ in one of the largest GWAS to date (Ripke et al., 2011). Micro RNAs (miRNAs) are small non-coding RNAs that have the ability to silence the expression of multiple target genes. miRNAs can affect multiple targets and,
consequently, can impact pathways controlling many biological processes. As MiRNAs can capably regulate gene expression, they therefore have the potential to act as disease modifiers (Lee et al., 2007; Williams et al., 2009).

*MIR137* regulates maturation and migration of adult neural stem cells (Szulwach et al., 2010) and is expressed in the subventricular layers and the subgranular layer of the hippocampus. It plays a critical role in neurogenesis and dendritic morphogenesis (Kwon et al., 2011) and has also been shown to regulate genes with genome-wide significance for SZ, including *CACNA1C* and *TCF4* (Kwon et al., 2011). *CACNA1C* may be relevant for WM integrity as abnormalities of calcium channels are associated with WM lesions in the brain (Matute, 2010). Calcium channel disruption can also play a role in increasing oxidative stress, which oligodendrocyte cells are very susceptible to (McTigue & Tripathi, 2008). *TCF4* is also pertinent to promoting oligodendrocyte differentiation during neurodevelopment (Emery, 2010). During oligodendrocyte differentiation, miRNAs can act to prevent the expression of genes that promote oligodendrocyte precursor cell maintenance to inhibit proliferation and thus promote differentiation (He et al., 2007). Kim et al. (2012a) recently demonstrated that ZFN804A could be silenced by *MIR137* in vitro. In addition, a recent post-mortem study found that the risk T allele of rs1625579 might be associated with decreased *MIR137* expression in the dorsolateral prefrontal cortex of patients (Guella et al., 2013). Furthermore, reduced *MIR137* levels in homozygous risk allele subjects correlated with an increase of *TCF4* (Guella et al., 2013). Moreover, Wright et al. (2013) found that in addition to *CSMD1*, *C10orf26*, *CACNA1C*, *TCF4*, and *ZNF804A*, other SZ associated genes may be targets of *miR-137*, including *ERBB4*, *GABRA1*, *GRIN2A*, *GRM5*, *GSK3B*, *NRG2*, and *HTR2C*. These genes are implicated in many neurological processes including synaptic long-term potentiation, receptor signaling and axonal guidance, processes that are disrupted in SZ.
Conjointly, imaging genetic studies have also found associations of \textit{MIR137} with SZ phenotypes. Potkin et al. (2009) reported that \textit{MIR137} was implicated in two GWAS imaging genetics studies of patients performing the Sternberg item recognition paradigm (SIRP) working memory task. Another study revealed that subjects at risk for SZ and bipolar who were homozygous risk carriers had reduced activity in the right posterior medial frontal gyrus region to increasing difficulty of sentence completion tasks in comparison to non-risk allele carriers (Whalley et al., 2012). This suggests that \textit{MIR137} may have a general effect on executive function.

\textit{MIR137} has also been implicated in variation of SZ symptomology. Green et al. (2013) revealed that the rs1625579 SNP genotype predicted membership of patients in a subgroup with severe cognitive deficits when combined with greater negative symptoms. Patients with more severe negative symptoms and the risk "T" allele were more likely to have been grouped in a cognitive deficit group (Green et al., 2013). Another study examining carriers of the risk allele among psychosis patients found that those homozygous for the "T" risk allele had lower scores for psychotic symptoms and a deficit in performance of episodic memory and attention control tasks (Cummings et al., 2012).

A recent investigation conducted by Lett et al. (2013) established that the \textit{MIR137} risk genotype significantly predicted an earlier age-at-onset of psychosis. Risk T allele homozygote patients also displayed larger lateral ventricle volume, reduced hippocampal volume and decreases in WM fractional anisotropy (FA). However, no differences in cortical thickness measures between genotypes were observed.

In summary, voxel-wise FA differences have already been reported for \textit{MIR137} in a patient sample but not in a sample of healthy controls. Considering miRNAs have the ability to promote oligodendrocyte development, and as \textit{MIR137} regulates other SZ genes that may be crucial for the development of WM, confirming whether \textit{MIR137}'s
effect on WM are general or specific to SZ patients remains an important question. The purpose of the present study was to address this question in a comparable sample of healthy participants stratified by MIR137 genotype. To expand on the previous study by Lett and colleagues (2013) region of interest (ROI) tractography on WM tracts known to be impacted by SZ, as well as whole-brain TBSS was conducted. Potential effects of this variant on alternative measures of WM microstructure more sensitive to axonal density and myelination (Narr et al. 2009; Song et al. 2002; Song et al. 2005) including axial, radial and mean diffusivity, as well as FA, were also evaluated. It is hypothesised that the risk ‘T’ allele at rs1625579 would be associated with compromised WM connectivity in those tracts that form frontal and temporal WM networks altered in SZ.

4.1.2.3 TCF4 - rs4309482 and rs9636107

Transcription factor 4 (TCF4), located on chromosome 18q21, codes for a basic helix-loop-helix transcription factor involved in neurodevelopment and diversification of dendritic cells in the immune system (Murre, 2005; Navarrete et al., 2012; Reizis, 2010). First, Stefansson et al. (2009) reported seven SNPs associated with SZ at a genome-wide level. The intronic SNP, rs4309482, located intergenically downstream of TCF4 and upstream of CCDC68, was reported in another large GWAS (Steinberg et al., 2011). Also, in a recent mega-analyses, two new TCF4 SNPs including rs12966547 that is in high LD with rs4309482, were identified as genome-wide significant for SZ (Ripke et al., 2011). Finally, another variant (rs9636107) within TCF4 was identified in the largest GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation).

TCF4 has also been associated with bipolar disorder (Del-Favero et al., 2002) and it is therefore possible that TCF4 risk variants may confer risk for psychosis phenotypes more generally. In addition, TCF4 variations have also been implicated in Pitt–Hopkins syndrome (Blake et al., 2010) characterized by severe mental retardation and developmental delay.
Cell and animal studies have indicated an important role of $TCF4$ in neuronal development. Forrest et al. (2013) investigated gene expression in $TCF4$-knockdown cells and identified an over-representation of genes involved in TGF-β signaling, and apoptosis. Altered expression of mental retardation genes such as $UBE3A$ (implicated in Angelman Syndrome), $ZEB2$ (implicated in Mowat-Wilson Syndrome) and $MEF2C$ was also found in these cells suggesting that $TCF4$ may regulate signalling pathways involved in cell differentiation and survival, as well as mental retardation genes (Forrest et al., 2013). $TCF4$ is also highly expressed in the embryonic central nervous system and the sclerotome of somites as well as the adult brain (de Pontual et al., 2009). $TCF4$ K/O mice have demonstrated significant disruption to the development of pontine nuclei (Flora et al., 2007) and overexpression of $TCF4$ in the forebrain of mice has resulted in cognitive impairments and deficits in pre-pulse inhibition (Brzózka et al., 2010). Finally, $TCF4$ is thought to be a negative regulator of myelin gene expression (He et al., 2007) and as expression of $TCF4$ is up-regulated in oligodendrocyte precursor cells following de-myelination, this transcription factor may be a potential regulator of oligodendrocyte differentiation process (Fancy et al. 2009).

In humans, $TCF4$ risk alleles are correlated with impaired sensorimotor gating and cognitive performance, mirroring established SZ endophenotypes (Quednow et al., 2012). Recently, Wirgenes et al. (2012) found that the rs12966547 and rs4309482 risk variants were associated with compromised verbal fluency in a sample of patients and controls. In an exploratory analysis, other $TCF4$ SNPs were significantly associated with negative symptoms, verbal learning, executive functioning and age at onset of psychosis. These variants were also associated with brain abnormalities including reductions in temporal cortical area, total brain volume and cerebellar volume across the total sample. The $TCF4$ mRNA expression level was also significantly increased in psychosis patients compared with controls and positively correlated with positive and
negative symptom severity. This demonstrates that TCF4 variants are associated with variation across key psychosis phenotypes and suggests possible mechanisms by which common SNPs in TCF4 may be involved in SZ pathology.

No significant associations between variants at TCF4 and WM microstructure have been reported in the literature to date. As TCF4 has been implicated in brain volume differences and as the transcription factor plays a role in oligodendrocyte differentiation, and as WM variation is an established endophenotype for SZ, it is predicted that the GWAS variants (rs4309482 and rs9636107) at TCF4 will be associated with variation in WM microstructure of healthy controls using ROI tractography and voxel-wise TBSS.

4.1.2.4 CSMD1 - rs10503253

In one of the largest SZ GWAS to date, which included over 51,000 cases and controls, seven risk loci were identified including five novel findings (Ripke et al., 2011). Two of the seven variants identified as meeting genome-wide significance had been previously implicated (6p21.32- p22.1 and 18q21.2). One of these variants, rs10503253, is located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2 This variant is of particular interest given previous evidence of CSMD1's association with multiple neurodevelopmental disorders including epilepsy and learning difficulties (Glancy et al., 2008; Håvik et al., 2011; Shimizu et al., 2003). CSMD2 has also been associated with SZ risk (Håvik et al., 2011) and related neurocognitive phenotypes (Stein et al., 2010). The precise mechanism of this variant remains unclear, however, CSMD1 has been implicated in immune function by playing a role in complement regulation (Kraus et al., 2006). Furthermore, CSMD1 is also thought to be involved in growth cone regulation (Kraus et al., 2006).
It is evident that immune molecules are implicated in neurodevelopment and brain function. In the rodent brain, complement components (C1q, C3) as well as MHC class I molecules are found at synapses where they play an important role in the refinement of synapses and neuronal connectivity (Datwani et al., 2009; Goddard et al., 2007; Stevens et al., 2007). Mice deficient in MHC class I signaling and classical complement cascade (C1q, C3 KOs) show deficits in synaptic pruning in the developing visual system which suggests that functional interactions occur between innate and adaptive immune molecules in the developing brain (Boulanger, 2009; Datwani et al., 2009; Stevens et al., 2007).

In addition, a significant upregulation of complement and MHC related genes are associated with neural injury and neurodegenerative diseases (J. J. Alexander et al., 2008; Amor et al., 2010). It is yet to be established how complement molecules affect the etiology of brain disorders, however, they may be involved in developmental synaptic pruning reactivation leading to brain degeneration (Stevens et al., 2007). Therefore molecules involved in complement regulation may be important for the protection of synapses from aberrant elimination during development and disease. Increased rates of synaptic pruning are thought to precede dysconnectivity and grey matter loss in SZ (Glantz & Lewis, 2000; Thompson et al., 2001b) as well as a reduction in synaptic and dendritic spines (Stephan et al., 2009). Therefore, it is possible that CSMD1 may be involved in such processes.

To investigate if this SNP (rs10503253) mediates SZ risk via deleterious effects on neurocognition, Donohoe et al. (2013) compared carriers and non-carriers of the risk 'A' allele on measures of neuropsychological performance impaired in SZ. Across two independent samples of cases and controls, the risk 'A' allele at CSMD1 was associated with poorer performance on neuropsychological measures of general cognitive ability and memory function but not attentional control. As a follow-up, Rose et al. (2013)
investigated if this variant affects brain structure and function underlying cognitive processes. In a sample of healthy controls, the risk “A” allele was associated with reduced cortical activations in the middle occipital gyrus and cuneus during a spatial working memory task. These brain regions are thought to support maintenance processes during performance of spatial working memory tasks. However, no significant structural differences in brain volume were observed. These data suggest that CSMD1 may be involved in neural mechanisms underlying memory and learning with the deleterious effects of the risk A allele on neurocognitive measures as well as brain activity, a possible part of the mechanism by which CSMD1 confers risk for SZ.

In summary, CSMD1 is implicated in deficits in learning and memory as well as variation in brain activation. The close relationship between WM integrity and cognition was outlined in Chapter 1 and therefore it is hypothesized that CSMD1 will affect WM integrity, particularly in WM bundles associated with learning and memory.

4.1.2.5 NOS1 - rs1607817

The NO synthase-1 (NOS1) gene maps to chromosome 12q24 and encodes for neuronal nitric oxide synthase (nNOS) that synthesises nitric oxide in the brain. Twelve alternate untranslated first exon and coding region consisting of 240 kilobases with 28 exons are involved in the transcription of this gene. NOS1 has been associated with several psychiatric illnesses including anxiety, depression, and SZ (Luciano et al., 2010; O’Donovan et al., 2008; Reif et al., 2006; Reif et al., 2011a). A NOS1 SNP, rs6490121, located in intron 10, was identified by O’Donovan et al. (2008) in a GWAS study as initially showing the strongest statistical evidence of association (p = 9.82 X 10^-6) although this finding was not replicated (Stefansson et al., 2009). More recently, an indel within NOS1, rs1607817, showed genome-wide association for SZ in the largest SZ GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation).
Nitric oxide (NO) regulates the release of classical neurotransmitters in many brain areas. Many effects of NO are mediated through soluble guanylyl cyclase, and the second messenger cyclic guanosine-3',5'-monophosphate (cGMP) that leads to a signaling cascade linked to the modulations of neurotransmitter release (Bernstein, Bogerts, & Keilhoff, 2005; Feil & Kleppisch, 2008). Postsynaptically, NOS1 is coupled to NMDA receptors and activation of this receptor sharply increases NO (Bernstein et al., 2005; Riccio et al., 2006).

Furthermore, it has been demonstrated that brain derived neurotrophic factor (BDNF) regulates the binding of the transcription factor CREB to DNA by initiating a nitric oxide-dependent signaling pathway (Riccio et al., 2006). NO can also undergo oxidative-reductive reactions to form toxic compounds (reactive nitrogen species) that cause cellular damage (Calabrese et al., 2007). Moreover, Li et al. (2005) reported that NO mediates microglial toxicity to oligodendrocytes. These functions of NOS1 are dependent on adaptor proteins to deliver NOS1 to multiple intracellular locations. Presynaptically, NOS1 is linked to the NOS1 (neuronal) adaptor protein gene (NOS1AP) and forms a complex with synapsin, which is involved in the assembly of synaptic vesicle clusters and synaptogenesis. Additionally, both NOS1AP and the synapsin II gene (SYN2) have been associated with SZ (Lee, 2005; Saviouk et al., 2007). Evidence has demonstrated abnormal distributions of nitrinergic neurons in frontal and temporal lobes in SZ (Akbarian, 1993; 1996), increased NO metabolites in the serum of patients with SZ (Imaz et al., 2007) as well as increased NOS1 messenger RNA in prefrontal cortex of postmortem patients (Baba et al., 2004).

NOS1 is also strongly associated with neurocognitive variation more generally. For example, NOS1 mouse knockout models have been associated with variance in cognition (Kirchner et al., 2004). In humans, Reif et al. (2006) reported that two of four genetic markers tested at the NOS1 locus were associated with variance in performance.
on a 'Go/No-Go' task that measures prefrontal function. Further associations between NOS1 polymorphisms, cognitive performance and prefrontal brain function in patients with SZ (Reif et al., 2011b) and healthy controls (Kopf et al., 2011) have been established in more recent studies. The putative risk 'G' allele identified by O'Donovan and colleagues at rs6490121 was associated with compromised verbal intelligence and working memory in SZ patients and healthy controls (Donohoe et al., 2009). For the same SNP, Rose et al. (2012) identified a significant reduction in ventromedial prefrontal grey matter volume in risk allele carriers during an ROI VBM analysis. Risk carriers also exhibited increases in activation of the fronto-parietal working memory networks as well as engagement of the default mode network during a spatial working memory task.

Down-regulation of NOS1 in WM neurons has also been reported in SZ, suggesting that NOS1 may be implicated in SZ-associated variation of WM microstructure (Connor et al., 2011). Considering that NO may also mediate microglial toxicity of oligodendrocytes (Li et al., 2005) it is predicted that the NOS1 indel (rs1607817) that reached genome-wide significance for SZ (Psychiatric Genetics Consortium, Manuscript in preparation), will affect WM integrity of healthy controls as a possible mechanism through which this variant may increase risk for SZ.

4.1.3 Summary, aims and hypotheses

ZNF804A has mainly been associated with changes in functional connectivity. Evidence also supports reduced cortical thickness between risk and non-risk carriers of this variant but there are conflicting reports on the effects of this zinc finger protein on WM microstructure. MIR137-associated differences in executive function, GM volume, as well as WM FA have been reported but no differences in cortical thickness have been elucidated. As only one study has reported WM differences associated with this variant, replication of these findings is warranted. TCF4 has been implicated in differences in verbal learning, executive function as well as decreases in temporal, cerebellar and...
whole brain volume. However, potential WM differences associated with this transcription factor have yet to delineated. **CSMD1** has impacted memory and learning and may possibly be exerting its effects through synaptic pruning processes. There are no studies published to date investigating the effects of **CSMD1** on WM with only one study reporting a negative grey matter volumetric finding. For **NOS1**, compromised verbal intelligence, working memory, cortical activation during a spatial working memory task as well as decreases in grey matter volume have been associated with variants in this gene. As NO may also play a role in oligodendrocyte toxicity, and as **NOS1** has shown to be down-regulated in WM in SZ, the potential effects of this gene on WM integrity warrants investigation.

**Aims and hypotheses**

Given the evidence reviewed above for each of the these GWAS identified risk variants in SZ, the purpose of the present study was to characterise the effects of these variants on WM microstructure, an established endophenotype for SZ. it is hypothesised that **ZNF804A** will not impact WM due to the failure of the original Lencz et al's (2010) FA finding to be replicated. As **MIR137**-associated differences in FA have already been identified, we hope to expand on the previous study by assessing alternative measures of WM microstructure as well as performing ROI tractography. For **TCF4**, it is hypothesised that both variants will be associated with changes in WM integrity due to the potential role of **TCF4** in oligodendrocyte differentiation. As **CSMD1** has been consistently associated with changes in learning and memory, it is hypothesised that these alterations may be mediated by changes to the microstructure of brain WM. Finally, It is also expected that the **NOS1** variant of interest may be associated with alterations in WM due to the association of nitric oxide with oligodendrocyte toxicity.
4.2 Methods

Parameters of DTI acquisition, participant recruitment, genotyping and methods implemented during preprocessing, atlas-based deterministic tractography and whole-brain TBSS are detailed in Chapter 3. Figure 12 depicts tract reconstruction for the ATR, CB, ILF, IFOF, UF and CC for both hemispheres.

4.2.1 Statistical analysis - Tractography

If group sample size allowed, the total sample was split into three genotype groups. However if the minor allele was too rare in our sample then the groups were collapsed into two. For tractography, the diffusivity indices (FA, MD, AD, RD) for each participant were exported to PASW statistical software (Release 18; SPSS Inc., Chicago, IL, USA) and subjected to a one-way multivariate analysis of covariance with genotype group as fixed factors. As FA, MD, RD and AD are constructed from the three eigenvalues of the tensor, an alpha level of \( p = .017 \) was applied to Bonferroni correct for testing of multiple diffusion measures arising from three eigenvalues. If age and gender did not significantly differ between genotype groups they were included as covariates of no interest in the analysis as a matter of standard protocol (Miller & Chapman, 2001).
Figure 12 Atlas-based deterministic tract reconstruction of the ATR (A. ii and i), CB (B. i and ii), IFOF (C i and ii), IFL (D i and ii) and UF (E i and ii) and the CC (F) for left and right hemispheres.
4.2.2 Statistical analysis – TBSS

Statistical analysis for TBSS was conducted using FSL’s ‘Randomise’ algorithm. Randomise is a permutation program that enables modelling and inference using standard general linear model (GLM) design setup. It can output voxelwise and cluster-based tests. Randomise incorporates a cluster-based thresholding option called Threshold-Free Cluster Enhancement (TFCE) (Smith & Nichols, 2009). TFCE is a proposed method to enhance cluster-like structures in an image without having to define an initial cluster-forming threshold or carry out a large amount of data smoothing. TFCE was selected using 5000 permutations per test. Threshold-free cluster enhancement produced an output image from a raw statistic image. The voxelwise values in this output image represented the amount of cluster-like local spatial support (smith & Nichols, 2009). Each TFCE score of each voxel was given by the sum of the scores of all supporting sections. For three genotype groups, a GLM f-test design was set-up, along with six one-tailed t-tests to compare between groups. For two genotype groups, two one-tailed t-tests were set-up using GLM. The statistical threshold was set at $p<0.05$, family-wise error corrected (FWE) corrected for multiple comparisons across the whole brain to find differences between genotype groups. T-tests were further bonferroni-corrected for the number of comparisons. Areas of significant differences were identified using the following FSL tools: the Harvard-Oxford Structural Atlas and the JHU ICBM-DTI and tractography atlases (www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#wm).

4.3 Results

4.3.1 Participant Demographics

Tables 11 – 16 below outline participant demographics based on each genotype.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSMD1 rs10503253</strong></td>
<td>130</td>
<td>58</td>
<td>72</td>
<td>32.38 (11.80)</td>
<td>17.02 (3.07)</td>
</tr>
<tr>
<td>CC</td>
<td>74</td>
<td>34</td>
<td>40</td>
<td>31.97 (11.73)</td>
<td>17.04 (3.30)</td>
</tr>
<tr>
<td>AC/AA</td>
<td>56</td>
<td>24</td>
<td>32</td>
<td>32.69 (11.97)</td>
<td>17 (2.77)</td>
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<tr>
<td><strong>MIR137 rs1625579</strong></td>
<td>123</td>
<td>58</td>
<td>65</td>
<td>32.30 (11.68)</td>
<td>16.97 (3.29)</td>
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<tr>
<td>GT/GG</td>
<td>41</td>
<td>17</td>
<td>24</td>
<td>31.63 (11.93)</td>
<td>16.9 (3.56)</td>
</tr>
<tr>
<td>TT</td>
<td>82</td>
<td>41</td>
<td>41</td>
<td>32.02 (11.62)</td>
<td>17.01 (3.17)</td>
</tr>
<tr>
<td><strong>NOS1 rs1607817</strong></td>
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<td>64</td>
<td>60</td>
<td>32.15 (11.53)</td>
<td>17.15 (3.19)</td>
</tr>
<tr>
<td>AC/AA</td>
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<td>40</td>
<td>32</td>
<td>31.5 (10.69)</td>
<td>17.23 (2.79)</td>
</tr>
<tr>
<td>CC</td>
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<td>17.04 (3.71)</td>
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<tr>
<td><strong>TCF4 rs4309482</strong></td>
<td>132</td>
<td>62</td>
<td>70</td>
<td>32.36 (11.81)</td>
<td>17.06 (3.17)</td>
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<tr>
<td>AG</td>
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<td>27</td>
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<td>17.66 (2.96)</td>
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<tr>
<td>GG</td>
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<td>27</td>
<td>31.49 (11.62)</td>
<td>16.74 (3.42)</td>
</tr>
</tbody>
</table>

Table 11 Participant demographics based on rs10503253 genotype

Table 12 Participant demographics based on rs1625579 genotype (G is minor allele; T is associated allele)

Table 13 Participant demographics based on rs1607817 genotype (A is minor allele; C is associated allele)

Table 14 Participant demographics based on rs4309482 genotype (G is minor allele; A is risk allele)
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
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<td>AA</td>
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<td>16</td>
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<td>16.96 (2.53)</td>
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<td>31</td>
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<td>17.16 (3.48)</td>
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<tr>
<td>GG</td>
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<td>132</td>
<td>62</td>
<td>70</td>
<td>32.34 (11.81)</td>
<td>17.08 (3.18)</td>
</tr>
</tbody>
</table>

Table 15 Participant demographics based on rs9636107 genotype (A is minor allele; G is associated allele)

<table>
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<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
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</thead>
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<td>7</td>
<td>10</td>
<td>34.71 (14.57)</td>
<td>16.76 (2.19)</td>
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<td>AC</td>
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<td>131</td>
<td>63</td>
<td>68</td>
<td>32.30 (11.64)</td>
<td>17.07 (3.16)</td>
</tr>
</tbody>
</table>

Table 16 Participant demographics based on rs1344706 genotype (C is minor allele; A is associated allele)

4.3.2 Atlas-based deterministic tractography and TBSS

No significant effects of variants at **CSMD1, MIR137, NOS1, TCF4 or ZNF804A** on measures of WM microstructure including FA, MD, AD and RD in WM fibre tracts of the ATR, CB, ILF, IFOF, UF or CC were observed (p > 0.05). Similarly, after FWE correction for multiple comparisons across the WM skeleton, no significant differences between genotype groups were observed for FA or MD data using TBSS.

4.4 Discussion

In this study we sought to characterize the effects of the GWAS SZ risk variants at **ZNF804A, MIR137, CSMD1, TCF4 and NOS1** on WM microstructure in healthy controls. Six WM tracts including the ATR, ILF, IFOF, UF, CB and CC were selected for analysis based on previous evidence of their association with SZ pathophysiology (Ellison-Wright
& Bullmore, 2009; Kubicki et al., 2007) and SZ related clinical symptom severity and
cognitive deficits (Ashtari et al., 2007; Mamah et al., 2010; Szeszko et al., 2007). However, no significant effects of variants at CSMD1, MIR137, NOS1, TCF4 or ZNF804A on these WM tracts were observed. Voxelwise TBSS of the FA and MD data was performed to determine if the genotype effects are specific to the tracts selected or if they were more generalized. Similar to the tractography results, no significant effects of variants at ZNF804A, MIR137, TCF4, CSMD1 or NOS1 were observed at a whole-brain level.

Firstly, there have been mixed reports in the literature regarding the effects of ZNF804A and the original finding (Lencz et al., 2010) has not been replicated. The differences reported in measures of functional connectivity between left and right hemispheres of the dorsolateral prefrontal cortex as well as variations in fronto-temporal connections (Esslinger et al., 2009) might not be a result of the underlying WM connectivity between these brain regions. ZNF804A may confer risk by interacting with neurotransmission or receptor density and affinity or through effects on grey matter, considering its association with cortical thickness variation (Voineskos et al., 2011). Furthermore, Sprooten et al., (2012) pointed out the possibility that the effects of ZNF804A on functional connectivity are sample specific as most of the cognitive and imaging data were derived from mostly overlapping samples (Esslinger et al., 2009; 2011; Walter et al., 2011) and replications have not been completely consistent with one replication not surviving multiple correction (Rasetti et al., 2011) and the other study failing to replicate the interhemispheric changes in connectivity (Paulus et al., 2011).

The rs1625579 SNP at MIR137 was the strongest genome-wide associated variant for SZ in the mega-analysis conducted by Ripke and colleagues (Ripke et al., 2011). The association of this polymorphism with variation in cognition, brain structure, function, age-of-onset of psychosis, and SZ symptomology suggests that this gene affects
established endophenotypes for SZ. As variation of WM connectivity is also considered a reliable SZ endophenotype, and as miRNAs are implicated in oligodendrocyte development, the present study investigated if this variant may also confer risk for SZ via an impact of WM microstructure in a sample of healthy controls. WM microstructural variations associated with \textit{MIR137} have been reported for SZ patients but not in healthy controls (Lett et al., 2013). Therefore we aimed to expand on the previous analysis by investigating whole-brain FA and MD, as well conducting ROI tractography to increase power in the hypothesized regions. However, this investigation failed to reveal significant differences for \textit{MIR137} genotype groups on measures of FA or MD at a whole-brain level or for FA, MD, AD and RD at an ROI tract-based level. This analysis concurs with the findings of Lett et al., (2013) The \textit{MIR137} finding by Lett et al. (2013) was observed in patients only and, similar to results reported here, no effects on WM were observed in the control sample. Therefore the effects of \textit{MIR137} on FA may be specific to a SZ population. Furthermore, a finding by Mothersill et al. (2013) of altered functional amygdala connectivity during a face processing task in a smaller but overlapping sample indicates that the functional connectivity differences between \textit{MIR137} genotype carriers may not be driven by alterations of structural connectivity.

\textit{TCF4} has been implicated in differences in verbal learning and executive function as well as decreases in temporal, cerebellar and whole brain volume. However, there are no reports in the literature delineating its effects on WM. Similarly, \textit{CSMD1} is implicated in deficits in learning and memory as well as variation in brain activation. The close relationship between WM integrity and cognition was outlined in Chapter 1 and therefore it was hypothesized that \textit{CSMD1} and \textit{TCF4} would affect WM integrity, particularly in WM bundles associated with learning and memory. As no significant effects of these variants on WM were found in this sample of healthy controls, it is possible that these genes confer risk for SZ without impacting WM or perhaps the effects that these genes may have on WM microstructure are too small to be detected by this sample size.
Considering TCF4 is important for oligodendrocyte differentiation during neurodevelopment (Emery, 2010) and, along with CSMD1 and CACNA1C, has been identified a target for MIR137, a multi-gene analysis of the potential combined effects of these variants on WM connectivity is warranted.

Finally, previous endophenotype research investigating the effects of NOS1 variants suggest that NOS1 has pleiotropic effects on the brain with the variant being associated with differences in verbal intelligence, working memory (Donohoe et al., 2009), cortical activation, grey matter volume (Rose et al., 2012) and P1 visual evoked potentials (O'Donoghue et al., 2011). However, there are no reports in the literature of the impact of NOS1 on WM. As NOS1 may also play a role in oligodendrocyte toxicity, the null result in this case was unexpected. It could be plausible that NOS1 exerts its effects on brain function and cognition without an effect on WM structural connectivity or, alternatively, the size of the effect that this variant may have on WM may be too small to be detected by the current sample size.

4.4.1 Limitations

Firstly, confirming these results in a larger group of healthy controls will be an important next step to establish if the effect that these variants might have on WM microstructure may be too small to detect with the current sample size. However, our sample size is greater than the smallest recommended sample of 80 participants for imaging genetics studies and therefore the current sample should be large enough to detect effects typical for MRI considering the high penetrance of genetic variants in neuroimaging (See Meyer-Lindenberg, 2010b for a review). Post-hoc power calculations are not recommended as power is directly related to the p-value and calculating power once the p-value associated with an analysis is known, adds no new information (See Hoenig & Heisey, 2001). As a result, the power of our study to detect a particular effect size was calculated using G* Power 3 software (Faul et al., 2007) and avoided the use of p values.
and effect sizes generated from our own analysis. Corresponding to a MANOVA test with \( p < 0.05 \) and with Cohen's \( f^2 \) effect size conventions of: Small = .01; Medium = .06; Large = .16 (Cohen's \( f^2 \) is one of many effect size measures used in the context of an F-test for ANOVA or multiple regression), it was calculated that the sample size of this study had just under 100% power to detect a large effect of Cohen's \( f^2 = .16 \) or greater. In addition, MANOVA tests comprising of three genotype groups (ZNF804A and TCF4) had 80% power to detect a medium effect size of .06. This is in line with effect sizes found in previous imaging genetics studies utilizing DTI (Mothersill et al., 2012). However, MANOVA tests consisting of two genotype groups had only 60% power to detect a medium effect. Finally, all MANOVA tests had just between 10-20% power to detect a small effect (See Figures 13-16), which indicates that the present analysis was underpowered to detect smaller effect sizes. Therefore, if the GWAS variants examined in this analysis had an effect on WM, comparable to what has been previously reported (Mothersill et al., 2012), then this study would be powered to detect the effects. However, as no significant results were identified, it can be concluded that either a) these genes do not affect WM microstructure or b) the effects that these variants have on WM are smaller than the average effect size of psychosis risk variants on structural connectivity (Mothersill et al., 2012).
Figure 13 Power analysis using G* Power 3 (Faul et al., 2007) for CSMD1 corresponding to a MANOVA test with two genotype groups, p < 0.05 and sample size of N = 130

Figure 14 Power analysis using G* Power 3 (Faul et al., 2007) for MIR137 corresponding to a MANOVA test with two genotype groups, p < 0.05 and sample size of N = 123
Figure 15 Power analysis using G* Power 3 (Paul et al., 2007) for NOS1 corresponding to a MANOVA test with two genotype groups, $p < 0.05$ and sample size of $N = 124$

Figure 16 Power analysis using G* Power 3 (Faul et al., 2007) for ZNF804A corresponding to a MANOVA test with three genotype groups, $p < 0.05$ and sample size of $N = 131$
Elucidation of the functional biology of these genome-wide associated variants is also likely to enhance our understanding of how increased risk may or may not relate to the variation in WM connectivity. Confirming these effects in patients with SZ will be an important next step in establishing the relevance of these findings to SZ risk. Similarly, repeating this analysis using high-angular resolution data will be useful for characterizing complex crossing fibers in brain WM bundles as measures such as FA, AD and RD are have found to be sensitive to the presence of crossing fibers (Alexander et al., 2001; Wheeler-Kingshott & Cercignani, 2009). Chapter 3 details the limitations associated with DTI analysis and the tensor model.

4.4.2 Conclusions

No significant effects of variants at ZNF804A, MIR137, TCF4, CSMD1 or NOS1 on WM microstructure were found during this analysis. It is plausible that the mechanism or mechanisms by which these variants confer risk for SZ does not involve variation of WM integrity. Alternatively, the effect that these variants might have on WM microstructure may be too small to detect with this sample size. Moreover, a multi-variant or pathway
approach is warranted to establish if these GWAS polymorphisms are conferring risk for
SZ via interactions with other genetic variants.
5. Investigating the Impact of Schizophrenia GWAS variants on Cortical Thickness, Surface area and Grey matter Volume using FreeSurfer
5.1 Introduction: Cortical volume or thickness – the importance of selecting the appropriate endophenotype

Although evidence suggests that measures of brain structure are heritable (Glahn et al., 2007; Peper et al., 2007; Thompson, et al., 2001a), and related to psychiatric disease including SZ (Goldman et al., 2008; 2009; McDonald et al., 2006; van der Schot et al., 2009), the biological mechanisms underlying these measurements and the particular genes that may influence these traits are still unknown. Winkler et al. (2010) examines issues associated with the use of grey matter measurements in imaging genetics and the importance of determining sensitive and appropriate endophenotypes.

Grey matter volume is the amount of grey matter between the grey-white boundary and the pia matter. Previous evidence indicates that grey matter is a function of cortical surface area and thickness and that these measures are both globally and regionally independent and all three measures are heritable (Panizzon et al. 2009). Furthermore, Panizzon et al. (2009) reported that surface area and cortical thickness are genetically distinct. Therefore, volume measurements are likely influenced by distinct genetic factors influencing cortical thickness and surface area. As a result, Winkler et al (2010) hypothesized that since different definitions of a phenotype can lead to different genetic findings (Rao, 2008 as cited in Winkler et al., 2010), measures of surface area or thickness would be more advantageous over volume measures for gene discovery.

In their study, Winkler et al. (2010) compared measurements obtained with voxel-based morphometry (VBM) (Ashburner & Friston, 2000), a straightforward method that quantifies the amount of grey matter existing in a voxel and allows for comparisons across subjects (Figure. 18, right), with measurements from surface-based representations of the brain in samples with genetic information. They assessed the genetic control over (1) grey matter volume computed using surface-based and volume-
based representations of the brain, (2) the cortical surface area and (3) the cortical thickness of 486 subjects from randomly ascertained extended pedigrees. They used quantitative genetic analyses techniques to estimate heritabilities and also investigated correlations between phenotypes.

![Surface-based representation vs. Volume-based representation](image)

**Figure 18** Cortical thickness, surface area and grey matter volume geometry (Adapted from Winkler et al., 2010). In the surface-based representation, the grey matter volume is a quadratic function of distances in the surfaces and a linear function of the thickness. In the volume-based representation, only the volumes can be measured directly and require partial volume-effects to be considered.

Findings from Winkler et al. (2010) indicate that there was more variability for measurements of surface area in comparison to cortical thickness, suggesting that the variability found in grey matter volume is more closely related to surface area than to thickness. Furthermore, grey matter volume was genetically and environmentally correlated with surface area and, to a much lesser extent, with thickness. All measures were found to be heritable, however, the pattern of heritabilities across the cortex for surface area and grey matter volume were similar whereas thickness followed a distinct pattern of heritability. There was also higher regional variability for thickness whereas the spatial distribution of heritabilities for surface area followed a more homogenous pattern.
The authors claim that while the geometric relationship between surface area and cortical thickness (Figure 18) is sufficient to demonstrate their spatial distinctiveness, it is not sufficient to characterize their biological independence. Their results indicate that surface area and cortical thickness have a weak genetic, environmental and phenotypic correlation both regionally and globally. They claim that this supports the hypothesis that area and thickness are from distinct genetic origins. Consistent with findings from Panizzon et al. (2009), Winkler et al. (2010) advocate that cortical thickness and surface area should be treated as separate traits of interest and should be preferred over grey matter volumes for imaging genetic studies. Grey matter volume measures may be less sensitive for gene identification than surface area or thickness separately. They claim that methods like VBM may be limiting for imaging genetics as such methods cannot discriminate genetically independent traits of thickness and area due to the contribution of distinct sets of genes contributing to thickness and surface area, as well as differences in heritability of these measures. However, the authors claim that volume-based techniques have their place in the analysis of grey matter for subcortical structures and for multivariate analysis that include voxel-based functional imaging, such as functional MRI or positron emission tomography (PET).

Various mechanisms may underlie the independent genetic control of thickness and area of the cortex. For example, Winkler et al. (2010) discuss that the ingrowth of the thalamo-cortical and cortico-cortical fibres during late fetal and perinatal development, and their myelination, that forms complex networks within and across cortical layers may have the potential of resulting in more pronounced horizontal than vertical growth (Kostović and Jovanov-Milošević, 2006 as cited in Winker et al. 2010). They also discuss the potential influence of the migration of interneurons within the cortex (tangential) (Polleux et al., 2002 as cited in Winkler et al., 2010) on both cortical thickness and surface area. In addition, the development of the neuropil of pyramidal cells (Zhang, 2004 as cited in Winkler et al., 2010) may not be uniform in all directions.
and may grow at different rates and reach different lengths vertically and horizontally. Therefore, there are a number of mechanisms that may underlie the genetic independence between area and thickness but these have yet to be fully delineated.

5.1.2 Subcortical volume
As well as variation in cortical volume measures, SZ is also associated with subcortical volumetric changes. Volumetric decreases in structures such as the superior temporal gyrus, hippocampus, amygdala, thalamus, frontal lobe, and temporal lobe (Ellison-Wright & Bullmore, 2010; Honea, 2005; I. C. Wright et al., 2000) are evident in SZ as well as increased ventricular, caudate, putamen, and globus pallidus volumes (Kempton et al., 2010). Decreases in bilateral hippocampal volume have been one of the most consistent findings in volumetric studies of SZ (Adriano et al., 2012).

5.1.2.1 Heritability of subcortical volume
Braber et al. (2013) reiterated the importance of disentangling the genetic and environmental factors that could explain the variation in measures of brain structure. The authors point out that the heritabilities of global (intracranial, total brain, grey matter, and white matter volumes) and regional (cortical) brain structures have been widely investigated, as reviewed in Blokland et al. (2012), and Peper et al. (2007), but only a small number of studies have focused on the heritability of the subcortical brain structures (Kremen et al., 2010; Wallace et al., 2006; I. C. Wright et al., 2002; Yoon et al., 2011). Blokland et al. (2012) conducted a meta-analysis of structural MRI data from 48 studies on over 1,250 twin pairs, and DTI data from 10 studies on 444 twin pairs. The proportion of total variance accounted for by genes (A), shared environment (C), and unshared environment (E), was calculated and it was revealed that additive genetic estimates were significantly different from zero for all meta-analyzed phenotypes with relatively high estimates of heritability for all subcortical structures, ranging from 52% for
the right thalamus to 82% for the right putamen (Blokland et al., 2012). The authors conclude that the high estimates suggest that the subcortical structures they investigated could have utility as endophenotypes and act as targets for GWAS investigations. They also found a significant influence of shared environment for many phenotypes. Braber et al. (2013) revealed high heritability estimates for subcortical structural volume, with the largest estimates observed for the thalamus, caudate nucleus, and putamen. They further investigated if heritability estimates were different for men and women, as so little is known about sex differences in heritability. However, they found no difference in heritability between males and females and therefore conclude that the same genes influence these brain phenotypes in males and females.

Evidence presented in the literature is inconsistent regarding suitability of subcortical volume as an endophenotype for SZ. Goldman et al. (2008) examined the heritability of morphological alterations in a sample of 221 healthy control subjects, 169 patients with SZ, and 183 unaffected siblings. They revealed that patients had decreased bilateral hippocampal and cortical grey matter volume and increases in bilateral dorsal striatum and right lateral ventricle in comparison to healthy control subjects. No significant volumetric differences were found in unaffected siblings compared with normal control subjects in any structure. They found strong evidence for heritability of reduced cortical volume and moderate evidence for hippocampal volume, whereas abnormal striatal and ventricle volumes showed no sign of heritability. These findings support evidence suggesting that brain volume is under genetic control, especially hippocampal and neocortical volume and familial influences of reduced cortical volume. However, as no significant differences in subcortical volume were observed between siblings and controls, the authors claim that the findings do not support the representation of measures of subcortical volumes as intermediate phenotypes.
However, in a sample of twenty-five studies including 1065 independent first-degree relatives of patients, 679 patients with SZ, and 1100 healthy control subjects, Boos et al. (2007) established that the largest difference between relatives and healthy control subjects was in hippocampal volume, with relatives having smaller volumes than controls. Grey matter was smaller and third-ventricle volume was larger in relatives compared with healthy control subjects. The authors concluded that abnormalities are present in nonpsychotic first-degree relatives of patients with SZ and are most pronounced in the hippocampus.

Therefore, evidence suggests that subcortical volume is heritable, particularly the thalamus, caudate nucleus and putamen. However, findings are inconsistent regarding the utility of subcortical volume as an endophenotype for SZ with one large study reporting no difference in subcortical volume measures between siblings and controls (Goldman et al., 2008) and a larger meta-analysis reporting strong differences in the hippocampus (Boos et al., 2007).

5.1.3 The present study
The aim of this present study is to examine the effect of GWAS identified variants associated with increased SZ risk on measures of cortical thickness, surface area and subcortical volume using cortical reconstruction in FreeSurfer (FS) v5.2, an automated method for MRI-based quantitative analysis of brain structure. FS is a program that carries out automated reconstruction of the brain's cortical surface and subcortical segmentation from MRI data and allows for the automatic labeling of a number of cortical and subcortical brain structures for both hemispheres. The reliability of FS cortical and subcortical reconstruction has previously been assessed (Han et al., 2006; Lee et al., 2006).
A detailed description of each gene of interest is documented in chapters 3 and 4. The sections below recap the function of each gene briefly and outline how each variant may confer risk for SZ via an association with variation in measures of cortical or subcortical structure. The exact function of these variants remains unclear and their effect on brain structure is uncertain. However, considering the evidence outlined below and the establishment of brain morphometry as a suitable endophenotype for SZ, the following hypotheses were devised (see below).

5.1.4 Genes of interest

5.1.4.1 ZNF804A

The rs1344706 SNP of the Zinc Finger Protein 804A gene (ZNF804A) was identified in the first large SZ GWAS as the most significant association (O'Donovan et al., 2008). In a VBM study with a sample of 70 patients and 38 healthy participants Donohoe et al. (2010) found for patients, but not for controls, that ‘AA’ risk carriers of this variant had relatively larger grey matter hippocampal volumes than heterozygous/homozygous non-carriers. These data are consistent with earlier behavioral data (Walters et al., 2010) suggesting that ZNF804A is delineating a SZ subtype characterized by relatively intact brain volume.

Schultz et al. (2013) conducted cortical thickness and folding analysis in 55 SZ patients and 40 healthy controls. In patients, homozygous risk allele carriers exhibited significantly increased cortical thickness in prefrontal and temporal regions and less disturbed superior temporal cortical folding, whereas the opposite effect was observed in healthy controls where the homozygous risk group had reduced cortical thickness and more alterations in cortical folding. This analysis concurs with previous analyses by suggesting that ZNF804A may mediate protective effects on cortical structure in patients. However, Cousijn et al. (2012) revealed in a large structural sample of 892
healthy young adults, using brain volumetry and VBM that neither rs1344706 nor the other SNPs of ZNF, individually or in combination, affected any volumetric or VBM measures. The authors concluded that the association of ZNF804A with psychosis seems unlikely to be mediated through an influence of rs1344706 or any other SNP on macroscopic brain structure. As measures of brain volume are a product of two genetically distinct measures of thickness and surface area, they may not be sensitive enough to determine subtle genetic effects. The current sample consists healthy controls and it is therefore predicted that ZNF804A will be associated with decreased thickness or surface area of the cortex.

5.1.4.2 MIR137 – rs1625579

A common variant, rs1625579, within an intron of the MIR137 gene showed the strongest genome-wide association for SZ in one of the largest genome-wide association study (GWAS) to date (Ripke et al., 2011). A recent investigation conducted by Lett et al. (2013) established that the risk T allele homozygote patients displayed larger lateral ventricle volume, reduced hippocampal volume and decreases in WM fractional anisotropy (FA). However, no differences in cortical thickness measures between genotypes were observed. Therefore this variant may be conferring risk via an impact on subcortical volumes. However, cortical surface area was not reported in this analysis. As surface area and thickness of the cortex are both genetically and environmentally distinct, MIR137 may have an effect on the area of the cortical surface without having an effect on cortical thickness.

5.1.4.3 TCF4 rs4309482 (PGC2) and rs9636107

Transcription factor 4 (TCF4), located on chromosome 18q21, codes for a basic helix–loop–helix transcription factor involved in neurodevelopment and diversification of dendritic cells in the immune system (Murre, 2005; Navarrete et al., 2012; Reizis, 2010).
First, Stefansson et al. (2009) reported seven SNPs associated with SZ at a genome-wide level. Another intronic SNP, rs4309482, was reported in another large GWAS (Steinberg et al., 2011). In a recent mega-analyses, two new TCF4 SNPs (rs17512836 (intron 3 of TCF4) and rs12966547 (in high LD with rs4309482) were identified as genome-wide significant for SZ (Ripke et al., 2011). Finally, another SNP (rs9636107) within TCF4 from the largest GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation).

Recently, Wirgenes and colleagues (2012) investigated the impact of five previously identified TCF4 SNPs (rs12966547 (Ripke 2011; in high LD with rs4209482), rs9960767 (Stefansson et al., 2009), rs2958182 (Li et al., 2010; in high LD with rs9960767), rs4309482 situated intergenically downstream of TCF4 and upstream of CCDC68 (Steinberg et al., 2011) and rs17512836 (Ripke 2011)) with endophenotypes associated with the illness brain structure in a sample of 480 consisting of patients and controls. In the 'schizophrenia SNP analysis', the risk alleles of rs12966547 and s4309482 were associated with larger ventricular volumes and the risk allele of rs9960767 was associated with a larger hippocampus and smaller ventricles, but these findings did not remain significant after correction for multiple testing. The 'exploratory analysis' showed that the minor allele of three SNPs (rs41396445, rs17514242 and rs10871582) in the same LD block were associated with reduced temporal cortical area in total sample. Out of these, minor alleles of two SNPs (rs17514242 and rs10871582) were associated with reduced total brain volume in the total sample. The major alleles of three SNPs (rs682245, rs12458118 and rs9951280) in the same LD block were associated with decreased cerebellar volume in patients with SZ. Although the findings for measures of cortical thickness and surface area were for other SNPs within TCF4 that are not of interest in this investigation, it is expected that the variants under analysis will be associated in variation in measures of cortical surface area or thickness as TCF4 may be of importance to processes involved in TGF-β signalling, and apoptosis as well as
cell differentiation and survival. This study will examine the effects of the previously identified SNP (rs4309482) from Steinberg et al. (2011) which is in high LD with rs12966547 from Ripke et al. (2011) and another SNP (rs9636107) from the largest GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation).

5.1.4.4 CSMD1

In one of the largest SZ GWAS to date, which included over 51,000 cases and controls, seven risk loci were identified including five novel findings (Ripke et al., 2011). Two of the seven variants identified as meeting genome-wide significance had been previously implicated (6p21.32- p22.1 and 18q21.2). One of these single nucleotide polymorphisms (SNPs), rs10503253, is located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2. In Rose et al's (Rose et al., 2013) investigation, the risk “A” allele was associated with reduced cortical activations in the middle occipital gyrus and cuneus during a spatial working memory task. However, no significant structural differences in brain volume using VBM were observed. As increased synaptic pruning is thought to one of the processes preceding grey matter loss and as molecules involved in complement regulation may be important for the protection of synapse from aberrant pruning, it is possible that CSMD1 may have subtle effects on cortical thickness or surface area that is VMB methods are not sensitive to. As increased synaptic pruning is thought to one of the processes preceding grey matter loss (Thompson et al., 2001; Glantz et al., 2009) and as molecules involved in complement regulation may be important for the protection of synapse from aberrant pruning (Schafer & Stevens, 2010; Stevens et al., 2007) it is possible that CSMD1 may have subtle effects on cortical thickness or surface area that VBM methods are not sensitive to.
5.1.4.5 CNNM2

In the Ripke et al. (2011) mega-analysis, five novel loci were identified. One of these loci on chromosome 10q34.33 contains two variants 130kb apart reaching genome-wide significance, including rs7914558, located in an intron of the CNNM2 gene \((P = 1.8 \times 10^{-9})\) and rs11191580 \((P = 1.1 \times 10^{-9})\), implicating a 0.5-Mb region containing multiple genes. In a sample of 173 patients and 449 healthy controls, Ohi et al. (2013) reported that the risk variant at CNNM2 was associated with variation of voxel-based grey matter volumes in the bilateral inferior frontal gyri. Subjects homozygous for the risk G allele rs7914558 had smaller grey matter volumes in the bilateral inferior frontal gyri than carriers of the non-risk A allele. Rose et al. (2014) conducted VBM to assess volumetric differences form CNNM2 risk carriers in areas associated with social cognition. In contrast to Ohi et al.’s (2013) study, the risk CNNM2 risk allele was associated with increased grey matter volume in the right temporal pole and the right anterior cingulate. As there are conflicting reports in the literature regarding the effects of this variant on volume measures, and as this variant has already been associated with differences in WM microstructure (Chapter 3), we aim to assess if these discrepancies can be explained by further exploring potential associations of CNNM2 with cortical thickness and surface area.

5.1.4.6 NOS1

The NO synthase-1 \((NOS1)\) gene maps to chromosome 12q24 and encodes for neuronal nitric oxide synthase \((nNOS)\) that synthesises nitric oxide in the brain. Twelve alternate untranslated first exon and coding region consisting of 240 kilobases with 28 exons are involved in the transcription of this gene. NOS1 has been associated with several psychiatric illnesses including anxiety, depression, and SZ (Luciano et al., 2010; O’Donovan et al., 2008; Reif et al., 2006; Reif et al., 2011a). A NOS1 SNP, rs6490121, located in intron 10, was identified by O’Donovan et al. (2008) in a GWAS study as initially showing the strongest statistical evidence of association \((p = 9.82 \times 10^{-6})\)
although this finding was not replicated (Stefansson et al., 2009). More recently, an indel within \textit{NOS1}, rs1607817, showed genome-wide association for SZ in the largest SZ GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation). For the putative risk ‘G’ allele identified by O’Donovan and colleagues at rs6490121, Rose et al. (Rose et al., 2012) identified a significant reduction in ventromedial prefrontal grey matter volume in risk allele carriers during an ROI VBM analysis. As a result, we expect that the \textit{NOS1} variant of interest may have been associated with variation in measures of prefrontal cortical thickness or surface area.

5.2 Methods

5.2.1 Overview

FreeSurfer (FS) processing can be divided into two streams: The surface-based stream and the volume-based stream. In the cortical surface stream, models of the boundary between WM and cortical grey matter, as well as the pial surface are constructed. Once these surfaces are delineated, many anatomical measures can be computed including cortical thickness, surface area and curvature at each point on the cortex. The surfaces can also be inflated for improved visualization. FS provides a cortical surface-based atlas that is based on average folding patterns mapped to a sphere. Individual surfaces can be aligned with this atlas using a high-dimensional nonlinear registration algorithm. The registration is based on aligning the cortical folding patterns and therefore directly aligns the anatomy instead of image intensities. A coordinate system is formed by the spherical atlas and allows point-to-point correspondence between subjects. This coordinate system can then be used to create group maps.

5.2.2 FreeSurfer Preprocessing

Dale (1999) and Fischl (1999) outline a full description of the FS reconstruction process. Firstly, the brain is 3-dimensionally rendered from the MRI and the MRI data is
converted into a format that is compatible with FS consisting of 256 coronal slices with 1mm x 1mm x 1mm voxels. The volume is affine registered with the Talairach (Talairach and Tournoux, 1988) atlas. Intensity normalization of the MRI volume is then conducted to remove variations in intensity due to radio frequency field inhomogeneities (Sled et al., 1998). The B1 bias field is a low frequency smooth signal that can corrupt MRI images as a result of the inhomogeneities in the magnetic fields of the MRI machine. In FS it is estimated by measuring the variation in the WM intensity. Voxels are then classified as WM or something other than WM (Figure 2B) based on intensity and neighbour constraints. Regions considered WM are differentiated based on their locations in Talairach space as well as on their intensity and the local neighbourhood intensities. The intensity at each voxel is then divided by the estimated bias field at that location in order to remove the effect of the bias field.

The skull and extra-meningeal tissues are removed using a hybrid watershed/surface deformation process (Figure 19; Ségonne et al., 2004). Cutting planes are chosen to separate the hemispheres from each other and also to remove the cerebellum and brain stem. The location of the cutting planes is based on the Talairach location of the corpus callosum and pons, as well as other algorithms that encode the expected shape of these structures.

Tessellation of the grey matter/white matter boundary then occurs and cerebral WM segmentation then takes place (Fischl et al., 2001; Ségonne et al., 2007). This is based on a linear combination of voxel intensities and local geometric information. On each hemisphere, the WM volume is tiled with a triangular tessellation to generate an initial surface. The surface is then deformed to follow intensity gradients between white and grey matter. This places the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class (Dale, 1999; Dale & Sereno, 1993; Fischl & Dale, 2000).
accurate and smooth representation of the grey-white matter boundary. The WM is nudged to follow the intensity gradients between grey matter and CSF to delineate the pial surface. The white and pial surfaces overlaid on the original T1-weighed image are shown in Figure 19. The average of the distance from the WM surface to the closest point on the pial surface and from that point back to the closest point on the WM surface gives us the thickness at each location of cortex (Fischl and Dale, 2000).

The cortical surface can then be inflated (Fischl, 1999) to expose entire cortical surface, including deep tissue. Automated cortical structure labelling is defined on this inflated surface using curvature information (Desikan et al., 2006; Fischl et al, 2004b). This surface can then be registered to the spherical atlas based on the folding patterns (Fischl et al., 1999). The local curvature, surface area, and sulcal depth can also be computed. A 3D view of the pial surface is shown in Figure 20. This surface can be inflated to show the areas in the sulci as shown in Figure 20.

Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004). Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han et al., 2006; Reuter et al., 2012).

![Figure 19 Three stages from the FreeSurfer cortical analysis pipeline. A. skull stripped](image-url)
image. B. white matter segmentation. C. surface between white and grey (yellow line) and between grey and pia (red line) overlaid on the original volume.

Figure 20 A. Pial surface. B. Inflated surface. Green indicates a gyrus. Red indicates a sulcus.

5.2.2.1 Sub-cortical segmentation, ASEG – volume-based stream

FreeSurfer calculates sub-cortical brain volumes by assigning a neuroanatomical label to each voxel in an MRI volume based on probabilistic information estimated automatically from a manually labeled training set (Fischl, et al., 2002). This process is called ASEG. Sub-cortical volumes are determined by the sum of the total voxels within the region of interest and multiplying by the voxel dimensions.

5.2.2.1.1 Construction of atlas labels

The same basic algorithm is used for both the cortical (Fischl et al., 2004) and the subcortical (Fischl et al., 2002) labelling (Figure 21). The segmentation is based on both a subject-independent probabilistic atlas and subject-specific measured values. The atlas is built from a set of subjects whose brains (surfaces or volumes) have been labelled by hand. This is called a training set. These labels are then mapped into a common space (Talairach space for volumes and spherical space for surfaces) to allow for point-to-point correspondence for all subjects. The label that was assigned accompanies each point in space for each subject and the measured value (or values) for each subject. Probabilities are then computed at each point. First, the probability that the point belongs to each class of labels is calculated. Then, the spatial configuration of labels that exist in the training set computes the second type of probability. This is called
the neighbourhood function. The neighbourhood function is the probability that a particular point belongs to a label given the classification of its neighbouring points. Third, the probability distribution function (PDF) of the measured value is estimated separately for each label at each point. For volume-based labelling, the measured value is the intensity at that voxel. For surface-based labelling, the measured value is the curvature in each of the principal directions at that vertex. A vertex is the triangular surface elements that constitute the surface of the reconstructed brain.

5.2.2.2 Cortical-based segmentation (APARC) – surface-based stream

The brain surface can then be automatically segmented into 34 gyral based regions of interest in each hemisphere. The segmentation process is called APARC and incorporates information about the individual’s sulcal and gyral geometry with spatial information provided by a FS atlas (See Figure 21). The FS atlas is created from a dataset of 40 MRI scans of young, middle aged and elderly control subjects as well as patients with Alzheimer’s disease. The cerebral cortex of each individual is manually divided into 34 regions by a trained operator (Desikan et al., 2006). When compared with manually defined ROIs, the automatically defined ROIs were highly accurate (Desikan et al, 2006).

Surface-based statistics such as cortical thickness, area, volume and curvature of individual regions may be determined using this map. Cortical thickness is calculated as the average of the distance from the WM surface to the closest point on the pial surface and from that point back to the closest point on the WM surface (Fischl & Dale, 2000). Mean thicknesses are then computed for each cortical structure. Averaging the thickness at that vertex, and multiplying it by the average white and pial surface areas of that face calculates the volume.
5.2.2.3 Vertex-based analysis

As the surface-based analysis in this thesis is exploratory without an a-priori hypothesis of particular regions-of-interest, a whole-surface vertex-based analysis was conducted. To perform a surface based vertex-wise group comparison the variability between subjects must be minimized by co-registering all MR images into a common space. Each individual reconstructed and inflated surface is mapped to a sphere that retains the individual’s cortical folding pattern. To facilitate cortical alignment, each individual curvature pattern is registered to a FS template. The FS spherical template represents an average of reconstructed spherical maps from 40 subjects. Each point on the template represents a mean pattern of curvature along with the variances of these values. Larger and more stable sulci contain a greater weighting in the registration process than the more variable, smaller sulci (Fischl, 1999). Before statistical analysis, the surfaces are smoothed by the averaging of neighbouring vertices.

Statistical tests can then be created at each vertex. The statistical analysis in FS fits the GLM to the data (Cortical thickness) from all subjects at each vertex. This identifies vertices where cortical thickness relates to covariates of interest (diagnosis, genotype).
It is necessary to correct for multiple comparisons in this type of analysis to reduce the probability of obtaining false positives (Type 1 errors). As there are approximately 150,000 vertices per hemisphere a significance of $p = 0.05$ would result in 7,500 false positive vertices. Monte Carlo permutation correction is a method of correction that provides strong control over type 1 errors. Permutation analysis computes multiple statistical tests at a set significance level (e.g. $p < 0.05$) where for each test, subjects are randomly assigned to each group. This generates a null distribution. If the statistically significant vertex exceeds those observed by chance in the permutation analysis, a significance value is assigned to an area and is said to be statistically significant. A random sample of possible permutations can be used, creating a Monte Carlo permutation test.

5.2.3 Participants

Healthy participants were recruited from the general population through local advertising. All participants were genetically Irish (i.e. had Irish born paternal and maternal grandparents), were between 18 and 65 years of age and were right-handed. Written, informed consent was obtained from all subjects in accordance with local ethics committee guidelines (see Appendix A). Exclusion criteria included a significant neurological or psychiatric history, a first-degree relative with a diagnosis of SZ or other psychosis, substance abuse in the preceding six months, pregnancy or other contraindication for MRI. Participants were not paid for participation but were offered reimbursement for study related expenses.

The FS dataset was comprised of 238 healthy participants. Eighty-two of these subjects were acquired as part of the Trinity College Institute for Neuroscience (TCIN) biobank project. The biobank subjects formed part of the CSMD1, CNNM2, MIR137 and ZNF804A analyses (see Table 24). However these subjects were not genotyped for NOS1 or TCF4. Individuals were selected for imaging analysis based upon structural
MRI data quality and successful genotyping. While the majority of the biobank sample was Caucasian Irish or other European, the lineage of 30 participants was not documented (See table 24). However, as the local population is quite homogenous, it is likely that these participants were also Caucasian Irish (92% of the population of Dublin city are of Irish/Caucasian background; www.cso.ie/census).

For subcortical analysis quality control, all ROIs with a volume larger or smaller than 1.5 times the inter quartile range (IQR) were identified and visually inspected by overlaying their segmentations on the subject’s anatomical images. Only ROI data for which segmentation was judged accurate upon visual inspection were subjected to statistical analyses (protocol obtained from ENIGMA consortium (http://enigma.ini.usc.edu/; Stein et al., 2012). All subjects were found to have good quality subcortical segmentation.

For cortical analysis, WM segmentation and the pial surfaces were visually inspected for each subject. Based on this visual inspection, 22 of the total 238 subjects were excluded from cortical surface analyses as a result of poor intensity normalisation resulting in aberrant WM segmentation.

Tables 17–23 below outline participant demographics based on each genotype.

<table>
<thead>
<tr>
<th>CSMD1 rs10503253</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>117</td>
<td>70</td>
<td>28.43 (10.38)</td>
<td>17.01 (3.16)</td>
</tr>
<tr>
<td>AC/AA</td>
<td>66</td>
<td>38</td>
<td>29.05 (10.60)</td>
<td>17.15 (2.37)</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>108</td>
<td>28.65 (10.43)</td>
<td>17.07 (2.88)</td>
</tr>
</tbody>
</table>

Table 17 Participant demographics based on rs10503253 genotype
<table>
<thead>
<tr>
<th>CNNM2 rs7914558</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>29</td>
<td>12</td>
<td>29.44 (10.89)</td>
<td>16.92 (2.73)</td>
</tr>
<tr>
<td>AG</td>
<td>73</td>
<td>28</td>
<td>27.41 (8.28)</td>
<td>17.01 (2.70)</td>
</tr>
<tr>
<td>GG</td>
<td>78</td>
<td>37</td>
<td>28.18 (11.00)</td>
<td>17.27 (2.99)</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>77</td>
<td>32.24 (11.71)</td>
<td>16.97 (2.92)</td>
</tr>
</tbody>
</table>

Table 18 Participant demographics based on rs7914558 genotype

<table>
<thead>
<tr>
<th>MIR137 - rs1625579</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT/GG</td>
<td>60</td>
<td>20</td>
<td>28.47 (10.83)</td>
<td>16.98 (3.12)</td>
</tr>
<tr>
<td>TT</td>
<td>123</td>
<td>57</td>
<td>28.04 (9.69)</td>
<td>17.15 (2.74)</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>77</td>
<td>28.19 (10.05)</td>
<td>17.10 (2.86)</td>
</tr>
</tbody>
</table>

Table 19 Participant demographics based on rs1625579 genotype (G is minor allele; T is associated allele)

<table>
<thead>
<tr>
<th>NOS1 - rs1607817</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC/AA</td>
<td>66</td>
<td>28</td>
<td>29.33 (8.60)</td>
<td>17.25 (2.88)</td>
</tr>
<tr>
<td>CC</td>
<td>48</td>
<td>21</td>
<td>31.81 (11.37)</td>
<td>17.24 (3.68)</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>49</td>
<td>30.38 (9.89)</td>
<td>17.24 (3.23)</td>
</tr>
</tbody>
</table>

Table 20 Participant demographics based on rs1607817 genotype (A is minor allele; C is associated allele)

<table>
<thead>
<tr>
<th>TCF4 -rs4309482</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>52</td>
<td>28</td>
<td>29.90 (9.24)</td>
<td>17.34 (2.98)</td>
</tr>
<tr>
<td>GG</td>
<td>26</td>
<td>10</td>
<td>34.81 (12.97)</td>
<td>17.64 (3.01)</td>
</tr>
<tr>
<td>AA</td>
<td>41</td>
<td>15</td>
<td>29.76 (10.68)</td>
<td>16.58 (3.61)</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>53</td>
<td>32.36 (11.81)</td>
<td>17.14 (3.22)</td>
</tr>
</tbody>
</table>

Table 21 Participant demographics based on rs4309482 genotype (G is minor allele; A is risk allele)
<table>
<thead>
<tr>
<th>TCF4 - rs9636107</th>
<th>N</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>25</td>
<td>9</td>
<td>16</td>
<td>33.8 (11.83)</td>
<td>16.90 (2.56)</td>
</tr>
<tr>
<td>AG</td>
<td>58</td>
<td>30</td>
<td>28</td>
<td>29.5 (9.36)</td>
<td>17.56 (3.31)</td>
</tr>
<tr>
<td>GG</td>
<td>35</td>
<td>11</td>
<td>24</td>
<td>29.09 (9.73)</td>
<td>17.11 (2.93)</td>
</tr>
<tr>
<td>Total</td>
<td>118</td>
<td>50</td>
<td>68</td>
<td>30.28 (10.12)</td>
<td>17.29 (3.18)</td>
</tr>
</tbody>
</table>

Table 22 Participant demographics based on rs9636107 genotype (A is minor allele; G is associated allele)

<table>
<thead>
<tr>
<th>ZNF804A - rs1344706</th>
<th>N</th>
<th>N males</th>
<th>N females</th>
<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>25</td>
<td>10</td>
<td>15</td>
<td>26.2 (10.30)</td>
<td>16.95 (2.47)</td>
</tr>
<tr>
<td>AC</td>
<td>73</td>
<td>50</td>
<td>23</td>
<td>28.53 (10.17)</td>
<td>17.73 (3.48)</td>
</tr>
<tr>
<td>AA</td>
<td>63</td>
<td>31</td>
<td>32</td>
<td>27.46 (8.99)</td>
<td>16.38 (3.48)</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>64</td>
<td>97</td>
<td>27.94 (10.21)</td>
<td>17.22 (2.94)</td>
</tr>
</tbody>
</table>

Table 23 Participant demographics based on rs1344706 genotype (C is minor allele; A is associated allele)

<table>
<thead>
<tr>
<th>CNNM2</th>
<th>CSMD1</th>
<th>MIR137</th>
<th>ZNF804A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>72</td>
<td>71</td>
<td>55</td>
</tr>
<tr>
<td>Unknown lineage</td>
<td>30</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Other European</td>
<td>7</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>UK</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Irish</td>
<td>33</td>
<td>32</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 24 Biobank subsamples

5.2.4 Genotyping

Genetic analyses were based on DNA extracted from saliva samples obtained using Oragene DNA self-collection kits (DNA Genotek; Ontario, Canada). All SNPs of interest were genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium (p > 0.05). Along with these
samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for each SNP for quality control purposes and were found to be concordant with available HapMap data for these SNPs.

5.2.5 MRI parameters

Structural images were acquired on a Philips Intera Achieva 3T MRI scanner with the acquisition of a 180 slice T1-weighted image using a turbo field echo (TFE) gradient echo pulse sequence (TR=8.4ms, TE=3.8ms, flip angle=8°, slice thickness=0.9mm, voxel size=0.9mm³, 180 slices, duration=6min).

5.2.6 Cortical Surface analysis

Automated surface reconstruction of T1 images was conducted using FreeSurfer (v.5.2.0) software (http://surfer.nmr.mgh.harvard.edu) developed at the Martinos Center for Biomedical Imaging as described in detail (Dale et al., 1999; Fischl et al., 1999a,b, 2001, 2002). The T1 volumes were processed through FreeSurfer’s recon-all preprocessing pipeline (http://surfer.nmr.mgh.harvard.edu/), which includes brain extraction, intensity normalization, segmentation, white and pial surface generation, correction of surface topology, inflation of surfaces to a sphere, and spherical registration to the fsaverage surface based on a measure of surface shape (Dale, 1999; Fischl, 1999; Fischl et al., 1999; Ségonne et al., 2004; Sled et al., 1998).

To investigate the difference of cortical thickness measurements between genotype groups we performed a surface-based group analysis using FS. First, the brain surface was smoothed using a full-width/half-maximum Gaussian kernel of 10 mm. This smoothing was done so that all subjects in this study could be displayed on a common template (an average brain as described at http://surfer.nmr.mgh.harvard.edu/) in order to perform and visualize a group analysis. For group statistics on the cortical surface, a
general linear model at each vertex in the cortex was fitted using FS's mri glmfit. The contrast matrix investigated the average differences between genotype groups on cortical thickness and surface area, which was a two-tailed t-test. Statistical parametric maps of significant group differences with a vertex-wise threshold (VWT) of $p < 0.05$ at each vertex in the cortical surface were corrected for multiple comparisons using Monte Carlo permutation cluster analyses with 10000 repeats. This results in a clusterwise probability (CWP) and is reported as P values throughout the results section. Finally, corrected clusters were displayed on the average subject’s inflated surface to facilitate the visualization of gyri and sulci simultaneously.

5.2.7 Subcortical volumetric analysis

Volumes of interest for both hemispheres including the lateral ventricles, thalamus, caudate, putamen, hippocampus, amygdala and nucleus accumbens for each participant were exported to PASW statistical software (Release 18; SPSS Inc., Chicago, IL, USA) and subjected to a one-way multivariate analysis of covariance with genotype group as fixed factors. Age and gender did not significantly differ between genotype groups and therefore these variables were included as covariates of no interest in the analysis as a matter of standard protocol (Miller & Chapman, 2001). Total intracranial volume was also added as a covariate to the analysis. As the caudate and putamen are part of the striatum we corrected for five comparisons and set an alpha level of 0.01 to Bonferroni correct for the testing of multiple comparisons.

5.3 Results

5.3.1 Cortical thickness and surface area

After Monte Carlo cluster-wise correction for multiple comparisons with 10,000 permutations, no clusters remained significant for either hemisphere in the CSMD1, CNNM2, NOS1, MIR137 or ZNF804A groups. For rs4309482 at TCF4 a significant
difference between homozygous A carriers and homozygous G carriers was observed in the superior frontal lobe for both the left hemisphere ($cwp = 0.011$) and the right hemisphere ($cwp = 0.013$) without a significant f-test difference. In both cases, risk AA carriers had increased cortical thickness in comparison to non-risk GG carriers (see Table 35 and Figure 22). For the second variant, rs9636107, at TCF4, a significant difference between homozygous A carriers and homozygous G carriers was found in the rostral middle frontal region with increased cortical thickness for risk G homozygotes in comparison to non-risk A homozygotes ($cwp = 0.013$; see Table 26 and Figure 23).

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>Size (mm$^2$)</th>
<th>MNI X</th>
<th>MNI Y</th>
<th>MNI Z</th>
<th>CWP</th>
<th>NVtxs</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>left</td>
<td>1123.1</td>
<td>-7.6</td>
<td>61.3</td>
<td>18.7</td>
<td>0.011</td>
<td>1700</td>
<td>Superiorfrontal</td>
</tr>
<tr>
<td>right</td>
<td>1111.5</td>
<td>11.4</td>
<td>38.9</td>
<td>24.7</td>
<td>0.013</td>
<td>2195</td>
<td>Superiorfrontal</td>
</tr>
</tbody>
</table>

Table 25 Significant clusters at cluster-wise probability ($cwp < 0.05$) in the left and right superiorfrontal region for comparison of AA and GG carriers for rs4309482 at TCF4.

Figure 22 Regions of increased cortical thickness for AA carriers in comparison to GG carriers for rs4309482 at TCF4 for left (A) and right (B) hemispheres.
Table 26 Significant clusters at cluster-wise probability (cwp) < 0.05 in the left and right superiorfrontal region for comparison of AA and GG carriers of TCF4.

![Figure 23 Region of increased cortical thickness for GG carriers in comparison to AA carriers for rs9636107 at TCF4.](image)

No significant differences were observed for measures of surface area for any genotype group.

5.3.2 Subcortical structure

After correction for multiple comparisons, no significant genotype differences in subcortical volume were found for the lateral ventricles, thalamus, caudate, putamen, hippocampus, amygdala, or nucleus accumbens (P > 0.01).

5.4 Discussion

In this study we sought to characterize the effects of GWAS SZ risk variants at CNNM2, CSMD1, MIR137, NOS1, TCF4 and ZNF804A on measures of cortical thickness and
surface area, as well as subcortical volume, in healthy controls. Seven volumes of interest for both hemispheres including the lateral ventricles, thalamus, caudate, putamen, hippocampus, amygdala and nucleus accumbens were selected for analysis based on previous evidence of their association with SZ pathophysiology (Ellison-Wright & Bullmore, 2010; Honea, 2005; Kempton et al., 2010; Wright et al., 2000).

5.4.1 Summary of results

No significant effects of genotype were observed for measures of cortical surface area or subcortical volume. For cortical thickness measures, however, both risk variants at TCF4 were associated with significant differences in post-hoc t-tests. For rs4309482 at TCF4 a significant difference between homozygous A carriers and homozygous G carriers was observed in the superior frontal lobe for both the left hemisphere and the right hemisphere without a significant f-test difference. In both cases, risk AA carriers had increased cortical thickness in comparison to non-risk GG carriers. For the second variant, rs9636107 at TCF4, a significant difference between homozygous A allele carriers and homozygous G carriers was found in the rostral middle frontal region with increased cortical thickness for risk G homozygotes in comparison to non-risk A homozygotes. These findings remained significant even after a relatively conservative Bonferroni correction for multiple testing. As TCF4 knockdown can result in altered expression of several mental retardation genes as well as over-representation of genes involved in TGF-β signaling and apoptosis (Forrest et al., 2013) - suggesting that TCF4 may regulate signaling pathways involved in cell differentiation and survival - we hypothesised that the TCF4 SZ risk variants would affect the thickness of the cortex.

Furthermore, the risk allele of rs4309482 has previously been associated with larger ventricular volumes and another TCF4 SNP has been associated with a larger hippocampus and smaller ventricles, although these findings did not remain significant
after correction for multiple testing (Wirgenes et al., 2012). In the same study, an ‘exploratory analysis’ showed that the minor allele of three $TCF4$ variants in the same LD block were associated with reduced temporal cortical area. Out of these, minor alleles of two SNPs were also associated with reduced total brain volume in the total sample. Furthermore, the major alleles of three SNPs in the same LD block were associated with decreased cerebellar volume in patients with SZ. Although the findings for measures of cortical thickness and surface area were for other SNPs within $TCF4$ that are not of interest in this investigation, it was hypothesized that the variants under analysis rs4309482 (identified in Steinberg et al. 2011) and rs9636107 from the largest SZ GWAS conducted to date (Psychiatric Genetics Consortium, Manuscript in preparation) would also be associated with variation in measures of cortical surface area or thickness as $TCF4$ may be of importance to processes involved in TGF-β signalling and apoptosis as well as cell differentiation and survival. Furthermore, $TCF4$ reached transcriptome-wide significance for predicting cortical grey matter thickness variability during normal aging (Kochunov et al., 2013).

5.4.2 Prefrontal cortex and schizophrenia

Both $TCF4$ variants were associated with variation of cortical thickness in areas of the dorsolateral prefrontal cortex (DLPFC), specifically the superior frontal cortex and the rostral middle frontal cortex. The DLPFC is a brain region that has featured in many studies of SZ and working memory. DLPFC primarily involves the superior frontal gyrus and middle frontal gyrus. The middle frontal gyrus can be further divided into rostral regions including anterior, middle, and posterior as well as caudal regions. The frontal lobes are under substantial genetic control with heritability estimates of between 0.70 and 0.92 (Cannon et al., 2006; Peper et al., 2007; Schmitt et al., 2007; White et al., 2002). Kikinis et al. 2010 investigated grey matter volume differences in sub regions of the DLPFC in a sample of 20 SZ patients and 20 healthy controls. Grey matter volumes
of mid rostral-middle frontal gyrus were then compared between groups, and a significant reduction in grey matter volume was observed but not in other areas of middle frontal gyrus (i.e., anterior or posterior rostral middle frontal gyrus, or caudal regions of middle frontal gyrus). The authors concluded that volumetric alterations in middle frontal gyrus grey matter are localized exclusively to middle rostral areas.

In a sample of 25 SZ patients, 29 first-degree relatives and 37 healthy controls, Oertel-Knochel et al. (Oertel-Knochel et al., 2012) found a significant reduction in cortical thickness in unaffected first-degree relatives in frontal, precentral, temporal, parietal, and limbic (insula, anterior cingulate, hippocampal gyrus/hippocampus) areas. The authors pointed out that temporal, parietal, and cingulate cortical thinning in unaffected relatives of SZ patients has been shown previously (Calabrese et al., 2008; Goghari et al., 2007a; 2007b; Goldman et al., 2009; Yang et al., 2010). However, the literature is contradictory with respect to altered frontal and especially prefrontal lobe structure (Goghari et al. 2007a, 2007b; Goldman et al. 2009). The authors listed reasons for such contradictory findings including sample selection, small sample sizes, heterogeneous study groups, and sensitivity of the imaging procedure (Oertel-Knochel et al., 2013).

Alterations of cortical thickness measures have also correlated with behavioural measures of working memory and IQ in SZ. Oertel-Knochel et al. (2013) found an association between subjective cognitive dysfunction (ESI) and perceptual-cognitive factor (SPQ) (only relatives) and thinning of frontal areas in patients and relatives. This corresponds with findings reporting a significant positive relationship between volume loss in frontal, temporal, and occipital regions and tests for verbal IQ, verbal learning, and executive functions in SZ patients (Ehrlich et al., 2012; Hartberg et al., 2010). Finally, Ehrlich et al. (2012) found strong associations with two independent measures of working memory and cortical thickness in the lateral prefrontal cortex of healthy control subjects, while patients exhibited associations between working memory and
cortical thickness in the right middle and superior temporal lobe. The authors claim that this study provides additional evidence for a disrupted structure-function relationship in SZ corresponding with the prefrontal inefficiency hypothesis.

From the evidence outlined above, it is apparent that alterations of volume and thickness in prefrontal areas are a characteristic of SZ with cortical thinning and reduced volume reported in the literature. Furthermore, the decline in cortical volume and thickness is also associated with variation in memory, cognition and IQ. Therefore, the association of TCF4's SZ risk alleles with increased prefrontal cortical thickness is unexpected. However this is not uncommon. For example, Walton and colleagues (2013) found that the non-risk allele of the GWAS variant, neurogranin, was associated with decreases in cortical thickness. They acknowledge that the risk allele structure can be inconsistent when comparing different studies and they outline possible reasons for this including multilocus effects, variation in local patterns of LD, population structure of study samples and differences in environmental exposure between study populations (Clarke & Cardon, 2010; Lin et al., 2007; Zaykin & Shibata, 2008). Furthermore, this analysis would also need to be repeated in a patient sample to determine if these variants delineate a subgroup of patients with preserved cortical grey matter.

5.4.3 Suitability of cortical thickness as an endophenotype for schizophrenia

Finally, some caution should be exercised when interpreting these results. Although cortical thickness and surface area are more sensitive endophenotypes due to their genetic distinctiveness (Winkler et al., 2011), cortical thickness may not necessarily be the most ideal endophenotype for SZ specifically. Goldman et al. (Goldman et al., 2009) examined the heritability of cortical thickness changes associated with SZ in a large sample of 196 healthy controls, 115 SZ patients, and 192 unaffected siblings. The study reported reduced cortical thickness in the patient sample, especially in the frontal and temporal lobes. The most pronounced thickness effects were seen in the frontal lobe,
with patients showing widespread reductions. The authors acknowledge that the prefrontal cortex is one of the most reliably implicated regions in studies of structural brain morphometry in SZ. Furthermore, healthy siblings had widespread relative risk for thickness reductions within the frontal lobe. Heritability was also observed throughout the brain including regions of the left superior and middle temporal gyrus, rostral middle frontal gyrus, inferior frontal gyrus (opercular part), banks of the superior temporal sulcus, inferior parietal lobule, and posterior cingulate and right caudal anterior cingulate.

However, the authors caution that widespread heritability of cortical thickness reductions alone does not necessarily indicate that reduced thickness is part of the genetic risk architecture of SZ. They state that findings like the ones reported in their investigation could also be found if siblings of healthy subjects with thin cortices were analysed. They go on to explain that in order to show that reduced thickness is also a marker of increased genetic risk for SZ, it has to be ‘enriched in the sample of people who are at increased genetic risk for this disease, ie, the sibling group’. However, they did not find significant cortical thickness reductions in their sibling sample. Although they acknowledge that smaller studies did find reductions in siblings, and in their sample, differences between the sibling and patient groups were less pronounced than those between controls and patients, suggesting that siblings did lie in an intermediate position between patients and controls. The authors speculate that larger samples might reveal a significant reduction of thickness in at risk healthy subjects. As they found no significant thickness reductions in unaffected siblings, the authors therefore concluded that reduced cortical thickness per se might not be ‘a major neural signature of the genetic risk architecture of schizophrenia’. However, they also cautioned that results must be considered in light of the technical limitations associated with MRI.
Finally, they also consider the more skeptical view of the SZ literature and suggest that, even though grey matter structure is heritable, it ‘may lie outside the core genetic risk architecture of schizophrenia’ and may be more associated with environmental effects related to disease state. However, from the present results, cortical thickness has proved to be a sensitive endophenotype for SZ GWAS variants with two variants within the same gene demonstrating effects on measures of cortical thickness. As TCF4 has also been associated with bipolar disorder and therefore may confer risk for psychosis phenotypes more generally, cortical thickness may be a sensitive endophenotype in this case. However this requires further confirmation.

5.4.4 Negative findings

There is conflicting evidence in the literature regarding the effects of ZNF804A on cortical morphology as well as subcortical structure. Using VBM, Donohoe et al. (2011) found for patients, but not for controls, that ‘AA’ risk carriers had relatively increased grey matter hippocampal volumes than heterozygous/homozygous non-carriers and suggests that ZNF804A is delineating a SZ subtype characterized by relatively intact brain volume. Shultz et al. (Schultz et al., 2013) conducted cortical thickness and folding analysis in 55 SZ patients and 40 healthy controls. In patients, homozygous risk allele carriers exhibited significantly increased cortical thickness in prefrontal and temporal regions and less disturbed superior temporal cortical folding, whereas the opposite effect was observed in healthy controls where the homozygous risk group had reduced cortical thickness and more alterations in cortical folding. This concurs with previous analyses by suggesting that ZNF804A may mediate protective effects on cortical structure in patients. Although our analysis consisted of a much larger healthy control sample, we failed to replicate these effects of ZNF804A on cortical thickness. However, Cousijn et al’s (2012) study of 892 healthy controls found no effect for ZNF804A on any volumetric or VBM measures. As this current analysis failed to find any effect of ZNF804A on measures of cortical thickness or surface area, we can conclude with the
authors of Cousijn et al. (2012) that the association of ZNF804A with psychosis seems unlikely to be mediated through an influence of rs1344706 on brain structure.

A recent investigation conducted by Lett et al. (2013) established that the risk T allele homozygote patients displayed larger lateral ventricle volume, reduced hippocampal volume and decreases in WM FA. However, no differences in cortical thickness measures between genotypes were observed. Therefore this variant may be conferring risk via an impact on subcortical volumes. However, cortical surface area was not reported so we hypothesised that as surface area and thickness of the cortex are both genetically and environmentally distinct, MIR137 may have an effect on the area of the cortical surface without having an effect on cortical thickness. We replicated the negative cortical thickness finding of Lett et al. (2013) and failed to find an effect on surface area. However, we did not replicate the subcortical findings in the Lett et al., (2013) study. As Lett et al's (2013) findings were specific to the patient sample, perhaps MIR137 does not affect subcortical volumes of healthy controls.

In Rose et al's (2010) investigation, although the risk “A” allele for CSMD1 was associated with reduced cortical activations in the middle occipital gyrus and cuneus, no significant structural differences in brain volume using VBM were observed. As increased synaptic pruning is thought to one of the processes preceding grey matter loss and as molecules involved in complement regulation may be important for the protection of synapse from aberrant pruning, it was hypothesised that CSMD1 may have subtle effects on cortical thickness or surface area that VMB methods are not sensitive to. However, grey matter alternations may not be part of the mechanism of how CSMD1 confers increased risk for SZ.

In a large sample of 173 patients and 449 healthy controls, Ohi et al. (2013) found that subjects homozygous for the risk G allele of rs7914558 at CNNM2 had smaller GM
volumes in the bilateral inferior frontal gyri than carriers of the non-risk A allele. However, in contrast to this, Rose et al. (2014) found that the risk CNNM2 risk allele was associated with increased GM volume in the right temporal pole and the right anterior cingulate using an ROI approach. Firstly, our sample was underpowered in comparison to the sample in Ohi et al. (2013) study and secondly, we did not use an ROI approach like Rose et al. (2014). Therefore, perhaps the effect that CNNM2 has on grey matter morphology is too small to be detected by this sample size.

For the putative risk 'G' allele identified by O'Donovan and colleagues at rs6490121 of the NOS1 gene, Rose et al. (2012) identified a significant reduction in ventromedial prefrontal GM volume in risk allele carriers during an ROI VBM analysis. As a result, we expected that the NOS1 indel identified in the latest and largest SZ GWAS (Psychiatric Genetics Consortium, Manuscript in preparation) might also be associated with variation in measures of prefrontal cortical thickness or surface area. However, it is possible that the effects identified in Rose et al.'s (2012) study may be specific to that particular NOS1 variant that is not in high LD with the variant of interest in the present study.

5.4.5 Conclusion

In conclusion, we utilised measures of cortical grey matter thickness and surface area, as well as subcortical volume as endophenotypes to delineate the potential effects of SZ GWAS variants on grey matter structure. Both TCF4 GWAS risk variants were associated with increased prefrontal cortical thickness. As reductions in prefrontal cortical thickness and volume have been associated with SZ, the direction of the effects in this case was unexpected. However this is not uncommon (Walton et al. 2013) and possible inconsistencies in the risk allele structure need to be considered (Clarke et al., 2010; Lin et al., 2007; Zaykin et al., 2008). It is also possible that the TCF4 variants have a protective effect on cortical grey matter; however, confirmation in a patient sample would be required to determine if these variants are delineating a subgroup of
patients with preserved prefrontal cortical thickness. Furthermore, the suitability of cortical thickness as an endophenotype for SZ has been debated and therefore environmental effects need to be taken into consideration during interpretation of results. Finally, no effects of the other SZ GWAS variants were found on measures of cortical thickness, surface area or subcortical volume. These variants may have effects too small to be detected in this sample or they may have another role to play in the pathophysiology of SZ that has implications for the understanding of the genetic underpinnings of the disorder. However, two of the seven variants within the same gene had an effect of cortical thickness demonstrating the utility of cortical thickness as a measure for characterising the effects of SZ GWAS variants on brain structure and further implicating \textit{TCF4} in the pathophysiology of SZ.
6. Discussion
The core research question that guided the individual studies of the present thesis asked, "Do SZ GWAS-identified genes impact WM microstructure and brain morphology?" In order to address this question, MRI endophenotypes that are validated in neuroimaging for the assessment of WM integrity and brain structure were utilised. The endophenotype approach in psychiatry (Gottesman & Gould, 2003) involves the identification of heritable, quantifiable characteristics that lie between genotype and disease phenotype which may be useful targets for genetic studies in order to establish how particular genes of interest are conferring risk for a disorder. The four experimental chapters of this thesis assessed how SZ GWAS variants impact WM microstructure and brain morphology. Chapter 2 investigated effect size differences in the literature between the association of common SZ risk variants with measures of structural and functional connectivity. Following from this, an empirical investigation of the impact of a novel SZ GWAS variant at *CNNM2* was assessed in Chapter 3. In light of these results, and the demonstration of DTI as a suitable endophenotype for GWAS variants, the same methodology was applied in Chapter 4 to assess the impact of other novel and established GWAS variants on WM including variants at *ZNF804A, MIR137, TCF4, CSMD1* and *NOS1*. Finally, Chapter 5 investigated the impact of these SZ GWAS variants on measures of brain morphology. As evidence suggests that measures of cortical volume may not be a suitable endophenotype (Winkler et al., 2011), measures of cortical thickness and surface area were assessed, as well as subcortical volume.

6.1 Interpretation and implication of thesis findings

To briefly summarise the four major findings of the present thesis: Chapter 2 revealed larger and more variable effect sizes associated with DTI measures in comparison to functional connectivity measures. Chapter 3 revealed that a SZ GWAS variant rs7914558 at *CNNM2* is associated with increased FA and decreased MD and RD in major WM tracts implicated in SZ. However, chapter 4 found no significant effects of other SZ GWAS variants at *CNNM2, CSMD1, MIR137, NOS1, TCF4* and *ZNF804A* on
WM microstructure. Finally, chapter 5 investigated the effects of these same GWAS variants on cortical thickness, surface area and subcortical volume and found that both variants within *TCF4* were associated with increased cortical thickness in areas of the DLPFC.

The findings of chapter 2 suggest that measures of structural connectivity, such as DTI, may be sensitive to a wider range of effects in comparison to functional connectivity measures, which may only be able to accurately detect large effects. This result may also suggest that structural connectivity is closer to the level of genes than functional connectivity. However, the average sample size of the studies utilizing DTI was smaller than that for the functional studies and therefore effect sizes may be over-inflated. Given the smaller sample sizes, the variability of effect sizes reported and the limited number of studies published to date, establishing the true effect sizes of DTI- and fMRI-based connectivity studies will require reanalysis as additional investigations, including those from the present work, are published.

The direction of effect in chapter 3 was unexpected, as previous investigations have shown that FA is globally decreased and MD is generally increased in SZ (Kubicki et al. 2007; Ellison-Wright & Bullmore, 2009; Lee et al. 2013). However, the relationship between FA and WM integrity in SZ is thought to be more complex, as increases in FA have been previously associated with increasing severity of positive and negative symptoms (Hubl et al. 2004; Seok et al. 2007; Shergill et al. 2007; Mulert et al. 2012; Szeszko et al. 2008; Lee et al. 2013). Increased FA is also evident in genetically high-risk (GHR) subjects in comparison to healthy controls (Kin et al., 2012; Hoptman et al., 2008). This may represent a compensatory mechanism as a result of increased genetic risk for the disease or global dysregulation of neural circuits (Kim et al., 2012; Alba-Ferrera & Erausquin, 2013) Therefore, CNNM2 may be associated with a pattern of highly integrous WM synonymous with compensatory mechanisms of GHR subjects,
or alternatively, may be delineating a subgroup of patients with increased symptom severity. However, replication in independent and patient samples is required to support these claims.

The negative findings in chapter 3 may indicate that variants at *CSMD1, ZNF804A, TCF4, NOS1* and *MIR137* do not confer risk for SZ via an impact on WM microstructure. Therefore further analyses utilizing other established intermediate phenotypes for SZ should be carried out to determine the mechanisms through which these genes increase disease risk. However, as the sample size of this study was underpowered to detect small effects (see section 6.2.2), definitive conclusions cannot be made regarding these negative findings. Furthermore, the lack of a patient sample is also problematic as the effects that these variants have on patients may differ to the effects they have on healthy controls. For example, the same variant at *MIR137* was previously associated with variation in FA of SZ patients (Lett et al., 2013; see section 6.3.2) but we failed to replicate this finding in healthy participants.

Findings from chapter 4 were also unanticipated. As decline in cortical volume and thickness has been reported in the SZ literature (Oertel-Knochel et al., 2013; Ehrlich et al., 2011; Hartberg et al., 2010), the association of *TCF4*’s SZ risk alleles with *increased* prefrontal cortical thickness was not hypothesized. However, this is not uncommon. A possible explanation is inconsistency in risk allele structure due to multilocus effects, variation in local patterns of LD, population structure of study samples and differences in environmental exposure between study populations (Clarke & Cardon, 2010; Ping-I Lin, 2007; Zaykin & Shibata, 2008). It is possible that the *TCF4* variants in question may have a flipped risk allele structure or, alternatively, TCF4 may have a protective effect on cortical grey matter. Nonetheless this is speculative and replication of this analysis in a patient sample is required to determine if these variants delineate a subgroup of patients with increased cortical grey matter. Additionally, increases cortical thickness may also
have a pathological basis as brain over-growth and increases in the thickness of the cortex during early childhood have been reported in the autism literature (Zielinski et al., 2014). Further cross-disorder analyses may reveal the extent of a shared genetic architecture for SZ and autism spectrum disorders.

Very little is known regarding the functional effects of these GWAS SNPs and therefore it is difficult to hypothesize the mechanism by which these variants act to increase risk for SZ. As one step towards addressing this, the findings in chapter 3 suggest a role for CNNM2 in structural connectivity in healthy controls while the findings in chapter 4 implicate TCF4 in cortical thickness variation. Highlighting the utility of DTI and structural MRI for elucidating the biological functions of GWAS identified variants in SZ, these data suggest that the CNNM2 and TCF4 risk variants investigated were associated with apparently increased WM integrity and cortical thickness respectively. The CNNM2 findings were consistent with the direction of association of an earlier CNNM2 study by our group, in which the risk allele was associated with preserved neuropsychological function and grey matter volume (Rose et al. 2013) (however, our findings require confirmation in independent samples using the same diffusion-based techniques). Although increased or 'preserved' WM integrity and cortical thickness are generally considered protective, patterns of brain over-growth and highly integrous WM may also have pathological foundations. Further elucidation of the functional biology of CNNM2 and TCF4 will help elucidate if increased SZ risk is related to the observed patterns of variation in WM microstructure and cortical thickness. As for MIR137, ZNF804A, CSMD1 and NOS1, it is possible that these variants are conferring risk for disease via alternative mechanisms. Further analyses utilizing other established endophenotypes for SZ, including functional connectivity analysis, neuropsychological assessment, as well as cellular and animal work, should be conducted to determine the biological pathways affected by these variants. It is also plausible that these variants may have effects on the brain that are too small to be detected by this sample size and therefore definitive
conclusions cannot be made about the negative findings until these analyses are replicated in a more statistically powerful sample.

6.2 Limitations and future directions

6.2.1 Diffusion parameters

The DTI data described throughout this thesis was acquired using only 15 diffusion gradients. To sensitize MRI images to diffusion of water molecules, instead of the application of a homogeneous magnetic field (like T1 sequences), the magnetic homogeneity is varied linearly by the application of a pulsed field gradient. Since precession of protons in water is proportional to the magnet strength, the protons precess at different rates and this results in the dispersion of the phase and signal loss. To refocus or rephrase the spins, another gradient pulse is then applied in the same magnitude but in the opposite direction. However, the refocusing will not be perfect for water protons that have moved (diffused) during the time interval between the pulses, and the signal measured by the MRI machine is reduced. This signal loss produces a contrast and allows for the quantification of the amount of diffusion occurring in a given structure. The more attenuated the image is at a given position, the greater diffusion there is in the direction of the diffusion gradient. In order to measure the tissue's complete diffusion profile the application of different directions of the diffusion gradient for each scan is necessary. The more directions applied, the more accurate a diffusion profile can be generated (Tournier et al., 2011). Therefore, repeating this analysis using high-angular resolution data will be useful for characterizing complex crossing fibers in brain WM bundles. Measures such as FA, AD and RD have been found to be sensitive to the presence of crossing fibers (Alexander et al., 2001; Wheeler-Kingshot et al., 2009). It is estimated that approximately 90% of WM voxels contain crossing fibres (Jeurissen, 2010), and therefore, Tournier et al. (2011) warn that it is unlikely that any tracts will remain unaffected throughout their entire course. In addition, Sprooten et al.
(2012) also point out that DTI is not sensitive to more small-scale differences in structural integrity given the scale at which FA is measured, especially close to the synapse or near the grey matter, further away from large fiber bundles. Therefore, the effects of CNNM2 on WM microstructure found in this thesis may be even more extensive than what is observed here using 15-direction DTI.

6.2.2 Study sample and statistical power

The sample sizes used in the various analyses described throughout this thesis exceed the smallest recommended sample of 80 participants for imaging genetics studies, which is considered large enough to detect effects typical for MRI given the high penetrance of genetic variants in neuroimaging (See Meyer-Lindenberg, 2010 for a review). It was calculated that the sample size of this study had almost 100% power to detect a large effect of Cohen’s $f^2 = .16$ or greater. In addition, MANOVA tests comprising of three genotype groups (ZNF804A and TCF4) had 80% power to detect a medium effect size of .06. This is in line with effect sizes found in previous imaging genetics studies utilizing DTI (Mothersill et al., 2012). However, MANOVA tests consisting of two genotype groups had only 60% power to detect a medium effect. All MANOVA tests had just between 10-20% power to detect a small effect (See Figures 1-6), which indicates that the present analysis was underpowered to detect smaller effect sizes. Notwithstanding the sufficient power of the current sample size to detect large effects commonly found in the imaging genetics literature (Mothersill et al., 2012), definitive conclusions cannot be made regarding the negative findings of this thesis as some GWAS variants may have small effects on WM microstructure or brain morphology. It should also be noted that neuroimaging in general has low power in comparison to other neuroscience methodologies, as highlighted by a recent review revealing that neuroimaging has a median statistical power of 8% in comparison to a median statistical power of 21% for neuroscience studies in general (Button et al., 2013). Further investigations need to examine the approximate variability of statistical
power across different neuroimaging methodologies including distinct methods of functional, structural and diffusion imaging.

Furthermore, this thesis comprised MRI data on healthy control subjects and lacked a large enough patient sample for imaging genetics. Although there are advantages associated with the utilization of healthy control samples including the lack of confounding factors such as medication or disease progression and severity, the replication of these analyses with a patient sample will be required to fully characterize the effects of these risk variants in SZ. The effects that these variants have on brain structure in healthy control subjects may contrast to the potential effects they may have in a patient sample. This pattern has been reported for ZNF804A whereby Walters et al. (2010) found that the ZNF804A genotype was associated with differences in episodic and working memory in patients but not in controls. Donohoe et al. (2010) also found for patients, but not for controls, that 'AA' risk carriers had relatively larger hippocampal grey matter volumes than heterozygous/homozygous non-carriers.

6.2.3 Future directions: advanced methods for neuroimaging genetics

This thesis investigated the effects of single SNPs on WM and brain structure. However, as GWAS sample sizes increase, further GWAS variants are reaching genome-wide significance for SZ. Ripke et al. (2013) estimated that 6,300–10,200 independent and mostly common SNPs contribute to the etiology of SZ and these account for at least 32% of the variance on liability. The authors expect that these genes will implicate one or more biological pathways fundamental to disease risk (Ripke et al., 2013). Therefore, as the number of SZ GWAS variants may grow into the thousands, the methodology employed in this thesis, whereby we explored the effects of single risk variants on brain structure, would be futile. Future neuroimaging genetics models should focus on multivariate approaches including independent components analysis (ICA) and partial least squares (PLS) that have the ability to simultaneously assess the effects of a group
of SNPs and neuroimaging phenotypes.

Single SNPs may also be interacting with other genetic variants to confer risk for SZ. For example, Wright et al. (2013) found that in addition to CSMD1, C10orf26, CACNA1C, TCF4, and ZNF804A, other SZ associated genes may be targets of miR-137, including ERBB4, GABRA1, GRIN2A, GRM5, GSK3B, NRG2, and HTR2C. These genes are implicated in many neurological processes including synaptic long-term potentiation, receptor signaling and axonal guidance, all of which are processes that become disrupted in SZ. Therefore, these variants may not be acting alone to confer risk for SZ and the assessment of how their potential interactions with other variants may affect brain structure will be a crucial next step in this research.

Gene pathway analysis may also be useful in characterizing the effects of genetic interactions on endophenotypes. Genetically complex diseases, like SZ, involve contributions from a large number of independent or interacting genetic variants (Lvovs et al., 2012) with many of these variants falling below the accepted levels of genome-wide significance. However, Hargreaves et al. (2013) found in their investigation of the cell adhesion molecule (CAM) pathway on cognition that the proportion of variance explained by the polygenic analysis versus the most strongly associated individual SNP analysis was comparable (~3%). An explanation for this is that polygenic scores may introduce as much noise as signal into the analysis (i.e. variation neither specific to cognition, nor to the target patient population). The entire genetic pathway may not be informative and could lead to the inclusion of genes that detract from the signal. Nonetheless, as this type of analysis is still in its infancy, further replication is required to fully determine how gene pathways may influence disease phenotypes.

The emergence of world-wide consortia efforts such as Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) and Cohorts for Heart and Ageing Research
in Genetic Epidemiology (CHARGE) with MRI data on over 20,000 individuals has made genome-wide association with MRI phenotypes possible (Stein et al. 2012). The DTI working group of ENIGMA is currently conducting genome-wide association with DTI-based phenotypes (Jahanshad et al. 2013) and this will expectantly lead to the localization of genetic effects in the brain and may provide new information on how genetic variants affect risk for disease.

As well as gene x gene interactions or multivariate analyses, the characterization of gene x environment interactions in SZ is imperative. Environmental risk factors for SZ include hypoxia, low birth weight, childhood trauma, cannabis exposure and urban upbringing. Decoster et al. (2011) found that cannabis use in female patients who carried the $\textit{BDNF}$ met-allele was associated with an earlier age at onset of psychosis in comparison to val/val carriers. It has also been revealed that val carriers of the $\textit{COMT}$ gene exposed to childhood abuse are more vulnerable to the psychosis-inducing effects of cannabis (Alemany et al., 2014). How genetic risk factors interact with environmental risk factors will be essential in predicting disease onset as well as prevention and therefore future research designs should incorporate both genetic and environmental data to fully delineate the risk architecture of SZ.

As the development of specific hypotheses for the potential effects of SZ GWAS variants on the brain remains problematic, multi-modal methods in imaging genetics should be considered to fully characterize the effects of risk variants on distinct neurological measures. Research designs incorporating both methods of structural connectivity and functional connectivity during a cognitive task or resting state would be advantageous in determining the aspects of connectivity (anatomical or functional) that are more sensitive to gene effects. Combined EEG-fMRI may also be beneficial in determining gene effects on electrical brain activity and hemodynamic changes. $\textit{In vivo}$ magnetic resonance spectroscopy (MRS) may also inform structural MRI by assessing the effects of genetic
variants on changes in brain structure and potentially corresponding metabolic changes. Connectomics – an emerging form of network analysis grounded in mathematic graph theory – may also complement DTI analyses by identifying specific brain networks that may be affected by risk variants. Furthermore, MRI techniques such as multicomponent relaxation (MCR) using T1 and T2 relaxation information that is specific to changes in myelination may also augment DTI analysis and assist in the interpretation of gene-associated changes in measures of FA and diffusivity.

Finally, this thesis is based on the common disease common variant hypothesis. In contrast to this, the common disease rare variant (CDRV) hypothesis emphasizes the role of rare but highly penetrant alleles in SZ (Manolio et al., 2009). Here, each disease case is caused by a single rare variant, and these variants can occur in different genes in different individuals. This model also states that new (or de novo) mutations in sporadic cases continuously generate highly penetrant alleles that may explain the high prevalence of SZ (Rodriguez-Murillo et al., 2012). Delineating the contribution of rare variants to SZ risk, as well as how they interact with other common or rare variants and environmental factors to confer risk for SZ will be crucial to understanding the genetic architecture of the disorder. The elucidation of the genetic architecture of SZ and how this architecture impacts established endophenotypes will help clarify the pathophysiology of SZ that will in turn facilitate the development of novel and more effective treatment and diagnostic tools.

6.3 Conclusion

One of the most prevalent issues associated with SZ GWAS is that most of the identified common variants lie within non-coding regions (introns or intergenic regions); how these variants are acting to confer risk for a disease is usually indiscernible. Rodriguez-Murillo et al (2012) acknowledge that, "linking these common variants to the function or expression of a gene and the pathophysiology of the disease is a daunting task."
Nevertheless, biological relevance needs to be established for a SNP, or a variant in linkage disequilibrium with the SNP, before positive correlations from GWAS are considered equivalent to disease risk. In a step towards addressing this problem, this thesis investigated if common SZ GWAS variants confer risk for SZ via an impact on measures of WM microstructure and brain morphology. Of the seven GWAS variants investigated in the thesis, one variant was found to be associated with changes in WM microstructure and another two variants within the same gene were found to be associated with differences in cortical thickness measures.

The work presented in this thesis may have several important implications. Firstly, we have shown that common genetic variants of large effect can impact upon brain structure in SZ, comparable to previous reports in the literature (Mothersill et al., 2012). Secondly, we found the role of genetic variants of smaller effect more difficult to characterize; this was possibly due to insufficient power, although the underlying genetic architecture of SZ is complex and the effects of individual variants are likely to be smaller than previously hypothesized. Consortia efforts such as ENIGMA will be crucial in determining the small effects of these common variants on different facets of brain structure. Thirdly, we found that several established GWAS genes may not necessarily impact upon brain structure in SZ, but may act via alternative biological pathways. This has implications for the understanding of the genetic underpinnings of the disorder. If a majority of variants found to be statistically associated with the disease are not impacting brain structure and WM microstructure (shown to be heritable and associated with SZ), then it is possible that their association with increased risk is independent of such effects. This lends support to the view that SZ is a complex and heterogeneous illness comprised of multiple genetic variants (See Rodriguez-Murillo et al., 2012). Indeed, the results of this thesis suggest that while two genome-wide SZ genes (CNNM2 and TCF4) impact upon WM microstructure and cortical thickness respectively,
the functional significance of other genes (ZNF804A, CSMD1, NOS1 and MIR137) may be as multifarious and enigmatic as the disease itself.

Undoubtedly, clinical translation is the ultimate goal of imaging genetics research. For example, polygenic models of risk prediction (which examine the collective effect of all nominally-associated SNPs) could be combined with neuroimaging to aid in the identification of high-risk individuals or family members, as well as disease prognosis (Voineskos et al., 2014). Particular brain circuits associated with genetic variation may also become targets of treatment innovation. For these aspirations to become reality, the future of imaging genetics should look towards (i) multivariate methods using large patient and healthy control samples from multi-modal consortia efforts or biobank initiatives (which would provide sufficient statistical power to characterize the effects of multiple SNPs), (ii) gene pathways and gene interaction effects on distinct brain measures and (iii) efficient ways to obtain and integrate environmental risk data in order to fully deconstruct the profoundly complex risk architecture of this highly heritable, costly and debilitating disorder.
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Appendix A: Ethics approval, Information letter and Consent form for the study
RE: A quantitative-trait based genetic association study of schizophrenia and related psychoses.

Please quote this reference in all communications regarding this study: 2002/7/15

Dear Dr. Corvin,

I refer to your letter dated 5-01-02 in which you sought Ethical approval for an amendment to the above study and with which you enclosed an Information and Consent form.

Dr. Michael Barry, Chairman of the Joint Research Ethics Committee, has, on behalf of the Committee, approved the amendment subject to the following condition:

0 The Committee’s Consent Form for Participation in Genetic Research (copy enclosed) should be used instead of the Consent Form supplied by you.

As already discussed, the Committee has recently authorised the use of the enclosed form as an interim measure pending finalisation of the design of this document.

Yours sincerely,

[Signature]
Daniel R. Lynch,
Senior Executive Officer
Re: Resource for Psychoses Genomics, Ireland

Please quote this reference in all communications regarding this study: 05/05/03: Chairman's Action

Dear Professor Gill,

The Vice-Chairman of the SJH/AMNCH Research Ethics Committee has noted from an initial perusal of the proposal to conduct the above study that it has already been approved by other ethics committees. He asked me to advice you therefore that it is not necessary to have this proposed study reviewed by the SJH/AMNCH Research Ethics Committee.

Yours sincerely,

Daniel R. Lynch, Secretary, SJH / AMNCH Research Ethics Committee.
Dear Applicant,

Further to a meeting of the Faculty of Health Sciences Research Ethics Committee 2007 - 2008, I am pleased to inform you that the above project has been approved without further audit.

Yours sincerely,

[Signature]

Dr. Orla Sheils
Chairperson
Faculty of Health Sciences Ethics Committee
CONFIDENTIAL

30th September 2009

Dr. Gary Donohoe
TCD Dept of Psychiatry
The Trinity Centre
St. James's Hospital
Dublin 8

Re: Addendum to Current Ethics Committee Protocol for:
Genetic Association of Schizophrenia and Related Psychoses
(Protocol No. 30/09)

Dear Dr. Donohoe,

Thank you for your application for Chairman’s approval of your study. I am pleased to inform you that your study has been granted approval and research can commence immediately.

For your information, copies of your application will be forwarded to all members of the Research Ethics Committee, and if any additional conditions are attached, we will inform you of these after the next Committee meeting, to be held on February 9, 2010.

May we remind you that this approval is subject to full adherence with the terms and conditions set out in your research protocol and the condition that you report back to the Research Ethics Committee no later than 12 months subsequent to this approval (September 30th, 2010), with a summary report on the progress of this research. This report should include, but is not limited to:

- Progress to date or outcomes in the case of a completed project
- A statement of compliance with the approved proposal and/or minor amendments to the proposal and a justification of these
- A description of measurements taken to maintain and secure personal information/records pertaining to the research

If there are any material changes to Protocol 30/09 in the next 12 months, you are required to contact the Research Ethics Committee for approval.

With very best wishes.

Yours sincerely,

JAMES V. LUCEY MD.,Ph.D.,FRCPI,FRCPSych.,
SECRETARY TO RESEARCH ETHICS COMMITTEE
CONSENT FORM

Title of Project:

"A structural and functional MRI investigation of genetics, cognition and emotion in schizophrenia"

About the consent form:

This consent form explains the research study in full. If you have any questions, please ask the researcher. If you are happy to be involved in this study, then please complete this consent form and make it available to the researcher(s).

If you have any questions regarding this research, please feel free to contact Dr. Emma Rose on 01-8962464 or 0833807395.

Information about the Project:

In this study we are seeking to gather information about brain function and structure. There is evidence to suggest that certain aspects of our genetic make-up influence how we think and feel, and may cause differences in the parts of our brains that control thoughts and feelings. In order to examine this, you will be asked to perform some tasks while we image your brain using Magnetic Resonance Imaging (described below). Prior to the imaging, you will be asked to practice the tasks so you will know what to do once in the scanner.
Task Description

You will be asked to complete two tasks. The first task is a working (or 'short-term') memory task. In this task you will be asked to remember the location of items on a computer screen. The second task is a reward task, in which you will try to win as much pretend money as possible. You will be given the information on how to complete the tasks. You will then practice the tasks on a computer. You must make sure that you understand the tasks before we start scanning. You will then do the same tasks while in the scanner. You will see the tasks presented on a screen and the instructor will show you how to respond. These tasks should take no more than 30 minutes to learn and will take less than an hour to complete in the scanner.

The study will take place at the MRI facility located at Trinity College Dublin. The session (including the MRI scanning) will take 2.0 hours. You may withdraw from the study at any time. All information gathered during the course of this research is confidential and is available to you upon request.

What is MRI?

The purpose of functional MRI scanning is to determine which brain regions are activated as someone performs certain tasks. The MRI scanner uses a combination of radio waves and a strong magnetic field to take pictures of your brain while you perform the tasks. While you are inside the scanner you head will be placed inside a special
device, known as the head coil. When you have been safely and comfortably placed in the head coil, the bed is moved slowly into the scanner. When your head is in the middle of the magnetic field, radio frequency pulses and magnetic fields are switched on and off to produce a signal which we use for measuring blood flow.

**What will I be asked to do while I am in the MRI scanner?**

Different types of MRI will be done while you are in the scanner. For some images you will be asked to lie still and relax. For others you will be asked to do the tasks you practiced outside the scanner while we take the brain pictures. You will be able to hear us while you are in the scanner and we will explain exactly what you need to do before we start each MRI test run. Individual MRI test runs will last no longer than 10 minutes and the entire testing session will be completed within 60 minutes. It is **very** important that you keep still and **do not move your head** while we are taking an image of your brain.

**What are the risks associated with MRI?**

When operated by appropriately qualified individuals, MRI presents virtually no risk, as there is **NO** exposure to x-rays or radioactivity with this procedure. The noise produced by an MRI exam can be very loud and you will be issued with protective headphones or earplugs to prevent damage to your hearing. The noise produced by the exam has been reported to produce temporary threshold shifts (i.e. decreased ability to hear quiet sounds) in a small percentage of people. Given the confines of an MRI machine, a small percentage of people in the past have reported feeling claustrophobic (fear of being closed in a tight space) when placed into an MRI scanner. Please let us know before we put you in the scanner if you have experienced claustrophobia in the past.
During MRI scanning, you will be in contact with the MRI operator via an auditory communication system. This will be used to regularly check your comfort and to allow you to inform us of any problems or concerns. You will also have a "panic button" which you may press at any time to indicate that you wish to stop the scanning procedure.

As the MRI involves a large magnetic field, it is essential that NO METAL BE BROUGHT INTO THE SCANNER WITH YOU.

Items that must be removed by individuals before entering the MRI facility include:

- Purse, wallet, money clip, credit cards, cards with magnetic strips;
- Electronic devices such as beepers or cell phones;
- Hearing aids;
- Metal jewellery (in all parts of the body), watches;
- Pens, paper clips, keys, coins;
- Hair barrettes, hairpins;
- Any article of clothing that has a metal zipper, buttons, snaps, hooks, under wire bras, or metal threads;
- Shoes, belt buckles safety pins.

Other objects that may be hazardous include:

- Metallic spinal rod
- Plates, pins, screws, or metal mesh used to repair a bone or joint
• Joint replacement or prosthesis

• Metal jewellery such as that used with body piercing.

• Some tattoos or tattooed eyeliner (these alter MR images, and there is a chance of skin irritation or swelling; black and blue pigments are the most troublesome)

• Bullet, shrapnel, or other type of metal fragment

• Metallic foreign body within or near the eye (such an object generally can be seen on an x-ray; metal workers are most likely to have this problem)

• Dental fillings (while usually unaffected by the magnetic field, they may distort images of the facial area or brain; the same is true for orthodontic braces and retainers)

If you have any of these items, please inform us immediately.

There may be additional or unknown risks associated with MRI. For example, in very rare cases, the strong magnetic field can induce nerve stimulation (e.g., switching the strong magnetic field gradients during imaging has been reported to cause twitching in the neck muscles). Also, in very rare cases, the radio signals have been reported to cause burns. There may be other risks associated with imaging that are not yet known.

Who shouldn’t undergo the MRI procedure?

Research participants who have the following items should not undergo an MRI procedure:

• Cardiac pacemaker or an implanted defibrillator
• Catheter that has metal components that may pose a risk of a burn injury

• A metal clip placed to prevent bleeding from an intra-cranial aneurysm

• A medication pump (such as that used to deliver insulin or a pain-relieving drug)

• A cochlear (inner ear) implant

It is essential that you inform the MR operator if you have any metal items in any of the above lists.

**Pregnancy and MRI**

For female participants it is also important that you tell us if there is any possibility that you are pregnant. To date there are no known risks of MRI during pregnancy, however as a precautionary safety measure pregnant individuals will not be included in the study. To participate in the current study women of child-bearing potential must be using one of the following acceptable methods of birth-control:

a. oral or transdermal contraceptives

b. barrier (diaphragm or condom) with spermicide

c. intrauterine progesterone contraceptive system

d. Levonorgestrel implant

e. Medroxyprogesterone acetate contraceptive injection

f. complete abstinence from sexual activity

**What if the brain imaging finds some abnormality in my brain?**

The brain images that are taken are not the kind that are used to look for problems in your brain. We will routinely check images for the presence of a brain abnormality.
Should an abnormality be detected, we will contact you immediately and will recommend that you contact your GP to arrange for a clinical-quality brain scan. To make sure that you can be contacted at a later date, you will be asked to provide a name and contact details for a next-of-kin.

Although a significant abnormality is extremely unlikely, you should be aware that if such an abnormality is detected and you are informed, then this knowledge might have consequences for you. Please take the time to consider carefully what it would mean to you if we told you of an abnormality in your brain which might, or might not, affect you later in life. Knowledge of an abnormality may affect your ability to work in certain professions, obtain life or health insurance and other facets of daily living. If you do not want to know, then it is better not to participate in this study.

**By providing my consent I agree that:**

I have been informed of the discomforts and risks that I may reasonably expect to experience as part of this study. I have been informed that if a brain abnormality is observed, that I will be contacted for a meeting with a radiologist. I have been informed that when used on appropriately qualified individuals, MRI presents virtually no risk. There will be no exposure to x-rays or radioactivity in this study. I understand that noise produced by this exam could be very loud, and that I will wear earplugs or headphones to prevent damage to my hearing. Even with earplugs, the noise produced by the exam may produce temporary threshold shifts (i.e., decreased ability to hear quiet sounds). I have been informed that I may experience some discomfort from lying in the MRI scanner such as claustrophobia (fear of being closed in a tight space) or tight sensations.
from having my head restrained to prevent movement. I have been informed that I will also be asked to perform some tasks that I have been trained on, prior to the MRI procedure, which should not cause undue distress.

I have been informed that other risks of injury due to MRI include damage to implanted electronic devices (such as pacemakers), haemorrhage if aneurysm clips are present and trauma if ferrous metal objects are brought too close to the scanner. However, these risks are minimal in a properly administered site. I do not have any of these items in my body.

I understand these risks and am agreeing to volunteer to participate in this research. I understand that I can withdraw at any time from the study.

PARTICIPANT'S NAME: ____________________________________________

PARTICIPANT'S SIGNATURE: _______________________________________

Date: ___________________________
rosse@tcd.ie

Mr. Omar Mothersill, Room 0.18, Trinity Centre, St. James’s Hospital, Dublin 8.

Tel: 01-8962464

rmotherso@tcd.ie
GENERAL MRI DATA CONSENT FORM

Trinity College Institute of Neuroscience, (TCIN) is performing research, utilising an MRI scanner at Trinity College, Dublin 2. These research scans, although not full clinical scans, will be read by a radiologist.

In the unlikely event of an irregularity being found, the radiologist, [Dr William Torreggiani of The Adelaide and Meath Hospital Incorporating the National Children’s Hospital (AMNCH), Tallaght] will inform the participants GP, that a proper clinical scan may be required to determine whether or not an irregularity is of clinical significance.

To enable us to perform the research scans the participant agrees to give consent/permission for:

(i) TCIN to conduct the MRI scan and store MRI scan data of participant;

(ii) TCIN or Principal Investigator, (PI) to contact participants GP;

(iii) TCIN radiographer to send MRI scan data to radiologist acting for TCIN;

(iv) Radiologist to store data in a hospital system with same care as other patient data ensuring participants confidentiality;

(v) Radiologist/ Clinician (acting for TCIN) to contact participants GP;

(vi) TCIN to store data on the study for a period of at least 5 years or as specified in the specific consent form.
A dated standard letter signed by the appropriate Principal Investigator will be sent to all participants' GP's, it is the responsibility of the Principal Investigator to ensure that this is sent at least two days before scanning to allow for postal delays. The principal investigator is responsible for their project at all times.

The TCIN designated radiologist will be sent data in a form that allows identification so that if a response is required he can act quickly (a copy of this is also held at TCIN). This will be stored in the hospital system with the same rigour and attention to confidentiality as all other medical data, as per the rules of that institution; a copy of this data will also be stored at TCIN. The raw scan data will be stored at TCIN in anonymous form for research purposes as agreed on the consent form of the specific research project.

I agree to the above points and understand that my data will be treated carefully at TCIN and in the hospital system.

Participant Name and Address ____________________________________________
Signed by Participant: ____________________________

Participants GP Name and Address

______________________________

______________________________

Date: _________________________
Saliva Sample Collection and Analysis

Letter of Consent

I agree to provide a saliva sample from which a sample of my DNA will be extracted.

The purpose of this study – to investigate genes that may be involved in brain function – has been explained to me. I have had the opportunity to read a letter of information about this project and to ask questions that I may have had.

I understand that I will not be paid for my participation and will not benefit personally from the study.

I understand that my personal details will be kept confidential and that my saliva sample will not be used for any other purpose than in studies of the genetics of brain function.

I understand that I can withdraw my consent for the study at any time and have contact details for the study organisers if I wish to contact them.

Signed:_______________________________ Date:__________________

Witnessed by:_________________________ Date:__________________
Appendix B: Atlas-based deterministic tractography ROI

“AND” gates
ROI "AND" inclusion gates for tracts of interest displayed in red and blue.

Figure B (1). ROI AND GATES for right Cingulum bundle displayed on FA image of representative subject

Figure (B) 2. ROI AND GATES for corpus callosum displayed on FA image of representative subject
Figure (B) 3. ROI AND GATES for right inferior longitudinal fasciculus bundle displayed on FA image of representative subject

Figure B (4). ROI AND GATES for right uncinate fasciculus displayed on FA image of representative subject
Figure B (5). ROI AND GATES for right inferior fronto-occipital fasciculus displayed on FA image of representative subject

Figure B (6). ROI AND GATES for right anterior thalamic radiation displayed on FA image of representative subject
Appendix C: Average FA, MD and RD values extracted from significant TBSS clusters
Figure C (1) Significantly increased FA in the bilateral IFOF, left ILF, right ATR and corticospinal tract for AG carriers in comparison to AA carriers of rs7914558 CNNM2 variant (p < .0027)

Figure C (2) Significantly increased FA in the bilateral IFOF/ILF, genu of CC and corticospinal tract for GG carriers in comparison to AA carriers of rs7914558 CNNM2 variant (p < .0027)

Figure C (3) Significantly increased MD in the forceps minor for AA carriers in comparison to GG carriers of rs7914558 CNNM2 variant (p < .0027)
Figure C (4) Significantly increased RD in the left IFOF for AA carriers in comparison to AG carriers of rs7914558 CNNM2 variant (p < .0027)

Figure C (5) Significantly increased RD in the forceps minor for AA carriers in comparison to GG carriers of rs7914558 CNNM2 variant (p < .0027)
Appendix D: TBSS scripts used for analysis on Linux server operated by the Trinity Centre for High Performance Computing (TCHPC)
TBSS scripts

**Tbss\_1\_preproc**: prepares FA data in the TBSS working directory in the right format

```bash
#!/bin/sh

#SBATCH -n 1 # 1 cores

#SBATCH -t 24:00:00 # 1 hour

#SBATCH -p compute # partition name

#SBATCH -U HPC\_11\_00168 # your project name - contact Ops if unsure what this is

#SBATCH -J TBSS\_step1 # sensible name for the job

cd ../data/

echo

"#####################################################################
###########"

echo "### tbss preprocessing"

tbss\_1\_preproc *\_nii\_gz
```

**Tbss\_2\_reg**: applies nonlinear registration of all FA images into standard space using the FMRIB58\_FA standard-space image as the target
SHELL - n | # cores

\#SBATCH -n 1

\#SBATCH -p compute # compile partition name

\#SBATCH -t 4:00:00 # 40 hours

\#SBATCH -n 1 # 1 cores

###

\#SBATCH -u

\#SBATCH -a

\#SBATCH -t

\#SBATCH -D

\#SBATCH -c

\#SBATCH -p

\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -y

\#SBATCH -l

\#SBATCH -o

\#SBATCH -p

\#SBATCH -t

\#SBATCH -c

\#SBATCH -N

\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -A

\#SBATCH -J

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\#SBATCH -N

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\#SBATCH -J

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\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -A

\#SBATCH -J

\#SBATCH -N

\#SBATCH -A
#SBATCH -t 24:00:00  # 24 hours

#SBATCH -p compute  # partition name

#SBATCH -U HPC_11_00168  # your project name - contact Ops if unsure what this is

#SBATCH -J CSMD1_tbss_step3_4  # sensible name for the job

cd ../data/FA

echo

"########################################################

##################"

echo "### tbss step 3 (post registration)"

tbss_3_postreg -S

tbss_4_prestats: projects all subjects' FA data onto the mean FA skeleton

#!/bin/sh
#SBATCH --exclusive

#SBATCH -p sla-o 2

#SBATCH -J loss develops scaling

echo "#SBATCH step 4 (scaling)"

echo "###"

###############################################################

echo
cd /data

#SBATCH -p compute # partition name

#SBATCH -l 2.4:0:0  # 24 hours

#SBATCH -n 1 # 1 core
###SBATCH -t 72:00:00  # 72 hours

###SBATCH -p compute  # partition name

###SBATCH -U HPC_11_00168  # your project name - contact Ops if unsure what this is

###SBATCH -J TBSSFA_stats_final  # sensible name for the job

```bash
cd ../data/stats

randomise -i all_FA_skeletonised -o tbss_FA -m mean_FA_skeleton_mask -d design_MIR137.mat -t design_MIR137.con -n 5000 -x --T2 -V
```

**Part1 non_FA data:** scales the non_FA data

```
#!/bin/sh

#SBATCH -n 1  # 1 cores

#SBATCH -t 24:00:00  # 1 hour

#SBATCH -p compute  # partition name

#SBATCH -U HPC_11_00168  # your project name - contact Ops if unsure what this is
```
#SBATCH -t 2:00:00  # 1 day and three hours

#SBATCH -n 1  # 1 cores

#SBATCH -j y # job name

skel

#!/bin/sh

skel

subject warped non FA data into a 4D file and projects this onto the original mean FA

TBS non FA: applies the original nonlinear registration to the non FA data, merges all

$ iss-1 -preproc -1 1000000 . . . . . . .

echo "### iss preprocessing"

####

########################################################

echo

cd $DATA/MD

#SBATCH -J MD-step  # sensible name for the job
#SBATCH -p compute  # partition name

#SBATCH -U HPC_11_00168  # your project name - contact Ops if unsure what this is

#SBATCH -J TBSS_step1  # sensible name for the job

echo

"#####################################################################
###########
###
tbss non FA"

echo "### tbss non FA"

tbss_non_FA MD

FSL Linux Commandline

- **Threshold TBSS output at p = .0027:** fslmaths

  tbss_FA_tfce_corrp_tstat**.nii.gz -thr .9973 <output image>

- **Identify significant clusters:** cluster --

  in=tbss_FA_tfce_corrp_tstat**.nii.gz --thresh=.9973 <table of significant clusters.txt>
• **Tbss_fill script** thickens the thresholded stats image to 'fill out' significant clusters onto skeleton for display purposes: *tbss_fill*

  *tbss_FA_tfce_corrp_tstat**.nii.gz 0.9973 mean_FA <output image>*

• **Binarize thresholded image**: *fslmaths <input image> -bin <output name>*

• **Extract FA measures from binary mask** in significant regions for every subject:

  *fslmeants -i all_FA_skeletonised -m <thresholded binary mask> -o “output_file.txt”*

---

**FreeSurfer scripts**

**Recon all preprocessing**

# -- Request 96 hours of time

#SBATCH -t 96:00:00

# -- Assign this to your project ID

#SBATCH -U HPC_11_00168

# -- Give the job a name for identification

#SBATCH -J FS_Subject_CON9095
# -- Ask for an email at beginning and end of job

#SBATCH --mail-type ALL

# -- Send the email to

#SBATCH --mail-user pald@tcd.ie

# -- Load the correct modules

. /etc/profile.d/modules.sh

module load tcin fsl/4.1.9

module load tcin freesurfer/5.2.0

# -- Now we can run the commands we would like.

# -- Set the SUBJECTS_DIR variable

export FREESURFER_HOME=/home/support/tcin/apps/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export SUBJECTS_DIR=*****/

recon-all -s 001 -all
FreeSurfer Group Analysis

1. **Qcache script:** resamples to average subject and smooths at various full-width at half maximum (FWHM).

```bash
#!/bin/bash

# -- Request ONE node in the cluster

#SBATCH -N 1

# -- Request a node in the 'compute' partition

#SBATCH -p compute

# -- Request 96 hours of time

#SBATCH -t 96:00:00

# -- Assign this to your project ID

#SBATCH -U HPC_11_00168

# -- Give the job a name for identification
```
#SBATCCH -J FS_Subject_CON7082

# -- Load the correct modules

  . /etc/profile.d/modules.sh

module load tcin fsl/4.1.9

module load tcin freesurfer/5.2.0

# -- Now we can run the commands we would like.

# -- Set the SUBJECTS_DIR variable

export FREESURFER_HOME=/home/support/tcin/apps/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export FREESURFER_HOME=/home/support/tcin/apps/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export SUBJECTS_DIR=/projects/pi-donoghug/HPC_11_00168/Freesurfer_data/

recon-all -s CON7054 -qcache

export SUBJECTS_DIR=/projects/pi-donoghug/HPC_11_00168/Freesurfer_data/

recon-all -s CON9004 -qcache

2. mris_preproc

#!/bin/bash
# -- Request ONE node in the cluster

#SBATCH -N 1

# -- Request a node in the 'compute' partition

#SBATCH -p compute

# -- Request 96 hours of time

#SBATCH -t 96:00:00

# -- Assign this to your project ID

#SBATCH -U HPC_11_00168

# -- Give the job a name for identification

#SBATCH -J FS_Subject_******

# -- Load the correct modules

. /etc/profile.d/modules.sh

module load tcin fsl/4.1.9

module load tcin freesurfer/5.2.0
# -- Now we can run the commands we would like.

# -- Set the SUBJECTS_DIR variable

export FREESURFER_HOME=/home/support/tcin/apps/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export SUBJECTS_DIR=/projects/pi-donoghug/HPC_11_00168/Freesurfer_data

mris_preproc --fsgd ****.fsgd --cache-in thickness.fwhm10.fsaverage --target fsaverage --hemi rh --out rh.*****.doss.10.mgh

3. **mri_glmfit script**: fits a general linear model at each vertex in the cortex

#!/bin/bash

# -- Request ONE node in the cluster

#SBATCH -N 1

# -- Request a node in the 'compute' partition

#SBATCH -p compute
# -- Request 96 hours of time

#SBATCH -t 96:00:00

# -- Assign this to your project ID

#SBATCH

# -- Give the job a name for identification

#SBATCH -J FS_Subject_******

# -- Load the correct modules

/etc/profile.d/modules.sh

module load tcin fsl/4.1.9

module load tcin freesurfer/5.2.0

# -- Now we can run the commands we would like.

# -- Set the SUBJECTS_DIR variable

export FREESURFER_HOME=/home/support/tcin/apps/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export SUBJECTS_DIR=/projects/pi-donoghug/HPC_11_00168/Freesurfer_data
mri_glmfit --y rh.*****.thickness.doss.10.mgh --fsgd *****.fsgd doss --C
<three_group_contrast1 mtx> --C <three_group_contrast2 mtx> --C
<three_group_contrast3 mtx> --C <three_group_ftest mtx> --surf fsaverage rh --cortex
--glmdir rh.*****.thickness.doss.glmdir

4. **mri_glmmsim script:** Corrects the statistical parametric maps of significant group differences with a vertex-wise threshold (VWT) of $p < 0.05$ at each vertex in the cortical surface for multiple comparisons using Monte Carlo permutation cluster analyses with 10000 repeats

#!/bin/bash

# -- Request ONE node in the cluster

#SBATCH -N 1

# -- Request a node in the 'compute' partition

#SBATCH -p compute

# -- Request 96 hours of time

#SBATCH -t 96:00:00

# -- Assign this to your project ID
#SBATCH -U HPC_11_00168

# -- Give the job a name for identification

#SBATCH -J FS_Subject_******

# -- Load the correct modules

/etc/profile.d/modules.sh

module load tci fsl/4.1.9

module load tci freesurfer/5.2.0

# -- Now we can run the commands we would like.

# -- Set the SUBJECTS_DIR variable

export FREESURFER_HOME=/home/support/tci/app/freesurfer/5.2.0

source $FREESURFER_HOME/SetUpFreeSurfer.sh

export SUBJECTS_DIR=/projects/pi-donoghug/HPC_11_00168/Freesurfer_data

mri_glmfit-sim --glmdir rh.*****.thickness.doss.glmdir --sim mc-z 10000 1.3 mc-z.abs --sim-sign abs --cwpvalthresh .999 --overwrite

Visualization using TKSURFER graphical user interface (GUI)

tksurfer fsaverage rh inflated -annotation

/Users/sinead/Documents/Freesurfer_data/*******_.thickness.doss.glmdir/three_group_c
ontrast2/mc-z.abs.sig.ocn.annot -fthresh 5 -overlay
/Users/sinead/Documents/Freesurfer_data/********.thickness.doss.glmdir/three_group_c
ontrast2/mc-z.abs.sig.cluster.mgh
Appendix E: Conference presentations, Faculty of 1000 evaluations and awards won
List of Conference Oral Presentations

1. Impact of schizophrenia risk variant rs7914558 at CNNM2 on white matter microstructure. *Inaugural Human Disease Mapping Meeting*, 30-31\textsuperscript{st} January, 2014, RCSI, Dublin

2. The impact of schizophrenia risk variant rs10503253 at CSMD1 on white matter connectivity. *5\textsuperscript{th} School of Medicine Postgraduate Research Day*, 20\textsuperscript{th} September, 2012

3. Impact of genome-wide associated schizophrenia risk variant rs10503253 at CSMD1 on structural brain connectivity. *7\textsuperscript{th} Annual Neuroscience Ireland Meeting*, 5\textsuperscript{th}-6\textsuperscript{th} September, 2012, RCSI, Dublin

4. The impact of common schizophrenia risk variants on structural brain connectivity. *Annual Irish Diffusion Imaging Group Symposium*, Feb 8\textsuperscript{th}, 2013, National University of Ireland Galway

List of Conference Posters

1. The effects of psychosis risk genes on functional and structural neural connectivity: a review. *TCD school of Medicine, Tercentenary symposium*, 4\textsuperscript{th} November, 2011, Trinity College Dublin

2. CSMD1 genome-wide associated schizophrenia risk variant rs10503253 and brain white matter integrity. *8\textsuperscript{th} Federation of European Neuroscience Societies Forum of Neuroscience*, 14\textsuperscript{th}-18\textsuperscript{th} July, 2012, Barcelona, Spain

3. Impact of schizophrenia risk variant rs10503253 at CSMD1 on white matter connectivity. *19\textsuperscript{TH Annual meeting of the Organisation for Human Brain Mapping*}, June 16\textsuperscript{th} – 13\textsuperscript{th}, 2013, Seattle, Washington
Awards won:

1. Runner-up short talk at *Inaugural Human Disease Mapping Meeting*, 30-31\textsuperscript{st} January, 2014, RCSI, Dublin

Faculty of 1000 evaluations:


Appendix F: Publications arising from this research and manuscripts in progress
List of publications


Manuscripts in progress


3. Van Erp, T.G.M. et al., A prospective meta-analysis comparing subcortical brain volume abnormalities in 2,083 patients with schizophrenia and 2,540 controls via the ENIGMA consortium
Impact of schizophrenia risk variant rs7914558 at CNNM2 on white matter microstructure

Sinead Kelly¹, ², Derek W. Morris¹, ⁶, Emma Jane Rose¹, ², ³, Omar Mothersill¹, ², Ciara Fahey¹, ², Carol O'Brien¹, ², Erik O'Hanlon⁴, Michael Gill¹, ², Aiden P. Corvin¹, ², Gary Donohoe¹, ², ⁵

1. Neuropsychiatric Genetics Group, Department of Psychiatry, Trinity College Dublin, Ireland.
2. Trinity College Institute for Neuroscience, Trinity College Dublin, Ireland.
3. Transdisciplinary Science and Translational Prevention Program (TSTPP), Research Triangle Institute, Baltimore, Maryland, United States.
4. Department of Psychiatry, Royal College of Surgeons in Ireland, Ireland.
5. School of Psychology, National University of Ireland Galway, Ireland.
6. Discipline of Biochemistry, School of Natural Sciences, National University of Ireland Galway, Ireland.

Running Title: Effects of rs7914558 on white matter

Corresponding author:

Prof. Gary Donohoe

Professor of Psychology

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Abstract

A single nucleotide polymorphism (SNP rs7914558) located at cyclin M2 (CNNM2) on 10q24.32 recently reached genome-wide significance for schizophrenia (SZ). It is unknown how risk may be mediated by this variant, particularly given the high linkage disequilibrium (LD) within the region. Given CNNM2's role in magnesium transport, which in turn is required for white matter (WM) myelination, we investigated whether this SNP was associated with variability in WM microstructure. Diffusion tensor imaging (DTI) was conducted on 125 healthy controls genotyped for rs7914558. Tractography analysis was carried out for the inferior longitudinal fasciculus (ILF), the uncinate fasciculus (UF), the inferior fronto-occipital fasciculus (IFOF), the anterior thalamic radiation (ATR), the cingulum bundle (CB) and the corpus callosum (CC). A dosage effect of the risk G allele at rs7914558 was observed for mean fractional anisotropy (FA) of the right ATR, such that the risk allele was associated with increasing FA (p < 0.05). A similar dosage effect was observed for mean diffusivity (MD) and radial diffusivity (RD) of the right ILF, UF, and the left IFOF, with the risk allele being associated with decreased MD and RD (p < 0.05). While requiring confirmation in independent samples, particularly in cases with SZ, these data suggest that changes in WM integrity may be one of the biological mechanisms associated with this schizophrenia-associated risk variant.

Keywords: DTI; schizophrenia; white matter; CNNM2; GWAS

1.1 Introduction

Compromised white matter (WM) integrity is frequently associated with schizophrenia (SZ) (Kubicki et al. 2007). Two major WM networks are thought to be disrupted in the illness; the first involves connections between the frontal lobe, thalamus and cingulate gyrus and the second involves WM interconnecting the frontal lobe, insula, hippocampus, amygdala and occipital lobe (Ellison-Wright and Bullmore, 2009). WM
abnormalities are apparent in first-degree relatives, individuals at high risk of SZ, and in patients during the early stages of illness, suggesting that these abnormalities may represent a stable characteristic of the disease under strong genetic control (Kochunov et al. 2010; Pérez-Iglesias et al. 2010; Witthaus et al. 2008;), and therefore a potential endophenotype for SZ (Mothersill et al. 2013).

In one of the largest SZ genome-wide association study (GWAS) to date, which included over 51,000 cases and controls, seven risk loci were identified including five novel findings (Ripke et al. 2011). One of the novel loci identified (10q34.33) contained two variants reaching genome-wide significance, including a single nucleotide polymorphism (SNP) rs7914558 located in region of high and complex linkage disequilibrium spanning four genes, including the cyclin M2 (CNNM2) gene, to which the variants is ascribed by Ripke et al (2011). CNNM2 is implicated in magnesium (Mg\(^{2+}\)) transport, which plays a crucial role in neural transmission and many other biological processes. Hypertension risk and altered serum Mg\(^{2+}\) blood levels are associated with sequence variation in CNNM2 (Meyer et al. 2010). Furthermore, mutations in CNNM2 are associated with hypomagnesaemia risk (Stuiver et al. 2011). We recently found evidence that the same allele was associated with variation in social cognition and increased grey matter volume of the temporal pole and anterior cingulate (Rose et al. 2014); however, the mechanism by which this variant increases SZ risk or causes variability in intermediate phenotypes related to SZ remains unknown.

As magnesium is required for production of myelin sheaths for WM (Gong et al. 2003), and as WM changes are evident in SZ (Davis et al. 2003), this variant may increase SZ risk via an impact on WM microstructure. We reasoned that if the identified CNNM2 variant increases risk via an effect on WM structure then a direct effect of rs7914558 on WM tracts known to be altered in SZ might be detectable. On this basis, the present study sought to characterise in vivo the effects of the SZ-associated risk 'G' allele at rs7914558 on variation of WM structural connectivity using diffusion tensor imaging.
(DTI) in a sample of healthy controls which overlapped substantially with those included in our earlier study (Rose et al. 2014). We hypothesised that the risk ‘G’ allele, identified in the Ripke et al. (2011) analysis, would be associated with altered WM connectivity in six tracts that form part of the previously identified frontal and temporal WM networks altered in SZ (Ellison-Wright and Bullmore, 2009), namely the inferior longitudinal fasciculus (ILF), the uncinate fasciculus (UF), the inferior frontal occipital fasciculus (IFOF), the anterior thalamic radiation (ATR) the cingulum bundle (CB) and the corpus callosum (CC).

2.1 Methods

2.1.1 Participants

The analysis was comprised of 125 healthy participants with a mean age of 32.24 years (SD = 11.71). Participant demographics are presented in Table 1. Healthy participants were recruited from the general population. All participants were genetically Irish (i.e. had Irish-born paternal and maternal grandparents), were between 18 and 65 years of age and were right-handed. Written, informed consent was obtained from all subjects in accordance with local ethics committee guidelines (see Supplementary material). Exclusion criteria included a significant neurological or psychiatric history, a first-degree relative with a diagnosis of SZ or other psychosis, substance abuse in the preceding six months, pregnancy or other contraindication for MRI.

>> INSERT TABLE 1 HERE <<

2.1.2 Genotyping

Genetic analyses were based on DNA extracted from saliva samples obtained using Oragene DNA self-collection kits (DNA Genotek; Ontario, Canada). The CNNM2 risk rs7914558 variant was genotyped using a Taqman® SNP genotyping assay on a
7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium (p > 0.05). Along with these samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for rs7914558 for quality control purposes and were found to be concordant with available HapMap data for this SNP.

2.1.3 DTI

Magnetic resonance images were collected using a 3-T Philips Achieva scanner. DTI images were acquired via a single-shot spin-echo echo planar imaging (EPI) with diffusion sensitizing gradients applied sequentially along 15 non-collinear directions with a b-value of 800s/mm² (TR= 12445ms TE= 52ms, FOV= 224mm x 224mm x 149mm, acquisition matrix = 112 x 112, reconstruction matrix =128 x 128, 60 slices, 2.2mm slice thickness, slice gap = 0.299mm, spatial resolution = 2mm x 2mm x 2.2mm, flip angle = 90°).

2.1.4 Preprocessing

Using ExploreDTI software (Leemans et al. 2009), diffusion data were converted to ExploreDTI*.mat files with a voxel size of 2x2x2mm. Diffusion tensor estimation was weighted linear and was based on the least-squares (LS) regression model (Basser et al. 1994). A cubic interpolation and robust estimation of tensors by outlier rejection (RESTORE) (Chang et al. 2005) was used to correct for subject motion and cardiac pulsation artefacts. Eddy Current correction and B-matrix rotation were also used when realigning the diffusion weighted images (Leemans and Jones, 2009). A quality check of the corrected diffusion data was then performed and residual and outlier profiles were also examined using ExploreDTI. Finally the motion correction parameters were inspected. Movement during scanning was less than 2mm in any direction and less than 3° rotation in axial, sagittal or coronal planes for all participants.

2.1.5 Atlas-based tractography
Atlas-based deterministic tractography was conducted using ExploreDTI4.8.3. All data were transformed into Montreal Neurological Institute (MNI) space. A whole-brain WM tract construction was then carried out for each participant using a linear interpolation. Seed point resolution was set at 2x2x2 mm with a seed fractional anisotropy threshold of 0.2 and an angle threshold of 45 degrees. Then, particular tracts implicated in SZ were isolated using the automated atlas based tractography segmentation tool shown to yield robust and reliable tract delineation (Verhoeven et al. 2010). These were identified as: the UF, the ILF, the IFOF, the ATR the CB and the CC (See supplemental material for a detailed account of ROI placement).

After performing deterministic tractography, diffusion metrics including mean fractional anisotropy (FA) mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) were extracted for each tract (See Figure 1).

2.1.6 Statistical analysis

The diffusivity indices for each participant were exported to PASW statistical software (Release 18; SPSS Inc., Chicago, IL, USA) and subjected to a one-way multivariate analysis of covariance with genotype group (AA, AG, GG) as fixed factors. Age and gender did not significantly differ between genotype groups and therefore these variables were included as covariates of no interest in the analysis as a matter of standard protocol (Miller and Chapman, 2001). As FA, MD, RD and AD are constructed from the three eigenvalues of the tensor, an alpha level of \( p = 0.017 \) was applied to Bonferroni correct for testing of multiple diffusion measures arising from three eigenvalues.

2.1.7 Whole-brain TBSS
Post-hoc voxelwise tract-based spatial statistics (TBSS, Smith et al. 2006) were performed using FSL v4.1.6 (Smith et al. 2004) to determine if any detected genotype effects on FA, MD, AD or RD were specific to our original six tracts of interest, or whether these effects extended to additional brain regions. TBSS pre- and post-processing, as described by Smith et al. (2006), was conducted on each genotype group (see supplementary material). A GLM F-test design was then constructed to examine voxelwise FA, MD, AD and RD differences between genotype groups. Six post-hoc t-tests were subsequently conducted to assess direction of effects between each group. The statistical threshold of $p<0.05$ was (i) family-wise error corrected for comparisons across the entire brain and (ii) Bonferroni-corrected for the six post-hoc t-tests multiplied by the three tensor eigenvalues from which FA, MD, AD and RD were derived, giving a new alpha level of 0.0027 (0.05/18).

3.1 Results

3.1.1 Tractography

After covarying for age and gender, an effect of genotype was observed for fibres of the right ATR ($F_{2, 114} = 4.51$, $p = .013$, partial $\eta^2 = .073$) with homozygous as well as heterozygous G allele carriers showing increased FA in comparison to A homozygotes (See Figure 2 (a)). An effect of genotype was observed for MD in the left IFOF ($F_{2, 113} = 6.62$, $p = .002$, partial $\eta^2 = .105$) and the right ILF ($F_{2, 113} = 7.54$, $p = .001$; partial $\eta^2 = .118$), with risk homozygote and heterozygote G allele carriers displaying decreased MD in comparison to non risk A homozygotes (see Figure 2 (b) and (c)). The right UF also displayed a genotype effect for MD ($F_{2, 113} = 4.82$, $p = .01$, partial $\eta^2 = .079$), with decreased MD in homozygous G carriers compared to A carriers (see Figure 2 (d)).
An effect of genotype on RD was noted for the left IFOF ($F_{(2,113)} = 7.59$, $p = .001$, partial $\eta^2 = .118$), and the right ILF for RD ($F_{(2,113)} = 6.92$, $p = .001$, partial $\eta^2 = .109$) due to decreased MD and RD in G allele carriers in comparison to homozygous A allele carriers. (see Figure 2 (e) and (f)). An effect of genotype for fibres of the right UF was also observed ($F_{(2,113)} = 4.76$, $p = .01$, partial $\eta^2 = .078$) with G homozygotes displaying decreased RD in comparison to A homozygotes (See Figure 2 (g)).

3.1.2 TBSS

After family-wise error (FWE) correction using threshold free cluster enhancement (TFCE, Smith and Nichols, 2009) and Bonferroni correcting for 6 comparisons x 3 diffusion eigenvalues, two of the six one-tailed t-tests remained significant. In comparison to AG carriers, A allele homozygotes had decreased FA in the left IFOF, left ILF, right corticospinal tract, right IFOF and right ATR ($p < 0.0027$) (See Table 2 and Figure 3(a)). A allele homozygotes also displayed decreased FA in comparison to homozygous G carriers in the right ILF, right IFOF, splenium of corpus callosum, forceps minor, left IFOF, left ILF and right corticospinal tract ($p = 0.0027$) (See Table 3 and Figure 3(b)).

For the MD data, homozygous A carriers had increased MD in a large cluster of the forceps minor and the ATR in comparison to G allele homozygotes ($p = 0.035$), however this did not remain significant after Bonferroni correction (See Table 4 and Figure 4).

Finally, two t-tests for the RD data remained significant after Bonferroni correction. Homozygous A carriers displayed increased RD in clusters of the left IFOF, ($p < .0027$) (See Table 5 and Figure 5 (a)). Similarly, homozygous A carriers displayed increased
RD in comparison to homozygous G carriers in clusters of the forceps minor (p < .0027), remaining significant after Bonferroni correction for multiple comparisons (See Table 6 and Figure 5 (b)).

<<INSERT FIGURE 3 HERE>>

<<INSERT TABLE 2 AND TABLE 3 HERE>>

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<<INSERT FIGURE 5 HERE>>

<<INSERT TABLE 5 AND TABLE 6 HERE>>

4.1 Discussion

In this study we sought to characterize the effects of the SZ risk variant rs7914558 located at CNNM2 on WM microstructure in healthy controls. Five WM tracts were selected for analysis based on previous evidence of their association with SZ pathophysiology (Ellison-Wright and Bullmore, 2009; Kubicki et al. 2007) and SZ related clinical symptom severity and cognitive deficits (Ashtari et al. 2007; Mamah et al. 2010; Szeszko et al. 2008). Based on a number of related diffusivity metrics, CNNM2 was associated with differences in diffusivity along four of the six WM tracts investigated. Differences in WM microstructure were observed using measures of MD and RD in (1) the right ILF, (2) the right UF and (3) the left IFOF, and using measures of FA (4) in the right ATR. In each case the risk G allele carriers showed significantly increased FA or decreased MD and RD in comparison to the non-risk homozygous group. In addition to observing an effect in four of the five tracts investigated (and in the same direction across these tracts) the robustness of CNNM2’s effects on WM connectivity was also

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illustrated by the size of effects observed. Based on partial $\eta^2$ values, these ranged from .073-0.118, indicating that CNNM2 rs7914558 explained 7-12% of WM variation in this sample, comparable to effect sizes found in previous imaging genetic studies using DTI (Mothersill et al. 2012).

Post-hoc voxelwise TBSS analyses were conducted to determine if our genotype effects were specific to the tracts selected or if they extended to additional WM fibres. Genotype effects on FA, MD and RD data were observed along the left IFOF, right ILF and ATR – supporting our tractography findings. However, these effects also extended to the corticospinal tract and the genu and forceps minor of the corpus callosum (CC). This suggests that although deterministic tractography of the entire CC initially failed to yield significant associations, prospective segmentation tractography decomposing the structure into sub-sections may reveal more subtle genotype effects.

In SZ, FA is usually decreased and MD and RD are generally increased in comparison to healthy controls (Kubicki et al. 2007; Ellison-Wright and Bullmore, 2009; Lee et al. 2013). In the present study, based on healthy participants carriers V non-carriers of the rs7914558 risk variant, the association fell in the opposite direction. However, the relationship between WM and SZ is complex. For example, in SZ patients, increased FA of fibres connecting temporal regions has been associated with increasing severity of hallucinations (Hubl et al. 2004; Mulert et al. 2012; Seok et al. 2007; Shergill et al. 2007; Rotarska-Jagiela and Oertel-K noeichel, 2009), while increased FA within the UF and IFOF has been correlated with increased positive and negative symptoms of SZ (Lee et al. 2013; Szeszko et al. 2008).

Furthermore, findings of increased FA and decreased MD and RD in risk carriers may also represent some sort of compensatory mechanism (Alba-Ferrera and de Erausquin 2013), with relatively preserved WM previously reported in siblings of SZ patients (Boos et al. 2013), participants at high risk (Hoptman et al., 2008) and participants at ‘ultra high-risk’ of psychosis (Bloeman et al. 2010). However, this is speculative and further
investigation of the functional biology of this variant is required to determine causality. More importantly, a number of previous studies from our group (e.g. ZNF804A (Walters et al. 2010; Hargreaves et al. 2011)) and others (e.g. DARPP-32 (Meyer-Lindenberg and Straub, 2007)) have previously found evidence of association between SZ risk alleles and preserved brain structure or function. In a recent study on an overlapping sample from our group (Rose et al. 2014) the CNNM2 risk allele was also associated with variation in grey matter and social cognitive performance. In that study, risk allele carriers showed increased grey matter volumes in regions implicated in social cognition. The direction of findings in the present study would appear consistent with these previous findings.

An important consideration in characterising the neural effects of risk variants such as CNNM2 is their potential interactions with other SZ genetic risk factors and/or environmental risk factors for disease. The SNP studied in the present analysis, rs7914558, is located on chromosome 10q24 in a large region of high and complex linkage disequilibrium (LD). Analysis of HapMap Phase II + III data (release 28 NCBI build 36) indicates that rs7914558, positioned at 104,765,898(hg18) is in high LD (r2>0.8) with 42 other SNPs in this region (including 22 SNPs at r2=1). These SNPs span a 322kb region from rs11191438 at position 104,627,854 to rs4307650 at position 104,949,842. This region contains four RefSeq genes: C10orf32, AS3MT, CNNM2 and NT5C2. Analysis of SNP x gene expression databases does not identify a direct functional link between rs7914558 (or proxy) and altered expression of any of the four genes in this region, and therefore all four remain candidate SZ loci.

4.1.1 Limitations

Very little is known regarding the functional effects of the CNNM2/rs7914558 SNP and therefore it is difficult to hypothesize the mechanism by which this variant acts to increase risk for SZ. As one step towards addressing this, the present study suggests a role for CNNM2 in structural connectivity in healthy controls. Confirming these effects in
a larger sample as well as in patients with SZ will be an important next step in establishing the relevance of these findings to SZ risk. Similarly, as the diffusion data consisted of just 15 diffusion gradient directions, repeating this analysis using high-angular resolution data will improve our characterization of complex crossing WM fibers and allow for greater sensitivity of diffusion measures including FA, AD and RD (Alexander et al. 2001; Wheeler-Kingshot and Cergiani, 2009). Tractography of projection and commissural tracts should also be conducted in light of our whole-brain TBSS results.

4.1.2 Conclusions

The present study suggests that a novel genome-wide associated risk variant for SZ, CNNM2 (rs7914558), is associated with variation in WM microstructure in a sample of healthy controls, with the risk G allele carriers displaying decreases in MD and RD as well as increases in FA. Highlighting the utility of DTI for elucidating the biological functions of GWAS identified variants in SZ, these data suggest that the CNNM2 risk variant investigated was associated with apparently preserved WM integrity. These findings were consistent with the direction of association of an earlier CNNM2 study by our group, in which the CNNM2 risk allele was associated with preserved neuropsychological function and grey matter volume (Rose et al. 2014). Nonetheless, the findings would benefit from replication using a similar diffusion-based approach. The clinical and cognitive consequences of this variant remain to be elucidated, and further studies of this variant's effects on both WM and other intermediate phenotypes are necessary to confirm its functional significance.
References


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Author disclosure

Role of funding source

This work was generously supported by Science Foundation Ireland (SFI grant 12/IP/1359 to GD and SFI08/IN.1/B1916-Corvin to AC), and the Health Research Board (HRA/2009/197 to GD). The authors report that there are no manuscript archiving requirements specified as conditions of these grant awards.

Contributors

Kelly S contributed to conception and design, acquisition of data, analysis/interpretation, writing of article, critical review and final approval for submission.

Morris D contributed to acquisition of data, critical review of article and final approval for publication.

Mothersill O contributed to acquisition of data, critical review of article and final approval for publication.
Rose E contributed to acquisition of data, critical review of article and final approval for publication.

Fahey C contributed to acquisition of data, critical review of article and final approval for publication.

O'Brien C contributed to acquisition of data, critical review of article and final approval for publication.

O'Hanlon E contributed to analysis and interpretation, critical review of article and final approval for publication.

Corvin A contributed to conception and design, acquisition of data, critical review of article and final approval for publication.

Gill M contributed to conception and design, critical review of article and final approval for publication.

Donohoe G contributed to conception and design, acquisition of data, analysis/interpretation, writing of article, critical review and final approval for submission.

**Conflict of Interest**

All authors have declared no conflict of interest in relation to the subject of this study.

**Acknowledgments**

We would like to thank all those individuals who participated in the study, and staff and students in the Trinity College Institute of Neuroscience involved in the collection of data. Finally, we would like to acknowledge data management support from the Trinity Centre for High Performance Computing. The data management system used for this work was BC|SNPmax v. 3.5-121 (Biocomputing Platforms Ltd, Finland).
Figure legends, tables, figures

Figure legends

**Figure 1.** Atlas-based deterministic tract reconstruction of the ATR (A. ii and i), CB (B. i and ii), IFOF (C. i and ii), IFL (D. i and ii), UF (E. i and ii) and CC (F) for left and right hemispheres.

**Figure 2.** Differences between rs7914558 genotype groups in measures of a) fractional anisotropy (FA) of the right anterior thalamic radiation, b) mean diffusivity (MD) of the left inferior fronto-occipital fasciculus (IFOF), c) MD of the right inferior longitudinal fasciculus (ILF), d) MD of the right uncinate fasciculus, (UF), e) radial diffusivity (RD) of the left IFOF, f) RD of the right ILF and g) RD of the right UF). * p < .05; ** p < .01. Error bars represent +/- 2 standard error

**Figure 3.** a) regions showing decreased FA for AA homozygotes in comparison to AG carriers (p < .0027 FWE corrected), b) regions showing decreased FA for AA homozygotes in comparison to GG homozygotes (p < .0027 FWE corrected)

**Figure 4.** Regions showing increased MD for AA homozygotes in comparison to GG homozygotes (p < .05 FWE corrected)

**Figure 5.** a) regions showing increased RD for AA homozygotes in comparison to AG carriers (p < .0027 FWE corrected), b) regions showing increased RD for AA homozygotes in comparison to GG homozygotes (p < .0027 FWE corrected)

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Table 1. Participant demographics based on rs7914558 genotype

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**Table 2.** Regions of decreased FA for AA carriers in comparison to AG carriers (p < .0027, FWE corrected)

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**Table 3.** Regions of decreased FA for AA carriers in comparison to GG carriers (p < .001, FWE corrected)

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**Table 4.** Regions of increased MD for AA carriers in comparison to GG carriers (p < .0027, FWE corrected)

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**Table 5.** Regions of increased RD for AA carriers in comparison to AG carriers (p < .0027, FWE Corrected)

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<td>-8</td>
<td>29</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 6.** Regions of increased RD for AA carriers in comparison to GG carriers (p < .0027, FWE corrected)

**Figures**
Figure 1. Atlas-based deterministic tract reconstruction of the ATR (A. ii and i), CB (B. i and ii), IFOF (C i and ii), IFL (D i and ii), UF (E i and ii) and CC (F) for left and right hemispheres.

Figure 2. Differences between rs7914558 genotype groups in measures of a) fractional anisotropy (FA) of the right anterior thalamic radiation, b) mean diffusivity (MD) of the left
inferior fronto-occipital fasciculus (IFOF), c) MD of the right inferior longitudinal fasciculus (ILF), d) MD of the right uncinate fasciculus, (UF), e) radial diffusivity (RD) of the left IFOF, f) RD of the right ILF and g) RD of the right UF). * p < .05; ** p < .01. Error bars represent +/- 2 standard error

Figure 3. a) regions showing decreased FA for AA homozygotes in comparison to AG carriers (p < .0027 FWE corrected), b) regions showing decreased FA for AA homozygotes in comparison to GG homozygotes (p < .0027 FWE corrected)
Figure 4. Regions showing increased MD for AA homozygotes in comparison to GG homozygotes (p < .05 FWE corrected)

Figure 5. a) regions showing increased RD for AA homozygotes in comparison to AG carriers (p < .0027 FWE corrected), b) regions showing increased RD for AA homozygotes in comparison to GG homozygotes (p < .0027 FWE corrected)
Genome-wide schizophrenia variant at MIR137 does not impact white matter microstructure in healthy participants

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Running Title: MIR137 and white matter microstructure

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Abstract

A single nucleotide polymorphism (SNP rs1625579) within the micro RNA 137 (MIR137) gene recently achieved strong genome-wide association with schizophrenia (SZ). However, how SZ risk may be mediated by this variant is unknown. As miRNAs have the potential to influence oligodendrocyte development, we investigated whether this SNP was associated with variability in white matter (WM) microstructure. Diffusion tensor imaging (DTI) was conducted on 123 healthy participants genotyped for rs1625579. The analysis consisted of whole-brain tract-based spatial statistics and atlas-based tractography analysis of the six major WM tracts known to be affected in SZ – the inferior longitudinal fasciculus, the uncinate fasciculus, the inferior fronto-occipital fasciculus, the anterior thalamic radiation, the cingulum bundle and the corpus callosum. No significant differences in either whole-brain fractional anisotropy or mean diffusivity between MIR137 genotype groups were observed (p > 0.05). Similarly, atlas-based tractography of particular tracts implicated in SZ failed to reveal any significant differences between MIR137 genotype groups on measures of WM connectivity (p > 0.05). In the absence of WM effects comparable to those reported for other SZ associated genes, these data suggest that this variant alone may not confer variability in these WM measures and therefore may not act in isolation for any effects that the variant may have on WM microstructure in SZ samples.
Introduction

Compromised white matter (WM) integrity is a widely replicated finding in schizophrenia (SZ) pathophysiology [1] with a meta-analysis revealing two aberrant WM networks, firstly in the frontal lobe consisting of WM interconnecting the frontal lobe, thalamus and cingulate gyrus and, secondly, a network in the temporal WM interconnecting the frontal lobule, insula, hippocampus-amygdala and occipital lobe [2]. An abundance of evidence suggests that these WM abnormalities SZ are heritable, for example WM abnormalities are apparent in first-degree relatives, individuals at high risk of SZ, and in patients during the early stages of illness [3-5]. Therefore, WM connectivity is potentially an appropriate endophenotype (i.e a variation occurring on the pathway between genes and disease phenotype) for delineating the mechanisms of genetic risk in SZ [6].

A single nucleotide polymorphism (SNP) rs1625579, within an intron of the micro RNA 137 (MIR137) host gene showed the strongest genome-wide association for SZ in one of the largest genome-wide association studies (GWAS) to date [7]. MIR137 regulates maturation and migration of adult neural stem cells [8] and is expressed in the subventricular layers and subgranular layer of the hippocampus [9]. It plays a critical role in neurogenesis and dendritic morphogenesis [9] and has also been shown to regulate other genes with genome-wide significance for SZ, including CACNA1C and TCF4 [10] that may be relevant for WM integrity and oligodendrocyte development [11, 12, 13]. In addition, miRNAs can act to prevent the expression of genes that promote oligodendrocyte precursor cell maintenance to inhibit proliferation and thus promote differentiation [14]. Therefore, evidence suggests that MIR137 may regulate genes that have the potential to affect WM development.

A recent investigation conducted by Lett and colleagues [15] found in a neuroimaging sample of 92 SZ patients and 121 healthy controls, that the risk T allele homozygote patients displayed decreases in whole-brain fractional anisotropy (FA) throughout the WM skeleton using tract-based spatial statistics (TBSS). This effect was observed in the
patient sample only. Considering miRNAs have the ability to promote oligodendrocyte
development, and as MIR137 regulates other SZ genes that may be crucial for the
development of WM, confirming whether MIR137's effect on WM are general or specific
to SZ patients remains an important question. The purpose of the present study was to
address this question in a comparable sample of healthy participants stratified by
MIR137 genotype. To expand on the previous study [15], we conducted region of
interest (ROI) tractography on WM tracts known to be impacted by SZ (i.e. the inferior
longitudinal fasciculus (ILF), uncinate fasciculus (UF), inferior frontal occipital fasciculus
(IFOF), anterior thalamic radiation (ATR) the cingulum bundle (CB) and the corpus
callosum (CC)), as well as whole-brain TBSS. We also evaluated potential effects of this
variant on alternative measures of WM microstructure more sensitive to axonal density
and myelination [16,17,18] including axial, radial and mean diffusivity, as well as FA. It
was hypothesised that the risk 'T' allele at rs1625579 would be associated with
compromised WM connectivity in those tracts that form frontal and temporal WM
networks altered in SZ [2].

Methods

Participants

The sample was comprised of 123 healthy participants recruited from the general
population with a mean age of 32.30 years (SD = 11.68). Participant demographics are
presented in Table 1. All participants were genetically Irish (i.e. had Irish born paternal
and maternal grandparents), were between 18 and 65 years of age and were right-
handed. Written, informed consent was obtained from all subjects in accordance with
local ethics committee guidelines. Exclusion criteria included a significant neurological or
psychiatric history, a first-degree relative with a diagnosis of SZ or other psychosis,
substance abuse in the preceding six months, pregnancy or other contraindication for MRI.

>>INSERT FIGURE 1 HERE<<

Genotyping

The MIR137 rs1625579 variant was genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems) (See supplementary material)

DTI

Magnetic resonance images were collected using a 3-T Philips Achieva scanner. DTI images were acquired via a single-shot spin-echo echo planar imaging (EPI) with diffusion sensitizing gradients applied sequentially along 15 non-collinear directions with a b-value of 800s/mm² (TR= 12445ms TE= 52ms, FOV= 224mm x 224mm x 149mm, acquisition matrix = 112 x 112, reconstruction matrix =128 x 128, 60 slices, 2.2mm slice thickness, slice gap = 0.299mm, spatial resolution = 2mm x 2mm x 2.2mm, flip angle = 90°).

Preprocessing

Using ExploreDTI software [19], diffusion data were converted to ExploreDTI*.mat files with a voxel size of 2x2x2mm. Diffusion tensor estimation was weighted linear and was based on the least-squares (LS) regression model [20]. A cubic interpolation and robust estimation of tensors by outlier rejection (RESTORE) [21] was
used to correct for subject motion and cardiac pulsation artifacts. Data processing also included eddy current correction and B-matrix rotation during realignment [22]. A visual inspection of the corrected diffusion data was then performed and residual and outlier profiles were also examined. Allowable movement during scanning was less than 2mm in any direction and less than 3° rotation in axial, sagittal or coronal planes for all participants. A further nine subjects were not included in the current sample due to excessive motion in the scanner.

**Atlas-based tractography**

Atlas-based deterministic tractography was conducted using ExploreDTI (v4.8.3). All data were transformed into Montreal Neurological Institute (MNI) space. A whole-brain WM tract construction was then carried out for each participant using a linear interpolation. Seed point resolution was set at $2^2 \times 2^2$ mm with a seed fractional anisotropy threshold of 0.2 and an angle threshold of 45 degrees. Particular tracts implicated in SZ (i.e. UF, ILF, IFOF, ATR, CB and CC) were isolated using the automated atlas based tractography segmentation tool known to yield robust and reliable tract delineation [23]. (See Figure 1).

The diffusivity indices including mean FA, AD, MD and RD for each participant were exported to PASW statistical software (Release 18; SPSS Inc., Chicago, IL, USA) and subjected to a one-way multivariate analysis of covariance with genotype group (GT/GG and TT) as fixed factors. The frequency of the minor 'G' allele is such that in our sample only two individuals were identified who were 'G' homozygotes. Therefore, GT and GG carriers were collapsed into one group as previously conducted [24]. Age and gender did not significantly differ between genotype groups and therefore these variables were included as covariates of no interest in the analysis as a matter of standard protocol [25]. As FA, MD, RD and AD are constructed from the three eigenvalues of the tensor, a Bonferroni corrected alpha level of $p = .017$ was applied for testing of multiple diffusion measures arising from three eigenvalues.
Whole-brain TBSS

Voxel-wise tract-based spatial statistics (TBSS [26]) of the FA and MD data was performed using FSL v4.1.6 to determine if any potential genotype effects are specific to the tracts selected or if they are more generalized. All subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT [27,28] that uses a b-spline representation of the registration warp field [29]. Next, the mean FA image was created and thinned to create a mean FA skeleton representing the centres of all tracts common to the group. Each subject's aligned FA data was projected onto this skeleton and the resulting data was fed into voxelwise cross-subject statistics. For MD data, FA images were used to achieve the nonlinear registration and skeletonisation stages, and also to estimate the projection from each individual subject onto the mean FA skeleton. The nonlinear warps and skeleton projection were then applied to MD data.

Statistical analysis for TBSS was conducted using FSL's 'Randomise' algorithm. Threshold-free cluster enhancement (TFCE) [30] was selected using 5000 permutations per test. For the two genotype groups, two one-way general linear model (GLM) t-test designs were set-up. The statistical threshold was set at $p<0.05$, family-wise error corrected (FWE) corrected for multiple comparisons across the whole brain to find differences between genotype groups.

Results

DTI

After FWE correction, no significant differences in whole-brain FA or MD between MIR137 genotype groups remained significant ($p > 0.05$). Similarly, atlas-based tractography of particular tracts implicated in SZ revealed no significant differences between MIR137 genotype groups on measures of FA, MD, AD or RD ($p > 0.05$).
Discussion

The rs1625579 SNP at MIR137 remains one of the strongest genome-wide associated variants for SZ [7]. The association of this polymorphism with variation in cognition, brain structure, function, age-of-onset of psychosis, and SZ symptomology suggests that this gene affects established endophenotypes for SZ. Since variation of WM connectivity is considered a reliable SZ endophenotype, and miRNAs are implicated in oligodendrocyte development, we investigated if this variant may also confer risk for SZ via an impact of WM microstructure. WM microstructural variations associated with MIR137 have been reported for SZ patients but not in healthy controls [15]. The present study aimed to expand on the previous work by investigating whole-brain FA and MD, and conducting regionally specific tractography in tracts that are relevant to the SZ disease phenotype. It was our aim to determine whether a more extensive battery of tests of WM integrity may be sensitive to the effects of SZ risk variants, like MIR137/rs1625579, in the absence of confounding disease-specific effects, i.e. in healthy individuals. Our analyses were not indicative of an association between MIR137 genotype and measures of FA, or MD, at a whole-brain level or for FA, MD, AD and RD at an ROI tract-based level. This expands on the previous findings [15] and lends support to the conclusion that MIR137 may not impact WM independently of SZ diagnosis. Additional factors associated with illness including the presence of other risk variants, medication, as well as severity and duration of illness may contribute to previous MIR137 patient effects [15].

Given the negative findings reported here, one important question to consider is whether our study was adequately powered to detect true differences associated with this variant. Post-hoc power analyse are not recommended [31], and therefore the power of our study to detect a particular effect size was calculated using G* Power 3 software [32] avoiding the use of p values and effect sizes generated from our own analysis. Corresponding to a one-tailed t-test with p < 0.05, it was calculated that the
sample size of this study had 100% power to detect an effect of Cohen's $d = .7$ or greater and 90% power to detect a medium effect size of .55. This is in line with effect sizes found in previous imaging genetics studies utilizing DTI [6]. However, this sample size had only 50% power to detect an effect size of .3. On this basis, while the present analysis was underpowered to detect smaller effects, it would appear to have been adequately powered to detect effects of the size previously reported for other risk variants [6].

Another factor that may have contributed to our outcomes and should be considered is that our DTI data included just 15 gradient directions. Repeating this analysis using high-angular resolution data may be useful for characterizing complex crossing fibers in brain WM bundles. Measures such as FA, AD and RD are found to be sensitive to the presence of crossing fibers [33]. Crossing fibers are particularly problematic for tractography based on the diffusion tensor [34] as they can potentially cause the tracking algorithm to venture off course into an adjacent WM tract and this can result in both false-positive and false-negatives connections [35]. In addition, Sprooten and colleagues [36] also point out that DTI is not sensitive to more small-scale differences in structural integrity given the scale at which FA is measured, especially close to the synapse or near the grey matter, further away from large fiber bundles.

Finally, the latest SZ GWAS has identified another SNP at the same locus in a larger sample [37]. The SNP considered in this current analysis was selected prior to publication of this latest GWAS, however, both SNPs are in high linkage disequilibrium ($r^2=0.76$) and therefore it is unlikely that a potential association between these variants and WM microstructure in healthy participants has been discounted.
Conclusions

No significant effects of rs1625579 at MIR137 were found in healthy individuals for either whole-brain TBSS and ROI tractography. This was true for measures of MD, RD and AD, as well as the more commonly used FA. This expands on the previous findings [15] and suggests that MIR137 may not impact WM independently of SZ diagnosis. A recent study by our group found an association with this variant and altered functional connectivity during face processing in healthy controls [24]. Understanding potentially differential effects of MIR137 on structural and functional connectivity will require a more detailed exploration of the variant's functional biology. Finally, a multi-variant or pathway approach is warranted to establish if this variant at MIR137 is conferring risk for SZ via regulation of other SZ associated genetic variants.
Acknowledgments

We would like to thank all those individuals who participated in the study, and staff and students in the Trinity College Institute of Neuroscience involved in the collection of data. Finally, we would like to acknowledge data management support from the Trinity Centre for High Performance Computing. The data management system used for this work was BC|SNPmax v. 3.5-121 (Biocomputing Platforms Ltd, Finland). This work was generously supported by Science Foundation Ireland (SFI grant 12/IP/1359 to GD and SFI08/IN.1/B1916-Corvin to AC), and the Health Research Board (HRA/2009/197 to GD).
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Figure 1. Atlas-based deterministic tract reconstruction of the anterior thalamic radiation (ATR) (A. ii and i), cingulum bundle (CB) (B. I and ii), inferior fronto-occipital fasciculus (IFOF) (C i and ii), inferior longitudinal fasciculus (ILF) (D i and ii), uncinate fasciculus (UF) (E i and ii) and the corpus callosum (CC) (F) for left and right hemispheres.
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<th>Age (years; mean (SD))</th>
<th>Education (years; mean (SD))</th>
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</thead>
<tbody>
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<td>24</td>
<td>31.63 (11.93)</td>
<td>16.9 (3.56)</td>
</tr>
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<td>82</td>
<td>41</td>
<td>41</td>
<td>32.02 (11.62)</td>
<td>17.01 (3.17)</td>
</tr>
<tr>
<td>Total</td>
<td>123</td>
<td>58</td>
<td>65</td>
<td>32.30 (11.68)</td>
<td>16.97 (3.29)</td>
</tr>
</tbody>
</table>

Table 1. Participant demographics based on rs1625579 genotype (G is minor allele; T is associated allele)
The effects of psychosis risk variants on brain connectivity: a review

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In light of observed changes in connectivity in schizophrenia and the highly heritable nature of the disease, neural connectivity may serve as an important intermediate phenotype for schizophrenia. However, how individual variants confer altered connectivity and which measure of brain connectivity is more proximal to the underlying genetic architecture (i.e., functional or structural) has not been well delineated. In this review we consider these issues and the relative sensitivity of imaging methodologies to schizophrenia-related changes in connectivity. We searched PubMed for studies considering schizophrenia risk genes AND functional or structural connectivity. Where data was available, summary statistics were used to determine an estimate of effect size (i.e., Cohen's d). A random-effects meta-analysis was used to consider (1) the largest effect and (2) all significant effects between functional and structural studies. Schizophrenia risk variants involved in neurotransmission, neurodevelopment and myelin function were found to be associated with altered neural connectivity. On average, schizophrenia risk genes had a large effect on functional (mean d = 0.76) and structural connectivity (mean d = 1.04). The examination of the largest effect size indicated that the outcomes of functional and structural studies were comparable (Q = 2.17, p > 0.05). Conversely, consideration of effect size estimates for all significant effects suggest that reported effect sizes in structural connectivity studies were more variable than in functional connectivity studies, and that there was a significant lack of homogeneity across the modalities (Q = 6.928, p = 0.008). Given the more variable profile of effect sizes associated with structural connectivity, these data may suggest that structural imaging methods are more sensitive to a wider range of effects, as opposed to functional studies which may only be able to determine large effects. These conclusions are limited by methodological considerations, and require further investigation involving larger samples, multiple genes, and novel analysis techniques for confirmation.

Keywords: schizophrenia, functional connectivity, structural connectivity, genotype, effect size

INTRODUCTION

EXAMINING THE FUNCTION OF SCHIZOPHRENIA (SZ) RISK VARIANTS

Schizophrenia is a complex genetic disorder affecting roughly 1% of the world's population (see Lewis and Lieberman, 2000 for a review). It is characterized by hallucinations and delusions, reduced emotion and cognitive impairment, and imposes a heavy cost on society (for example, the total cost of psychotic disorders in Europe in 2010 was recently calculated as €93.9 billion; (justivsson et al., 2011). While there is no consensus about its exact causes, the heritability of SZ is estimated to be about 80% (Sullivan et al., 2003). Genome-wide association (GWAS) and copy number variation studies have identified several common and rare gene variants associated with the disorder (O'Donovan et al., 2008). Understanding the function of these variants could, therefore, lead to a greater understanding of disease pathogenesis, which could direct new treatments.

Schizophrenia patients present with variable symptom profiles and distinct disease trajectories. This heterogeneity may be in part due to the complex genetics of SZ, which in turn poses significant problems for understanding the mechanisms by which genetic variants confer risk for this disease. In an attempt to address this complexity, researchers have focused on so-called "intermediate phenotypes," which are measurable variations that occur on the pathway between genes and disease, and as such may be closer to the underlying genetic architecture than clinical symptoms (see Gottesman and Gould, 2003 for a review). Possible intermediate phenotypes for SZ include changes in brain chemistry, structure, function (Braff et al., 2007) and connectivity (Meyer-Lindenberg, 2009).

While changes in functional and structural connectivity may be a critical aspect of the SZ disease profile, there has been little systematic evaluation of the relative sensitivity of these different indices to genetic risk for SZ. In this meta-analysis we outline empirical investigations that have utilized functional magnetic resonance imaging (fMRI) or diffusion tensor imaging (DTI) to investigate the effects of SZ risk variants on functional and structural brain connectivity. We also consider the relative magnitude...
of these effects, in order to determine the extent of the genetic impact on brain connectivity.

**ALTERED FUNCTIONAL CONNECTIVITY IN SZ**

In the early 20th century, German neurologist Carl Wernicke proposed that SZ arises from altered neural connectivity (or dysconnectivity) rather than from abnormalities in specific parts of the brain (see Stephan et al., 2009 for a review). One hundred years later, advances in neuroimaging technology have enabled scientists to empirically consider dysconnectivity as a key component of SZ pathogenesis.

Two or more brain regions are said to be functionally connected if they show a correlation of activity over time (Friston et al., 1993). The hypothesis that functional connectivity is altered in SZ is supported by positron emission tomography (PET; e.g., Friston and Frith, 1993; Meyer-Lindenberg et al., 2001, 2005) and fMRI studies (Lawrie et al., 2002), which reveal abnormal prefrontal-temporal connectivity in SZ patients while they perform cognitive tasks. Electroencephalograph (EEG) research also demonstrates abnormal functional connectivity patterns in patients with SZ (Breakspear et al., 2003), and one genetic mouse model of SZ reveals decreased hippocampal-prefrontal connectivity during a T-maze task (Sigurdsson et al., 2010).

Support for the role of functional connectivity as an intermediate phenotype for psychiatric disorders includes a fMRI study by Pazos et al., 2003. These authors examined the effects of a 5-HTTLPR polymorphism that is associated with anxiety and depression, on functional connectivity between the amygdala and cingulate cortex. It was reported that variant-associated changes in connectivity predicted almost 30% of the variance in the behavioral effects of this polymorphism. Behavioral variability was also in fact better predicted by changes in connectivity than changes in regional brain activation.

Understanding the underlying biological causes of altered functional connectivity has the potential to lead to a better understanding of SZ pathogenesis, but so far the etiology of functional dysconnectivity remains unclear. However, different mechanisms have been proposed, which we will discuss in the following sections.

**THE "DISCONNECTION" HYPOTHESIS OF SZ**

The "disconnection" hypothesis was first proposed by Karl Friston and colleagues in the 1990s (Friston, 1998). This hypothesis postulates that SZ is primarily caused by abnormal N-methyl-D-aspartate (NMDA)-receptor mediated synaptic plasticity, which in turn, is caused by dysregulation of these receptors by neurotransmitters such as dopamine. Support for the role of the NMDA receptor in SZ comes from several studies. Firstly, drugs that block the NMDA-receptor, such as ketamine and phencyclidine, can induce psychotic symptoms in healthy controls (see Javitt, 2010 for a review). Similarly, ketamine administration induces sensory processing deficits in controls similar to deficits seen in patients, suggesting a role for NMDA receptors in these deficits (Umbricht et al., 2000). Activity of midbrain dopaminergic neurons is partially regulated by glutamatergic projections from the prefrontal cortex (PFC), acting via NMDA receptors, and NMDA receptor-blockade enhances amphetamine-induced increases in striatal dopamine in controls, similar to increases seen in SZ patients (Kegeles et al., 2000). Finally, genetic variants that play a role in NMDA-signaling have been associated with increased SZ risk in candidate gene studies [e.g., G72,GRM3 and RGS4; see Harrison and Weinberger (2005) for a review].

**FUNCTIONAL CONNECTIVITY MRI ANALYSIS TECHNIQUES**

Functional connectivity can be measured with a variety of tools (e.g., PET, EEG), but this review will focus on papers using fMRI to measure the phenotype in healthy controls and patients with SZ. Using the blood oxygen level dependent (BOLD) response as an indirect measure of neuronal activity (Ogawa et al., 1990), there are a range of approaches to analysis. This review will focus on seeded connectivity and psychophysiological interaction (PPI).

A seeded connectivity analysis begins with the selection of a seed region, which can be a voxel, or cluster of voxels in the fMRI time-series (Nallamani and Tuo, 2011). The mean time-course for the seed region is then correlated with all other voxels in the brain. Voxels that pass a certain threshold are considered to be functionally connected with the seed region, resulting in a functional connectivity map. While the PPI approach also measures the co-variation of the BOLD signal in voxels across the brain (Friston et al., 1997), it also measures changes in the interactions between brain regions in response to different psychological tasks.

**STRUCTURAL CONNECTIVITY AND SZ**

White matter (WM) contains myelinated nerve cells that connect various gray matter (GM) areas of the brain to each other, and carry nerve impulses between neurons. Compromised WM integrity is evident in SZ (Kubicki et al., 2007; Ellison-Wright and Bullmore, 2009). Moreover, WM abnormalities are apparent in individuals at high risk of SZ and also in patients during the early stages of illness, suggesting that these abnormalities may be a stable characteristic of the disease (Witthaus et al., 2008; Perez-Iglesias et al., 2010). There are two key postulations regarding the nature of WM deficits in SZ: The "global theory" and the "macro-circuit theory." The global theory of WM disruption in SZ suggests that WM is compromised uniformly throughout the brain, whereas the macro-circuit theory proposes that specific WM tracts are compromised, which may be a cause or consequence of abnormalities in the gray matter regions these tracts connect (Buchbaumer et al., 2006; Konrad and Winterer, 2008).

**Diffusion tensor imaging**

Diffusion tensor imaging is a method used to measure the diffusion of water molecules in brain WM. Healthy brain WM has a complex axonal structure and, therefore, water diffusion will be restricted along the direction of the axons. This is known as anisotropic diffusion. However, if brain WM is compromised water diffusion can become less restricted (i.e., isotropic). A common measure derived from DTI to describe the degree of anisotropy during diffusion is fractional anisotropy (FA). However, other measures of diffusion such as radial and axial diffusivity can also be obtained. Based on the voxel-wise information provided by DTI, fiber tracking algorithms can be implemented in regions of interest to reconstruct the underlying three-dimensional WM pathways. While caution must be exercised when interpreting measures of anisotropy (Jones, 2008, 2010; Tournier et al., 2011), such measures are thought to index structural integrity of WM tracts and,
thus, may be reasonably considered to be implicit indices of brain connectivity.

**WM integrity and SZ: evidence from DTI investigations**

A review by Kubicki et al. (2007) noted that the most frequent positive findings of DTI studies in SZ were decreased FA within the prefrontal and temporal lobes, as well as abnormalities within the fiber bundles connecting these regions. WM tracts within these regions that were found to be affected included: (a) the uncinate fasciculus that connects parts of the limbic system with areas in the frontal cortex; (b) the cingulum bundle; and (c) the arcuate fasciculus that connects parts of the temporoparietal junction with the frontal cortex and is thought to be part of the superior longitudinal fasciculus. Ellison-Wright and Bullmore (2009) conducted a meta-analysis of 15 DTI studies, which included a total of 407 patients with SZ and 383 comparison subjects. Results identified two regions of FA decreases in SZ subjects in comparison to controls. The first region was in the left frontal deep WM, which is traversed by WM tracts interconnecting the frontal lobe, thalamus and cingulate gyrus. The tracts include: (a) anterior thalamic radiation (ATR); (b) corticobulbar tracts; (c) inter-hemispheric fibers running through the genu of the corpus callosum; (d) the inferior fronto-occipital fasciculus; (e) the cingulum bundle. The second region was in the left temporal deep WM that is traversed by WM tracts interconnecting the frontal lobe, insula, hippocampus–amygdala, temporal and occipital lobes. These tracts include: (a) inter-hemispheric fibers running through the splenium of the corpus callosum; (b) the inferior fronto-occipital fasciculus; (c) the inferior longitudinal fasciculus; (d) the fornix/striatomegali. These two reviews of the current DTI/SZ literature suggest that specific networks of WM are disrupted in SZ providing support for the macro-circuit theory of WM disruption in the disease.

**Pathophysiological mechanisms of compromised WM**

As the integrity of axons is dependent on myelination and factors influencing myelination, it is possible that myelin and oligodendroglial function also plays a role in the pathophysiology of SZ (Davis et al., 2003). Since myelination also impacts synaptic plasticity, oligodendrocyte abnormality and subsequent myelin dysfunction may contribute to the development of SZ by altering synaptic function and information processing (Fields, 2008). Conduction velocity along axons is also thought to be essential for learning processes (Fields, 2008) and disruption of this has the potential to lead to the range of cognitive impairments observed in SZ (Tanaka et al., 2009). Furthermore, oligodendrocyte and myelin dysfunction also impacts neuronal activity that is relevant to SZ, such as glutamate and dopamine signaling. Evidence from psychotic episodes of multiple sclerosis (MS) patients and experimentally induced demyelination suggests that altered myelin function leads to altered dopamine signaling (Takahashi et al., 2011). Similar analyses have also revealed increased levels of glutamate in brains of MS patients as well as increased expression of glutamate receptors on oligodendrocytes (Takahashi et al., 2011). Glutamate transporters are also present on oligodendroglia and are thought to regulate glutamate concentrations to prevent glutamate-induced excitotoxicity (Pitt et al., 2003). Over activation of oligodendrogial glutamate receptors is excitotoxic and can result in oligodendrocyte death (Davis et al., 2003).

Recently, attention has turned toward the consideration of genes that influence oligodendrocyte architecture and how these genes may also be associated with SZ risk. Hakak et al. (2001) examined the expression of 6500 genes derived from postmortem cortical tissue of SZ patients and controls. The expression levels of six myelin-related genes were significantly down regulated for SZ patients in comparison to control subjects. These genes included: myelin-associated glycoprotein (MAG), CNP, myelin and lymphocyte protein (MAL), gelosin (GSN), ErbB3, and transferring. Down regulation of these genes supports the view that oligodendrocytes, the cell type from which all these genes derive in the brain, contribute to the pathophysiology of SZ.

**USING IMAGING GENETICS TO EXAMINE NEURAL CONNECTIVITY**

Two previous meta-analyses have considered the magnitude of the impact of gene variants on brain function, each reporting large effect sizes. Munafò et al. (2008) examined the effect sizes of the 5-HTTLPR polymorphism and amygdala activation, while Mier et al. (2010) examined the magnitude of effect of the catechol-O-methyltransferase (COMT) Val158Met polymorphism on brain function, reporting association between this variant and activation of the PFC. However, to our knowledge no studies have specifically considered the effect size of gene variants in studies of functional and structural connectivity, or compared effect sizes between these phenotypes. Consideration of the relative impact of these two measures of brain connectivity will help us to better delineate whether or not one phenotype is more proximal to the underlying genetics, and thus preferential as an intermediate phenotype for studies of SZ. This could not only aid our theoretical understanding of the SZ disease trajectory but may also have significant practical implications for future investigations.

**METHODS**

We searched for relevant papers based on the criteria of studies that included genes implicated in SZ risk and measures of either DTI or functional connectivity. PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used to search for relevant functional and structural connectivity papers published until June 2011. The following search terms were included in this search: [schizophrenia OR schiz*] AND [genetic or gene*] AND [MRI OR DTI] AND [connectivity] AND [structural OR functional]. This literature search was supplemented with a review of the references from each of the papers identified. In total 24 studies meeting these search criteria were retrieved, including 12 DTI studies and 12 functional connectivity studies. Individual studies differed slightly in terms of MRI acquisition and analysis parameters (e.g., voxel size, size of Gaussian function used for smoothing). However, all studies were included regardless of these differences, due to the small number of studies available. Where the data presented were insufficient for effect size calculations, a request for supplementary data was sent to the corresponding author. This led to data being available for 19 out of the 24 studies identified (10 DTI studies and 9 functional connectivity studies).

Effect size calculations were performed using two online effect size calculators http://www.uccs.edu/~faculty/lbecker/ and
A total of 24 effect sizes were calculated for structural connectivity were also observed for all the other studies examining the impact of a specific variant or group of variants. Therefore, direction of effect was not included in the analysis and all effect sizes were considered positive.

A random effects meta-analyses considering the relative difference in the impact of SZ risk genes on functional and structural connectivity was carried out using the comprehensive meta-analysis (CMA; software package v2; www.meta-analysis.com). For the purposes of this analysis, Hedge’s $g$ and its associated variance were calculated for the outcome of each significant effect in each study. As with prior estimates of Cohen’s $d$, $g$ was calculated using a variety of input variables including descriptive and inferential statistics. In the first analysis, the largest effect for each study was chosen so to reflect the maximal sensitivity to gene effects within each investigation. In a secondary analysis, all of the effects for each significant result in each paper were taken into account. This strategy allowed us to account for both variability in the number and range of significant effects reported across methodologies.

RESULTS
Overall, 8 fMRI and 10 DTI studies were included in the meta-analysis. Summary information from all of these studies is presented in Tables 1 and 2.

FUNCTIONAL CONNECTIVITY
A total of 44 effect sizes were calculated from the functional connectivity studies. Effect sizes (i.e., Cohen’s $d$) ranged from medium to large ($d = 0.46$–$1.65$) with an average effect size of $0.76$ (SD $\pm 0.23$). The largest effect size ($d = 1.65$) was reported for the impact of a single nucleotide polymorphism (SNP) in ZNF804A on functional connectivity within the right PFC in SZ patients (Rasetti et al., 2011). While large effect sizes ($d > 0.7$) were also calculated in other studies examining the effects of this SNP on functional connectivity (Esslinger et al., 2009, 2011; Rasetti et al., 2011) these results were not consistent: the smallest effect size was also reported for this SNP ($d = 0.46$; Paulus et al., 2011).

STRUCTURAL CONNECTIVITY
A total of 24 effect sizes were calculated for structural connectivity investigations. Effect sizes ranged from small to large ($d = 0.38$–$1.95$) with an average effect size of $1.04$ (SD $= 0.42$). The largest effect size was revealed for the impact of NRG1 SNP on WM integrity in the left ATR (Spruoten et al., 2009). Large effect sizes were also observed for all the other studies examining the impact of NRG1 on WM integrity (all $d > 0.80$). Similar effect sizes were revealed for studies investigating the ErbB4 gene, with Cohen’s $d$ for these studies ranging from $0.81$ to $1.41$. Both the MTHFR gene and the 5-HTT gene had large effect sizes of $1.29$ and $0.92$ respectively. The smallest effect size of $0.38$ was computed for the effect of a COMT haplotype on left prefrontal WM integrity. Cohen’s $d$ for the COMT papers ranged from $0.38$ to $0.76$.

DISCUSSION
The aim of this review was to consider the nature and magnitude of effect of SZ risk variants on functional and structural connectivity. Our focus was the overall magnitude of such effects, rather than delineating the direction of effect of a specific variant. Therefore, we focused on the size, rather than the direction of individual results. Examining the effect of risk variants on connectivity in fMRI and DTI studies, we found that variation in genes implicated in neurotransmission, plasticity, development and myelin function are associated with altered neural connectivity. Meta-analyses of effect size data revealed that there was no significant difference between the effect sizes of functional and structural studies when the largest effect size of each study was analyzed. However, when all effects were taken into consideration, the effect sizes for the structural studies were larger than in the functional connectivity studies, and there was a significant lack of homogeneity across the modalities. Mostly likely, given the absence of difference when only the largest effects from each set of studies are considered, this difference reflects the greater variation in effect sizes in structural studies compared to fMRI studies.

META-ANALYSIS
In our first meta-analysis we considered only the largest effect sizes in each study (Figure 1; Table 3). This analysis revealed no significant difference in outcome variability between the effect sizes for functional and structural studies ($Q = 2.171, p = 0.141$). Our second analysis examined all of the effects for each result in each paper (Figure 2; Table 3). This analysis revealed a significant difference between effect sizes in functional and structural studies ($Q = 6.928, p = 0.008$).
## Table 1 | Details of the functional connectivity studies included in this meta-analysis.

<table>
<thead>
<tr>
<th>First author and date</th>
<th>Gene of interest</th>
<th>Connectivity</th>
<th>Method</th>
<th>Statistic</th>
<th>n</th>
<th>Cohen's d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meyer-Lindenberg et al. (2007)</td>
<td>PPP1R1B</td>
<td>L. PFC - striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
<td>SC</td>
<td>4.41*</td>
<td>126</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. PFC - striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
<td>SC</td>
<td>4.57*</td>
<td>126</td>
<td>0.82</td>
</tr>
<tr>
<td>Kempl et al. (2008)</td>
<td>PRODH</td>
<td>DIPFC - striatum, reference haplotype carriers &gt; protective haplotype carriers</td>
<td>SC</td>
<td>3.91*</td>
<td>103(108)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DIPFC - striatum, risk haplotype carriers &gt; protective haplotype carriers</td>
<td>SC</td>
<td>2.88*</td>
<td>48(108)</td>
<td>0.87</td>
</tr>
<tr>
<td>Di Giorgio et al. (2008)</td>
<td>DISC1</td>
<td>R. hippocampus - R. dIPFC, Ser/Ser &gt; Cys carriers</td>
<td>PPI</td>
<td>3.58*</td>
<td>80</td>
<td>0.81</td>
</tr>
<tr>
<td>Esslinger et al. (2009)</td>
<td>ZNF804A</td>
<td>R. dIPFC - L. hippocampus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.94*</td>
<td>115</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. dIPFC - R. dIPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.05*</td>
<td>115</td>
<td>0.77</td>
</tr>
<tr>
<td>Esslinger et al. (2011)</td>
<td>ZNF804A</td>
<td>R. dIPFC - L. MFG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>5.09*</td>
<td>111</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. dIPFC - R. SFG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.6*</td>
<td>111</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. dIPFC - L. MFG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.9*</td>
<td>111</td>
<td>0.74</td>
</tr>
<tr>
<td>Walter et al. (2011)</td>
<td>ZNF804A</td>
<td>R. dIPFC - L. inferior frontal gyrus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.77*</td>
<td>109</td>
<td>0.73</td>
</tr>
<tr>
<td>Paulus et al. (2011)</td>
<td>ZNF804A</td>
<td>R. dIPFC - L. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.3*</td>
<td>94</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. dIPFC - R. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.22*</td>
<td>94</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. dIPFC - R. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.85*</td>
<td>94</td>
<td>0.59</td>
</tr>
<tr>
<td>Rasetti et al. (2011)</td>
<td>ZNF804A</td>
<td>Controls: R. dIPFC - L. HF, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>2.72*</td>
<td>96</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls: R. dIPFC - L. dIPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.65*</td>
<td>96</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls: R. dIPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.21*</td>
<td>96</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls: R. dIPFC - L. hippocampus, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>3.74*</td>
<td>96</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls: R. dIPFC - R. hippocampus, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>2.89*</td>
<td>96</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Siblings: R. dIPFC - R. hippocampus, AA - abnormal coupling</td>
<td>SC</td>
<td>2.53*</td>
<td>83</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Siblings: R. dIPFC - L. PFC, AA &lt; C carriers</td>
<td>SC</td>
<td>2.77*</td>
<td>83</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Siblings: R. dIPFC - R. dIPFC, AA - greater task-related modulation of coupling</td>
<td>PPI</td>
<td>4.36*</td>
<td>83</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients: R. dIPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.58*</td>
<td>33</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients: R. dIPFC - L. hippocampus, AA &lt; C carriers</td>
<td>PPI</td>
<td>3.56*</td>
<td>33</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients: R. dIPFC - L. PFC, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>2.84*</td>
<td>33</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patients: R. dIPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>3.40*</td>
<td>33</td>
<td>1.22</td>
</tr>
</tbody>
</table>

n, sample size; SC, seeded connectivity; PPI, psychophysiological interaction; dIPFC, dorsolateral prefrontal cortex; MFG, middle frontal gyrus; SFG, superior frontal gyrus; TPJ, temporo-parietal junction; MTG, middle temporal gyrus; LG, lingual gyrus; IFG, inferior frontal gyrus; *p-value is uncorrected for multiple comparisons; family wise error corrected within region of interest. 

false discovery rate corrected within region of interest; false discovery rate corrected for whole brain; family wise error corrected within region of interest.
single studies to date (DISCI; Di Giorgio et al., 2008; PRODH; Kempf et al., 2008; PPP1R1B; Meyer-Lindenberg et al., 2007).

In 10 of the 12 IMRI studies included in this review, SZ risk variants were reported to affect functional circuits that included the PFC during the performance of a variety of tasks, such as memory encoding and retrieval, working memory, emotion processing, and during rest. These findings reflect the PFC’s dominant role in many processes related to higher cognitive functioning, making it consistently implicated in SZ pathogenesis (Callicott et al., 2008). There are several possibilities for why PFC function is making it consistently implicated in SZ pathogenesis (Callicott et al., 2008; Kemppf et al., 2008;二级B BDNF and DISCI). Almost all variants considered here were associated with significant variation in FA scores using DTI.

The average effect size for these studies was large, with the largest effect size computed for the impact of NRG1 on FA in the left ATR (Sprooten et al., 2009). The NRG1 gene codes for the NRG1 protein, that is involved in growth and differentiation of neuronal and glial cells and is necessary for the normal development of the nervous system. ErbB4 is a receptor for the NRG1 protein. It is thought that NRG1 may mediate its effects on SZ susceptibility through functional interaction with ErbB4 (Norton et al., 2006). Interestingly, ErbB4 was also observed here to show effects on WM integrity that would be considered to be in line of large magnitude. The role of these genes in myelin function suggests a mechanism by which they confer risk for SZ. The relatively large impact of these genes on structural connectivity, noted here, suggests that genetically mediated “disconnectivity” in SZ results from macro-circuit WM abnormalities in addition to micro-circuit synaptic plasticity. However, since oligodendrocyte dysfunction may also impact synaptic function and information processing via a myelin-dependent impact on synaptic plasticity (Fields, 2008), it remains to be established if the influence of these variants is specific to structural connectivity or if they also impact upon functional connectivity.

**Table 2 | Details of the structural connectivity studies using DTI included in this meta-analysis.**

<table>
<thead>
<tr>
<th>First author and date</th>
<th>Gene</th>
<th>Connectivity</th>
<th>Statistic (t or F)</th>
<th>N</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>McIntosh et al. (2008)</td>
<td>NRG1 SNP8NRG243177</td>
<td>Reduced FA in ALIC</td>
<td>t = -2.65*</td>
<td>43</td>
<td>0.83</td>
</tr>
<tr>
<td>Winterer et al. (2008)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in MF subcortical WM</td>
<td>t = -4.67***</td>
<td>50</td>
<td>1.35</td>
</tr>
<tr>
<td>Sprooten et al. (2009)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in left ATR</td>
<td>t = 5.52***</td>
<td>28</td>
<td>1.95</td>
</tr>
<tr>
<td>Vang et al. (2009)</td>
<td>NRG1 SNP8NRG221533</td>
<td>Reduced FA in anterior cingulum</td>
<td>F = 5.27*</td>
<td>31</td>
<td>0.86</td>
</tr>
<tr>
<td>Konrad et al. (2009)</td>
<td>ErbB4 rs707264</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 8.18***</td>
<td>34</td>
<td>1.54</td>
</tr>
<tr>
<td>ErbB4 rs756440</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -4.24***</td>
<td>50</td>
<td>1.22</td>
<td></td>
</tr>
<tr>
<td>ErbB4 rs839541</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -2.81***</td>
<td>50</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>ErbB4 rs839523</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -4.31***</td>
<td>50</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>G-T-G-T versus lower risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -4.73***</td>
<td>50</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>G-T-G-T versus all other</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -3.86***</td>
<td>32</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>All other versus non-risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = -3.2***</td>
<td>50</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Zuliani et al. (2011)</td>
<td>ErbB4 rs4673628</td>
<td>Reduced FA in right ALIC</td>
<td>t = 3.48*</td>
<td>36</td>
<td>1.19</td>
</tr>
<tr>
<td>ErbB4 rs4673629</td>
<td>Reduced FA in left ALIC</td>
<td>t = -3.98*</td>
<td>36</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>Thomason et al. (2010)</td>
<td>COMT val158met</td>
<td>Main effect of genotype on FA, AD, RD in GCC</td>
<td>F = 3.04*</td>
<td>40</td>
<td>0.76</td>
</tr>
<tr>
<td>COMT val158met</td>
<td>Main effect of genotype on FA, AD, RD in ATR</td>
<td>F = 2.79*</td>
<td>40</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>COMT val158met</td>
<td>Main effect of genotype on FA, AD, RD in UF</td>
<td>F = 2.47*</td>
<td>40</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Liu et al. (2010)</td>
<td>COMT val158met</td>
<td>Decreased FA in right CST for Val/Val carriers</td>
<td>F = 5.19*</td>
<td>79</td>
<td>0.51</td>
</tr>
<tr>
<td>LPS, HPS and APS haplotypes</td>
<td>Association with mean FA in left PF lobe</td>
<td>F = 2.79*</td>
<td>68</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>LPS, HPS and APS haplotypes</td>
<td>Association with mean FA in right PF lobe</td>
<td>F = 3.58*</td>
<td>68</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>LPS, HPS and APS haplotypes</td>
<td>Association with mean FA in right UF</td>
<td>F = 3.507*</td>
<td>68</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Roffman et al. (2011)</td>
<td>MTHFR 677T</td>
<td>Reduced FA in bilateral DACC</td>
<td>F = 6.59*</td>
<td>18</td>
<td>1.29</td>
</tr>
<tr>
<td>Pacheco et al. (2009)</td>
<td>5-HTTLPR</td>
<td>Increasing number of low expressing alleles – decreasing FA in left FUF</td>
<td>t = -3.03*</td>
<td>37</td>
<td>-0.92</td>
</tr>
</tbody>
</table>

n, sample size; FA, fractional anisotropy; MF, medial frontal; WM, white matter; ALIC, anterior limb of internal capsule; AD, axial diffusivity; RD, radial diffusivity; ATR, anterior thalamic radiation; UF, uncinate fasciculus; GCC, genu of corpus callosum; CST, corticospinal tract; PF, prefrontal; DACC, dorsal anterior cingulate cortex; FUF, frontal uncinate fasciculus; **p < 0.001; ***p < 0.001; *p < 0.05 family wise error corrected.

**Structural Connectivity Studies**

A number of candidate SZ risk genes have been investigated in terms of their effects on structural connectivity. This includes genes that are involved in myelination (NRG1, ErbB4) neurotransmission (COMT, MTHFR, 5-HTTLPR) and neurodevelopment (BDNF and DISCI).
FIGURE 1 | Forest plot reporting Hedges’ $g$ and 95% CI for the analysis showing the largest effect size in each paper. CI, confidence interval; g, Hedges’ $g$; SE, standard error.

Table 3 | Results of random-effects meta-analysis comparing the relative difference in the impact of variants on functional and structural connectivity.

<table>
<thead>
<tr>
<th>Effect size and 95% confidence interval</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of studies</td>
<td>Point estimate</td>
</tr>
<tr>
<td>MAXIMUM ESTIMATE</td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>9</td>
</tr>
<tr>
<td>Structural</td>
<td>10</td>
</tr>
<tr>
<td>Total between</td>
<td></td>
</tr>
<tr>
<td>ALL ESTIMATES</td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>44</td>
</tr>
<tr>
<td>Structural</td>
<td>24</td>
</tr>
<tr>
<td>Total between</td>
<td></td>
</tr>
</tbody>
</table>
Other gene variants associated with variation in WM connectivity included COMT, MTHFR, 5-HTTLPR, BDNF and DISC1. Results for both the *MTHFR* gene and the *5-HTT* gene had large effect sizes. The *MTHFR* gene codes for the an enzyme that plays
a role in the regulation of intracellular methylation reactions and may influence dopamine signaling (Roffman et al., 2008). The hypofunctional 677T variant of this gene has been associated with increased SZ risk (Gilbody et al., 2007). The reuptake of serotonin to the presynaptic neuron for recycling or degradation after serotonin release is regulated by the serotonin transporter (5-HTT). Although the 5-HTTLPR polymorphism has been found to be associated with SZ in a South India population (Vijayan et al., 2009), other genetic association studies have given conflicting results (Rao et al., 1998). The smallest effect size was computed for the effect of a COMT haplotype on left prefrontal WM integrity (Liu et al., 2010). The COMT gene codes for an enzyme that is involved in the degradation of dopamine. Therefore, the evidence from DTI studies investigating the impact of the COMT gene on WM indicates that neurosignalling processes involved in SZ may also impact structural connectivity. Finally, BDNF (Chiang et al., 2011) and DISCI (Sprooten et al., 2011) which are genes that are crucial for neurodevelopment, were also associated with WM connectivity in SZ. However, as insufficient data was available, effect sizes for these studies were not calculated.

META-ANALYSIS

The papers included in this review most commonly report a large effect of gene variants on functional and structural connectivity. This result is similar to previous meta-analyses in imaging genetics, which reported large effect sizes of gene variants (i.e., 5-HTTLPR polymorphism and the COMT Val158Met polymorphism) on brain function (Munafo et al., 2008; Mier et al., 2010). This result is also consistent with the intermediate phenotype hypothesis that common SZ risk variants will show small effects on behavior and disease risk, but large effects at the level of the brain (Ilost et al., 2011).

When the maximum effect size value for each paper in our meta-analysis was compared between fMRI and DTI studies, no significant difference was found between these measures. As only a small number of studies were obtained, there may be a lack of power to detect such differences. However, examination of effect sizes for all significant effects indicate that structural connectivity studies were associated with overall larger and more variable effect sizes. This suggests that measures of structural connectivity, such as DTI, may be sensitive to a wider range of effects compared to functional connectivity measures, which may only be able to accurately detect large effects. This result may also indicate that structural connectivity is closer to the level of genes than functional connectivity.

LIMITATIONS

A number of limitations need to be considered in evaluating the findings of the present study. Firstly, many of the studies included in the meta-analysis have examined the effects of polymorphisms that do not have consistent association with SZ phenotypes. This makes it difficult to determine the relevance of these genes for our understanding of SZ pathogenesis (for a review, see Meyer-Lindenberg, 2010). Secondly, it should also be noted that the sample sizes included in these studies are relatively small and thus, are under powered to detect differences in brain connectivity conferred by individual variants. Due to the interplay between sample size, power, and effect size, smaller studies generally show larger effects in meta-analyses (Sterne et al., 2009) and may lack sufficient power to detect smaller effects. Related to the general issue of sample size, it is important to note that the average sample size of the studies utilizing DTI was smaller than that for the functional studies. As a result, the effect sizes for the structural papers may be over-inflated. However, the results of our meta-analysis suggest that despite smaller samples, the structural imaging studies were associated with a wider range of effects, suggesting that sample size is not the only factor at play here.

Due to the under-representation of publications with negative results, the studies included in this review may not be representative of connectivity research in its entirety, but rather a bias toward only publish papers showing statistically significant results. Therefore, while our effect size findings are calculated on the basis of published effect sizes, it is possible that the true effect sizes are smaller, and to an extent that is unknown. Similarly, it is also unclear to what extent differences in scanning parameters between the studies included in this meta-analysis influenced results. More systematic investigation of these differences will in the future be possible with the accumulation of more studies.

An additional limitation in the studies considered here is that each investigation examined the effects of only one particular variant. However, the true function of these genes may be affected by additive or epistatic interactions with other variants. As such, the results presented in this review may be incomplete without taking these interactions into account (Nicosia et al., 2010). Finally, it is probable that these results could be impacted by differences in functional and structural methodological approaches. For example, a number of analysis methods can be employed to measure functional connectivity between brain regions. However, we are not currently aware of the relative strengths and weaknesses of these different approaches. There are also various approaches used to quantify WM connectivity using DTI, which also pose different strengths and limitations (see Jones, 2010 for a review).

CONCLUSIONS AND FUTURE DIRECTIONS

In a short period of time, imaging genetics has made important progress in delineating genetic effects on neural connectivity. In particular, it has established neural connectivity as a key intermediate phenotype for SZ, which can be used to explore the complex trajectory from genetic risk to clinical symptoms. Despite the progress that has taken place, we believe important advances can be made in this research field in four key areas. Firstly, future studies should examine the effects of gene variants on neural connectivity in larger sample sizes, as this can provide the extra statistical power that may be necessary to detect smaller effects of these genes. Multi-site research projects, such as the IMAGEN project in Europe, may be particularly suited for compiling imaging and genetic databases of thousands of subjects (Schumann et al., 2010). Secondly, future studies should examine additive and epistatic effects of gene variants on neural connectivity, as these variants are unlikely to be working in isolation. Thirdly, future studies should examine the effects of risk variants in healthy controls and SZ patients as the opposite effects of these genes on connectivity can be found in these different groups (e.g., Prata et al., 2009).
Finally, future studies could benefit from the novel application of recently developed analysis techniques to imaging genetics. For example, DCM hold potential for constructing models of changing brain interactions that also take into account genetic variation (Meyer-Lindenberg, 2009). Other recent advances include the use of parallel ICA to simultaneously analyze independent components derived from fMRI and genetic data (Li et al., 2009). For example, Meda et al. (2010) used this technique in a pilot study to identify simultaneous independent components of fMRI data and SNP data, derived from a sample of 35 controls and 31 SZ patients. The authors found correlations between different neural networks and a number of SNPs, including polymorphisms involved in altered dopamine transmission. While the authors only included a small number of SNPs and a small sample size, this research suggests a powerful new approach for future studies examining the effects of SZ risk variants on functional brain networks. Similarly, more advanced DTI techniques could be implemented that use high angular resolution to account for multiple crossing fibers within a single voxel. Such imaging techniques include Q-space approaches and mixture models (Tournier et al., 2011). These models provide mathematical alternatives to the tensor model for the characterization of diffusion processes. Furthermore, Jones (2010) recommends the integration of DTI with other measures of WM such as measures of axon density and myelination that can be acquired using techniques such as magnetization transfer or multicomponent relaxometry.

In conclusion, the present meta-analysis examined the nature and magnitude of effect of SZ risk variants on functional and structural connectivity. Gene variants impacting upon both synaptic plasticity and axonal connectivity have been associated with altered neural connectivity in patients and healthy controls. As such, it is likely that both mechanisms make important contributions to SZ pathogenesis. On average, risk variants exert a large effect on functional and structural connectivity. There is also more variability in the effects of variants on structural connectivity, compared to functional connectivity. While imaging genetics has made considerable progress in the field of neural connectivity in a short period of time, important advances are still to be made. It is hoped that this research will lead to a better understanding of the biological mechanisms mediating genetic risk for SZ, which can then be used to direct novel treatments for the disorder.

ACKNOWLEDGMENTS

This work was supported by a Science Foundation Ireland research award to Professor Aiden Corvin (SFI08/IN.1/B1916-Corvin).

REFERENCES


Effects of MIR137 on fronto-amygdala functional connectivity

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ARTICLE INFO
Article history:
Accepted 11 December 2013
Available online xxxx

Keywords:
MIR137
Schizophrenia
Functional connectivity
Amygdala
Emotion

ABSTRACT
Background: MIR137 is implicated in brain development and encodes a microRNA that regulates neuronal maturation and adult neurogenesis. Recently, a common genetic variant within MIR137 showed genome wide evidence of association with schizophrenia, and with altered amygdala activation in those at genetic risk for schizophrenia. Following this evidence, we investigated the effects of MIR137 genotype on neuronal activity during face processing.

Methods: By grouping 81 healthy participants as carrier or non-carriers of the MIR137 rs1625579 risk allele associated with schizophrenia, we investigated MIR137’s effects on altered cortical response during an fMRI face processing task and altered functional connectivity using the amygdala as a seed region.

Results: Homozygous carriers of the risk allele were observed to show relatively increased functional connectivity between the right amygdala and frontal regions that play a key role in emotion processing and regulation (e.g. the cingulate and prefrontal cortex).

Conclusions: Our findings provide the first evidence that the rs1625579 variant affects fronto-amygdala functional connectivity, providing further evidence that MIR137 may contribute to forms of psychosis in which affective symptoms are more prominent.

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Introduction
MIR137 is one of a group of genes that encode microRNAs (miRNA)—small non-coding RNA molecules modulating gene expression. MIR137 is highly expressed in the brain, particularly in medial temporal regions, and plays an important role in neurogenesis and dendritic morphogenesis (Smrt et al., 2010). In a meta-analysis of genome-wide association studies (Ripke et al., 2011) (GWAS), a common single nucleotide polymorphism (SNP), rs1625579, within the MIR137 gene showed the strongest genome-wide evidence for schizophrenia. The mechanisms by which the rs1625579 variant increases schizophrenia risk are unknown; however, in animal studies, altered expression of other miRNAs has been reported in key components of the brain’s emotional network(s). For example, changes in miRNA expression in the amygdala and medial prefrontal cortex—in response to acute stress and maternal deprivation, suggest a role for this class of molecule in emotion regulation (O’Connor et al., 2011). In support of this hypothesis, we recently reported an association between this variant and mood congruent psychotic symptoms in a large sample of patients with psychosis, despite relatively subtle effects observed on cognition (Cummings et al., 2012). This implies that MIR137 may be associated with forms of psychosis in which affective symptoms are more prominent.

Emotion processing deficits have been proposed as a core clinical feature of schizophrenia (Aleman et al., 2005) and may be related to Q2 genetic risk (Gur et al., 2007). Variation in amygdala activation, a brain region that plays an important role in assigning emotional value to stimuli and in forming emotional memories, has recently been associated with MIR137 (Whalley et al., 2012). In this study a genotype-by-group interaction on activation in the amygdala during the Hayling sentence completion task was observed. This task is typically associated with deactivation of the amygdala (Whalley et al., 2011); however, among participants with high genetic risk for schizophrenia, homozgyous risk allele carriers showed comparatively less deactivation in the amygdala compared to homozgyous and heterozygous non-risk carriers. The authors suggest that this finding may reflect a misattribution of emotional salience in the high-risk homozgyous risk group to the stimuli presented in the task, which were considered to be non-emotional. However, effects of MIR137 genotype on brain function during a task designed to measure emotion processing have yet to be reported. Face processing tasks may be particularly useful for examining genetic effects on emotion processing, as evidence suggests that impairments in processing emotional information from facial stimuli may be related to the genetic architecture of schizophrenia (Gur et al., 2007).
MIR137 has been shown to play a role in the shaping of dendrites, raising the possibility that this gene may affect functional connectivity in the brain, which has been proposed as a possible etiological mechanism in the pathogenesis of schizophrenia (Friston, 1998; Stephan et al., 2009). Altered dendritic morphology has been suggested as a factor contributing to the aberrant functional connectivity observed in schizophrenia (Meyer-Lindenberg et al., 2005) as it may affect synaptic plasticity between groups of neurons (Stephan et al., 2009). A recent study by Lett et al. (2013) reports that schizophrenia patients homozygous for the rs1625579 risk allele have relatively reduced fractional anisotropy, an index of structural brain connectivity, throughout the brain compared to non-risk carriers. While the exact relationship between white matter integrity and functional connectivity is not fully understood, congruent results between the two modalities have been reported (Danoiseaux and Greicius, 2009), suggesting that global effects of rs1625579 on white matter integrity may also have effects on functional connectivity.

The purpose of the present study was to investigate the impact of the rs1625579 variant within MIR137 on brain activity during emotion processing in a sample of healthy individuals. We employed a widely-used face processing task that includes both angry and neutral facial stimuli (Grosbras and Paus, 2006; Schneider et al., 2011; Tahmasebi et al., 2012; Thyeau et al., 2012). We considered both brain activation and functional connectivity of the amygdala using an established seed-based correlation approach (Erk et al., 2010; Esslinger et al., 2009; Paulus et al., 2013) with the aim of delineating the role of rs1625579 genotype on the neurobiological underpinnings of emotion processing.

In doing so we sought to test the hypothesis that the MIR137 risk allele is associated with significant differences in amygdala activity and functional connectivity during emotion processing. Testing this hypothesis is important because of the evidence both that emotional processing is aberrant in schizophrenia and that dysconnectivity is a significant feature of the disorder. Showing that MIR137 is related to both is important for understanding (1) the genetic basis of schizophrenia and (2) the genetic architecture of emotion processing.

Material and methods

Participants

In total, 98 healthy volunteers participated in the study. Inclusion criteria required that participants be right-handed, aged 18 to 65, have no history of co-morbid psychiatric disorder, no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Participants were recruited using local media advertisements. In addition to satisfying the above criteria, participants were screened for family history of schizophrenia. Volunteers were of Irish ancestry (i.e. had Irish maternal and paternal grandparents) and all provided written informed consent in accordance with local ethics committee guidelines.

MRI

Participants were imaged using a Philips Intera Achieva 3T MR system. Functional imaging consisted of whole-brain BOLD EPI in which 40, 2.4 mm slices were acquired with a 1 mm slice gap and the following imaging parameters: TR = 2200 ms; TE = 30 ms; FOV = 220 x 220 mm; and flip angle = 75°. The duration of functional scanning was 160 TRs. Structural imaging consisted of a T1-weighted image (180 slices; duration 6 min) using a TFE gradient echo pulse sequence, with a slice thickness of 0.9 mm and 230 x 230 FOV.

Face processing task

During fMRI, subjects performed face processing task designed by Grosbras and Paus (2006) and adapted for the IMAGEN study (Schneider et al., 2011; Schumann et al., 2010; Tahmasebi et al., 2012; Thyeau et al., 2012) (http://www.imagen-europe.com/). In this task, subjects were asked to passively watch a series of 2-5 second black-and-white video clips of faces showing neutral or angry facial expressions, or moving circles (i.e. control condition). Videos were presented in 18 second blocks, with 4-7 video clips presented per block. In the course of the task 5 neutral face blocks were presented and 5 angry face blocks were presented; every second block was a control block of which there were 9, resulting in 19 blocks in total. All subjects performed the same task, i.e. the total number of exposures to each condition was the same between subjects. After scanning, subjects completed a brief task where they were shown pictures of faces and asked to determine whether these matched faces seen during the task. Of the 5 pictures presented in this follow-up task, subjects who answered correctly for 4 or 5 pictures were included in further fMRI analysis. 8 subjects were excluded due to poor performance (<4 correct answers) or missing data for this follow-up task.

Genotyping

Genetics analysis was carried out using DNA obtained from saliva samples that were collected using Oragene DNA self-collection kits (DNA Genotek). The rs1625579 SNP was genotyped on a TaqMan® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for TaqMan® genotyping was >95% and the samples were observed to be in Hardy–Weinberg Equilibrium.

Data processing and analysis

After realignment of raw EPI data (fMRI section), graphical plots of estimated time series of translations and rotations were carefully inspected for excessive motion in each subject, defined as >3 mm translation and/or >3° rotation in any direction. Overall, 1 subject exhibited rotation >3° and was excluded from further fMRI analysis. 8 additional subjects were excluded due to bad quality MRI data and/or significant artefacts. Of the 81 remaining subjects, there were 1 'GG' homozygote, 25 'GT' heterozygotes and 55 'TT' homozygotes. Due to the relative infrequency of 'GG' homozygotes, we compared subjects carrying 0 or 1 copy of the risk allele ('GG/ GT'; N = 26) with homozygous risk 'TT' allele carriers ('TT'; N = 55). The allele frequencies observed in our sample were as expected (the risk 'T' allele was reported as the common allele in Ripke et al. (2011)) and we used the same grouping strategy as was used in other imaging genetics investigations of this SNP (Lett et al., 2018; Whalley et al., 2012).

fMRI

Image processing and statistical analyses were conducted using Statistical Parametric Mapping (SPM 8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) running on MATLAB R2011b (v7.13; http://www.mathworks.co.uk/). Functional images were realigned to the mean functional image, spatially normalised into a standard space (Montreal Neurological Institute (MNI) template) with a voxel size of 3 mm x 3 mm x 3 mm and were subsequently smoothed with a 10 mm FWHM (full width at half maximum) isotropic Gaussian filter (i.e. a kernel width 2–3 times greater than the original voxel size).

Statistical analysis was performed using a standard general linear model (GLM) in SPM 8 (Friston et al., 1994). For each condition, a boxcar function representing stimulus presentation was created and convolved with a haemodynamic response function (HRF) to model neural responses at each voxel. The first-level GLM included these convolved condition regressors, plus 6 regressors modelling head motion and/or >3° rotation in any direction. Overall, 1 subject exhibited rotation >3° and was excluded from further fMRI analysis. 8 additional subjects were excluded due to bad quality MRI data and/or significant artefacts. Of the 81 remaining subjects, there were 1 'GG' homozygote, 25 'GT' heterozygotes and 55 'TT' homozygotes. Due to the relative infrequency of 'GG' homozygotes, we compared subjects carrying 0 or 1 copy of the risk allele ('GG/ GT'; N = 26) with homozygous risk 'TT' allele carriers ('TT'; N = 55). The allele frequencies observed in our sample were as expected (the risk 'T' allele was reported as the common allele in Ripke et al. (2011)) and we used the same grouping strategy as was used in other imaging genetics investigations of this SNP (Lett et al., 2018; Whalley et al., 2012).

Please cite this article as: Mothersill, O., et al., Effects of MIR137 on fronto-amygdala functional connectivity, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.12.019
each subject (the same contrasts used in previous studies using this task, e.g. Schneider et al., 2011):

1.) All faces (angry and neutral) versus control to model face-specific activation.

2.) Angry faces versus neutral faces to model emotion-specific activation.

Individual SPMs were then entered into a second-level random effects model to determine task effects at the group level (one-sample t-test across the sample and independent t-test between genotype groups). For the comparison of genotype groups, a region of interest (ROI) analysis of the amygdala was also employed, using a bilateral amygdala mask constructed using the automated anatomical labelling atlas within the Wake Forest University Pickatlas (Maldjian et al., 2003, 2004; Tzourio-Mazoyer et al., 2002). Due to the previously reported effects of gender on amygdala function (Kilpatrick et al., 2006), and the trend for significant differences in the distribution of the sexes between the two genotype groups (see Subject demographics section), gender was added to the analyses of genotype effects as a covariate.

Functional connectivity analysis

Functional connectivity was assessed using a seed based correlation approach, similar to that used by Esslinger et al. (2009), Erik et al. (2010), and Paulus et al. (2013), to examine the effects of GWAS psychosis risk variants on functional connectivity. Amygdala masks were obtained as described above (MNRI section). Both right and left amygdalae were used as seed regions in two separate connectivity analyses. Time series from the amygdala were extracted using first eigenvectors from all voxels within the amygdala mask (Esslinger et al., 2009). This time series was temporally filtered using a high-pass filter of 128 s to remove low-frequency signals and task-related variance was removed by applying an effects-of-interest F-contrast of the six movement parameters (Esslinger et al., 2009; Paulus et al., 2013). Noise was excluded from this seed by selecting voxels active for the faces versus control contrast at a threshold of p < 0.05 (Esslinger et al., 2009); this threshold was not used for statistical inference. We chose the faces versus control contrast as there was no significant effect of the angry versus neutral faces contrast on amygdala activity across our group, similar to previous studies using this task (Schneider et al., 2011). One subject did not show right or left amygdala activation at this threshold; this subject was excluded from further connectivity analysis.

To account for noise, first eigenvectors from all voxels within masks of white matter (WM) and cerebrospinal fluid (CSF) were extracted, and entered, together with task and movement regressors, into a new fixed-effects GLM with the amygdala time-series as the regressor of interest. Task-related variance was also removed from WM/CSF time series by applying an effects-of-interest F-contrast of the six movement parameters. The WM and CSF masks were kindly provided by Esslinger, C. and Paulus, F. (personal correspondence). These masks have previously been used in imaging genetics studies examining the effects of GWAS psychosis risk variants on functional connectivity (Esslinger et al., 2009; Paulus et al., 2013). Individual connectivity maps produced by the analysis were then compared between genotype groups using an independent t-test in SPM 8. Gender was also added to second-level functional connectivity analyses as a covariate. For all analyses, statistical parametric maps were initially thresholded at a level of p < 0.001 (uncorrected) and regions were considered significant at a cluster level of p < 0.05, corrected for multiple comparisons across the whole brain using the family-wise error rate (FWE). MNI coordinates of results were converted to Talairach space using BrainMap GingerALE 2.1 (Eickhoff et al., 2009; Turkeltaub et al., 2012) and anatomical localisation of these coordinates were performed using Talairach Client 2.4.3 (Lancaster et al., 1997, 2000).

Results

Subject demographics

Independent t-tests were performed to compare age and years of education between genotype groups; a Pearson's chi-squared test was performed to compare gender frequencies between genotype groups. There were no significant differences between genotype groups for age or years of education (p > 0.05) with a trend for significant differences in the distribution of the sexes (p = 0.07; see Table 1).

Functional activation

Across our sample, the faces versus baseline contrast was associated with significant neural activation in clusters incorporating key regions involved in face processing including the middle temporal gyrus and amygdala, consistent with previous studies using this task (Grosbras and Paus, 2006; Schneider et al., 2011) (t(81) = 23.44, p < 0.05 corrected; see Table 2 and Fig. 1). Several of these brain regions, including the bilateral amygdala, also survived correction for multiple comparisons at a voxel-level FWE-corrected threshold (see Supplemental Table 1). The angry versus neutral faces contrast was associated with significant neural activation in a cluster incorporating the left cingulate gyrus/BA 32 (t(81) = 4.67, p < 0.05, corrected; see Table 2 and Fig. 1). These activations did not differ between genotype groups for either the faces versus control or angry versus neutral face contrasts. In addition, ROI analysis within the bilateral amygdala did not reveal significant differences between genotype groups for the faces versus control or angry versus neutral face contrasts, both at a threshold of p < 0.05 FWE-corrected at the cluster level, and at an exploratory threshold of p < 0.05 uncorrected.

Functional connectivity

'T' homozygotes showed significantly increased functional connectivity between the right amygdala and two clusters incorporating (1) the right cingulate gyrus/BA 31 and left BA 24; and (2) the right inferior frontal gyrus/BA 47 (t(81) = 5.17, p < 0.05, corrected; see Table 3 and Fig. 2). There were no significant left amygdala connectivity differences between genotype groups. As an additional data quality check, in each individual the average parameter estimates of all voxels were calculated for each cluster that showed a significant connectivity difference between genotype groups. Next, average parameter estimates were checked in SPSS (19.0.0) for the presence of outliers. No outliers were identified.

Discussion

This study investigated the functional effects of the genome-wide associated schizophrenia risk variant rs165579 within MIR137 on functional activation in healthy participants. A functional connectivity analysis of this data revealed an effect of genotype on amygdala functional connectivity. Compared to subjects carrying one or no copies of the...
Table 2
Clusters, including individual peaks, showing significantly increased functional activation during face (angry and neutral) versus control, and angry versus neutral face conditions, corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>p value</th>
<th>Condition</th>
<th>Cluster peaks</th>
<th>t-Value</th>
<th>Z-value</th>
<th>Peak coordinates (MNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5318</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right middle temporal gyrus/BA 22</td>
<td>23.44</td>
<td>&gt;8</td>
<td>54 − 40 7</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Right middle frontal gyrus/BA 46</td>
<td>18.39</td>
<td>&gt;8</td>
<td>48 23 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right cerebellum</td>
<td>17.21</td>
<td>&gt;8</td>
<td>42 − 49 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left cerebellum</td>
<td>15.39</td>
<td>&gt;8</td>
<td>−15 − 76 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>14.47</td>
<td>&gt;8</td>
<td>−42 − 49 20</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>Left middle temporal gyrus/BA 21</td>
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<td>−60 − 49 10</td>
</tr>
<tr>
<td>2</td>
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<td>&lt;0.001</td>
<td>Faces</td>
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<td>11.41</td>
<td>&gt;8</td>
<td>−39 11 25</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Left inferior frontal gyrus/BA 47</td>
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<td>7.42</td>
<td>−39 32 2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Left middle frontal gyrus/BA 6</td>
<td>8.16</td>
<td>6.94</td>
<td>−42 2.40</td>
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<td>&gt;8</td>
<td>6 14 58</td>
</tr>
<tr>
<td>3</td>
<td>1288</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Left superior frontal gyrus/BA 8</td>
<td>5.03</td>
<td>4.68</td>
<td>9 44 43</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Left anterior cingulate/BA 32</td>
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<td>−12 29 19</td>
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<td>Left cingulate/BA 32</td>
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<td>Left cingulate/BA 32</td>
<td>4.46</td>
<td>4.20</td>
<td>−9 23 37</td>
</tr>
</tbody>
</table>

a p values are FWE-corrected for multiple comparisons at the cluster level; Faces = All faces (angry and neutral) versus control condition; Angry = Angry versus neutral faces condition.

The significant right amygdala activation reported in the main text is contained within this cluster.

The significant left amygdala activation reported in the main text is contained within this cluster.

Fig. 1. Functional activation associated with face processing task. Red-yellow: Brain regions showing increased activation during the face (angry and neutral) versus control condition (N = 81; one-sample t-test; significance is set at p < 0.001 uncorrected without a cluster threshold for display purposes; d.f. = 80). Green: Brain regions showing increased activation during the angry versus neutral face condition (N = 81; one-sample t-test; significance is set at p < 0.001 uncorrected without a cluster threshold for display purposes; d.f. = 80). Colour bars represent t-values. Each 2D sagittal slice is labelled with an x-coordinate (MNI space). Clusters are rendered on the 'ch256' brain template using MRcroGL (http://www.mccauslandcenter.sc.edu/mrcrogl/). Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and Paint.NET v3.5.10.

Please cite this article as: Mothersill, O., et al., Effects of MIR137 on fronto-amygdala functional connectivity, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.12.019
risk allele ('GG/GT' carriers), homozygous risk allele (T) carriers showed increased functional connectivity between the right amygdala and frontal regions involved in emotion processing and regulation, including the cingulate and prefrontal cortex.

Emotion processing in the brain can be conceptualised as being mediated by two distinct, yet interconnected pathways/systems (Phillips et al., 2003). The ventral system, which includes the amygdala and insula, is thought to be responsible for attaching emotional significance to stimuli and producing an affective state; the dorsal system, which includes the lateral prefrontal cortex and supragenual/posterior cingulate, is thought to play a role in emotion regulation, the ability to alter one's reaction to an emotional stimulus (Ochsner and Gross, 2005). This is achieved in part through an inhibitory effect on neuronal firing in the amygdala (Stein et al., 2007). Since altered functional connectivity has been proposed as a key etiological factor in the pathogenesis of schizophrenia (Friston, 1998), altered connectivity between the regions that comprise these systems may contribute to emotional deficits, a key clinical feature of the disorder. For example, altered fronto-amygdala functional connectivity has been observed in schizophrenia patients relative to healthy controls during emotion perception (Das et al., 2007) and in psychosis prone subjects during emotional reappraisal (Modinos et al., 2010).

Although our original aim was to examine differences in amygdala activation in response to emotional faces, the face processing task used in the present study was not associated with increased amygdala activation while viewing angry faces compared to viewing neutral faces. As such, the amygdala activity observed in our sample may represent face processing, rather than emotion processing per se. However, the lack of a significant amygdala response to the angry faces compared to the neutral faces may also reflect participants' emotional responses to both types of facial stimulus. Healthy subjects have responded similarly to both emotional and neutral faces (Lee et al., 2008) and reported neutral faces as emotional stimuli (ille et al., 2011) during other face processing tasks. Participants may interpret neutral faces as emotional stimuli due, for example, to their structural properties (e.g. high or low eyebrows (Adams et al., 2012)) or presentation context (e.g. depending on the types of faces/stimuli preceding the neutral faces in the task) (Wieser and Borsch, 2012).

The present finding of increased connectivity between the amygdala and key regions involved in emotion regulation may reflect an increased regulatory response in the risk group while processing the facial stimuli. However, this conclusion is speculative due to the fact that we observed an altered pattern of connectivity over an experimental period that also included non-facial stimuli. As such, we cannot rule out the possibility that this effect is stationary and face processing independent. Future studies could use psychophysiological interaction (PPI) analysis to examine gene effects on functional connectivity related to specific experimental conditions (e.g. face processing) (Friston et al., 1997). Our study, based on a sample size which was in the average range for the type of analyses conducted, may not be sufficiently powered for PPI due to the low statistical power associated with this technique, which results in high incidence of false negatives (O'Reilly et al., 2012).

Although we observed a significant increase in amygdala connectivity in risk allele homozygotes, we observed no risk allele effects on amygdala activation in the present study, despite highly significant bilateral activation in this region across our sample in response to facial stimuli. This is in contrast to Whalley et al., who reported increased amygdala activation in MIR137 risk allele homozygotes during a

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**Table 3**

Clusters showing significantly increased functional connectivity with the right amygdala in rs1625579 'TT' homozygotes compared to 'GG/GT' carriers.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>p value*</th>
<th>Cluster peaks</th>
<th>t-Value</th>
<th>Z-value</th>
<th>Peak coordinates (MNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>651</td>
<td>&lt;0.001</td>
<td>Right cingulate gyrus/BA 31</td>
<td>5.17</td>
<td>4.78</td>
<td>12 - 43 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left cingulate gyrus/BA 24</td>
<td>4.56</td>
<td>4.28</td>
<td>-12 - 1 - 49</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>0.036</td>
<td>Left cingulate gyrus/BA 31</td>
<td>4.28</td>
<td>4.04</td>
<td>0 - 28 37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right inferior frontal gyrus/BA 47</td>
<td>4.60</td>
<td>4.31</td>
<td>39 20 - 11</td>
</tr>
</tbody>
</table>

* p values are FWE-corrected for multiple comparisons at the cluster level.

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**Fig. 2** Effects of MIR137 variation on fronto-amygdala functional connectivity. Red-yellow: Brain regions showing relatively increased connectivity with the right amygdala in risk 'T' homozygotes relative to 'C' carriers (N = 80; independent t-test between genotype groups: significance is set at p < 0.001 uncorrected without a cluster threshold for display purposes; d.f. = 77). Colour bars represent t-values. Each 2D sagittal slice is labelled with an x-coordinate (MN1 space). Clusters are rendered on the 'ch256' brain template using MRIcroGL (http://vmw.mccauslandcenter.sc.edu/mricrogl/). Bar graphs were constructed as described in the Functional connectivity section; a.u. = arbitrary units. Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and Paint.NET v3.5.10.

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Please cite this article as: Mothersill, O., et al., Effects of MIR137 on fronto-amygdala functional connectivity, NeuroImage (2013), dx.doi.org/10.1016/j.neuroimage.2013.12.019
sentence completion task in first degree relatives of patients with schizophrenia but not in relatives of patients with bipolar disorder and subjects at low genetic risk. Besides the different paradigms, this is an important difference between our study which consisted of healthy controls without a family history of schizophrenia and overall lower genetic risk of the disorder compared to the sample examined in Whalley et al. It has previously been suggested that functional connectivity may represent a more sensitive intermediate phenotype in identifying neural circuits affected by schizophrenia risk variants compared to measures of neural activation (Meyer-Lindenberg, 2009).

As such, while we were unable to detect differences in cortical activation in our healthy control sample, the use of functional connectivity may have enabled us to already detect rs1625579 specific effects on amygdala function in individuals with a comparably lower genetic risk for the disorder. Potential interactions with other environmental and genetic risk factors in first degree relatives of patients with schizophrenia might then further impact these effects on the level of neural system connectivity and contribute to the finding of altered amygdala activation.

While patients with schizophrenia show consistent differences in amygdala function (Aleman and Kahn, 2005; Shayaneg and Stahl, 2005), the degree to which the genetic basis of these differences is schizophrenia specific, or relate to psychosis more broadly is unknown (Rasett et al., 2009). While MIR137 was associated with schizophrenia but not bipolar disorder in the Ripke et al. (2011) study, whether the effects of MIR137 on amygdala connectivity observed in the present study of healthy participants are only relevant to schizophrenia risk is uncertain. miRNAs are suggested to represent novel therapeutic targets for emotion-related disorders such as anxiety and depression (O'Connell et al., 2011). mRNA levels are altered in these disorders, and both antidepressants and mood stabilisers alter mRNA levels in the brain. It is interesting to speculate about whether the present finding that MIR137 variation may affect emotional networks in a manner that has relevance for other psychiatric disorders also.

The impact of rs1625579 on measures of brain function and connectivity is likely to interact with, and be influenced by, other variants that confer risk for schizophrenia. For example, several genome-wide associated psychosis risk genes, including ZNF804A, CACNA1C, TCF4 and CSMD1, are targets of MIR137. ZNF804A was the first variant to show an effect on functional connectivity, and also showed increased amygdala-related connectivity with other cortical regions. As such, an important direction for future imaging genetics studies will be to examine the possible additive or epistatic effects of variants in these genes on functional connectivity of neural circuits during face processing (Nicodemus et al., 2010). Finally, functional connectivity between the amygdala and cingulate is also sensitive to environmental stress, such as urban upbringing (Lederbogen et al., 2011). Whether and how the functional connectivity effects of MIR137 observed are mediated by environmental experience will be an important question for future imaging genetics studies.

Conclusions

In conclusion, our study reports for the first time the effects of a genome-wide associated schizophrenia risk variant, rs1625579, within MIR137, on functional connectivity between the amygdala and (1) the cingulate and (2) the prefrontal cortex, brain regions that play an important role in emotion processing and regulation. This is the first study to demonstrate effects of MIR137 on functional connectivity, and provides further evidence that the rs1625579 variant may contribute to forms of psychosis in which affective symptoms are more prominent, building on previous findings that the variant is associated with mood congruent psychotic symptoms. Further research on this variant may uncover novel molecular pathways associated with illness risk, which may inform future treatment strategies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.12.019.

Acknowledgments

We wish to thank Dr. Frieder Paulus and Dr. Christine Esslinger for advice on functional connectivity analysis. We also wish to thank all those individuals who participated in the study, and staff and students in the Trinity College Institute of Neuroscience involved in the collection of data. Finally, we would like to acknowledge data management support from the Trinity Centre for High Performance Computing. The data management system used for this work was BC(Sp)max v. 3.5-121 (Biocomputing Platforms Ltd, Finland).

This work was generously supported by Science Foundation Ireland (SFI grant 12/IP/1359 to GD and SF108/1N.1/B1916-Corvin to AC), and the Health Research Board (HR/2009/197 to GD).

Conflict of interest

All authors have declared that there are no conflicts of interest in relation to the subject of this study.

References


Please cite this article as: Mothersill, O., et al., Effects of MIR137 on fronto-amygdala functional connectivity, NeuroImage (2013), http://dx.doi.org/10.1016/j.neuroimage.2013.12.019