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The Impact of Schizophrenia

Genome Wide Association Study Genes on

Functional Connectivity:

A Functional Magnetic Resonance Imaging Study

By

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2014

A dissertation submitted for the degree of Doctor of Philosophy to the

University of Dublin, Trinity College.

Department of Psychiatry, Trinity College Dublin
Declaration

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Summary

Genome-wide association studies (GWAS) have identified several genetic variants associated with increased schizophrenia risk. However, the specific mechanisms by which these variants increase risk are not fully understood. Altered communication between brain regions, described as functional dysconnectivity, has been consistently observed in schizophrenia patients relative to healthy controls, suggesting that risk variants may exert some of their effects on this phenotype. This thesis aims to characterise the effects of schizophrenia GWAS risk variants on functional connectivity in healthy volunteers, using functional magnetic resonance imaging (fMRI).

Chapter 1 provides an overview of the genetic component of schizophrenia and the evidence that functional connectivity provides a useful intermediate phenotype for studying schizophrenia risk variants. Chapter 2 presents a meta-analysis comparing schizophrenia risk variant effects on functional and structural connectivity based on recent imaging genetics studies. This study suggests that structural connectivity studies are associated with a wider range of effect sizes relative to studies of functional connectivity. Chapter 3 outlines the MRI methods used in this thesis.

Chapter 4 presents a phenotypic study examining neural activation in schizophrenia patients and healthy controls during performance of an emotional face processing task using fMRI. In this study, patients showed weaker deactivation of the medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC), compared to controls. Chapters 5,
6 and 7 present studies examining the effects of three individual schizophrenia GWAS risk variants on functional connectivity. The first of these studies indicated that homozygous carriers of the rs1625579 variant, within MIR137, have increased functional connectivity between the right amygdala and the cingulate and right inferior frontal gyrus, brain regions involved in emotion processing and regulation, compared to non-risk allele carriers. The next study showed that homozygous carriers of a GWAS insertion and deletion (indel) variant within NOS1 show increased functional connectivity between the right dorsolateral prefrontal cortex and the right middle frontal gyrus, right superior temporal gyrus and right mPFC, providing further evidence that NOS1 increases risk through a mechanism of altered prefrontal function. The final imaging genetics study reported no significant effects of the rs1344706 variant, within ZNF804A on functional connectivity, suggesting that any effects of this variant may be smaller than expected.

In conclusion, this thesis makes a significant contribution to the psychiatric imaging genetics literature by reporting the first evidence that two recent GWAS risk variants are associated with altered functional connectivity in healthy volunteers. These results extend GWAS findings with neurobiological data, and suggest altered functional connectivity as a mechanism of risk. In addition, the finding of altered mPFC activity in schizophrenia patients during face processing may provide a useful translational model for future studies examining novel therapies for social cognitive deficits in the disorder. It is hoped that further research on schizophrenia GWAS genes and related pathways will help identify novel molecular targets to improve cognitive function in this disorder.
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Note

This thesis includes research that forms part of a collaborative project on psychosis. The author was involved in recruiting and testing schizophrenia patients and healthy control participants for the neuroimaging component of this project in 2011 and 2012 with Sinead Kelly. Prior to 2011, recruitment and testing of study participants was undertaken by other researchers in the Neuropsychiatric Genetics Research Group, Trinity College Dublin, including Dr. Emma Jane Rose.

All imaging analysis reported in this thesis was undertaken by the author. Recruitment of schizophrenia patients for the psychosis study was undertaken by other members of the Neuropsychiatric Genetics Research Group, including Dr. Eric Kelleher, Denise Hogan, Catherine Delaney and Christina Mooney. All genotyping and genetics analysis reported in this thesis was undertaken by Prof. Derek Morris and colleagues in the Neuropsychiatric Genetics Research Group. IT support was provided by Dr. Carlos Pinto and the Trinity Centre for High Performance Computing. Finally, MRI scanning was undertaken in the Trinity College Institute of Neuroscience (Principal Physicist: Mr. Christian Kerskens; Radiographer: Mr. Sojo Joseph).
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Glossary of Terms

ACC = Anterior cingulate cortex

AD = Axial diffusivity

ALIC = Anterior limb of internal capsule

ATR = Anterior thalamic radiation

a.u. = Arbitrary units

BA = Brodmann Area

BOLD = Blood-oxygen-level dependent

CD-CV = Common disease-common variant

CI = Confidence interval

CMA = Comprehensive Meta-Analysis

CNV = Copy-number variant

CSF = Cerebrospinal fluid

CST = Corticospinal tract

DACC = Dorsal anterior cingulate cortex

DCM = Dynamic causal modelling

d.f. = Degrees of freedom

DLPFC = Dorsolateral prefrontal cortex

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DNA = Deoxyribonucleic acid

DSM = Diagnostic and Statistical Manual of Mental Disorders

DTI = Diffusion tensor imaging

EEG = Electroencephalogram

EPI = Echo-planar imaging

FA = Fractional anisotropy

fMRI = Functional magnetic resonance imaging

FOV = Field of view

FUF = Frontal uncinated fasciculus

FWE = Family-wise error

FWHM = Full width at half maximum

GCC = Genu of corpus callosum

GLM = General linear model

GWAS = Genomewide association study

HRF = Haemodynamic response function

ICA = Independent component analysis

IFG = Inferior frontal gyrus

Indel = Insertion and deletion

LG = Lingual gyrus
MF = Medial frontal

MFG = Middle frontal gyrus

MIST = Montreal Imaging Stress Task

MNI = Montreal Neurological Institute

mPFC = Medial prefrontal cortex

MRI = Magnetic resonance imaging

mRNA = Messenger ribonucleic acid

MTG = Middle temporal gyrus

NMDA = N-methyl-D-aspartate

nNOS = Neuronal nitric oxide synthase

OR = Odds ratio

PET = Positron emission tomography

PFC = Prefrontal cortex

PGC = Psychiatric Genetics Consortium

PPI = Psychophysiological interaction

qPCR = Quantitative polymerase chain reaction

RD = Radial diffusivity

RF = Radio frequency

ROI = Region of interest
SANS = Scale for the Assessment of Negative Symptoms

SAPS = Scale for the Assessment of Positive Symptoms

SC = Seed voxel correlation analysis

s.d. = Standard deviation

s.e. = Standard error

SENSE = Sensitivity encoding

SFG = Superior frontal gyrus

SNP = Single nucleotide polymorphism

SPM = Statistical Parametric Mapping

SWM = Spatial working memory

TE = Echo delay time

TFE = Turbo Field Echo

TPJ = Temporo-parietal junction

TR = Repetition time

TTL = Transistor-transistor logic

UF = Uncinate fasciculus

VNTR = Variable number of tandem repeats

WM = White matter
Chapter 1

Introduction
1.1 Cognitive dysfunction in schizophrenia

Schizophrenia is a complex, devastating psychiatric disorder affecting between 0.5 and 1% of the world’s population (Lewis and Lieberman, 2000; van Os and Kapur, 2009), and is characterised by the following symptom clusters:

1. Hallucinations and delusions, also known as positive symptoms.
2. Altered drive and volition, including reduced motivation and social withdrawal, also known as negative symptoms.
3. Cognitive deficits, including impaired memory and disorganised thinking.
4. Impaired emotion regulation resulting in depressive states.

Schizophrenia is associated with low employment (Marwaha et al., 2004), homelessness (Folsom et al., 2005) and premature mortality relative to the general population (Saha et al., 2007), predominantly caused by increased rate of physical health problems and suicide (Bushe et al., 2010; Hor and Taylor, 2010). The disorder also imposes a heavy cost on society. For example, psychotic disorders were estimated to cost €93.9 billion in Europe in 2010 (Gustavsson et al., 2011).

A core feature of schizophrenia is cognitive dysfunction (Lewandowski et al., 2011), including deficits in attention, processing speed, memory, problem solving, and social cognition (Nuechterlein et al., 2004). Cognitive dysfunction is generally stable over a patient’s life and frequently precedes the emergence of other symptoms such as auditory hallucinations. It is also one of the strongest predictors of functional outcome in schizophrenia patients in employment, relationships with other people, independent
living, and response to psychiatric rehabilitation (McGurk et al., 2007; Lewandowski et al., 2011).

As such, efforts to improve cognitive function are likely to result in significant improvements in prognosis. However, large clinical trials of second-generation antipsychotics (currently the main class of drug used to treat schizophrenia) have reported only modest improvements in cognition (Hill et al., 2010). As a result, a current research priority is to identify novel molecular targets relevant to cognition (Carter and Barch, 2007). Identifying new molecular targets for psychiatric therapies is also critical considering that several pharmaceutical companies, including GlaxoSmithKline and Pfizer, have reduced investment in this area in recent years due to the perceived complexity of brain disorders (Abbott, 2011; Wegener and Rujescu, 2013). As schizophrenia has a large genetic component (see section 1.2), it is hoped that a better understanding of the genetic architecture of the disorder will help in this process. In fact, Novartis, another pharmaceutical company, has recently announced plans to increase investment in research on the genetics of psychiatric disorders, including schizophrenia (Abbott, 2013). The next section outlines the heritability of schizophrenia and progress that has been made in elucidating specific risk genes associated with the disorder.

1.2 Schizophrenia heritability

For any given population, heritability ($h^2$) is an estimation of the variance in a specific trait that is attributable to genetic differences, e.g. $h^2 = 0.50$ means that 50% of the
variance in the trait is attributable to genetic factors (Visscher et al., 2008). Estimates of $h^2$ can be made from similarities observed in people who vary in the amount of genes they share. For example, monozygotic twins share 100% of their genes, while dizygotic twins and siblings share 50% of their genes, and half-siblings share 25%. Therefore, if a trait is more similar amongst monozygotic twins compared to dizygotic twins, this suggests an important genetic factor in the trait. Schizophrenia is highly heritable, with current estimates of $h^2 = 0.64$ in a national family study (Lichtenstein et al., 2009), and $h^2 = 0.81$ in a meta-analysis of twin studies (Sullivan et al., 2003). However, it should be noted that in twin and family studies, a limitation might be the shared environment in families. As a result, genetic heritability described might also represent environmental factors. Population studies also suggest that the sibling recurrence risk ratio ($\lambda_{sib}$) of schizophrenia is 8.6, i.e. if a person has schizophrenia, the risk of a sibling developing the disorder is 8.6 times the population risk (Lichtenstein et al., 2006).

Given this strong genetic component, a priority for schizophrenia research is to identify specific genes associated with risk in order to elucidate the complex neurobiology of the illness. Doing so is expected to help identify new molecular targets for pharmacological therapies. However, unlike simple genetic disorders caused by a single mutated gene such as Huntington's disease, schizophrenia is a complex genetic disorder affected by many genes, and is far more common in the population (~1 in 100 in schizophrenia compared to ~1 in 15,000 in Huntington's disease, for example) (Walker, 2007).
A prominent hypothesis for explaining the genetics of common disorders suggests that they are a result of the cumulative impact of many common genetic variants of small effect interacting with other biological factors (Chakravarti, 1999). This is known as the common disease-common variant (CD-CV) hypothesis. Common variants typically take the form of single nucleotide polymorphisms (SNPs), changes in a single base-pair of the deoxyribonucleic acid (DNA) sequence that occur frequently in the human genome (Bush and Moore, 2012). SNPs can affect amino acid sequences, messenger ribonucleic acid (mRNA) transcripts and transcription factor binding. Other common variants include small insertions and deletions (indels), changes in the DNA sequence in which base-pairs are added or deleted, resulting in small structural differences in chromosomes (Albers et al., 2011).

Strategies used to identify risk genes for disease include:

1. **Candidate gene studies**: Candidate gene studies are a type of genetic association study in which genes are selected due to an expected role in the disease mechanisms under investigation (Patnala et al., 2013). SNPs are then identified that affect regulation of this gene or the gene product. In a candidate gene study, the SNP is verified for trait (or disease) association by observing how frequently it occurs in a sample of random test participants having the trait (or disease), and selected control participants.

For example, the gene encoding catechol-O-methyltransferase, *COMT*, has previously been postulated as a candidate gene for schizophrenia due to its role in the metabolism of dopamine, a neurotransmitter implicated in the disorder.
COMT is highly expressed in the prefrontal cortex (PFC), a brain region critical for cognitive functions disrupted in schizophrenia such as working memory (see section 1.7). A SNP in this gene (rs4680) leads to less COMT activity, and as a result, increased dopamine in the PFC. This effect has been detected and replicated using functional neuroimaging, which has shown a pattern of increased prefrontal activation during working memory in healthy volunteers associated with the risk allele. This is thought to reflect inefficient processing in this brain region in risk carriers, which may increase illness risk through a mechanism of disrupted cognition.

2. Genome-wide association studies (GWAS): The utility of candidate gene studies for understanding the neurobiology of schizophrenia has been questioned because their association with the categorical disease phenotype is inconsistent (Williams et al., 2007; Meyer-Lindenberg, 2010). Alternatively, an unbiased, hypothesis-free way to identify genetic variants associated with schizophrenia is through GWAS, in which hundreds of thousands of variants are tested for association with a trait or disease in hundreds of thousands of individuals (Feero et al., 2010). Large-scale GWAS has been made possible due to advances in molecular genetic technology over the past decade, permitting affordable genotyping of up to 1 million SNPs from an individual’s DNA. GWAS is particularly relevant for developing new treatments, as researchers need to study factors significantly associated disease risk.
Limitations of the GWAS approach include the multiple testing problem arising with the number of SNPs that are analysed (> 500,000) (Corvin et al., 2010). As a result, GWAS results must be replicated in at least one independent sample, and the combined effect from the discovery and replication(s) must be associated with a $p < 5 \times 10^{-8}$, similar to a Bonferroni correction of $p < 0.05$ for a million tests (Chanock et al., 2007). These issues have led to collaborations between laboratories across the world in order to produce sample sizes necessary to detect effects passing these criteria, e.g. the Psychiatric Genetics Consortium (PGC) (Ripke et al., 2011).

The first schizophrenia risk variant identified using GWAS was a SNP within an intron (a non-coding region of DNA) of the gene encoding zinc finger protein 804a, (ZNF804A), rs1344706 (O'Donovan et al., 2008), a gene thought to play a role in gene regulation and neurodevelopment (see section 7). More recently, a mega-analysis of GWAS studies identified five new loci associated with schizophrenia risk, including a SNP within an intron of MIR137, rs1625579, which encodes a microRNA also involved in neurodevelopment (Ripke et al., 2011). Four other SNPs identified in this analysis are thought to contain targets of MIR137, suggesting disruption of MIR137 molecular pathways as a new etiological mechanism in schizophrenia pathogeneses. In addition, the most recent GWAS undertaken has identified a further ~100 genetic risk variants for schizophrenia (Psychiatric Genetics Consortium, Manuscript in preparation).
Some mutations predisposing to schizophrenia may be highly penetrant, rare in the population, and recent (Walsh et al., 2008). These rare variants may include structural variations known as copy-number variants (CNVs), which involve microduplications and microdeletions of genomic material. The most established schizophrenia CNVs are reported to account for ~2-4% of risk (Sebat et al., 2009), smaller than the ~30% of schizophrenia risk thought to be accounted for by the combined actions of common variants (Stone et al., 2008; Purcell et al., 2009). However, it is expected that improved technologies will see identification of further rare variants (e.g. Williams et al., 2012). Overall, genetic risk for schizophrenia in the population is most likely caused by a combination of large numbers of common variants with small effects and individual rare variants with large effects.

1.3 Intermediate phenotypes in schizophrenia

In Huntington’s disease, a Mendelian disorder (caused by a single gene), changes in the genetic code can be closely associated with functional effects on the brain (Braff et al., 2007). However, schizophrenia is a complex disorder affected by many genes, in addition to epigenetic, environmental, medication and random factors, and it is defined using behavioural criteria. Given that GWAS variants are simply associated with diagnosis of schizophrenia, elucidating specific mechanisms by which these variants confer risk is particularly challenging.

Researchers have attempted to address this issue by taking an intermediate phenotype (or endophenotype) approach (Gottesman and Gould, 2003) (figure 1.1). An
intermediate phenotype for a disorder is a heritable, measurable variation that is closer to the level of genes than clinical symptoms and may mediate genetic effects on the broader illness phenotype (Pearlson and Calhoun, 2009). An intermediate phenotype is expected to show increased genetic penetrance compared to diagnostic criteria, with biological phenotypes expected to show increased penetrance compared to behavioural ones (Mier et al., 2010). Identifying intermediate phenotypes for schizophrenia can lead to the following advantages (from Braff et al., 2007):

1. Intermediate phenotypes may lead to better diagnostic classification, e.g. subgroups of patients diagnosed based on shared biological disease pathogenesis, which may lead to more specific treatments.

2. New molecular targets for drug therapies may be identified using intermediate phenotypes (known to be associated with illness and heritable) that are linked to specific genetic risk mechanisms. Resulting drug therapies may be specific for the intermediate phenotype associated with genetic risk (e.g. a cognitive deficit, altered connectivity within a specific network).
Figure 1.1: Diagram illustrating the intermediate phenotype hypothesis, in which genetic loci and environmental risk factors impact upon intermediate phenotypes, which themselves impact upon clinical outcomes (from Carlson et al., 2004)

Gottesman and Gould (2003) suggested five criteria for assessing the potential utility of an intermediate phenotype in psychiatric genetics:

1. The intermediate phenotype is associated with the illness in the population.
2. The intermediate phenotype is heritable.
3. The intermediate phenotype is mainly clinical state-independent.
4. The intermediate phenotype and illness co-segregate within families.
5. The intermediate phenotype is found in unaffected family members at a higher rate than in the population.

Candidate intermediate phenotypes for schizophrenia have been proposed, including sensory motor gating, working memory, glutamatergic mechanisms and functional connectivity (Gottesman and Gould, 2003; Meyer-Lindenberg, 2009).
1.4 The ‘disconnection’ hypothesis of schizophrenia

This thesis considers functional connectivity as a candidate intermediate phenotype for schizophrenia. The origins of connectivity research can be traced back to the nineteenth century and the theory of connectionism proposed by physiologist Friedrich Goltz (Frackowiak et al., 2004). Goltz suggested that healthy brain function depended on communication between different areas, not just specific areas themselves. In the early twentieth century, German neurologist Carl Wernicke proposed that psychosis arises from altered neural connectivity (or dysconnectivity) rather than from abnormalities in specific parts of the brain (Stephan et al., 2009). A similar idea was later put forward by Eugene Bleuler, who invented the word ‘schizophrenia’ to describe ‘splitting’ of mental domains. Over the past twenty years, advances in neuroimaging technology have led scientists to reconsider dysconnection as a key component of schizophrenia pathogenesis.

This consideration is unsurprising given that a high proportion of the brain’s energy is used by endogenous oscillations within neural networks (Raichle et al., 2001). Connectivity within these networks is a stable feature of the brain, so it is likely to be affected by brain disorders and associated risk genes (Meyer-Lindenberg, 2009).

Functional connectivity has been defined by Karl Friston and Chris Frith (1995) as “the temporal correlation between two neurophysiological measurements.” Altered functional connectivity between brain regions has been observed in schizophrenia.
patients relative to healthy controls using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Friston and Frith, 1995; Meyer-Lindenberg et al., 2001; Lawrie et al., 2002; Meyer-Lindenberg et al., 2005). Electroencephalogram (EEG) research also demonstrates abnormal functional connectivity patterns in patients with schizophrenia (Breakspear et al., 2003), and a genetic mouse model of schizophrenia has revealed decreased hippocampal-prefrontal connectivity during a T-maze task (Sigurdsson et al., 2010).

Understanding the underlying biological causes of altered functional connectivity has the potential to lead to a better understanding of schizophrenia pathogenesis, but so far the aetiology of functional dysconnectivity remains unclear. However, different mechanisms have been proposed. For example, Karl Friston and colleagues have proposed the ‘disconnection hypothesis’ of schizophrenia, suggesting that the disorder 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 (Friston, 1998; Stephan et al., 2009).

Support for the role of the NMDA receptor in schizophrenia comes from several studies. Firstly, drugs that block the NMDA receptor, such as ketamine and phencyclidine, can induce psychotic symptoms in healthy controls (Javitt, 2010). 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).
The altered functional connectivity observed in schizophrenia might also be caused by aberrant structural connectivity, i.e. the white matter tracts connecting brain regions. Compromised white matter integrity has been observed in schizophrenia patients relative to healthy controls in studies using diffusion tensor imaging (DTI) (Kubicki et al., 2007; Ellison-Wright and Bullmore, 2009). DTI measures the diffusion of water molecules in the brain (Kubicki et al., 2007). A common measure of white matter integrity derived from this method is fractional anisotropy (FA), describing how diffusion of water molecules is restricted along the length of fibre tracts in healthy white matter. If white matter is compromised, diffusion can become less restricted, resulting in reduced FA. White matter abnormalities are also apparent in individuals at high risk of schizophrenia and 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).

It is likely that genetic risk in schizophrenia is mediated in part by effects of specific variants on functional and structural connectivity. Although it is expected that genetic effects on these phenotypes will be larger than on behaviour, it is unknown whether effects are larger on functional or structural connectivity, or if the range of effects observed are comparable across these modalities.
1.5 Altered functional connectivity as an intermediate phenotype for schizophrenia

Imaging genetics combines neuroimaging data with genetic data to identify genetic variation associated with specific differences in brain structure and function. This emerging field has been used to examine the utility of brain-based intermediate phenotypes, including structural connectivity and functional connectivity, which are expected to show higher genetic penetrance compared to cognitive or behavioural intermediate phenotypes (Rose and Donohoe, 2013).

Evidence that functional connectivity could be a particularly useful intermediate phenotype for schizophrenia comes from multiple lines of evidence:

(1) Significantly altered functional connectivity has been consistently observed in schizophrenia across a range of neuroimaging modalities (see above).

(2) There is evidence that functional connectivity patterns are moderately to highly heritable. For example, a resting-state fMRI family study of 333 individuals from 29 extended pedigrees estimated $h^2 = 0.424 \pm 0.17$ for default-mode network functional connectivity (Glahn et al., 2010). Two twin studies using graph theoretical analyses estimate $h^2 = 0.6$ and $h^2 = 0.42$, respectively (the second study in children) (Fornito et al., 2011; van den Heuvel et al., 2012). In addition, twin studies using EEG have suggested $h^2$ ranging from 0.23 to 0.89 for different functional connectivity measures (Posthuma et al., 2005; Smit et al., 2008; Schutte et al., 2013).
(3) Functional connectivity patterns of specific networks may be clinical state independent. For example, healthy students with high risk of developing a schizophrenia-spectrum disorder (high psychosis proneness) have shown reduced prefrontal-amygdala functional connectivity relative to students at low risk (Modinos et al., 2010).

(4) Altered functional connectivity has been observed in unaffected siblings of schizophrenia patients in a similar pattern seen in patients, compared to healthy controls without a family history, using fMRI (Rasetti et al., 2011; Khadka et al., 2013).

(5) Functional connectivity has been shown to explain more variance in behaviour than brain activity or structure, suggesting it may be particularly well-placed to examine mechanisms linking genetic variation to behavioural outcomes. For example, in a study by Pezawas et al. (2005), healthy volunteers underwent fMRI while performing an emotion recognition task and were genotyped for the 5-HTTLPR polymorphism, a variant in the serotonin transporter gene, SLC6A4, associated with anxiety. Carriers of the risk variant showed decreased functional connectivity between the amygdala, which plays an important role in emotion, and the anterior cingulate cortex (ACC), which plays a role in emotion regulation. Connectivity within this network accounted for 30% of the variance in behavioural measures of trait anxiety (the harm avoidance subset of the Tridimensional Personality Questionnaire (TPQ), the only behavioural measure used), significantly more than brain activity (blood-oxygen-level dependent (BOLD) signal; see section 1.6) or grey matter volume. Although this specific finding has not been replicated, more recent studies also report an association
between higher harm avoidance scores and altered amygdala-prefrontal functional connectivity (Buckholtz et al., 2008; Li et al., 2012).

1.6 Using fMRI to measure *in vivo* functional connectivity

This project used fMRI to measure functional connectivity. This technique was originally developed by Seiji Ogawa and colleagues in 1990 as an extension of anatomical magnetic resonance imaging (MRI) to provide information on brain function. fMRI takes advantage of the fact that oxygenated haemoglobin in the blood has different magnetic properties compared to deoxygenated haemoglobin, leading to a specific MR signal in the tissues surrounding blood vessels, the blood-oxygenation-level dependent (BOLD) signal (Logothetis and Wandell, 2004). Neuronal activity is metabolically expensive, so increased firing requires increased oxygen ($O_2$) transport from surrounding blood vessels. Cerebral blood flow then increases, overcompensating for the reduced $O_2$, which leads to a higher concentration of oxygenated haemoglobin and increased BOLD signal. In this way, the BOLD signal can be used as a proxy measure of neuronal activity. Research by Nikos Logothetis and colleagues (2001) has provided evidence that the BOLD signal directly reflects neural responses elicited by a stimulus by showing correlations of monkey visual cortex activity simultaneously measured using fMRI and EEG.

Advantages of fMRI compared to other measures of brain function (e.g. EEG, evoked potentials) include high spatial resolution and increased signal to noise (Mier et al., 2010). The penetrance of genetic variants on brain function is also particularly high for
functional neuroimaging. For example, meta-analysis on genetic effects on brain
function using EEG reported only a small compound effect size (Cohen's $d = 0.02$)
(Flint and Munafò, 2007). In contrast, meta-analyses of fMRI studies report large
effects, which are also larger than effects reported for behavioural measures, consistent
with the intermediate phenotype hypothesis (see section 2).

The most commonly used fMRI method to examine functional connectivity in imaging
genetics is seed voxel correlation analysis (Biswal et al., 1995; Pezawas et al., 2005;
Meyer-Lindenberg, 2009; Esslinger et al., 2009; Bedenbender and Paulus et al., 2011).
First, the fMRI signal is extracted from a region-of-interest, called a seed region. Then
the temporal correlation between this signal and the signals of all the other voxels in the
brain is determined. The first study to use this method to analyse fMRI data was
conducted by Biswal et al. (1995), who reported temporal correlations between the
premotor cortex and the same region in the contralateral hemisphere at rest.

The current implementation of this technique can also be applied to data acquired
during a task. If a task is performed during the scan, regional brain activation related to
the task can be subtracted from the data using a general linear model (GLM) (see
section 3.3 for a detailed description of this procedure, as implemented using SPM and
MATLAB). A disadvantage with this technique if used during a task is that it examines
connectivity across a period of time that includes multiple cognitive states. Thus, even
after removal of task related activation from estimates of functional connectivity
statistically, some residual task effects on neural networks may remain. However,
Meyer-Lindenberg (2009) points out that changing cognitive states are also associated
with resting brain function, and this does not preclude a reliable determination of 
resting-state connectivity.

Seed voxel correlation analysis may have greater sensitivity to detect genetic effects 
compared to other measures of functional connectivity, such as psychophysiological 
interaction analysis (PPI) (as PPI is considered to lack power relative to other methods - 
O’Reilly et al., 2012; see section 8.5) or independent component analysis (ICA) (which 
examines the time-course of a whole network; this may not be as sensitive as examining 
the relationship between a small group of voxels and other voxels - von dem Hagen et 
al., 2012).

So far, the seed voxel correlation method has successfully identified significant effects 
of several schizophrenia risk variants on functional connectivity, including effects of 
variants in candidate genes PRODH and PPP1R1B (Meyer-Lindenberg et al., 2007; 
Kempf et al., 2008). However, to date effects of only one schizophrenia risk variant 
showing GWAS-association have been examined on functional connectivity (the 
rs1344706 variant within an intron of ZNF804A; Esslinger et al., 2009; Esslinger et al., 
2011; Walter et al., 2011; Rasetti et al., 2011; Paulus et al., 2013a; Mohnke et al., 
2013).

1.7 Cognitive tasks used in this thesis

This thesis examined genetic effects on functional connectivity during performance of 
an emotional face processing task and a spatial working memory task. Inclusion of
these tasks also allowed the project to examine genetic effects on regional brain activation. These tasks were chosen as they target prominent cognitive functions disrupted in schizophrenia: social cognition and working memory.

Social cognition is a broad construct consisting of cognitive processes that allow people to perceive, interpret and store information about themselves and others (Penn et al., 2008; Van Overwalle, 2009). Examples include recognising emotions from facial expressions or tone of voice, or thinking about the thoughts and goals of others. In schizophrenia, social cognitive deficits are a defining feature, affecting quality of life and functional outcomes (Brekke et al., 2005). For example, a recent meta-analysis by Fett et al. (2011) suggests that social cognition predicts more variation in social and occupational functioning than cognitive performance alone.

One aspect of social cognition that is significantly impaired in schizophrenia is processing the emotional content of faces (Li et al., 2009). For example, patients have been reported to have difficulties recognising emotions from faces (Aleman and Kahn, 2005), but are also more sensitive to negative facial expressions such as anger and fear compared to healthy controls (Mandal et al., 1998; Evans et al., 2011). Excessive threat detection from facial expressions has been hypothesised to contribute to the development of persecutory delusions (Green and Philips, 2004), which are associated with patient distress (Freeman et al., 2002) and predict admission to hospital (Castle et al., 1994).
Working memory is a construct containing cognitive processes that allow people to maintain, store, and manipulate information over a short period of time (Baddeley, 1992). Working memory is highly related to general intelligence, and holding information in short-term memory and being able to manipulate it is necessary for a variety of complex cognitive tasks such as understanding language, learning, reasoning and problem-solving (Süß et al., 2002; Baddeley, 2003; Colom et al., 2004; Bühner et al., 2008; Colom et al., 2008). Spatial working memory specifically refers to the maintenance, storage and manipulation of spatial information, employed for example when playing chess (Glahn et al., 2003).

Impaired working memory capacity (including spatial working memory) is a core feature of schizophrenia and is also a predictor of functional outcomes in work, relationships, etc. (Park and Holzman, 1992; Lee and Park, 2005; Bowie et al., 2008). Spatial working memory impairment has been observed in schizophrenia patients before and after hospitalisation, suggesting it as a stable marker of the disorder, and correlations between spatial working memory impairment and negative symptoms have been reported (Carter et al., 1996; Park et al., 1999; Glahn et al., 2003).

1.8 Project aims

This PhD project consists of five studies. In the first study (section 2), a literature review was undertaken to examine effect sizes of schizophrenia risk genes on functional and structural connectivity. A random-effects meta-analysis was then used to compare
the range of effect sizes observed between modalities in order to assess the relative impact of risk genes across these measures.

The second study was a schizophrenia phenotype study. This study used fMRI to examine neural activity in patients compared to healthy controls during emotional face processing in order to further characterise functional differences between these groups and further elucidate the neurobiology of social cognitive deficits in this disorder (section 4).

Since effects of only one GWAS schizophrenia risk variant have been examined on functional connectivity, examination of other variants is warranted, as well as replication of previous findings. The final three studies used fMRI to examine the effects of GWAS schizophrenia risk variants on functional connectivity in healthy volunteers during performance of a relevant cognitive task (emotional face processing or spatial working memory) in order to further characterise these effects (sections 5-7).

Specifically, this project aimed to test the following hypotheses:

(1) Schizophrenia risk variants have different effect sizes on functional versus structural connectivity, with a different range of effects observed for each measure.

(2) Patients with schizophrenia show altered limbic activity while passively viewing dynamic angry and neutral facial expressions, compared to healthy controls.
(3) GWAS schizophrenia risk variants are associated with altered neural activity and functional connectivity patterns in healthy volunteers.

Given the altered functional connectivity observed in schizophrenia and the heritability of this phenotype, showing that specific variants are associated with altered connectivity can extend and support GWAS statistical evidence with neurobiological data and identify mechanisms mediating risk (Bigos and Weinberger, 2010). This information will be important for the identification of new molecular targets for pharmacological treatments.
Chapter 2

Examining effect sizes of psychosis risk variants on functional and structural connectivity: A meta-analysis
Abstract

Background: Meta-analyses in imaging genetics indicate that schizophrenia risk variants exert large effects on brain structure and function, consistent with the intermediate phenotype hypothesis. This study examined effect sizes of variants on functional and structural connectivity, and compared variability of effect sizes between these modalities to examine the relative sensitivity of imaging methodologies to schizophrenia-related changes in connectivity.

Methods: PubMed was used to search for studies considering schizophrenia risk genes and functional or structural connectivity. Where data were 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.

Results: On average, schizophrenia risk genes exerted a large effect on functional (mean $d = 0.76$) and structural (mean $d = 1.04$) connectivity. 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 suggests 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 ($Q = 6.928, p = 0.008$).
Discussion: 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.

2.1 Introduction

Meta-analysis is a statistical method of combining data across multiple studies (Egger et al., 1997). Meta-analysis of a group of studies may provide a more precise estimate of a treatment effect than an individual study, and may be used to identify important differences in treatment effects across groups of studies. These analyses typically combine data from studies using estimates of effect size, a statistic that measures the strength of a deviation from the null hypothesis (Friston, 2012).

A common measure of effect size is Cohen’s $d$, which is defined as the difference between two means divided by the pooled standard deviation (Cohen, 1992). Cohen’s $d$ can be categorised as small ($d < 0.3$), medium ($d = 0.5$) or large ($d > 7$), with $d = 1$ meaning that two groups differ by one standard deviation (note however that there is no upper limit on this measure).

In the field of imaging genetics, the first two meta-analyses conducted reported medium to large effects of risk variants on brain function: Munafo et al. (2008) examined effect sizes of the 5-HTTLPR polymorphism on amygdala activation (pooled effect size $d = 0.54$), and Mier et al. (2010) examined effect sizes of the COMT Val158Met
polymorphism on prefrontal activation (pooled effect size $d = 0.73$). Rose and Donohoe (2013) compared effect sizes of schizophrenia risk variants on cognitive phenotypes with effect sizes on imaging phenotypes, reporting that variants show medium to large effects on brain structure and function, with larger effects on imaging phenotypes compared to cognition. These studies support the intermediate phenotype hypothesis in psychiatric genetics, which predicts high penetrance of genetic effects on the brain compared to behaviour or symptomatology (Meyer-Lindenberg, 2010).

However, no previous studies have specifically examined effect sizes of risk variants on functional or structural connectivity, or compared effect sizes between these connectivity phenotypes. Examining effects on connectivity is particularly important for our understanding of schizophrenia given that (1) altered functional and structural connectivity have been proposed as aetiological mechanisms in the pathogenesis of the disorder and (2) functional connectivity has been suggested as a better intermediate phenotype than brain activation, and may better-account for behavioural effects of genetic variation (see section 1).

The current study examined the magnitude of gene effects on functional and structural connectivity, using Cohen’s $d$ as a measure of effect size. Random effects meta-analysis was then undertaken to examine the heterogeneity of effects between these methodologies. Consideration of the relative impact of variants on these two connectivity measures could help us better delineate whether or not one phenotype is more proximal to the underlying genetic risk architecture relative to the other. This
could (1) aid our theoretical understanding of the schizophrenia disease trajectory and (2) have significant practical implications for future imaging genetics studies.

2.2 Methods

Relevant papers were searched for based on the criteria that studies included genes implicated in schizophrenia risk and measures of either functional connectivity or DTI. PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used to search for all 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. 24 studies meeting these search criteria were retrieved in total (12 functional connectivity studies and 12 DTI studies). Individual studies differed slightly in terms of MRI acquisition and analysis parameters (e.g. voxel size, size of Gaussian function 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 18 out of the 24 studies identified (eight functional connectivity studies and 10 DTI studies).

Effect size calculations were performed using two online effect size calculators:
Estimates of effect size were calculated based upon either descriptive data (i.e. mean, standard deviation, sample size), or statistical data (i.e. t-statistic or F-test statistic). The purpose of this study was to estimate differences in effect size rather than differences in direction of effect. That is, this study was 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-analyses considering the relative difference in the impact of schizophrenia risk genes on functional and structural connectivity was carried out using the Comprehensive Meta-Analysis (CMA) software package v2 (http://www.meta-analysis.com). In a random effects meta-analysis, effect sizes included are assumed to be a random sample drawn from a distribution of population effects (Borenstein et al., 2007). The combined effect estimates the mean effect in this distribution, and heterogeneity of effect sizes between two groups of studies can be estimated using a Cochran’s Q test.
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. Hedge’s $g$ is another measure of effect size, which was originally developed to remove a small positive bias affecting the calculation of Cohen’s $d$ (Hedges et al., 1985). 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 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

Figure 2.1 shows a flow diagram highlighting studies for which necessary statistical information was available when conducting the meta-analysis, and studies for which this statistical information was unavailable. Overall, eight fMRI and 10 DTI studies reported necessary statistical information and were included in the meta-analysis. Summary information from all of these studies is presented in table 2.1 and table 2.2.

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 $0.76$ (s.d. ± 0.23). The largest effect size ($d = 1.65$) was reported for the impact of the rs1344706 variant in ZNF804A on functional connectivity within the right PFC in schizophrenia 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; Esslinger et al., 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., 2013a).

Figure 2.1: Flow diagram highlighting studies reporting necessary statistical information to be included in the meta-analysis and studies for which this information was unavailable.
Table 2.1: Details of the functional connectivity studies included in the 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 2007</td>
<td>PPPIRIB</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>
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<td>R. PFC - striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
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<td>Researcher</td>
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<td>p-value</td>
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<tr>
<td>Kempf</td>
<td>PRODH</td>
<td>DLPFC</td>
<td>SC</td>
<td>3.91*</td>
<td>103</td>
<td>0.79</td>
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<td>2008</td>
<td></td>
<td>- striatum, reference haplotype carriers &gt; protective haplotype carriers</td>
<td>(108 total)</td>
<td></td>
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<td></td>
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<td>- striatum, risk haplotype carriers &gt; protective haplotype carriers</td>
<td>(108 total)</td>
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<td>Di Giorgio</td>
<td>DISC1</td>
<td>R. hippocampus- R. DLPFC, Ser/Ser&gt;Cys</td>
<td>PPI</td>
<td>3.58*</td>
<td>80</td>
<td>0.81</td>
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<td>Esslinger</td>
<td>ZNF804A</td>
<td>R. DLPFC - L. hippocampus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.98§</td>
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<td>2009</td>
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<td>R. DLPFC - R. DLPFC, CC &gt; CA &gt; AA</td>
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<td>4.05§</td>
<td>115</td>
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<td>R. DLPFC - L. DLPFC, CC &gt; CA &gt; AA</td>
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<td>115</td>
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<tr>
<td>Walter</td>
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<td>L. TPJ - L. inferior frontal gyrus, AA &gt; CA &gt; CC</td>
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<td>2011</td>
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<td>L. TPJ - L. cuneus, AA &gt; CA &gt; CC</td>
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<td>2013a</td>
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<td>R. DLPFC - L. HF, AA &gt; CA &gt; CC</td>
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<td>R. DLPFC - L. DLPFC, AA &gt; CA &gt; CC</td>
<td>SC 3.42*</td>
<td>94</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. DLPFC - L. DLPFC, AA &gt; CA &gt; CC</td>
<td>SC 2.26*</td>
<td>94</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. DLPFC - R. DLPFC, AA &gt; CA &gt; CC</td>
<td>SC 2.39*</td>
<td>94</td>
<td>0.5</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>R. DLPFC - R. DLPFC, AA &gt; CA &gt; CC</td>
<td>SC 2.33*</td>
<td>94</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. DLPFC - R. DLPFC, CC &gt; CA &gt; AA</td>
<td>SC 2.43*</td>
<td>94</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: R. DLPFC - L. HF, C &gt; CA &gt; AA</td>
<td>SC 2.72*</td>
<td>96</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: R. DLPFC - L. DLPFC, C &gt; CA &gt; AA</td>
<td>SC 3.65*</td>
<td>96</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: R. DLPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td>SC 3.21*</td>
<td>96</td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: PPI R. DLPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td>PPI 3.74§</td>
<td>96</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls:</td>
<td>Siblings:</td>
<td>Siblings:</td>
<td>Siblings:</td>
<td>Patients:</td>
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</tr>
<tr>
<td></td>
<td>PPI</td>
<td>SC</td>
<td>SC</td>
<td>PPI</td>
<td>SC</td>
<td>PPI</td>
</tr>
<tr>
<td></td>
<td>2.89†</td>
<td>2.53*</td>
<td>2.77*</td>
<td>4.36§</td>
<td>4.58§</td>
<td>3.56§</td>
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<tr>
<td></td>
<td>96</td>
<td>83</td>
<td>83</td>
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<td>33</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.57</td>
<td>0.62</td>
<td>0.98</td>
<td>1.65</td>
<td>1.28</td>
</tr>
<tr>
<td>Patients:</td>
<td>PPI</td>
<td>2.84*</td>
<td>33</td>
<td>1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>-------</td>
<td>----</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. DLPFC - L. PFC, CC &gt; CA &gt; AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients:</td>
<td>PPI</td>
<td>3.40*</td>
<td>33</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. DLPFC - R. PFC, CC &gt; CA &gt; AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: n, sample size; SC, seed voxel correlation analysis; PPI, psychophysiological interaction; DLPFC, 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 2.2: 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 2008</td>
<td>$NRGI$ SNP8NRG243177</td>
<td>Reduced FA in ALIC</td>
<td>$t = 2.65^{**}$</td>
<td>43</td>
<td>0.83</td>
</tr>
<tr>
<td>Winterer 2008</td>
<td>$NRGI$ SNP8NRG221533</td>
<td>Reduced FA in MF</td>
<td>$t = 4.67^{***}$</td>
<td>50</td>
<td>1.35</td>
</tr>
<tr>
<td>Sprooten 2009</td>
<td>$NRGI$ SNP8NRG221533</td>
<td>Reduced FA in left ATR</td>
<td>$t = 5.52^{***}$</td>
<td>28</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>$NRGI$ 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 2009</td>
<td>$NRGI$ SNP8NRG221533</td>
<td>Reduced FA in anterior cingulum</td>
<td>$F = 5.27^{*}$</td>
<td>31</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>$NRGI$ SNP8NRG221533</td>
<td>Reduced FA in anterior cingulum</td>
<td>$F = 18^{***}$</td>
<td>34</td>
<td>1.54</td>
</tr>
<tr>
<td>Konrad 2009</td>
<td>$ErbB4$ rs707284</td>
<td>Reduced FA in temporal lobe WM</td>
<td>$t = 4.24^{***}$</td>
<td>50</td>
<td>1.22</td>
</tr>
<tr>
<td>SNP</td>
<td>Region</td>
<td>Statistical Test</td>
<td>t Value</td>
<td>df</td>
<td>p Value</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------</td>
<td>------------------</td>
<td>---------</td>
<td>----</td>
<td>---------</td>
</tr>
<tr>
<td>ErbB4 rs758440</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 rs839541</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>ErbB4 rs839523</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 v lower risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 3.85***</td>
<td>32</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>G-T-G-T v all other</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>All other v non risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>t = 3.66***</td>
<td>50</td>
<td>1.057</td>
<td></td>
</tr>
<tr>
<td>Zuliani 2011 ErbB4 rs4673628</td>
<td>Reduced FA in right ALIC</td>
<td>t = 3.48*+</td>
<td>36</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>Genotype/Region</td>
<td>Effect</td>
<td>Statistic</td>
<td>Degree of Freedom</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
<td>-----------</td>
<td>------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>ErhB4 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 2010</td>
<td>Main effect of genotype on</td>
<td>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>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>FA, AD, RD in UF</td>
<td>$F = 2.47^{*}$</td>
<td>40</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Liu 2010</td>
<td>Decreased FA in right CST for Val/Val carriers</td>
<td>$F = 5.197^{*}$</td>
<td>79</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>COMT val158met</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>Association with mean FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS, HPS and APS haplotypes in right UF</td>
<td>$F = 3.507^*$</td>
<td>68</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roffman 2011</td>
<td>Reduced FA in bilateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$MTHFR\ 677T$ in bilateral DACC</td>
<td>$F = 6.59^*$</td>
<td>18</td>
<td>1.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacheco 2009</td>
<td>Increasing number of low expressing alleles -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$5-HTTLPR$ decreasing FA in left FUF</td>
<td>$t = -3.03^*$</td>
<td>37</td>
<td>-0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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; * $p < 0.05$; *** $p < 0.001$; *i $p < 0.05$ family-wise error corrected
Overall, 24 effect sizes were calculated for the structural connectivity studies. These effects ranged from small to large ($d = 0.38-1.95$) with an average effect of $d = 1.04$ (standard deviation = 0.42). The largest effect size was the impact of a $NRG1$ variant on white matter integrity of the left anterior thalamic radiation (Sprooten et al., 2009). Large effect sizes were observed for all studies examining $NRG1$ effects on white matter integrity (all $d > 0.80$). Similar effect sizes were observed across studies investigating $ErhB4$ (Cohen's $d$ for these studies ranged between 0.81 and 1.41). Both $MTHFR$ and $5-HTT$ had large effects of $d = 1.29$ and $d = 0.92$, respectively. The smallest effect ($d = 0.38$) was the impact of a $COMT$ haplotype on left prefrontal white matter integrity. Cohen's $d$ for $COMT$ studies ranged from 0.38 to 0.76.

The first meta-analysis considered only the largest effect sizes in each study (see figure 2.1 and table 2.3). This analysis revealed no significant difference in outcome variability between the effect sizes for functional and structural studies ($Q = 2.171$, $p = 0.141$). The second analysis examined all of the effects for each result in each paper (see figure 2.2 and table 2.3). This analysis revealed a significant difference between effect sizes in functional and structural studies ($Q = 6.928$, $p = 0.008$).
Figure 2.2: Forest plot reporting Hedges’ $g$ and 95% CI for the analysis showing the largest effect size in each paper. CI, confidence interval; $g$, Hedges’ $g$; s.e., standard error
Figure 2.3: Forest plot reporting Hedges' $g$ and 95% CI for each functional and structural connectivity analysis. CI, confidence interval; g, Hedges' $g$; s.e., standard error
Table 2.3: Results of random-effects meta-analysis comparing the relative difference in the impact of variants on functional and structural connectivity; d.f. = degrees of freedom

<table>
<thead>
<tr>
<th>Estimation</th>
<th>Effect Size and 95% Confidence Interval</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of studies</td>
<td>Point Estimate</td>
</tr>
<tr>
<td>Functional</td>
<td>9</td>
<td>0.812</td>
</tr>
<tr>
<td>Structural</td>
<td>10</td>
<td>1.067</td>
</tr>
<tr>
<td><strong>Total between</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>44</td>
<td>0.687</td>
</tr>
<tr>
<td>Structural</td>
<td>24</td>
<td>0.934</td>
</tr>
<tr>
<td><strong>Total between</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4 Discussion

This study compared effect sizes of schizophrenia risk variants on functional versus structural connectivity in order to examine which phenotype may be closer to the underlying genetic risk architecture of the disorder. Meta-analyses of effect size data revealed that there was a significant lack of homogeneity across the modalities when all significant effects were taken into account (p<0.008). This difference likely reflects greater variation in effect sizes in structural studies compared to fMRI studies.

On average, schizophrenia risk variants were reported to exert large effects on both functional (mean $d = 0.76$) and structural connectivity (mean $d = 1.04$). This result is consistent both with previous meta-analyses in imaging genetics and with the intermediate phenotype hypothesis (see section 1 and 2.1). 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.
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 schizophrenia phenotypes. This makes it difficult to determine the relevance of these genes for our understanding of schizophrenia pathogenesis (Meyer-Lindenberg, 2010). Secondly, it should be noted that the sample sizes included in these studies are relatively small, and thus may be underpowered to detect some of the 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 and may lack sufficient power to detect smaller effects (Sterne et al., 2000). Related to the general issue of sample size, it is important to note that the average sample size of the studies utilising 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 this 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 tendency of journals to accept positive 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 the 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 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 and differences in sample demographics. 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 (see section 1). Due to the small number of studies available, this meta-analysis included studies that used different methods to assess functional connectivity, including seed-based methods (e.g. Esslinger et al., 2009) and psychophysiological interaction analysis (PPI) (e.g. Di Giorgio et al., 2008). There are also various approaches used to quantify white matter connectivity using DTI, which also pose different strengths and limitations (Jones, 2010). This meta-analysis also included studies that examined healthy volunteers only (e.g. Esslinger et al., 2009) and studies that examined both healthy volunteers and schizophrenia patients (e.g. Rasetti et al., 2011). Future meta-analyses including more studies should specifically examine functional and structural studies that use similar analysis methods and sample characteristics in order to minimize the potential confounding effects of these additional variables.
2.5 Conclusions

In conclusion, the present meta-analysis examined the magnitude of effect of schizophrenia risk variants on functional and structural connectivity. On average, risk variants exert a large effect on functional and structural connectivity, consistent with previous meta-analyses in imaging genetics and with the intermediate phenotype hypothesis. There is also more variability in the effects of variants on structural connectivity, compared to functional connectivity. It is hoped that future meta-analyses in imaging genetics, including (1) more studies, (2) studies with larger samples sizes and (3) studies examining multi-genetic effects, will help define the neurobiological mechanisms mediating genetic risk, which can help inform new treatment and prevention strategies.
Chapter 3

MRI materials and methods
3.1 Emotional face processing paradigm

Sample characteristics: 108 healthy participants were assessed using the emotional face processing task. All participants met the following inclusion criteria: they were right-handed, aged 18 - 65 and had no history of co-morbid psychiatric disorder, substance abuse in the preceding six months, head injury associated with a loss of consciousness or seizures. Participants were recruited using posters placed around Dublin and through the Trinity College Department of Psychiatry website:

http://www.medicine.tcd.ie/neuropsychiatric-genetics/volunteers/.

Participants were also screened for family history of schizophrenia. All participants were of Irish ancestry, having Irish maternal and paternal grandparents, and all provided written informed consent in accordance with local ethics committee guidelines (see Appendix A for consent forms used in 2011 and 2012).

Task description: During fMRI, participants performed an emotional face processing task designed by Grosbras and Paus (Grosbras and Paus, 2006; Grosbras et al., 2007). In their original study, participants watched a series of video clips of angry or neutral/ambiguous facial expressions or angry or neutral hand gestures. As we were exclusively interested in face processing, we used a modified version of this task omitting the hand gesture component. This version of the task has been used in the IMAGEN project, a multi-site European imaging genetics project examining risk factors for mental illness in 2,000 14 year-old healthy adolescents (Schumann et al., 2010). Stimuli were presented via specialised software developed for the IMAGEN project.
This task employed a block design, in which stimuli of the same type are presented together in blocks (Buckner et al., 1996). Block designs have increased efficiency relative to event-related designs, and are generally associated with larger effects, as a greater proportion of the task time is taken up with the condition of interest (Friston et al., 1999; O’Reilly et al., 2012).

Participants watched a series of two-five second black and white video clips of faces starting from a neutral expression, and then turning into an angry expression or displaying a neutral expression (e.g. licking of lips; see figure 3.1). Taylor et al. (2003) have previously reported that rating emotional stimuli during a task can modulate neural activation in limbic regions (e.g. the amygdala). As participants passively watched the video clips this potential confound was avoided. A baseline condition consisted of videos of black and white concentric circles expanding and contracting; these roughly matched the contrast and motion of the faces (Schneider et al., 2011). Video clips were displayed at 30 frames per second. Each block was 18 seconds long and consisted of four-seven video clips. The duration of the task was ~six minutes, consisting of 19 blocks in total: five angry face blocks, five neutral face blocks and nine baseline blocks (every second block was baseline). The total number of exposures to each condition was the same between participants. To ensure that the task was synchronised to the scan, a signal sent from the scanner at the beginning of the scan was converted into a transistor-transistor logic (TTL) pulse which was sent to the computer displaying the videos to trigger the start of the task.
Figure 3.1: Stimuli presentation during emotional face processing task. Figure courtesy of Tahmasebi et al., 2012

To ensure that they had paid attention to the videos, participants completed a follow-up face recognition task after scanning in which they were shown static images of faces and asked if these matched the faces seen during the task (see figure 3.2). Participants who answered correctly for four or five of the five pictures presented were included in further fMRI analysis. Four subjects were excluded due to poor performance (< four correct answers) and four were excluded due to missing data for this follow-up task.
MRI acquisition parameters for face processing paradigm: Participants were imaged using a Philips Intera Achieva 3-Tesla MR system (Philips Medical Systems, Best, The Netherlands) with a SENSE (Sensitivity encoding) 8-channel head coil, in the Trinity College Institute of Neuroscience. SENSE is a parallel imaging technique using multiple radiofrequency (RF) receiver coils to reduce scanning time (Pruessmann et al., 1999). Participants lay supine in the MRI scanner and stimuli were projected onto a screen behind the participant and viewed in a mirror positioned above their face. BOLD signal changes were measured using a whole-brain EPI (echo-planar imaging) sequence in which 40 2.4 mm slices were acquired with a 1 mm slice gap and the following imaging parameters: TR (repetition time) = 2200 ms; TE (echo delay time) = 30 ms; FOV (field of view) = 220 x 220 mm; flip angle = 75°; spatial resolution = 3.4 x 3.4 x 2.4 mm$^3$. The duration of functional scanning was 160 TRs/352s.
Structural imaging (for more accurate normalisation during pre-processing) consisted of a T1-weighted image (180 slices; duration: 6 minutes) using a TFE (Turbo Field Echo) gradient echo pulse sequence, with a slice thickness of 0.9 mm, 230 x 230 mm FOV and a spatial resolution of 0.9 x 0.9 x 0.9 mm³.

**fMRI spatial pre-processing and statistical analysis:** Spatial pre-processing and statistical analysis of functional images were conducted using Statistical Parametric Mapping (SPM8, revision 4290; http://www.fil.ion.ucl.ac.uk/spm/) and MATLAB R2011b (version 7.13; http://www.mathworks.co.uk/products/matlab/) running on an Intel Quad Core PC with 8GB of RAM, running Windows 7 Ultimate 64-bit Service Pack 1. Pre-processing consisted of the following steps (default parameters were used in SPM8 unless otherwise stated):

1. EPI images were realigned to the mean functional image to reduce variance due to movement (Friston *et al.*, 1996a).

2. The participant’s structural image was co-registered to the mean functional image to achieve a more precise spatial normalisation of the functional images using the anatomical image.

3. Functional images were normalised to MNI (Montreal Neurological Institute) space using the unified segmentation approach with a voxel size of 3 x 3 x 3 mm³ to allow comparison of activation in the same region across participants and report results in a standard co-ordinate system (Ashburner and Friston, 2005).

4. Images were smoothed using a 10 mm FWHM (full width at half maximum) isotropic Gaussian filter (i.e. a kernel width two-three times greater than the
original voxel size) to increase signal to noise ratio, allow for remaining
differences in brain morphology after normalisation, and make the data more
normally distributed so that it conforms to the parametric assumptions of the
General-Linear Model (GLM; Friston et al., 1995a; Friston et al., 1995b).

After pre-processing, graphical plots of estimated time series of translations and
rotations were carefully inspected for excessive motion in each participant, defined as >
3mm translation and/or >3° rotation in any direction. Overall, one participant exhibited
rotation >3° and was excluded from further fMRI analysis. In addition, nine individuals
were excluded due to bad quality MRI data and/or significant artefacts.

Statistical analysis on pre-processed images was performed using a standard GLM in
SPM8 at two levels (Friston et al., 1994).

(1) A first-level fixed effects analysis estimated task-associated activity in each
individual. For each experimental condition (i.e. angry faces, neutral faces, and
baseline), a boxcar function representing stimulus presentation was created and
convolved with a canonical haemodynamic response function (HRF) to model
neural responses at each voxel. This first-level GLM included these convolved
condition regressors, plus six regressors modelling head movement to reduce
remaining movement-related variance after realignment. A high-pass filter of
128 seconds was used to remove low-frequency signals (e.g. scanner drifts,
physiological noise) (Frackowiak et al., 1997), and serial correlations in the
fMRI time-series were accounted for by an autoregressive AR(1) model.
Condition effects at each voxel were then calculated using a t-contrast,
producing a statistical parametric map (SPM) of the following contrasts for each subject:

(a) Faces (angry and neutral combined) versus baseline to model face-specific activation

(b) Angry faces versus neutral faces to model emotion-specific activation

(2) Individual SPMs were then entered into a second-level random-effects analysis to determine task effects at the group level (Holmes and Friston, 1998). For all analyses, an initial voxel-level threshold of $p<0.001$ (uncorrected) was set. To reduce the probability of obtaining false positive results, a cluster-level family-wise error (FWE) rate of $p<0.05$ was then calculated using Gaussian Random Field theory (Worsely et al., 1992; Friston et al., 1993). The FWE rate is the probability of obtaining false positive results when performing multiple comparisons; clusters large enough to pass the $p<0.05$ threshold were considered statistically significant. Cluster-level FWE-thresholds have been used in previous imaging genetics studies (e.g. Whalley et al., 2012), and may be more sensitive to genetic effects on brain function compared to voxel-level corrections (Meyer-Lindenberg et al., 2008). However, it should be noted that this technique does not allow inference on the significance of individual voxels (Friston et al., 1996b).

MNI coordinates of results were converted to Talairach space using the Lancaster transform, icbm2tal, as implemented in BrainMap GingerALE 2.1
Neural activation during face processing: Viewing faces (angry and neutral) was associated with significantly increased neural activity across the face network, including the bilateral amygdala, fusiform gyrus and superior temporal gyrus, similar to previous studies using this task (Schneider et al., 2011; Tahmasebi et al., 2012; Marečková et al., 2012) \((t_{90}) = 24.17; p<0.05, \text{corrected}; \text{see table 3.1 and figure 3.3})\. In addition, viewing angry faces was associated with significantly increased neural activity in regions important for emotion processing and regulation, including the anterior cingulate, relative to viewing neutral faces \((t_{90}) = 5.15; p<0.05, \text{corrected})\. NB - given that the face processing task was associated with large clusters of activation incorporating several brain regions, Appendix B additionally presents these results at a voxel-level threshold of \(p<0.05, \text{FWE-corrected}, \text{to show in more detail the specific regions activated by this task}}.
Table 3.1: Clusters, including individual peaks, showing significantly increased neural activation during face (angry and neutral combined) versus baseline, 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(^b)</td>
<td>5404</td>
<td>&lt;0.001</td>
<td>Faces(^c)</td>
<td>Right superior temporal gyrus/ BA(^d) 22</td>
<td>24.17</td>
<td>&gt;8</td>
<td>54 -43 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right middle frontal gyrus/ BA 46</td>
<td>19.11</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>18.25</td>
<td>&gt;8</td>
<td>42 -49 -20</td>
</tr>
<tr>
<td>2(^c)</td>
<td>3846</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Left fusiform gyrus/ BA 37</td>
<td>15.77</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>15.56</td>
<td>&gt;8</td>
<td>-15 -76 -35</td>
</tr>
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<td></td>
<td></td>
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<td>6 14 58</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Right superior frontal gyrus/ BA 8</td>
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<td>9 47 40</td>
</tr>
<tr>
<td>4</td>
<td>1371</td>
<td>&lt;0.001</td>
<td>Angry</td>
<td>Left cingulate gyrus/ BA 32</td>
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<td>4.81</td>
<td>-9 23 37</td>
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<td></td>
<td></td>
<td></td>
<td>Left anterior cingulate gyrus/ BA 32</td>
<td>4.98</td>
<td>4.67</td>
<td>-9 29 22</td>
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<td></td>
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<td>4.21</td>
<td>-12 41 10</td>
</tr>
<tr>
<td>5</td>
<td>145</td>
<td>0.020</td>
<td>Angry</td>
<td>Left Cuneus/ BA 17</td>
<td>4.96</td>
<td>4.65</td>
<td>-24 85 16</td>
</tr>
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<td>4.58</td>
<td>-30 88 22</td>
</tr>
<tr>
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<td>0.011</td>
<td>Angry</td>
<td>Right lingual gyrus/ BA 18</td>
<td>4.68</td>
<td>4.42</td>
<td>18 91 11</td>
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<td></td>
<td></td>
<td>Right fusiform gyrus/ BA 19</td>
<td>4.34</td>
<td>4.12</td>
<td>27 67 8</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Right lingual gyrus/ BA 17</td>
<td>3.51</td>
<td>3.39</td>
<td>12 91 7</td>
</tr>
</tbody>
</table>

*p values are FWE-corrected for multiple comparisons at the cluster level*
The significant right amygdala activation reported in the main text is contained within this cluster.

Faces = Faces (angry and neutral combined) versus baseline; Angry = Angry versus neutral faces

BA = Brodmann Area

The significant left amygdala activation reported in the main text is contained within this cluster.
Figure 3.3: Neural activation associated with face processing

Red-yellow: Brain regions showing increased activation during the face processing (angry and neutral combined) versus baseline (N=90; one-sample t-test; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 89).

Green: Brain regions showing increased activation during angry versus neutral face processing (N=90; one-sample t-test; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 89).
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 MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and/or Paint.NET v3.5.10.

3.2 Spatial working memory paradigm

Sample characteristics: 141 healthy participants were examined using the spatial working memory task (a sample partially overlapping with the face processing sample). All participants met the inclusion criteria described in section 3.1.

Task description: During fMRI, participants performed a spatial working memory task that has previously been used to examine effects of common genetic variants on brain activity in healthy participants (Rose et al., 2012a; Rose et al., 2012b; Rose et al., 2013). This task was developed and presented using Presentation software (Neurobehavioural Systems, Albany, California, USA). In this block design task, participants were required to maintain the spatial locations of visual items in working memory over a number of seconds. Participants were asked to determine whether a white dot (the target) and a red circle (the probe) were in the same location on a computer screen relative to a white fixation cross. All participants performed a practice version of the task prior to the scan to ensure they understood how to respond. The task consisted of three conditions (see figure 3.4):

1) Baseline: The target and the probe appear simultaneously.
(2) 1 dot: The target and the probe are separated by a 3 second delay.

(3) 3 dots: The target now consists of 3 white dots. The target and probe are separated by a 3 second delay, and participants are asked to determine whether the probe was in the same position as any one of the white dots.

To account for the potentially confounding effect of block order on brain activity across the scan, there were two predetermined block orders and participants were allocated to one or the other prior to the scan. Participants were given left- and right-hand MRI compatible response units. They pressed the left button for a 'match' and the right button for 'no match'. In addition to neural activation, task accuracy (number of correct trials out of 72) and reaction time were recorded. These values were compared between groups of interest using IBM SPSS Statistics version 19.0.0 (Armonk, New Jersey, USA). Five participants missing this behavioural data were excluded from fMRI analysis and one participant was excluded due to an error with the task that caused it to run for several seconds after the scan finished.

The start of the task was synchronised to the first TTL pulse sent from the scanner at the start of the scan. However, for some participants the start of the task was not synchronised to the TTL pulse due to an experimental error; for these participants the task was run manually at the start of the sequence. Given the block design nature of the task, any small discrepancies in timing resulting from this error are unlikely to affect results.
Figure 3.4: Spatial working memory task

MRI acquisition parameters: For the spatial working memory task, a whole-brain EPI sequence was employed in which 32 non-contiguous, axial 3.5 mm slices were acquired with a 0.35 mm slice gap and the following imaging parameters: TR = 2000 ms; TE = 35 ms; FOV (field of view) = 224 x 224 mm at a 64 x 64 matrix; flip angle = 90°; spatial resolution = 3.5 x 3.5 x 3.5 mm³. The duration of functional scanning was 220 TRs/440s. Structural imaging is described in section 3.1.

fMRI spatial pre-processing and statistical analysis: Spatial pre-processing and statistical analysis of functional images followed the steps outlined in section 3.1. Four participants were excluded from further fMRI analysis due to excess motion and 21 individuals were excluded due to bad quality MRI data and/or significant artefacts. At the first-level GLM, the following contrasts were used:

(1) Spatial working memory (1 dot and 3 dots) to measure working memory specific activation
(2) 3 dots versus 1 dot to measure effects of increasing working memory load

**Neural activation during spatial working memory:** Spatial working memory (1 dot and 3 dots combined) was associated with significantly increased neural activation in the fronto-parietal attention network (Fox *et al.*, 2005; Toro *et al.*, 2008), including, bilaterally, the dorsolateral prefrontal cortex (BA 9 and 46; $t_{(109)} = 19.09; p<0.05$ corrected; see table 3.2 and figure 3.4). In addition, the 3 dots condition was associated with increased activity across several of these frontal and parietal regions compared to the 1 dot condition ($t_{(109)} = 13.80; p<0.05$, corrected). NB - given that the spatial working memory task was associated with large clusters of activation incorporating several brain regions, Appendix B additionally presents these results at a voxel-level threshold or $p<0.05$, FWE-corrected, to show in more detail the specific regions activated by this task.
Table 3.2: Clusters, including individual peaks, showing significantly increased neural activation during spatial working memory (1 dot and 3 dots combined) versus baseline, and 3 dots versus 1 dot conditions, corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Condition</th>
<th>Cluster peaks</th>
<th>t-value</th>
<th>Z-value</th>
<th>Peak coordinates (MNI)</th>
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<td>1</td>
<td>15501</td>
<td>&lt;0.001</td>
<td>SWM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Right precuneus/ BA 7</td>
<td>19.09</td>
<td>&gt;8</td>
<td>15 -64 55</td>
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<td>16.95</td>
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<td>27 -4 49</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Left superior parietal lobule/ BA 7</td>
<td>16.91</td>
<td>&gt;8</td>
<td>-27-55 55</td>
</tr>
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<td>2</td>
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<td>3 dots</td>
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<td>13.80</td>
<td>&gt;8</td>
<td>21 -64 58</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>Right inferior parietal lobule/ BA 40</td>
<td>11.82</td>
<td>&gt;8</td>
<td>39 -40 46</td>
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<td></td>
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<td>Right precuneus/ BA 31</td>
<td>11.41</td>
<td>&gt;8</td>
<td>30 -73 31</td>
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<td>3</td>
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<td>&lt;0.001</td>
<td>3 dots</td>
<td>Left precuneus/ BA 7</td>
<td>11.02</td>
<td>&gt;8</td>
<td>-21 -64 58</td>
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<td></td>
<td></td>
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<td>7.78</td>
<td>-27 -73 31</td>
</tr>
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<td></td>
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<td>Left inferior parietal lobule/ BA 40</td>
<td>8.02</td>
<td>7.09</td>
<td>-36 -43 46</td>
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<td>27 -4 49</td>
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<tr>
<td>6</td>
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<td>5.32</td>
<td>54 11 28</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>Right precentral gyrus/ BA 6</td>
<td>5.16</td>
<td>4.87</td>
<td>45 5 25</td>
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<td>Right middle frontal gyrus/ BA 46</td>
<td>4.28</td>
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<td>7</td>
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<td>4.78</td>
<td>4.55</td>
<td>-48 8 28</td>
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</tbody>
</table>

*p values are FWE-corrected for multiple comparisons at the cluster level; SWM = Spatial working memory (1 dot and 3 dots combined) versus baseline; 3 dots = 3 dots versus 1 dot condition*
Figure 3.5: Neural activation associated with spatial working memory

Red-yellow: Brain regions showing increased activation during spatial working memory (1 dot and 3 dots combined) versus baseline (N=109; one-sample t-test; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 108).

Green: Brain regions showing increased activation during the 3 dots condition compared to the 1 dot condition (N=109; one-sample t-test; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 108).
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 MRIcroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and/or Paint.NET v3.5.10.

3.3 Functional connectivity analysis

To examine functional connectivity between different brain regions, seed voxel correlation analysis was used, the most commonly used functional connectivity analysis in imaging genetics (see section 1 for an overview of previous studies using this analysis technique). Seed regions were chosen a priori based on the cognitive task and genetic variant under investigation (see section 5-7). Next, a nominal threshold was applied to this seed region to reduce noise (e.g. p<0.5; Esslinger et al., 2009; see section 5-7). The first eigenvariates of all voxels included in the seed region were then extracted using singular value decomposition in SPM8 (Di Giorgio et al., 2008). The eigenvariate was used as a summary measure of activity in the seed region rather than the mean, as positive or negative voxels in the seed may have cancelled each other out, affecting the final time-course; the eigenvariate is more robust to this heterogeneity (Ashburner et al., 2013).

The extracted time course was temporally filtered using a high pass filter of 128 seconds (performed during the first-level analysis, see section 3.1) to remove low frequency signals and task related variance was removed by applying an effects of interest correction with an F-contrast of the six movement parameters (e.g. Esslinger et
al., 2009, Paulus et al., 2013a). This was performed in order to remove the contribution of task-based co-activation to the measurement of functional connectivity. To account for noise, first eigenvariates from masks covering cerebrospinal fluid (CSF) and white matter (WM) were extracted, and entered, along with task and movement regressors, into a new fixed-effects GLM with the seed time series as the regressor of interest. Task-related variance was also removed from CSF/WM time series by applying an effects-of-interest F-contrast of the six movement parameters. The CSF and WM masks were kindly provided by Esslinger, C. and Paulus, F. (personal correspondence), and have been previously used in imaging genetics studies examining the effects of GWAS psychosis risk variants on functional connectivity (e.g. Esslinger et al. 2009; Paulus et al. 2013a). Individual functional connectivity maps produced by the analysis were then compared between groups of interest using a random effects analysis in SPM8.

3.4 Statistical power of imaging genetics studies

It has previously been suggested that sample sizes above 70 are sufficient to detect significant genetic effects on brain function (Walter et al., 2011). This is based on pooled effect sizes from two previous meta-analyses in imaging genetics (Munafo et al. 2008, pooled effect size $d = 0.54$; Mier et al., 2010, pooled effect size $d = 0.73$), which suggested that samples $> 70$ provide 80% power to detect genetic effects at a $p<0.05$ (uncorrected) level. In this project, the smallest sample size used to examine genetic effects on regional activation and functional connectivity was 80, which is above this sample size and comparable to other imaging genetics studies.
To examine the statistical power of this sample further, a post-hoc sensitivity power analysis was conducted in G*Power 3.1.7 (Faul et al., 2007; Faul et al., 2009), which has previously been used to examine power in imaging genetics (e.g. Jansen et al., 2010). At a more conservative threshold of $p<0.001$ (uncorrected) and 80% power, a sample size of 80 would be able to detect effect sizes of $d \sim 0.96$, assuming equal group numbers. This suggests that the current sample is underpowered to detect small to medium effects at this threshold. Although this is a limitation of the current sample, the sample is nonetheless similarly powered to other imaging genetics studies. Moreover, genetic effects on brain function are expected to be larger than on behaviour or symptomatology, a prediction that has been supported by recent meta-analyses (Munafo et al., 2008; Mier et al., 2010; Mothersill and Kelly et al., 2012; Rose and Donohoe, 2013).
Chapter 4

Altered medial prefrontal activity during dynamic face processing in schizophrenia
Abstract

**Background:** Processing the emotional content of faces is recognised as a key deficit of schizophrenia, associated with poorer functional outcomes and possibly contributing to the severity of clinical symptoms such as paranoia. At the neural level, fMRI studies have reported altered limbic activity in response to facial stimuli. However, some studies may be limited by the use of cognitively demanding tasks and static facial stimuli. The current study used a face processing task involving implicit face processing and dynamic stimuli to further characterise neural activity differences in emotional brain regions in schizophrenia patients relative to healthy controls.

**Methods:** Functional MRI was used to examine neural activity in 25 patients with a DSM-IV diagnosis of schizophrenia and 21 age- and gender-matched healthy controls while they participated in a face processing task designed by Grosbras and Paus (2006), which involved viewing video clips of angry and neutral facial expressions, and a non-biological baseline condition.

**Results:** While viewing faces (angry and neutral combined versus baseline), patients showed significantly weaker deactivation of the medial prefrontal cortex, including the anterior cingulate, and decreased activation in the left cerebellum, compared to controls. Patients also showed weaker medial prefrontal deactivation while viewing the angry faces relative to the baseline condition.
**Discussion:** Given that the anterior cingulate plays a role in processing negative emotion, weaker deactivation of this region in patients while viewing faces may contribute to an increased perception of social threat, which may in turn contribute to paranoia. Further examination of the neurobiology of social cognition in schizophrenia using fMRI may help establish targets for treatment interventions.

4.1 Introduction

Deficits in processing the emotional content of faces is a key feature of schizophrenia (see section 1.7). For example, inability to recognise particular emotions from facial expressions may lead to impairments in understanding the emotions of other people during social interactions, which could contribute to negative functional outcomes in work and relationships. Similarly, excessive threat detection from facial expressions may lead to paranoid delusions.

At the neural level, emotional face processing activates limbic regions including the amygdala, which is important for detecting the emotional salience of a stimulus and generating an affective response, and the medial prefrontal cortex (mPFC) and anterior cingulate (ACC), which are important in expressing negative emotion and regulating other limbic regions (Etkin *et al.*, 2011). Meta-analysis of fMRI studies by Li *et al.* (2010) indicates that schizophrenia patients generally show reduced activation of the bilateral amygdala in response to emotional faces compared to healthy controls, which may contribute to difficulties understanding the emotions of other people. Similarly, Hempel *et al.*, (2003) and Habel *et al.*, (2010) report decreased activation of the ACC in
patients viewing emotional faces. However, increased activation of the amygdala and ACC have been reported in response to neutral faces in patients versus controls, which may result in patients mistakenly attaching emotional salience to non-emotional expressions (Hall et al., 2008; Habel et al., 2010).

There are two limitations, however, with studies of face processing in schizophrenia to date. One limitation is that many studies have used explicit face processing tasks, where participants must judge the emotional content of the faces presented and select an emotion from a list of two or more. Taylor et al., (2003) have previously shown that explicit emotional face processing during a task can reduce neural activity in limbic regions. Some studies have tried to overcome this limitation, for example, by instructing participants to determine the gender of faces to ensure attention to the task but also to make sure that the emotion recognition component of the task was implicit (e.g. Phillips et al., 1999). However, this type of task may also modulate limbic activity, given the widespread dysconnectivity and cognitive deficits observed in schizophrenia (Stephan et al., 2009; Meyer-Lindenberg et al., 2005); e.g. these tasks may influence limbic responses in ways that vary with connectivity, cognitive ability and/or task difficulty (Holt et al., 2006). Therefore, implicit face processing tasks with minimal additional demands may provide a more accurate measure of neural activity during face processing.

A second limitation is that many of the previous studies of face processing in schizophrenia have used static stimuli, such as Ekman’s Pictures of Facial Affect (Ekman et al., 1975) (e.g. Holt et al., 2006). However, human faces and facial
expressions are dynamic in nature, and temporal cues contribute to the recognition of facial expressions (e.g. Sato et al., 2004). Therefore, tasks that include dynamic facial expressions may more accurately reflect real world social interactions. fMRI has revealed that several brain regions, including the amygdala, are more active while viewing dynamic facial expressions compared to static images (Sato et al., 2004), and task-induced functional connectivity between the amygdala and cingulate is also increased in response to dynamic facial expressions compared to static stimuli (Foley et al., 2012). Similar to studies using static images, schizophrenia patients show poorer ability to recognise dynamic facial expressions compared to healthy controls during neuropsychological examination (Johnston et al., 2010). Also, patients have been reported to show increased limbic activation while watching dynamic fearful faces compared to controls under fMRI (Russell et al., 2007).

The purpose of the present study was to further explore and characterise activation differences between schizophrenia patients and healthy controls during face processing. The study used a dynamic face processing task designed by Grosbras and Paus (2006), which is also being used to examine the effects of cognitive remediation therapy for psychosis (Cognitive Genetics and Remediation (CogGene) Laboratory, Ireland), and in the IMAGEN project, a Europe-wide longitudinal imaging genetics project examining risk factors for mental illness in 14-year-old adolescents (Schumann et al., 2010). The study examined neural activation in patients with schizophrenia/schizoaffective disorder and age- and gender-matched healthy controls during passive viewing of dynamic angry and neutral faces. Specifically, the study tested the hypothesis that patients with schizophrenia or schizoaffective disorder would show altered limbic activity while
passively viewing dynamic angry and neutral facial expressions, compared to healthy controls.

Identifying differences in these regions in patients during a task that involves both (1) implicit face processing, and (2) dynamic face stimuli is important for better understanding the neurobiological correlates of social cognitive deficits in schizophrenia and identifying targets for further treatment.

4.2 Methods

Sample characteristics: 39 patients with a DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) diagnosis of schizophrenia or schizoaffective disorder were recruited for the present study. All subjects were right-handed, aged between 18 and 65, had no history of substance misuse in the preceding six months, no prior injury to the head associated with a loss of consciousness of more than a few minutes and provided consent in compliance with the local ethics committee. Five patients were excluded due to excessive movement during functional imaging (> 3mm translation and/or 3° rotation), seven patients were excluded due to bad quality MRI data and/or significant artefacts and two patients were excluded due to missing data for the faces follow-up task (see section 3.1), yielding a total of 25 patients. These 25 patients were then compared to a group of 21 age- and gender-matched healthy controls from the sample described in section 3.1. A description of the face-processing task and MRI acquisition parameters is provided in section 3.1.
fMRI spatial pre-processing and statistical analysis: MRI data acquisition, pre-processing and statistical analysis are described in section 3.1. In keeping with previous studies of emotion recognition in schizophrenia (e.g. Habel et al., 2010, Holt et al., 2006), we examined group effects during emotional and neutral face conditions separately using two t-contrasts (angry faces versus baseline and neutral faces versus baseline) in addition to the contrasts described in section 3.1.

4.3 Results

Participant demographics: Independent t-tests were performed to compare age and years of education between groups in SPSS (19.0.0); a Pearson’s chi-squared test was performed to compare gender frequencies between groups. There were no significant differences between groups for age or gender (p>0.05; see table 4.1). As there were significant differences between groups for years of education, the effects of this variable were examined across the sample for all contrasts examined (multiple regression with years of education as covariate of interest). There were no significant effects of education observed on neural activation.
Table 4.1: Emotional face processing task schizophrenia patients versus healthy controls participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean age (s.d.(^a))</th>
<th>Mean years of education (s.d.(^b))</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia patients (N=25)</td>
<td>42.88 (10.99)</td>
<td>14.13 (4.56)</td>
<td>20:5</td>
</tr>
<tr>
<td>Healthy controls (N=21)</td>
<td>38.24 (8.62)</td>
<td>18.00 (3.48)</td>
<td>16:5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic(^c)</th>
<th>t=1.571</th>
<th>t=3.168</th>
<th>(\chi^2=0.097)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p value</td>
<td>0.123</td>
<td>0.003</td>
<td>0.755</td>
</tr>
</tbody>
</table>

\(^a\) s.d. = standard deviation; \(^b\) Education available for 45 of 46 participants

\(^c\) t value derived from independent t-tests between groups; \(\chi^2\) value derived from Pearson’s chi-squared test with variables group and gender

Mean medication (and s.d.) in patient group (chlorpromazine equivalent in mg/day) = 377.52 (270.82)

Mean SAPS in patient group (Scale for the Assessment of Positive Symptoms (Andreasen, 1984(a))) = 9 (s.d. unavailable)

Mean SANS in patient group (Scale for the Assessment of Negative Symptoms (Andreasen, 1984(b))) = 21 (s.d. unavailable)

Note - SAPS/SANS scores listed above are mean scores for the schizophrenia patient sample recruited for neuroimaging as part of a psychosis research project in Trinity College, of which the 25 patients included in this fMRI study are a subset.
Neural activation: Relative to controls, patients showed weaker deactivation of the bilateral ACC and left medial frontal gyrus while viewing faces (angry and neutral combined) \( t(46)=4.85; p<0.05 \), corrected; see Table 4.2 and Figure 4.1). Patients also showed reduced activation of the left cerebellum relative to controls \( t(46)=4.49; p<0.05 \), corrected; see Table 4.2 and Figure 4.1). No significant differences were observed when the groups were compared using a contrast of angry versus neutral faces at a threshold of \( p<0.05 \), corrected.

Examining the angry and neutral face conditions separately, patients also showed weaker deactivation of the mPFC/ACC during the angry faces versus baseline condition \( t(46)=5.94; p<0.05 \), corrected; see Table 4.3 and Figure 4.1), while altered mPFC activity was only observed at uncorrected thresholds \( p<0.001 \) during the neutral faces versus baseline condition.

As an additional data quality check, in each individual the average parameter estimates of all voxels was calculated for each cluster that showed a significant activation difference between groups. Next, average parameter estimates were checked in SPSS (19.0.0) for the presence of outliers. One outlier was identified for the faces versus baseline contrast; as removal of this participant did not significantly affect results, results are reported with all participants included.
**Table 4.2:** Clusters, including individual peaks, showing significant activity differences between schizophrenia patients and controls during face processing (angry and neutral faces combined, relative to baseline), corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>Direction of effect</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>699</td>
<td>Patients &gt; controls</td>
<td>Right anterior cingulate/ BA 24</td>
<td>4.85</td>
<td>4.32</td>
<td>6 32 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left medial frontal gyrus/ BA 10</td>
<td>4.71</td>
<td>4.22</td>
<td>-9 56 4</td>
</tr>
<tr>
<td>2</td>
<td>168</td>
<td>Patients &lt; controls</td>
<td>Left cerebellum</td>
<td>4.49</td>
<td>4.05</td>
<td>-39 -70 -23</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>4.03</td>
<td>3.70</td>
<td>-18 -76 -35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>3.72</td>
<td>3.45</td>
<td>-24 -79 -23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p values are FWE-corrected for multiple comparisons at the cluster level*
Table 4.3: Clusters, including individual peaks, showing significant activity differences between schizophrenia patients and controls during angry face processing compared to baseline, corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>$p$ value$^a$</th>
<th>Direction of effect</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>813</td>
<td>&lt;0.001</td>
<td>Patients $&gt;$ controls</td>
<td>Left medial frontal gyrus/BA 10</td>
<td>5.94</td>
<td>5.06</td>
<td>-9 56 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right anterior cingulate gyrus/ BA 32</td>
<td>5.37</td>
<td>4.68</td>
<td>12 38 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left anterior cingulate gyrus/ BA 24</td>
<td>5.02</td>
<td>4.44</td>
<td>-3 41 1</td>
</tr>
</tbody>
</table>

$^a$p values are FWE-corrected for multiple comparisons at the cluster level
**Figure 4.1:** Altered neural activity in schizophrenia patients during face processing

Red-yellow: Brain regions showing altered activity during face processing in patients relative to healthy controls (N=46; independent t-test between groups; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 44).

Colour bars represent t-values. Each 2D sagittal slice is labelled with a MNI-coordinate. Clusters are rendered on the ‘ch256’ brain template using MRICroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Bar graphs constructed using average parameter estimates, obtained as described in section 4.3. Faces = Faces (angry and neutral faces combined) versus baseline contrast; Angry = Angry versus baseline contrast.
contrast; a.u. = arbitrary units; mPFC = medial prefrontal cortex. Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and/or Paint.NET v3.5.10.

4.4 Discussion

This study examined neural activity in patients with schizophrenia and healthy controls during a dynamic face-processing task. While passively viewing faces (regardless of emotional content), patients showed weaker deactivation of the mPFC/ACC compared to controls, and decreased left cerebellum activation. While viewing the angry faces specifically, patients also showed a pattern of weaker mPFC deactivation relative to controls. Average parameter estimates were examined within clusters showing altered activity between groups, revealing a pattern of decreased mPFC activity in the control group while viewing faces (Figure 4.1). In contrast, average parameter estimates were closer to 0 within this cluster in the patient group. Decreased BOLD response relative to baseline is thought to reflect decreased neural activity, although this relationship is not fully understood (Shumel et al., 2006). As such, it is important to note that the altered pattern of activation observed here may also indicate relatively increased neural activity in patients in response to facial stimuli.

Although schizophrenia patients show deficits in facial emotion perception, they are hypersensitive to expressions of fear and anger (Mandal et al., 1998; Evans et al., 2011). For example, Evans et al. (2011) have previously reported that schizophrenia patients show increased aversion to angry faces during an associative learning task. Our
finding of weaker mPFC/ACC deactivation during angry face processing in patients compared to healthy controls supports this view, given the role of the ACC in processing negative emotion. Increased detection of social threat (or cortical reaction to anger in others) may contribute to the development of paranoia.

Notably, our finding contrasts with one previous imaging study (Habel et al., 2010) which reported reduced ACC activity in schizophrenia patients compared to controls while viewing angry faces; however, Habel et al. used an explicit emotional face processing task which the authors suggested may not be comparable to implicit tasks due to differing cognitive demands; e.g. explicit face processing tasks may modulate neural activity in limbic regions. Further research using different tasks will be required to elucidate specific differences between explicit and implicit face processing in schizophrenia.

An alternative interpretation of the present results is that the weaker mPFC deactivation observed in patients reflects altered function of the default mode network, a network of brain regions that are more active when a person is not engaged in a cognitive task, i.e. at rest (Raichle et al., 2001). The default mode network, which includes the mPFC, has been hypothesised to play a role in a variety of functions such as self-referential processing that may be important for social cognition (Buckner et al., 2008).

Garrity and colleagues (2007) have previously shown that patients with schizophrenia show altered default mode activity relative to healthy controls. In their study,
independent component analysis was first used to identify the default mode network during an auditory oddball task. The spatial extent of this network was then compared between groups to examine default mode activity during the task, with patients showing altered spatial extent (or 'activity') across several default mode regions. Given these results, the present finding of weaker deactivation of the mPFC in patients during face processing may reflect a reduced ability to disengage the default mode network when attending to the faces. This may lead to impairments in accurately processing the facial stimuli as patients may not be able to devote the same cognitive resources to the task. Future studies could incorporate a behavioural component to examine if mPFC activity was correlated with reduced task accuracy or reaction time. This could be performed using task stimuli, but after the scan, in order to minimise confounding effects of explicit face processing on neural activation.

Although the causes of altered mPFC/ACC activity observed in schizophrenia are not fully understood, the aberrant function observed in these regions may partially result from altered brain structure and cell density. For example, studies of post-mortem brains of schizophrenia patients report reduced expression of astrocyte markers in the deep layers of the ACC, suggesting a population of these cells are adversely affected in the ACC in schizophrenia patients (Katsel et al., 2011). This could lead to altered neural activity, given the suggested role of astrocytes in neuronal signalling (Navarrete et al., 2013).

Altered ACC function may also result from altered grey matter volume in this region, and reduced structural connectivity to other parts of the cortex. For example, Zhou et
al., (2005) and Fujiwara et al., (2007) report decreased ACC volume, and Fujiwara et al. also report decreased white matter integrity in the cingulum (which connects the ACC to other cortical regions), in schizophrenia patients compared to healthy controls. Fujiwara and colleagues also reported that reduced ACC volume was associated with significantly impaired emotional face processing in the same sample of patients and healthy controls, further highlighting the importance of this region for social cognition.

These differences in both form and function are likely to be driven by a combination of environmental and genetic factors. For example, Lederbogen et al. (2011) used fMRI and the Montreal Imaging Stress Task (MIST) to examine neural activation in healthy volunteers with an urban or rural upbringing. In the MIST, participants perform arithmetic tasks within a limited amount of time, while also receiving negative feedback on performance by study investigators. Urban upbringing was associated with increased ACC activation during social stress, suggesting that activity in this region may be particularly sensitive to early-life environmental factors when perceiving social threat. Early life stress may affect ACC function via increased levels of the stress hormone cortisol, high levels of which have previously been associated with reduced ACC volume in other psychiatric disorders (Treadway et al., 2009).

The ACC may be susceptible to genetic variation also. For example, Voineskos et al., (2011) report that healthy volunteers carrying two copies of the GWAS-associated schizophrenia risk variant, rs1344706, within ZNF804A, show reduced grey matter cortical thickness in this region. A priority for future studies will be to examine
possible gene × environment (G × E) interactions on face processing and other measures of social cognition.

The cerebellum also plays an important role in social cognition (Van Overwalle et al., 2013), including processing negative facial emotions (Ferrucci et al., 2012). It will also be a priority of future imaging studies of schizophrenia to examine how altered activity in this region during face processing might also affect social cognitive performance.

One limitation with the current study is the heterogeneity of the patient sample, which includes patients with a diagnosis of schizophrenia or schizoaffective disorder, with different doses of anti-psychotic medication, and heterogeneous positive and negative symptom severity. Future studies examining dynamic face processing in patient subgroups, critically with larger sample sizes in each subgroup, will help determine whether patterns of neural activity observed here are consistent across these groups (e.g. grouping schizophrenia and schizoaffective disorder separately, examining patient groups with similar medication doses and symptoms).

4.5 Conclusions

In conclusion, this study reports significantly weaker mPFC/ACC deactivation and decreased cerebellum activity in schizophrenia patients during implicit processing of dynamic facial stimuli relative to healthy controls. Further examination of the neurobiology of social cognition in schizophrenia using fMRI may help establish targets to probe effects of emerging treatments (e.g. cognitive remediation therapy), and/or help
identify genetic or environmental risk factors for illness, which may help in the development of prevention strategies.
Chapter 5

Effects of *MIR137* on fronto-amygdala functional connectivity
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, this study investigated the effects of *MIR137* genotype on neuronal activity during face processing.

**Methods:** By grouping 81 healthy participants as carriers of (1) no copies/one copy of the *MIR137* rs1625579 risk allele or (2) two copies of the risk allele, this study 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 showed 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).

**Discussion:** These 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.
MIR137 is one of a group of genes that encode microRNAs - small non-coding RNA molecules modulating gene expression. In a mega-analysis of GWAS studies (Ripke et al., 2011), a common SNP within an intron of a primary transcript for MIR137, rs1625579, showed the strongest genome-wide evidence for schizophrenia (N of combined GWAS sample = 51,695; p = 1.6 x 10^{-11}; odds ratio = 1.12). Further evidence implicating MIR137 in schizophrenia risk includes a previous association identified using gene set enrichment analysis, which examines if a group of genes is overly-enriched with disease trait associated SNPs relative to the genome (Potkin et al., 2010).

Although the mechanisms by which the rs1625579 variant increases schizophrenia risk are unknown, the MIR137 gene plays an important role in neurodevelopment. For example, expression studies in mice have shown that MIR137 regulates neural stem cell proliferation (Sun et al., 2011), adult neurogenesis (Szulwach et al., 2010), dendritic morphogenesis and spine development (Smrt et al., 2010). In humans, real-time quantitative polymerase chain reaction (qPCR) has shown that MIR137 is expressed across several brain regions, but is most highly expressed in the amygdala, a brain region that plays an important role in assigning emotional value to stimuli and in forming emotional memories, with a 3.03 and 2.00 fold increase in expression relative to the DLPFC and hippocampus, for example (Guella et al., 2013).

In animal studies the altered expression of other microRNAs has been reported in key components of the brain's emotional network(s). For example, changes in microRNA
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., 2012). In support of this hypothesis, Cummings et al., (2012) 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. 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 and Kahn, 2005) and may be related to genetic risk (Gur et al., 2007). In this context, it is interesting to note that variation in amygdala activation 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 a deactivation of the amygdala (Whalley et al., 2011); however, among participants with high genetic risk for schizophrenia, homozygous risk allele carriers showed comparatively less deactivation in the amygdala compared to homozygous and heterozygous non-risk carriers. The authors suggest that this finding may reflect a misattribution of emotional salience in the high-risk homozygous 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 and dendritic spines, 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 (see section 1). 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). Additionally, a recent study by Lett et al. (2013) reports that schizophrenia patients homozygous for the rs1625579 risk allele have relatively reduced fractional anisotropy 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 (Damoiseaux et al., 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. The study 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; see section 3.1). The study considered both brain activation and functional connectivity of the amygdala using seed voxel correlation analysis (Esslinger et al., 2009; Paulus et al., 2013a; see section 3.3) with the aim of delineating the role of the rs1625579 variant on the neurobiological underpinnings of emotion processing. In doing so this study 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.

5.2 Methods

Sample characteristics: In total, 81 healthy volunteers with good quality face processing task data, successfully genotyped for MIR137, were included in this study. Participant inclusion criteria are described in section 3.1.

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.
MRI data acquisition, spatial pre-processing and statistical analysis: MRI data acquisition, pre-processing and statistical analysis are described in section 3.1. Overall, there was one GG homozygote, 25 GT heterozygotes and 55 TT homozygotes. Due to the relative infrequency of GG homozygotes, subjects carrying no copies or one copy of the risk allele (GG/GT; N=26) were compared with homozygous risk T allele carriers (TT; N=55). The allele frequencies observed in this sample were as expected (the risk T allele was reported as the common allele in Ripke et al. (2011)) and this study used the same grouping strategy as was used in other imaging genetics investigations of this SNP (Whalley et al., 2012; Lett et al., 2013).

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 (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003; Maldjian et al., 2004). 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 section 5.3), gender was added to the analyses of genotype effects as a covariate.

Functional connectivity was examined as outlined in section 3.3. Amygdala masks were obtained as described above. Both right and left amygdalae were used as seed regions in two separate connectivity analyses. Noise was excluded from the amygdala seed by selecting voxels active for the faces (angry ad neutral combined) versus baseline contrast at a threshold of p<0.5 (Esslinger et al. 2009); this threshold was not
used for statistical inference. We chose the faces (angry and neutral combined) versus baseline 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. Gender was also added to second-level functional connectivity analyses as a covariate.

5.3 Results

**Participant 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 5.1).
Table 5.1: *MIR137* face processing analysis participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean age (s.d.)</th>
<th>Mean years of education (s.d.)</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG/GT (N=26)</td>
<td>28.50 (9.52)</td>
<td>17.88 (3.65)</td>
<td>10:16</td>
</tr>
<tr>
<td>TT (N=55)</td>
<td>27.95 (8.04)</td>
<td>17.39 (3.12)</td>
<td>33:22</td>
</tr>
<tr>
<td>Statistic $^b$</td>
<td>t = 0.273</td>
<td>t = 0.629</td>
<td>$\chi^2 = 3.289$</td>
</tr>
<tr>
<td>p value</td>
<td>0.786</td>
<td>0.531</td>
<td>0.070</td>
</tr>
</tbody>
</table>

$s.d.$ = standard deviation

$^b$ t value derived from independent t-tests between groups comparing age and years of education; $\chi^2$ value derived from Pearson’s chi-squared test with variables group and gender

**Neural activation:** Regional brain activation did not differ between genotype groups for either the faces (angry and neutral combined) versus baseline 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 (angry and neutral combined) versus baseline 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 cingulate gyrus/BA 24; and (2) the right inferior frontal gyrus/BA 47 (t(80) = 5.17, p<.05, corrected; see **table 5.2** and **figure 5.1**). 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 was 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.
Table 5.2: Clusters, including individual peaks, 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>
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<td></td>
<td></td>
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<td>4.28</td>
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<td>0 -28 37</td>
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<td>2</td>
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<td>0.036</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*
Figure 5.1: 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 ‘G’ 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 (MNI space). Clusters are rendered on the ‘ch256’ brain template using MRICroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Bar graphs constructed using average parameter estimates, obtained as described in section 5.3. a.u. = arbitrary units; IFG = inferior frontal gyrus. Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and/or Paint.NET v3.5.10.
5.4 Discussion

This study investigated the functional effects of the genome-wide associated schizophrenia risk variant rs1625579 within MIR137 on neural activation in healthy participants. A functional connectivity analysis of this data revealed an effect of genotype on amygdala functional connectivity. Compared to participants carrying no copies or one copy of the risk allele (GG/GT carriers), homozygous risk allele (TT) carriers showed increased functional connectivity between the right amygdala and frontal regions involved in emotion processing and regulation, including the cingulate and PFC.

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 the original aim of this study 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 Brosch, 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 an altered pattern of connectivity was observed over an experimental period that also included non-facial stimuli. As such, the possibility that this effect is stationary and face processing independent cannot be ruled out. 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). This 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; this issue is described in more detail in section 8.5).

Although a significant increase in amygdala connectivity in risk allele homozygotes was observed, no risk allele effects on amygdala activation were observed in the present study, despite highly significant bilateral activation in this region across this 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 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 the present 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 this study was unable to detect differences in cortical activation in this healthy control sample, the use of functional connectivity may have enabled the study 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 systems connectivity and contribute to the finding of altered amygdala activation.

While patients with schizophrenia show consistent differences in amygdala function (Aleman and Kahn, 2005, Shayegan and Stahl, 2005), the degree to which the genetic basis of these differences are schizophrenia specific, or relate to psychosis more broadly, is unknown (Rasetti et al., 2009). While MIR137 was associated with schizophrenia but not bipolar disorder in the Ripke et al study (Ripke et al., 2011), whether the effects of MIR137 on amygdala connectivity observed in the present study of healthy participants are only relevant to schizophrenia risk is uncertain. MicroRNAs are suggested to represent novel therapeutic targets for emotion-related disorders such as anxiety and depression (O'Connor et al., 2012). MicroRNA levels are altered in these disorders, and both antidepressants and mood stabilisers alter microRNA 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. It will also be a priority for future studies to examine any possible association between MIR mRNA in the peripheral system and brain function or structure, in order to better understand effects of this class of molecule on the brain.

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 GWAS 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). Interactions between MIR137 and environmental risk will be an important topic for future imaging genetics studies to examine, particularly given the role of this gene in processes affected by environmental stress (e.g. epigenetic regulation of adult neurogenesis; Szulwach et al., 2010; Hsieh and Eisch, 2010).

5.5 Conclusions

In conclusion, this study reports for the first time the effects of a GWAS schizophrenia risk variant, rs1625579, within MIR137, on functional connectivity between the amygdala and (1) the cingulate and (2) the right inferior frontal gyrus, 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.
Chapter 6

Effects of a GWAS schizophrenia risk variant within *NOS1*

on prefrontal functional connectivity
Abstract

Background: *NOS1* plays an important role in neurotransmission and synaptic plasticity, and variation in this gene has been associated with altered cognition and prefrontal function. Recently, a common indel within *NOS1* showed genome-wide association with schizophrenia risk. However, the mechanisms by which this variant increases risk are unknown.

Methods: This study examined neural activity and prefrontal functional connectivity in (1) carriers of no copies or one copy of the *NOS1* risk allele and (2) risk allele homozygotes, during two cognitive tasks: spatial working memory (N = 97) and emotional face processing (N = 80).

Results: During emotional face processing, risk allele homozygotes showed relatively increased functional connectivity between the right dorsolateral prefrontal cortex and (1) the right middle frontal gyrus, (2) the right superior temporal gyrus and (3) the right superior frontal gyrus/medial frontal gyrus, relative to non-risk carriers.

Discussion: This study provides the first evidence that this indel affects functional connectivity, and provides further evidence that *NOS1* variation affects prefrontal function.
6.1 Introduction

The NOS1 gene encodes neuronal nitric oxide synthase (nNOS), an enzyme that produces nitric oxide (NO) in the brain (Khaldi et al., 2002). NO is a neurotransmitter and plays diverse roles in the central nervous system: it is involved in neurodevelopment, response to injury, glutamate signalling and NMDA-mediated synaptic plasticity (Jaffrey and Snyder, 1995; Brenman and Bredt, 1997; Khaldi et al., 2002; Akyol et al., 2004; Hopper and Garthwaite, 2006).

The NOS1 gene may play an important role in psychiatric illness as variation in this gene has been associated with anxiety, depression, impulsivity and schizophrenia (Reif et al., 2006; Reif et al., 2009; Luciano et al., 2010). Evidence that NOS1 variation contributes to schizophrenia risk comes from:

1. Positive candidate gene associations (e.g. Shinkai et al., 2002; Fallin et al., 2005; Tang et al., 2008).

2. Significant associations between rare missense variants (minor allele frequency <1%) in NOS1 and schizophrenia, identified using next-generation sequencing (Delaney et al., 2012).

3. Genome-wide associations between common variants in NOS1 and schizophrenia risk. For example, O’Donovan et al. (2008) reported a significant association between the rs6490121 variant in NOS1 and schizophrenia risk (p = 9.82 x 10^-6), although this finding was not subsequently replicated (Stefansson et al., 2009). More recently, an indel variant within NOS1 showed association with schizophrenia risk at a genome-wide significant level, in the largest genome-wide association study yet undertaken (Psychiatric Genetics Consortium, Manuscript in preparation).
Although the mechanisms by which this indel increases schizophrenia risk are unknown, it is likely to play a role in cognitive dysfunction, a core feature of the disorder that remains one of the strongest predictors of outcome (Lewandowski et al., 2011). Effects of the NOS1 gene on cognition is supported across modalities, particularly effects on cognitive functions that rely on the PFC, a brain region consistently implicated in schizophrenia pathogenesis (Callicott et al., 2000). For example, rat NOS1 knockout models show impairments in working memory and treatment of rats with a NOS1 inhibitor, l-NAME, reverses phencyclidine induced spatial learning deficits (Wass et al., 2006; Zoubovsky et al., 2011).

In humans, a NOS1 risk-haplotype has been associated with altered performance on a continuous performance test in psychosis patients (Reif et al., 2006). This test measures sustained attention, which relies on the PFC. Similarly, the rs6490121 variant has been associated with poorer verbal IQ and working memory performance in a sample of Irish schizophrenia patients and healthy controls (Donohoe et al., 2009). This finding has also been replicated in an independent sample of German patients and controls. The rs6490121 variant has also been associated with reduced prefrontal volume (measured using MRI and voxel-based morphometry) and altered prefrontal activation (measured using BOLD fMRI) in healthy carriers during a spatial working memory task (Rose et al., 2012a). Altered prefrontal activity (haemoglobin oxygenation measured using near-infrared spectroscopy) has also been associated with a variable number of tandem repeats (VNTR) polymorphism within the NOS1 gene in healthy carriers during both working memory and response inhibition (Kopf et al., 2011; Kopf et al., 2012). Using
the same method, altered PFC function has been associated with another NOS1 variant, rs41279104, in schizophrenia patients during a working memory task (Reif et al., 2011).

Some of these effects may result from altered expression of nNOS, which has been reported in post-mortem PFC samples of schizophrenia patients compared to healthy controls across multiple studies (Baba et al., 2004; Silberberg et al., 2010).

NOS1 effects on cognition may be mediated by effects on functional connectivity between groups of neurons, which has previously been shown to explain more variance in behaviour than brain activity or structure (Pezawas et al., 2005; see section 1). This gene is likely to play an important role in functional connectivity for three reasons:

1. nNOS acts as a second messenger in NMDA-receptor signalling, which plays a central role in synaptic plasticity between neurons (Fang et al., 2000; Stephan et al., 2009; Silberberg et al., 2010; Hayashi-Takagi and Sawa 2010).

2. nNOS may affect axon guidance in the human brain, as NO signalling plays an important role in axon guidance during brain development in other animals (Bicker 2005). The formation of functional networks in the brain critically depends on axonal guidance during development, in which axons migrate through the nervous system to find specific synaptic targets (Tessier-Lavigne and Goodman, 1996).

3. Down-regulation of NOS1 in white matter neurons has been reported in schizophrenia, suggesting possible effects of this gene on structural connectivity, e.g. white matter integrity (Connor et al., 2011). As discussed in section 5.1, congruent results between measures of functional connectivity and white matter
integrity have been reported (Damoiseaux et al., 2009), suggesting that genetic effects on white matter may also affect functional connectivity.

The purpose of the present study was to investigate the impact of the GWAS indel within NOS1 on neural activity and functional connectivity. Specifically, this study sought to examine connectivity of the PFC, given that previous variants in NOS1 have been consistently associated with altered PFC structure and function, suggesting that this region may be particularly sensitive to NOS1 variation. Additionally, this region was significantly activated by both the face processing and the spatial working memory tasks (p<0.05, corrected; see section 3). The right dorsolateral prefrontal cortex (DLPFC) was chosen as a seed region, using a mask that has previously been used to examine genetic effects on prefrontal functional connectivity (e.g. Esslinger et al., 2009). Neural activity and functional connectivity were examined during these two cognitive tasks. Specifically, this study tested the hypothesis that the PGC2 GWAS identified NOS1 indel is associated with significant differences in neural activity and prefrontal functional connectivity in healthy volunteers.

Given the putative role of functional connectivity as an intermediate phenotype in schizophrenia (see section 1), evidence that the NOS1 indel variant has measurable effects on this phenotype could:

(1) Support GWAS statistical evidence for this variant

(2) Provide further information on molecular mechanisms affecting functional connectivity in the brain.
6.2 Methods

**Sample characteristics:** In total, 97 healthy volunteers with good quality spatial working memory data, and an overlapping sample of 80 healthy volunteers with good quality emotional face processing data, successfully genotyped for \textit{NOS1}, were included in this study. Participant inclusion criteria are described in \textbf{section 3.1}.

**Genotyping:** Genetics analysis was carried out using DNA obtained from saliva samples that were collected using Oragene DNA self-collection kits (DNA Genotek). Because of difficulties with typing this indel on the basis of the platform used in this sample, a single nucleotide polymorphism (SNP; rs1607817) 486 base-pairs from the \textit{NOS1} indel on chromosome 12 and in high linkage disequilibrium \((r^2 = 0.954)\), was genotyped as a proxy for the indel. This SNP is an A/C polymorphism, with the C allele being the risk associated allele. The rs1607817 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.

**MRI data acquisition, spatial pre-processing and statistical analysis:** MRI data acquisition, pre-processing and statistical analysis for both tasks are described in \textbf{section 3.1}. For the spatial working memory sample, there were nine AA carriers, 48 AC carriers and 40 CC carriers. For the emotional face processing sample, there were nine AA carriers, 36 AC carriers and 35 CC carriers. Due to the relative infrequency of
AA carriers, participants carrying no copies or one copy of the risk allele were compared with homozygous risk C allele carriers.

Functional connectivity of the right DLPFC was examined as outlined in section 3.3. Masks of the DLPFC were kindly provided by Esslinger, C. and Paulus, P. (personal correspondence) and consisted of right BA 9 and the lateral section of right BA 46, smoothed with a 9 mm Gaussian function. This mask was created using the Marina toolbox (www.bion.de). For the spatial working memory task, first eigenvariates were extracted from all voxels within a 6 mm sphere centred on each individual’s most active voxel within the right DLPFC passing a nominal threshold of p<0.05, uncorrected during spatial working memory (1 dot and 3 dots combined versus baseline). This is known as the global maximum approach for selection of seed voxels (Esslinger et al., 2009; Bedenbender and Paulus et al., 2011). Two participants showed no significant DLPFC activity at this threshold and were thus excluded from subsequent connectivity analysis. For the emotional face processing task, first eigenvariates were extracted from all voxels within a 6 mm sphere centred on the individual’s most active voxel within the right DLPFC passing a nominal threshold of p<0.05, uncorrected during face processing (angry and neutral faces combined versus baseline).

Following recommendations by Bedenbender and Paulus et al. (2011), right DLPFC functional connectivity was examined using two seed voxel selection methods to examine whether genotype effects on connectivity were robust across different parameters. In the second method, the most active voxel within the right DLPFC was first identified at the group level (this was found at MNI coordinates 51, 5, 31, for the
spatial working memory task, and MNI coordinates 54, 23, 25, for the emotional face processing task). In each subject, the nearest local maximum within the right DLPFC, surviving a threshold of \( p < 0.05 \), uncorrected, was then identified. This is known as the next local maximum approach (Bedenbender and Paulus et al., 2011). Frequency distributions of MNI-coordinates from seed voxels identified using both methods are presented in Appendix C to help enable a better comparison between different imaging genetics studies (Bedenbender and Paulus et al., 2011). For both seed voxel selection methods, seed voxel coordinates from each orthogonal direction were also added as three covariates of no interest (x, y and z coordinates for each individual) to all second-level connectivity analyses to account for individual differences in seed location, due to the reported effects of individual differences in seed location on functional connectivity results (Bedenbender and Paulus et al., 2011).

6.3 Results

Participant 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, years of education, spatial working memory accuracy, spatial working memory reaction time or gender (\( p > 0.05 \)) (table 6.1 and 6.2).
Table 6.1: NOSI spatial working memory sample participant demographics

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (s.d.)</th>
<th>Mean years of education (s.d.)</th>
<th>Mean SWM accuracy (s.d.)</th>
<th>Mean SWM reaction time (s.d.)</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/AC</td>
<td>29.09</td>
<td>17.54</td>
<td>63.84</td>
<td>8533.34</td>
<td>25:32</td>
</tr>
<tr>
<td>(N=57)</td>
<td>(8.47)</td>
<td>(2.74)</td>
<td>(7.03)</td>
<td>(2243.98)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>29.28</td>
<td>17.63</td>
<td>63.18</td>
<td>8752.67</td>
<td>17:23</td>
</tr>
<tr>
<td>(N=40)</td>
<td>(10.26)</td>
<td>(3.93)</td>
<td>(10.93)</td>
<td>(2109.74)</td>
<td></td>
</tr>
</tbody>
</table>

Statistic\(^c\) $t = 0.098$ $t = 0.138$ $t = 0.366$ $t = 0.486$ $\chi^2 = 0.018$

p value $0.922$ $0.891$ $0.715$ $0.628$ $0.894$

\(^a\) s.d. = standard deviation

\(^b\) Education available for 93 of 97 participants

\(^c\) SWM = spatial working memory

\(^c\) t value derived from independent t-tests between groups comparing age and years of education; $\chi^2$ value derived from Pearson’s chi-squared test with variables group and gender
Table 6.2: *NOS1* emotional face processing sample participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean age (s.d.ᵃ)</th>
<th>Mean years of education (s.d.)</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/AC (N=45)</td>
<td>28.56 (7.87)</td>
<td>17.63 (2.73)</td>
<td>24:21</td>
</tr>
<tr>
<td>CC (N=35)</td>
<td>26.80 (7.05)</td>
<td>17.63 (3.94)</td>
<td>18:17</td>
</tr>
</tbody>
</table>

Statisticᵇ  
| t = 1.036 | t = 0.006 | $\chi^2 = 0.029$ |

p value  
| 0.304     | 0.995     | 0.866          |

ᵃs.d. = standard deviation

ᵇ t value derived from independent t-tests between groups comparing age and years of education; $\chi^2$ value derived from Pearson’s chi-squared test with variables group and gender

**Neural activation:** Regional brain activation did not differ between genotype groups during spatial working memory or emotional face processing under any of the contrasts examined (i.e. 1 dot and 3 dots combined versus baseline; 3 dots versus 1 dot; angry and neutral faces combined versus baseline; angry versus neutral faces).

**Functional connectivity:** Right DLPFC functional connectivity did not significantly differ between genotype groups during spatial working memory using either seed voxel selection method.
During the emotional face processing task, C homozygotes showed significantly increased functional connectivity between the right DLPFC and three clusters incorporating (1) the right middle frontal gyrus/BA 8; (2) the right superior temporal gyrus/BA 39; and (3) right superior frontal gyrus/BA 6 and the right medial frontal gyrus/BA 8 ($t_{(80)} = 5.74$, $p<0.05$, corrected; see Table 6.3 and Figure 6.1). As an additional data quality check, in each individual the average parameter estimates of all voxels was 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. Two outliers were detected. As results did not change significantly after excluding these participants from the analysis, results are presented with all participants included. Finally, since the AA and AC carriers were analysed as one group, it was important to ensure that these genotype groups showed comparable patterns of functional connectivity. To examine this further, average parameter estimates extracted from each significant cluster were plotted in bar graphs for each genotype group separately. These graphs are presented in Figure 6.2. The graphs indicate that the AA and AC groups show similar patterns of functional connectivity.

Under the next local maximum seed voxel selection method (Bedenbender and Paulus et al., 2011), no significant effects of genotype on functional connectivity were detected.
Table 6.3: Clusters, including individual peaks, showing significantly increased functional connectivity with the right DLPFC in rs1607817 CC carriers relative to AA/AC 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>211</td>
<td>0.001</td>
<td>Right middle frontal gyrus/ BA 8</td>
<td>5.74</td>
<td>5.21</td>
<td>33 23 34</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>0.007</td>
<td>Right superior temporal gyrus/ BA 39</td>
<td>5.03</td>
<td>4.65</td>
<td>45 -49 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right superior temporal gyrus/ BA 39</td>
<td>4.76</td>
<td>4.43</td>
<td>51 -55 34</td>
</tr>
<tr>
<td>3</td>
<td>244</td>
<td>&lt;0.001</td>
<td>Right superior frontal gyrus/ BA 6</td>
<td>4.69</td>
<td>4.38</td>
<td>12 23 49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right medial frontal gyrus/ BA 8</td>
<td>4.54</td>
<td>4.25</td>
<td>12 29 43</td>
</tr>
<tr>
<td>Region</td>
<td>Z</td>
<td>T</td>
<td>Cluster Size</td>
<td>p</td>
<td></td>
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<tr>
<td>------------------------</td>
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<td>--------------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right medial frontal gyrus/ BA 9</td>
<td>4.26</td>
<td>4.02</td>
<td>12 41 28</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FWE-corrected for multiple comparisons at the cluster level
Figure 6.1: Effects of NOS1 variation on right DLPFC functional connectivity

Red-yellow: Brain regions showing relatively increased connectivity with the right DLPFC in risk C homozygotes relative to AA/AC carriers using the global maximum seed voxel selection method (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. = 75).

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 MRicroGL (http://www.mccauslandcenter.sc.edu/microgl/). Bar graphs constructed using average...
parameter estimates, obtained as described in section 6.3. a.u. = arbitrary units; MFG = middle frontal gyrus; STG = superior temporal gyrus; SFG = superior frontal gyrus. Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and/or Paint.NET v3.5.10.

Figure 6.2: Effects of *NOS1* variation on right DLPFC functional connectivity (bar graphs present connectivity parameter estimates for three genotype groups)

Bar graphs constructed using average parameter estimates, obtained as described in section 4.3. a.u. = arbitrary units; MFG = middle frontal gyrus; STG = superior temporal gyrus; SFG = superior frontal gyrus.

6.4 Discussion

This study investigated the effects of a GWAS schizophrenia risk variant within *NOS1* on neural activity and prefrontal functional connectivity. Using a SNP in high LD with the indel risk variant as a proxy, no significant effects on activity or connectivity during spatial working memory were observed. However, significant effects on right DLPFC functional connectivity were observed during the emotional face processing task, with
participants carrying two copies of the risk C allele showing relatively increased functional connectivity between the right DLPFC and the right middle frontal gyrus, right superior temporal gyrus and right mPFC.

The emotional face processing task used presently was associated with significant neural activity in a large cluster occupying several regions of the lateral PFC, including the DLPFC (see section 3.1 for overall task effects). Although the exact functions of the DLPFC during face processing are not completely understood, this region may play several important roles. For example:

1. Through extensive connectivity with posterior cortical regions, the DLPFC plays a critical role in top-down regulation of attention (Arnsten and Rubia, 2012). During face processing, the DLPFC may play a role in attending to facial stimuli (e.g. focusing on the informative regions such as the eyes, nose and lips) and inhibiting the processing of irrelevant information during the task.

2. The mirror-neuron network, comprising the superior temporal sulcus, inferior parietal lobe, and parts of the PFC, is hypothesised to help people identify goals underlying the movements of others by matching them to their own behaviours and the goals the person most commonly associates with these behaviours (Rizzolati et al., 2001; Keysers and Perrett, 2004; Van Overwalle, 2009; Tahmasebi et al., 2012). The DLPFC, through connections with this network, has been hypothesised to play a role in imitative learning (Iacoboni, 2005).

3. The DLPFC plays an important role in emotion regulation through connections with limbic regions such as the medial PFC (Phillips et al., 2003; Chai et al., 2011).
The pattern of right DLPFC hyperconnectivity observed in the present study may represent relatively inefficient processing of the facial stimuli and/or altered emotion regulation in risk homozygotes. However, as with the functional connectivity analysis presented in section 5, this conclusion is speculative due to the fact that functional connectivity was examined over a period of time that also included non-facial stimuli. Thus, this effect may face processing independent (see sections 5.4, 8.4 and 8.5 for further discussion of this issue and possible solutions in future studies).

It is also important to note that genotype effects on functional connectivity observed in the present study did not survive correction for multiple comparisons when using the next local maximum seed voxel selection method proposed by Bedenbender and Paulus et al., (2011). As such, the present results should be interpreted with caution. There is currently no standard seed voxel selection method used in imaging genetics, and different methods have been implemented in the literature. An advantage of the global maximum approach in this study is that it ensures that the seed region is most strongly associated with a particular individual's working memory or face processing network (Esslinger et al., 2011). However, it should be noted that there was more variability in the location of the seed region across individuals using this method.

To account for possible inter-individual variance in seed region location, MNI coordinates of seed voxel locations were included as three covariates of no interest in random-effects analyses for both methods. In addition, histograms of seed voxel coordinates derived from both methods are included in Appendix C to inform future replication studies and/or meta-analyses; such studies may require detailed information
on specific parameters used in the analysis, and details on the robustness of findings across parameters (Bedenbender and Paulus et al., 2011).

The lack of significant effects of this variant during spatial working memory may indicate that the variant increases risk via effects on right DLPFC functional connectivity only during specific cognitive states (Esslinger et al., 2011). However, it is important to note that the current spatial working memory sample of 97 has 80% power to detect significant effects of $d = 0.83$ at a $p<0.001$ level (post-hoc sensitivity power analysis performed using G*Power 3.1.7 for a one-tailed independent t-test between a group of 57 and a group of 40). If population effects of this indel on functional connectivity during spatial working memory are smaller in nature, the current sample may have been underpowered to detect these effects at a significant threshold. Future studies, in larger samples and using additional connectivity assessments (e.g. PPI, see section 5.4 and section 8.5) may be required to more fully elucidate the effects of NOS1 variation on prefrontal functional connectivity across different cognitive states.

6.5 Conclusions

In conclusion, our study reports effects of a genome-wide associated schizophrenia risk variant within NOS1 on prefrontal functional connectivity during an emotional face processing task, providing the first evidence that this variant impacts functional connectivity, and further evidence that NOS1 variation affects this cortical region. Further research on NOS1 may uncover novel molecular pathways associated with schizophrenia risk, which may inform future treatment strategies.
Chapter 7

No significant effects of ZNF804A (encoding zinc finger protein 804A) observed on neural activity or functional connectivity in a sample of healthy volunteers
Abstract

Background: ZNF804A encodes a zinc-finger protein thought to be involved in gene regulation and neurodevelopment. A common SNP within this gene, rs1344706, has shown genome-wide association with psychosis risk. However, the mechanisms by which this variant increases risk are not completely understood. The present study aimed to extend and partially replicate results of previous fMRI examinations of this variant.

Methods: Effects of the rs1344706 risk allele were examined on neural activation and functional connectivity in healthy volunteers during performance of a spatial working memory task (N = 100) and an emotional face processing task (N = 82). The right DLPFC was used as a seed region for the spatial working memory task, while the right amygdala was used as a seed region for the emotional face processing task, based on findings from previous imaging genetics studies of this variant (e.g. Esslinger et al., 2009).

Results: No significant differences in neural activity or functional connectivity between genotype groups were observed at a p<0.05 corrected threshold, for either spatial working memory or emotional face processing.

Discussion: With the power available in this study, which was comparable to previous imaging genetics studies, no significant effects were found. This suggests that effects of
this variant on functional connectivity during spatial working memory and/or emotional face processing, if they exist, may be smaller than expected.

7.1 Introduction

*ZNF804A* encodes a protein expressed throughout the brain that is thought to play a role in gene regulation and neurodevelopment (Klug, 2010; Chung *et al.*, 2010). A SNP within the second intron of this gene, rs1344706, was the first variant to ever show GWAS-association with schizophrenia (O’Donovan *et al.*, 2008). This association was even stronger when bipolar disorder was also included in the sample studied, supporting the view that these disorders show overlapping genetic risk (Craddock *et al.*, 2005). This finding has since been replicated (Riley *et al.*, 2009) and meta-analysis by Williams *et al.* (2010) has confirmed this variant as showing one of the strongest associations with psychosis so far (p = 2.5 x 10^{-11}, OR = 1.10 when schizophrenia patients were included; p = 4.1 x 10^{-13}, OR = 1.11 when both schizophrenia and bipolar disorder patients were included).

Hill and Bray (2011) recently reported that rs1344706 is associated with altered DNA binding using human neural progenitor cells, and reduced *ZNF804A* expression during the first/second trimester of gestation in the foetal brain, suggesting this variant affects gene expression. However, the mechanisms by which this variant increases psychosis risk remain unknown.
Consistent with the view of functional connectivity as an intermediate phenotype for schizophrenia, several studies have shown effects of the rs1344706 variant across several neural networks. In the first imaging genetics study of the variant, Esslinger et al. (2009) reported an association between the risk A allele and (1) decreased functional connectivity within the right DLPFC and between the right DLPFC and contralateral DLPFC, and (2) increased functional connectivity between the right DLPFC and left hippocampus during an N-back task (N = 115; p < 0.05, FWE-corrected). Altered DLPFC-hippocampus connectivity has also been observed during an n-back task in patients with schizophrenia (Meyer-Lindenberg et al., 2005; Rasetti et al., 2011) and unaffected siblings of patients (Rasetti et al., 2011) compared to healthy controls, suggesting altered connectivity of this network may be a useful intermediate phenotype for the disorder.

Paulus et al. (2013a) later reported an association between rs1344706 and increased DLPFC-hippocampal connectivity during an N-back task in an independent sample, albeit at a lower statistical threshold (N = 94; p < 0.05, uncorrected, k ≥ 20). This was an important finding, further supporting the role of prefronto-hippocampal disconnection during working memory in schizophrenia risk, but suggesting that the true effects of the rs1344706 variant on connectivity may be smaller than previous estimates. Rasetti et al. (2011) have also reported an association between the rs1344706 variant and altered DLPFC-hippocampus connectivity during an N-back task.

However, effects of this gene on brain function may be different for different types of working memory (Esslinger et al., Rasetti et al. and Paulus et al. used N-back tasks to
probe gene effects). For example, Linden et al. (2013) reported an association between the ZNF804A risk allele and altered DLPFC activity during working memory for faces, in contrast with previous studies which reported no significant effects on neural activation. Deficits in spatial working memory also constitute a core feature of schizophrenia (see section 1). However, neural effects of ZNF804A during spatial working memory have not yet been reported.

The first Esslinger et al. study also reported a significant association between ZNF804A and increased right amygdala functional connectivity to several brain regions, including the bilateral PFC, bilateral ACC and right insula, during a face-matching task (N = 115; p<0.05, FWE-corrected). The authors suggested this pattern of altered connectivity may increase emotional instability. However, effects of ZNF804A have not been examined during other face processing tasks. As discussed in section 1, altered face-processing is a key feature of schizophrenia and may be related to genetic risk.

The purpose of this study was to examine the effects of ZNF804A on neural activity and functional connectivity during spatial working memory and emotional face processing tasks in healthy participants in order to extend the previous imaging genetics findings of the variant. Although not a complete replication of previous studies, this study is comparably powered to other recent imaging genetics studies of ZNF804A (e.g. Paulus et al., 2013a) and used similar cognitive tasks (working memory and face processing) that engage an overlapping set of brain regions to the tasks employed in previous imaging genetics studies of this variant (e.g. the N-back task and the face-matching task). The right DLPFC was used as a seed region for the spatial working memory task,
while the right amygdala was used as a seed region for the face processing task, based on previous findings (Esslinger et al., 2009). Specifically, this study tested the hypotheses that the rs1344706 variant is associated with altered neural activity and right DLPFC functional connectivity during spatial working memory, and altered neural activity and right amygdala functional connectivity during face processing.

Showing that rs1344706 is associated with altered neural activity/connectivity during spatial working memory and emotional face processing is important for understanding the mechanisms by which this variant increases risk at the level of neural systems, particularly given the role of disrupted spatial working memory and emotional face processing in schizophrenia. Additionally, showing that the risk allele is associated with altered connectivity could support previous findings.

7.2 Methods

Sample characteristics: In total, 100 healthy volunteers with good quality spatial working memory data and an overlapping sample of 82 healthy volunteers with good quality emotional face processing data, successfully genotyped for ZNF804A, were included in this study. Participant inclusion criteria are described in section 3.1.

Genotyping: Genetics analysis was carried out using DNA obtained from saliva samples that were collected using Oragene DNA self-collection kits (DNA Genotek). The rs1344706 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.

**MRI data acquisition, spatial pre-processing and statistical analysis:** MRI data acquisition, pre-processing and statistical analysis are described in section 3.1. For the spatial working memory sample, there were 13 CC homozygotes, 42 AC heterozygotes, and 45 AA homozygotes. For the emotional face processing task, there were seven CC homozygotes, 34 AC heterozygotes and 41 AA homozygotes. Due to the low number of CC homozygotes for the faces sample, we compared subjects carrying no copies or one copy of the risk allele (CC/AC; N = 41) with homozygous risk A allele carriers (AA; N = 41).

For spatial working memory, functional connectivity of the right DLPFC was examined as described in section 6.2 (the most active voxel within the right DLPFC was also found at MNI coordinates 51, 5, 31 across the overlapping sample included in ZNF804A analysis). Four participants did not show significant activity in this region at the p<0.05 threshold used. For face processing, functional connectivity of the right amygdala was examined as described in section 5.2. One subject did not show significant right amygdala activity at the threshold used (p<0.5). These participants were excluded from further connectivity analyses. Gender was added to all second-level emotional face processing analyses as a covariate, given the previously reported effects of gender on amygdala function (see section 5.2).
7.3 Results

**Participant demographics:** For the spatial working memory sample, a one-way analysis of variance (ANOVA) was performed to compare age, years of education, spatial working memory accuracy and reaction time, between the three 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, years of education, accuracy/reaction time or gender (p>0.05) (see table 7.1).

For the emotional face processing sample, 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, years of education or gender (p>0.05) (see table 7.2).
Table 7.1: ZNF804A spatial working memory sample participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean age (s.d.)</th>
<th>Mean years of education (s.d.)</th>
<th>SWM accuracy (s.d.)</th>
<th>SWM reaction time (s.d.)</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CC (N=13)</strong></td>
<td>30.69 (12.17)</td>
<td>16.38 (2.25)</td>
<td>63.00 (8.85)</td>
<td>8892.90 (2148.44)</td>
<td>7:6</td>
</tr>
<tr>
<td><strong>AC (N=42)</strong></td>
<td>30.07 (9.11)</td>
<td>18.20 (3.92)</td>
<td>63.50 (7.89)</td>
<td>8311.34 (1900.06)</td>
<td>16:26</td>
</tr>
<tr>
<td><strong>AA (N=45)</strong></td>
<td>29.38 (10.48)</td>
<td>16.95 (2.64)</td>
<td>63.16 (9.84)</td>
<td>8745.50 (2365.91)</td>
<td>22:23</td>
</tr>
</tbody>
</table>

**Statistic**

<table>
<thead>
<tr>
<th></th>
<th>F = 0.103</th>
<th>F = 2.283</th>
<th>F = 0.023</th>
<th>F = 0.597</th>
<th>$\chi^2$ = 1.495</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p value</strong></td>
<td>0.902</td>
<td>0.108</td>
<td>0.977</td>
<td>0.552</td>
<td>0.474</td>
</tr>
</tbody>
</table>

*a.s.d. = standard deviation

*bEducation available for 96 of 100 participants

*cSWM = Spatial working memory

*dF-statistic derived from one-way ANOVA comparing age, years of education, spatial working memory accuracy and reaction time between genotype groups; $\chi^2$ value derived from Pearson’s chi-squared test with variables group and gender
### Table 7.2: ZNF804A emotional face processing sample participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean age (s.d. &lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Mean years of education (s.d.)</th>
<th>Gender (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC/AC (N=41)</td>
<td>27.51 (6.85)</td>
<td>18.13 (3.81)</td>
<td>21:20</td>
</tr>
<tr>
<td>AA (N=41)</td>
<td>29.39 (9.87)</td>
<td>17.02 (2.66)</td>
<td>22:19</td>
</tr>
<tr>
<td><strong>Statistic&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>t = 1.001</td>
<td>t = 1.529</td>
<td>χ² = 0.049</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.320</td>
<td>0.130</td>
<td>0.825</td>
</tr>
</tbody>
</table>

<sup>a</sup>s.d. = standard deviation

<sup>b</sup>t value derived from independent t-tests between groups comparing age and years of education; χ² value derived from Pearson’s chi-squared test with variables group and gender

**Neural activation:** Regional brain activation did not differ between genotype groups during spatial working memory or emotional face processing under any of the contrasts examined (i.e. 3 dots and 1 dot combined versus baseline; 3 dots versus 1 dot; angry and neutral faces combined versus baseline; angry versus neutral faces).

**Functional connectivity:** During spatial working memory, the global maximum seed voxel selection method revealed a pattern of increased functional connectivity between the right DLPFC and the left fusiform gyrus associated with increasing risk allele...
dosage. However, inspection of parameter estimates in this cluster revealed three statistical outliers. When these individuals were removed, no significant effects were observed. Overall, no significant effects of ZNF804A were observed on functional connectivity during spatial working memory or emotional face processing.

7.4 Discussion

This study examined the effects of a GWAS-associated psychosis risk variant, rs1344706, on neural activity and functional connectivity during (a) spatial working memory and (b) emotional face processing. However, no significant effects of this variant were observed. This section outlines the main methodological issues that may account for the lack of significant results.

The most likely reason for the lack of significant effects is sample size. The smallest sample used in this study would have power to detect large effects of the ZNF804A risk allele ($d \sim 0.9$) at $p<0.001$ and 80% power (post-hoc sensitivity power analysis performed using G*Power 3.1.7 for a one-tailed independent t-test between groups of equal size). Recent meta-analyses suggest that schizophrenia risk variants are generally associated with large effects on brain function (e.g. Rose and Donohoe, 2013; Mothersill and Kelly et al., 2012). Nevertheless, many of the individual effects of ZNF804A reported in Esslinger et al. (2009) were medium, and it has been suggested that population effects of the ZNF804A variant may be smaller in nature than previously thought (Paulus et al., 2013a). This suggests that if there are true effects of the
ZNF804A risk allele on functional connectivity during spatial working memory or emotional face processing, they may be smaller than expected.

Esslinger et al. (2011) reported differing effects of rs1344706 on prefrontal-hippocampal functional connectivity under different cognitive states (during working memory, face processing and rest), suggesting that certain genotype effects on connectivity may be cognitive state dependent and only detectable during particular tasks. Both the spatial working memory and face processing tasks differed from previous tasks used to examine ZNF804A. For example, unlike the face-matching task used by Esslinger et al., the face processing task used in the present study did not require participants to actively match facial stimuli, and presented video clips of faces instead of static images. Such differences may render one task more sensitive to ZNF804A effects on functional connectivity compared to the other. For example, face matching involves explicit labelling of facial expressions, which not only modulates amygdala activity (see section 4.1) but may affect interactions between the amygdala and other cortical regions. Further studies examining genotype effects on functional connectivity during multiple tasks, in the same sample, would be required for confirmation (as examined in Esslinger et al., 2011).

Finally, due to the small number of CC homozygotes in our face processing sample (N=7), this study compared subjects carrying 0 or 1 copy of the risk allele (CC/AC; N=41) with homozygous risk A allele carriers (AA; N=41) using an independent t-test. Different statistical models have been applied in different imaging genetics studies, with some studies comparing three genotype groups, and others using two groups, and
models are usually chosen for practical reasons such as ensuring adequately powered group sizes rather than an underlying theory of the genetic risk mechanism(s) (Paulus et al., 2013b). Previous studies have also reported different results when different statistical models are applied, with some reporting significant results only for one particular model (e.g. Bigos et al., 2010). As such, it is possible that the model used for the emotional face processing analysis in this study is less sensitive to genetic effects compared to the additive genetic model used in e.g. Esslinger et al. (2009). Future studies will be required to examine the effects of using different statistical models to estimate genotype-related effects on neural connectivity. This will require larger samples to ensure adequately sized samples in each genotype group.

7.5 Conclusions

This study examined the effects of the rs1344706 risk variant, within ZNF804A, on neural activity and functional connectivity during spatial working memory and emotional face processing. This sample (N = 82 - 100) has successfully been used to identify significant effects of other variants on functional connectivity (e.g. variants in MIR137 and NOS1; see sections 5 and 6), is large for an fMRI study (Carp, 2012) and is comparable to samples sizes used in recent imaging genetics studies of ZNF804A (e.g. Paulus et al., 2013a). The lack of significant effects observed with the power available suggests that the true effects of ZNF804A on functional connectivity during spatial working memory or face processing, if they exist, are likely to be smaller than expected.
Chapter 8

General Discussion
8.1 Summary of main findings

This thesis examined how functional connectivity in the human brain is impacted by schizophrenia genetic risk variants identified by GWAS. Altered functional connectivity is observed in schizophrenia patients and unaffected siblings relative to volunteers without a family history of the disorder, and this phenotype has shown moderate to high heritability, suggesting a relationship between functional connectivity and genetic risk (see section 1.5). This thesis sought to further examine this relationship.

To date, effects of several schizophrenia candidate genes have been reported on functional connectivity (see section 2). However, among GWAS-identified schizophrenia risk variants, which have been more strongly associated with schizophrenia than common variants previously implicated using positional cloning strategies, effects of only one GWAS schizophrenia variant on functional connectivity have been examined so far. The work undertaken during this doctoral thesis has therefore extended the psychiatric imaging genetics literature by examining the functional connectivity effects of three GWAS risk variants in healthy volunteers, two of which have not previously been examined in the context of functional connectivity. In doing so, this thesis tests the hypothesis that GWAS schizophrenia risk variants are associated with altered functional connectivity in healthy carriers.

A review of the imaging genetics literature was first undertaken to examine the strength and range of previous schizophrenia risk gene effects on functional connectivity.
Random-effects meta-analysis was then used to examine differences in the range of effect sizes observed using functional or structural connectivity methods. Overall, imaging studies of putative schizophrenia risk associated variants have shown large effects on functional connectivity (mean \( d = 0.76 \)). Significant effects of GWAS variants observed in this thesis were also categorised as large (mean \( d = 1.16 \), calculated from the t-statistic associated with the most significant peak within each significant cluster reported in sections 5-6, and d.f., using methods outlined in section 2).

The finding of large effects of schizophrenia variants on functional connectivity is consistent both with findings of other meta-analyses in imaging genetics, and with the intermediate phenotype hypothesis, which predicts that variants will show increased penetrance at the level of the brain relative to behaviour, owing to the brain’s increased proximity to the level of genes (section 2).

However, meta-analysis revealed significant heterogeneity in the range of effect sizes across imaging modalities, with structural studies showing a wider range of effects (Q = 6.928, \( p = 0.008 \)). This suggests that functional connectivity methods may be less powerful and lack sensitivity to detect smaller effects relative to structural methods. This is a possible limitation with functional connectivity studies that should be considered when interpreting results (see section 8.3).

This thesis characterised the effects of schizophrenia risk-associated common variants on functional connectivity (and neural activation) during performance of two tasks that
assess cognitive functions disrupted in schizophrenia: emotional face processing and spatial working memory (section 3). As a point of departure from the main research question, the next section of the thesis examined one of these tasks, emotional face processing, in a sample of patients with a DSM-IV diagnosis of schizophrenia. This phenotype study focussed on emotional face processing in schizophrenia to better understand the neurobiological correlates of social cognitive dysfunction in the disorder, which significantly affects quality of life and functional outcomes (see section 1.7). While watching faces (angry and neutral conditions combined or angry faces specifically), patients showed significantly weaker deactivation of the mPFC/ACC relative to controls (N = 46, p<0.05, corrected).

Given the role of the ACC in salience detection and the processing of negative emotion, altered function of this region in patients may result in an increased attribution of negative salience to others, which may contribute to persecutory delusions. It is interesting to note that both MIR137 and NOS1 variants examined in this thesis were associated with altered connectivity of the mPFC, an area that also shows cellular, grey and white matter abnormalities in schizophrenia (section 4.4).

The final three sections of this thesis examined three schizophrenia risk variants identified by recent GWAS: a SNP within an intron of MIR137, rs1625579, an indel within NOS1, and a SNP within an intron of ZNF804A, rs1344706.
Homozygous carriers of the *MIR137* risk allele showed increased functional connectivity between the right amygdala and the cingulate and right inferior frontal gyrus, regions that play a role in emotion regulation (N = 80; p<0.05, corrected; section 5.3). This study provides the first evidence that the rs1625579 variant affects functional connectivity, and supports a possible role of the variant in more affective forms of psychosis, extending a previous study that suggested *MIR137* is associated with mood congruent psychotic symptoms.

The next section reported that homozygous carriers of the PGC2 GWAS *NOS1* indel showed increased functional connectivity between the right DLPFC and the right middle frontal gyrus, right superior temporal gyrus and right superior frontal gyrus/medial frontal gyrus (N = 80; p<0.05, corrected; section 6.3). These results provide further evidence that *NOS1* variation affects prefrontal function, building on a body of literature that spans different imaging modalities (see section 6.1). Observation of these results during face processing but not during spatial working memory also suggests that these effects may be cognitive state dependent.

In contrast, no significant effects of *ZNF804A* were observed on functional connectivity, conflicting with previous findings for this gene. The sample used is comparably large for an fMRI study, and similar to samples used in other imaging genetics studies. For the power available with this sample, the lack of significant findings may indicate that effects of *ZNF804A*, if they exist, are smaller than expected (the issue of sample size is discussed further in section 8.3).
8.2 Translational value of this research

As GWAS statistical association does not establish biological significance, this thesis provides neurobiological support for the role of specific genes in the pathogenesis of schizophrenia by providing evidence that these variants have functional effects on the brain in a manner relevant to schizophrenia (i.e. by affecting functional connectivity, which is altered in the disorder) (Bigos and Weinberger, 2010). This thesis also provides important information about the neurobiology of both schizophrenia and functional connectivity in the human brain. For example, microRNA and nNOS molecules likely play a role in illness pathogenesis through effects on critical neural processes such as synaptic plasticity (a process that contributes to functional connectivity between groups of neurons) (see sections 5.1 and 6.1).

It is hoped that further research on molecular pathways that incorporate MIR137 and NOS1 will help identify novel molecular targets for pharmacological therapies to treat cognitive deficits. In this context it is interesting to note that existing drugs affecting microRNAs or nNOS have been suggested as having therapeutic potential to treat cognitive deficits in psychiatric illness (Wass et al., 2006; O'Connor et al., 2012).

Since all cognitive functions require functional integration of spatially remote brain regions, and functional connectivity has been shown to explain a proportion of variation in behaviour (Pezawas et al., 2005), knowing that a particular gene or pathway is related to functional connectivity is important for understanding mechanisms mediating risk. To speculate on one example, novel therapies that have beneficial effects on functional
connectivity within a particular network (a corticolimbic network) may lead to selective improvements in cognition (emotion regulation) leading to reduced symptom severity (reduced mania).

In addition, similar patterns of disrupted connectivity in particular networks (frontoparietal, default mode, corticolimbic, etc.) have been observed across psychiatric disorders, leading to the suggestion that these patterns may contribute to transdiagnostic symptoms (e.g. executive dysfunction) (Buckholtz and Meyer-Lindenberg, 2012). As such, increased understanding of genetic effects on functional connectivity may have relevance for a wide range of other psychiatric disorders also.

Further examination of genetic effects on functional connectivity and the relation of these effects to cognition may help in the development of translational models for characterising and predicting the influence of therapeutic agents on cognitive function. These may include pharmacotherapy, deep brain stimulation, transcranial magnetic stimulation, cognitive behavioural therapy and cognitive remediation therapy (Millan et al., 2012). For example, both antipsychotic and cognitive remediation treatment have been associated with functional connectivity changes in schizophrenia patients, changes associated with clinical or cognitive improvements (Lui et al., 2010; Penadés et al., 2013). Whether and how these effects are affected by specific variants or molecular pathways will be important questions for future studies. Similarly, the phenotype study presented in this thesis supports the role of altered mPFC/ACC function in schizophrenia; a finding that may be used in future studies to probe the effects of specific treatments.
8.3 Strengths of this thesis

Methodology: The first strength of this project is the methodology employed, including the use of fMRI to examine effects of schizophrenia risk variants. As there is no known long-term health risk associated with being exposed to the static magnetic fields in MRI, this method can be used to safely examine brain function in living humans (Schenck, 2000). In addition, this method has higher spatial resolution compared to EEG or evoked potentials (≤ 3mm$^3$) and increased signal to noise ratio, as discussed in section 1.

Another methodological strength with this thesis is the use of functional connectivity as an intermediate phenotype. As discussed in section 1, functional connectivity is a promising intermediate phenotype for schizophrenia for several reasons: functional connectivity is moderately to highly heritable, it is altered in schizophrenia patients and healthy siblings, it may be clinical state independent, and has been shown to explain more variance in behaviour compared to brain activity or structure. Also, given that neither MIRI37 nor NOS1 were associated with altered neural activation, the use of functional connectivity enabled this project to examine genetic effects that would not have otherwise been detectable.

Finally, to analyse functional connectivity from BOLD data, the seed voxel correlation method has a number of strengths: it might be more sensitive relative to other methods (section 1.6), it is widely used in imaging genetics, and has been successfully used to
identify several psychiatric risk variant effects on functional connectivity (e.g. Pezawas et al., 2005; Esslinger et al., 2009; Paulus et al., 2013a).

**Examination of GWAS schizophrenia risk variants:** The second strength of this thesis is the examination of schizophrenia risk variants identified using GWAS. As discussed in section 1, GWAS studies have a significant advantage over candidate gene studies as they are less biased (being hypothesis-free) and more strongly associated with the categorical schizophrenia illness phenotype (Williams et al., 2007; Meyer-Lindenberg, 2010). Moreover, recent GWAS studies (that first identified the variants examined in this thesis) have included samples as large as tens of thousands of individuals, significant associations have been replicated in independent samples, and these associations have met stringent statistical significance criteria (e.g. \( p < 5 \times 10^{-8} \) for the combined effect from the discovery and replication samples) (Corvin et al., 2010).

8.4 Limitations of this thesis

**Sample size:** The first limitation with this thesis is sample size. Sample size is an important determinant of statistical power, the probability that a study will detect a genuine difference between populations when one exists (Taborsky, 2010). Therefore, a low sample size increases the probability of erroneously claiming two populations are not different when in fact they are (a Type II error). In order to be able to detect small to medium effects, larger sample sizes are preferable. According to the CD-CV hypothesis, schizophrenia is caused by small effects of many common variants (in addition to other factors). Indeed, recent GWAS studies have identified strong genetic
associations in samples of tens of thousands. However, each variant is only associated with a small increase in risk (e.g. for the rs1625579 variant, the odds ratio = 1.12, i.e. a 1.12% increase in schizophrenia risk associated with carrying the risk allele; see section 5.1). Larger sample sizes are also important considering that functional connectivity measures may have reduced power to detect significant effects relative to other connectivity measures (e.g. DTI; section 2.4).

It is currently impractical to study genetic association with neurobiological variables using fMRI in similarly large samples due to the significant costs this would imply. For example, typical research MRI scans cost over €400 per hour for a single participant (Voos and Pelphrey, 2013). The imaging genetics studies in this thesis used a sample size ranging from 80 to 100 (mean N = 88). This is a comparatively large sample for an fMRI study (Carp, 2012), and a similarly-sized sample to recent fMRI examinations of GWAS variants (e.g. Paulus et al., 2013a). Nevertheless, post-hoc sensitivity power analysis reported in section 3.4 indicates that such a sample would be unable to detect effects less than $d \sim 0.96$ at a $p<0.001$ threshold at 80% power (the minimum level of power that should be used, as proposed by Cohen, 1992).

Genetic effects on brain function are expected to be larger than effects on a complex psychiatric phenotype such as schizophrenia, which likely emerges from the interactions of many such genes and environmental factors (Mier et al., 2010). Nevertheless, some genetic effects may be small in nature, even on brain-based phenotypes such as functional connectivity (Paulus et al., 2013a). This makes it difficult to draw conclusions about the negative findings reported in this thesis, as the sample used may simply be underpowered to detect certain genetic effects at significant thresholds.
**Seed voxel correlation method:** The second limitation in this thesis arises from use of the seed voxel correlation method to examine functional connectivity. This method examines connectivity across a period of time that includes multiple cognitive states. Thus, even after removal of task related activation from estimates of functional connectivity statistically, some residual task effects on neural networks may remain.

Meyer-Lindenberg (2009) points out that changing cognitive states are also associated with resting brain function, and this does not preclude a reliable determination of resting-state connectivity networks. However, it is important to be aware of this issue in the interpretation of results. For example, it is unclear whether functional connectivity results observed are associated with the task used or whether they are task-independent. This issue could be addressed in future studies using psychophysiological interaction analysis (PPI), discussed in section 8.5.

**Lack of replication:** The final limitation is the lack of replication of significant results in an independent sample, which can help address both Type I and Type II errors in fMRI research (Lieberman and Cunningham, 2009).

**8.5 Suggested future directions for this research**

**Examination of genetic effects on functional connectivity in larger samples and replication of significant effects across independent samples:** It is important for future fMRI studies examining effects of GWAS variants on functional connectivity to
use larger sample sizes for increased statistical power. For example, sample sizes of 1000 would have 80% power to detect small effects \((d = 0.25)\) at a \(p < 0.001\) level (post-hoc sensitivity power analysis performed using G*Power 3.1.7 for a one-tailed independent t-test between groups of equal size). This will be important in order to reduce the Type II error rate and more fully characterise the range of effects of GWAS variants on functional connectivity. Larger imaging genetics samples will also allow for replication of significant results across independent samples, provided that each sample is sufficiently powered. Finally, larger samples will allow examination of interactions between genetic variables and environmental variables (e.g. urban upbringing, exposure to specific treatments, etc.).

Large international imaging genetics consortia are ideally positioned to meet these challenges. For example, the IMAGEN project includes ~2,000 14-year-old adolescents with whole-genome data and a battery of fMRI tasks, including the emotional face processing task used in this project (Schumann et al., 2011). Similarly, the ENIGMA consortium has examined effects of genetic variants on brain structure in a sample over 20,000 using meta-analysis (Stein et al., 2012).

**Examination of the effects of multiple genetic variants on functional connectivity:**

All of the genes examined in this thesis are part of molecular pathways that include multiple risk genes for schizophrenia. For example, \(\textit{ZNF804A}\) is a target of \(\textit{MIR137}\), while \(\textit{NOS1}\) is part of the NMDA-glutamate pathway (Nicodemus et al., 2010; Kim et al., 2012).
It is likely that individual psychiatric risk variants interact with other variants in complex molecular pathways to affect risk. As such, future imaging genetics studies should examine the interactive (epistatic) effects of multiple variants on functional connectivity. This is important in order to characterise the specific risk arising from interactions between different variants.

For example, a previous fMRI study of multiple genes in healthy controls provided important biological validation of increased schizophrenia risk arising from epistasis between multiple risk variants (Nicodemus et al., 2010). In addition, it has recently been shown that increased risk allele load on the ZNF804A pathway explains more variance in neuropsychological scores such as IQ than variance explained by any single schizophrenia risk variant on these measures (Hargreaves and Nicodemus et al., 2014). However, examining the effects of multiple variants will require larger sample sizes in order to ensure adequately powered samples in each of the (increased number of) groups.

Examination of genetic effects on context-dependent functional connectivity:

Future studies should also examine functional connectivity using additional analysis techniques. For example, PPI analysis can be used to examine context-dependent changes in functional connectivity during a task (Friston et al., 1997). This is important for accurately determining the effects of genetic variants on connectivity patterns specifically related to a task, e.g. emotional face processing. This provides more information on the association between the variant and specific cognitive states (e.g.
increased salience detection), which may help further elucidate the variant's role in cognition and behaviour.

PPI analysis consists of a multiple regression with a psychological regressor (modelling the task condition of interest), a physiological regressor (modelling the seed time course) and a PPI regressor (created by multiplying the two together). Since the PPI time course is likely to be similar to the other regressors, less unique variance can be attributed to it (O'Reilly et al., 2012). As a result, PPI is associated with reduced power to detect significant effects and a high proportion of false negatives are likely. As such, larger samples may be necessary in order to have sensitivity to detect significant results, especially for a field concerned with genetic effects, some of which may be small in nature.

**Examination of genetic effects on effective connectivity**: Future studies should also examine effects of GWAS variants on effective connectivity, defined as the effect of one group of neurons on another (Friston et al., 2003). Compared to functional connectivity, effective connectivity also examines the direction of information flow through a network, ultimately providing more information on the specific effects of a given genetic variant on neural networks. Effective connectivity between brain regions can be measured using dynamic causal modelling (DCM), in which a number of network models are defined *a priori* (e.g. based on anatomical data and prior imaging data), and Bayesian statistics are used to estimate which model is best accounted for by the BOLD fMRI data.
Multimodal examination of the effects of variants on functional and structural connectivity: Finally, future studies should integrate functional connectivity data with structural connectivity data (e.g. white matter integrity measured using DTI), as this may help determine whether functional connectivity effects are mediated by genetic effects on white matter integrity. Moreover, genetic effects on functional connectivity could be supported by observation of significant effects on structural connectivity within the same networks. White matter abnormalities have been observed in individuals at high risk for schizophrenia, and a number of schizophrenia risk variants, including \textit{NRG1}, have been associated with altered white matter integrity (Sprooten \textit{et al.}, 2009). These effects are likely to affect functional connectivity between brain regions considering that congruent results between these methods have been reported (Damoiseaux \textit{et al.}, 2009).

8.6 Conclusions

This thesis reports the first evidence that two recent GWAS-associated schizophrenia risk variants, within \textit{MIR137} and \textit{NOS1} respectively, are associated with altered functional connectivity in healthy volunteers, extending GWAS results with neurobiological data and identifying a mechanism of risk. However, no effects of a GWAS variant within \textit{ZNF804A} were observed, suggesting that effects of this variant may be smaller than expected. Finally, this thesis also reports weaker mPFC deactivation in schizophrenia patients relative to healthy controls during emotional face processing, suggesting that this pattern of neural activity may be a useful translational model for future studies examining novel therapies for social cognitive deficits in the disorder.
It is hoped that future studies on these GWAS genes and related pathways will help identify novel molecular targets for new drugs which may beneficially affect particular brain networks leading to improved cognition and symptom relief. Moreover, it is hoped that increased understanding of genetic effects on functional connectivity and the relation of these effects to cognitive dysfunction will lead to new translational models to test novel therapies including pharmacotherapy and cognitive remediation therapy.


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Appendix A

Ethics approval and study consent forms used in 2011-2012
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 5-03-02 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:

- 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,

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.
Tuesday, 15 January 2008

Study Title
Whole genome study of schizophrenia using RGPI resource

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

Noelle Cosgrave
Dr. Orla Sheils
Chairperson
Faculty of Health Sciences Ethics Committee
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.,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 sign this consent form and make it available to the researcher(s).

If you have any questions regarding this research before or after taking part, please feel free to contact any member of the research team (Note: contact information is given at the end of this form).

Information about the Project:
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 this study we are interested in looking at how your genes influence your brain structure and function. In order to examine this we will take picture of your brain using Magnetic Resonance Imaging (described below).

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 be still and relax. For others you will be asked to do tasks while we take the brain pictures (see description below). 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.

**Task Description**

You will be asked to complete two tasks during scanning. 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. You will practice this task on a computer before doing the same task in the scanner. For the second task, you will view a series of video clips showing different facial expressions. You will be asked to watch the video clips carefully. You will be given the information on how to complete the tasks prior to the scan. You must make sure that you understand the tasks before we start scanning. 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 15 minutes to learn and will take less than an hour to complete in the scanner.

**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. However, there are some potential side effects. 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. You will be issued with protective headphones and earplugs to prevent damage to your hearing. 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;
- 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 jewelry 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

Genetics testing

If you have not done so already, you will be asked to provide a saliva sample. We will extract DNA from this sample and use it for genetics testing. This sample will be stored by us and may be used in the future for other studies. The sample will not be used for any purpose other than to look at how genes impact brain structure and function.

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: ____________________

WITNESS'S NAME: ________________________________

WITNESS'S SIGNATURE: ________________________________

Date: ____________________

Research Team:

Dr. Gary Donohue, Room 0.19 Trinity Centre, St. James's Hospital, Dublin 8.
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donoghue@tcd.ie

Dr. Emma Jane Rose, Room 0.18, Trinity Centre, St. James's Hospital, Dublin 8.
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Mr. Omar Mothersill, Room 0.18, Trinity Centre, St. James's Hospital, Dublin 8.
Tel: 01-8962464
motherso@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

______________________________________________

______________________________________________

MRI-Consent.doc 1/2
Signed by Participant:__________________

Participants GP Name and Address

____________________________________

____________________________________

Date:______________________________
Appendix B

Tables of clusters showing significantly increased neural activation during cognitive tasks, corrected for multiple comparisons at the voxel-level
Table B1: Clusters, including individual peaks, showing significantly increased neural activation during face (angry and neutral combined) versus baseline, and angry versus neutral face conditions, corrected for multiple comparisons at the voxel-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>
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<td>1</td>
<td>4410</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right superior temporal gyrus/ BA</td>
<td>24.17</td>
<td>&gt;8</td>
<td>54 -43 10</td>
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<td></td>
<td></td>
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<td></td>
<td>BA 22</td>
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<td>2</td>
<td>1347</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right middle frontal gyrus/ BA 46</td>
<td>19.11</td>
<td>&gt;8</td>
<td>48 23 22</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right cerebellum</td>
<td>18.25</td>
<td>&gt;8</td>
<td>42 -49 -20</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Left fusiform gyrus/ BA 37</td>
<td>15.77</td>
<td>&gt;8</td>
<td>-42 -49 -20</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Left cerebellum</td>
<td>15.56</td>
<td>&gt;8</td>
<td>-15 -76 -35</td>
<td></td>
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<td></td>
<td></td>
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<td>Left middle temporal gyrus/</td>
<td>14.73</td>
<td>&gt;8</td>
<td>-60 -49 10</td>
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<tr>
<td>BA 21</td>
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<td>3 359</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Left amygdala</td>
<td>13.24</td>
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<td>-21 7 -14</td>
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<td>-30 -4 -20</td>
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<td>4 1045</td>
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<td>Faces</td>
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<td>&gt;8</td>
<td>-42 14 25</td>
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<td>9</td>
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<td>&lt;0.001</td>
<td>Faces</td>
<td>Left inferior frontal gyrus/ BA</td>
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<td>47</td>
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<tr>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Left middle frontal gyrus/ BA</td>
<td>8.80</td>
<td>7.45</td>
<td>-42 2 49</td>
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<td>5 205</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right superior frontal gyrus/ BA</td>
<td>11.14</td>
<td>&gt;8</td>
<td>6 14 58</td>
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<td>BA 6</td>
<td></td>
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<td></td>
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<tr>
<td>6 37</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right superior parietal lobule/ BA</td>
<td>6.49</td>
<td>5.86</td>
<td>36 -52 43</td>
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<td>7</td>
<td>19</td>
<td>&lt;0.001</td>
<td>Faces</td>
<td>Right lingual gyrus/ BA18</td>
<td>6.35</td>
<td>5.75</td>
<td>21 -91 -8</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>0.003</td>
<td>Faces</td>
<td>Left superior temporal gyrus/</td>
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<td>5.06</td>
<td>-54 -1 -14</td>
</tr>
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<td>8</td>
<td>0.009</td>
<td>Faces</td>
<td>Left caudate</td>
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<td>4.85</td>
<td>-15 5 10</td>
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<td>10</td>
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<td>Right superior frontal gyrus/</td>
<td>5.08</td>
<td>4.75</td>
<td>9 47 40</td>
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<td>11</td>
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<td>0.010</td>
<td>Angry</td>
<td>Left cingulate gyrus/ BA 32</td>
<td>5.15</td>
<td>4.81</td>
<td>-9 23 37</td>
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<td></td>
<td></td>
<td>0.019</td>
<td>Angry</td>
<td>Left anterior cingulate gyrus/</td>
<td>4.98</td>
<td>4.67</td>
<td>-9 29 22</td>
</tr>
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<td>12</td>
<td>6</td>
<td>0.021</td>
<td>Angry</td>
<td>Left Cuneus/ BA 17</td>
<td>4.96</td>
<td>4.65</td>
<td>-24 -85 16</td>
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<tr>
<td>13</td>
<td>2</td>
<td>0.028</td>
<td>Angry</td>
<td>Left middle occipital gyrus/</td>
<td>4.87</td>
<td>4.58</td>
<td>-30 -88 22</td>
</tr>
</tbody>
</table>

BA 19

*p values are FWE-corrected for multiple comparisons at the cluster level; *The significant right amygdala activation reported in the main text is contained within this cluster; ^Faces = Faces (neutral and angry combined) versus baseline; Angry = Angry versus neutral faces; ^BA = Brodmann Area
Table B2: Clusters, including individual peaks, showing significantly increased neural activation during spatial working memory (1 dot and 3 dots combined) versus baseline, and 3 dots versus 1 dot conditions, corrected for multiple comparisons at the voxel-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>
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<td>1</td>
<td>9941</td>
<td>&lt;0.001</td>
<td>SWM</td>
<td>Right precuneus/ BA 7</td>
<td>19.09</td>
<td>&gt;8</td>
<td>15 -64 55</td>
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<td>Right middle frontal gyrus/ BA</td>
<td>16.95</td>
<td>&gt;8</td>
<td>27 -4 49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>SWM</td>
<td>Left superior parietal lobule/ BA 7</td>
<td>16.91</td>
<td>&gt;8</td>
<td>-27 -55 55</td>
</tr>
<tr>
<td>2</td>
<td>159</td>
<td>&lt;0.001</td>
<td>SWM</td>
<td>Left thalamus</td>
<td>6.97</td>
<td>6.32</td>
<td>-21 -28 13</td>
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<td></td>
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<td>SWM</td>
<td>Left globus pallidus</td>
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<td></td>
<td></td>
<td>0.003</td>
<td>SWM</td>
<td>Left caudate</td>
<td>5.33</td>
<td>5.01</td>
<td>-21 -7 22</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SWM</td>
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<td>3</td>
<td>170</td>
<td>&lt;0.001</td>
<td>Right middle frontal gyrus/ BA 22</td>
<td>6.74</td>
<td>6.15</td>
<td>33 38 25</td>
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<td>4</td>
<td>128</td>
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<td>Left claustrum</td>
<td>6.35</td>
<td>5.84</td>
<td>-27 29 1</td>
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<td></td>
<td></td>
<td>&lt;0.001</td>
<td>Left claustrum</td>
<td>6.12</td>
<td>5.66</td>
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<td>5</td>
<td>30</td>
<td>0.002</td>
<td>Right superior temporal gyrus/ BA 22</td>
<td>5.47</td>
<td>5.13</td>
<td>54 -43 13</td>
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<td>21 -64 58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>Right inferior parietal lobule/ BA 40</td>
<td>11.82</td>
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<td>39 -40 46</td>
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<tr>
<td></td>
<td></td>
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<td>Right precuneus/ BA 31</td>
<td>11.41</td>
<td>&gt;8</td>
<td>30 -73 31</td>
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<tr>
<td>8</td>
<td>1010</td>
<td>&lt;0.001</td>
<td>3 dots Left precuneus/ BA 7</td>
<td>11.02</td>
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<td>-21 -64 58</td>
<td></td>
</tr>
<tr>
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<td></td>
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<td>3 dots Left precuneus/ BA 31</td>
<td>9.05</td>
<td>7.78</td>
<td>-27 -73 31</td>
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</tr>
<tr>
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<td>3 dots Left inferior parietal lobule/ BA 40</td>
<td>8.02</td>
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<tr>
<td>9</td>
<td>214</td>
<td>&lt;0.001</td>
<td>3 dots Right middle frontal gyrus/ BA 6</td>
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<td>27 -4 49</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>5.42</td>
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<td>53</td>
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<td>54 11 28</td>
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<td>3 dots Right precentral gyrus/ BA 6</td>
<td>5.16</td>
<td>4.87</td>
<td>45 5 25</td>
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<tr>
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<td></td>
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<td></td>
<td>Left medial frontal gyrus/ BA</td>
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<td>13</td>
<td>58</td>
<td>0.001</td>
<td>3 dots</td>
<td>5.70</td>
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<td>0 26 43</td>
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<td>0.026</td>
<td>3 dots</td>
<td>4.78</td>
<td>4.55</td>
<td>-48 8 28</td>
<td></td>
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</tbody>
</table>

*p values are FWE-corrected for multiple comparisons at the cluster level; *SWM = Spatial working memory (1 dot and 3 dots combined) versus baseline; 3 dots = 3 dots versus 1 dot condition*
Appendix C

Frequency distributions of MNI-coordinates from dorsolateral prefrontal seed voxels identified using the global maximum and next local maximum selection approaches
**Figure C.1:** Frequency distributions of MNI-coordinates from dorsolateral prefrontal seed voxels identified using the global maximum and next local maximum approaches for all spatial working memory task participants genotyped for the $NOS1$ indel variant (see section 6.2)

Global maximum approach seed distribution coordinates (top row: x, y; bottom row: z):
Next local maximum approach seed distribution coordinates

(top row: x, y; bottom row: z):
Figure C.2: Frequency distributions of MNI-coordinates from dorsolateral prefrontal seed voxels identified using the global maximum and next local maximum approaches for all emotional face processing task participants genotyped for the *NOS1* indel variant (see section 6.2)

Global maximum approach seed distribution coordinates

(top row: x, y; bottom row: z):
Next local maximum approach seed distribution coordinates

(top row: x, y; bottom row: z):
Figure C.3: Frequency distributions of MNI-coordinates from dorsolateral prefrontal seed voxels identified using the global maximum and next local maximum approaches for all spatial working memory task participants genotyped for the rs1344706 variant within ZNF804A (see section 6.2 and section 7)

Global maximum approach seed distribution coordinates

(top row: x, y; bottom row: z):
Next local maximum approach seed distribution coordinates

(top row: x, y; bottom row: z):
Appendix D

Publications arising from this thesis
List of publications arising from this thesis

   connectivity. Neuroimage. Advance online publication. doi:

   psychosis risk variants on brain connectivity: a review. Frontiers in Psychiatry,
   3(18).

MANUSCRIPTS IN PROGRESS

   Donohoe, G. Altered medial prefrontal activity during dynamic face processing
   in schizophrenia.
Effects of MIRI37 on fronto-amygdala functional connectivity

Omar Mothersill 1,2, Derek W. Morris 1,2, Sinead Kelly 1,2, Emma Jane Rose 1,2,3, Cara Fahey 1,2, Carol O’Brien 1,2, Ronan Lyne 2,3, Richard Reilly 4, Michael Gill 2,6, Aidan P. Corvin 4, Gary Donohoe 4,5

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ARTICLE INFO

Abstract

Background: MIRI37 is implicated in brain development and encodes a microRNA that regulates neuronal maturation and adult neurogenesis. Recently, a common genetic variant within MIRI37 showed genome-wide significant evidence of association with schizophrenia, and with altered amygdala activation in these at genetic risk for schizophrenia. Following this evidence, we investigated the effects of MIRI37 genotype on neural activity during face processing.

Methods: By grouping healthy participants as carriers or non-carriers of the MIRI37 rs1625579 risk allele 27 associated with schizophrenia, we investigated MIRI37's effects on altered cortical response during an MRI 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 lesser activation and 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 cortices).

Conclusions: Our findings provide the first evidence that the rs1625579 variant affects fronto-amygdala functional connectivity, providing further evidence that MIRI37 may contribute to forms of psychosis in which affective symptoms are more prominent.

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Introduction

MIRI37 is one of a group of genes that encode microRNAs (miRNA) — small non-coding RNA molecules modulating gene expression. MIRI37 is highly expressed in the brain, particularly in medial temporal regions, and plays an important role in neurogenesis and dendritic morphogenesis (Vidal et al., 2010). In a meta-analysis of genome-wide association studies (Björk et al., 2011) (GWAS), a common single nucleotide polymorphism (SNP), rs1625579, within the MIRI37 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 MIRI37 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 risk genetic factors (Gar et al., 2017). 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 MIRI37 (Whalley et al., 2012). In this study a genotype-by-group interaction on activation in the amygdala during the Hoyalg sentence completion task was observed. The task is typically associated with a decrease in the amygdala (Whalley et al., 2011); however, among participants with high genetic risk for schizophrenia, homozygous risk allele carriers showed comparatively less deactivation in the amygdala compared to homoygous and heterozygous non-risk carriers. The authors suggest that this finding may reflect a misattribution of emotional salience in the high-risk homozygous risk group to the stimuli presented in the tasks, which were considered to be non-emotional. However, effects of MIRI37 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 (Gar et al., 2007).

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MRS137 has been shown to play a role in the shaping of dentrites, raising the possibility that this gene may affect functional connectivity in the brain, which has been proposed as a possible biological mechanism in the pathogenesis of schizophrenia (Friston, 1998; Stephan et al., 2009). Altered dentrite 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 Lenti et al. (2013) reports that schizophrenia patients homozygous for the r1625579 risk allele have relatively reduced fractional anisotropy, an index of structural brain connectivity, throughout the brain compared to normocannental. While the exact relationship between white matter integrity and functional connectivity is not fully understood, congruent results between the two modalities have been reported (Damoiseaux 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 MRS137 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 (Groenras and Paus, 2006; Schneider et al., 2011; Talibas et al., 2012; Thyreau et al., 2012). We considered both brain activation and functional connectivity of the amygdala using an established seed-based correlation approach (Erik et al., 2010; Esslinger et al., 2009; Paulus et al., 2013) with the aim of delineating the role of r1625579 genotype on the neurobiological underpinnings of emotion processing. In doing so, we sought to test the hypothesis that the MRS137 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 isotropy, an index of intrinsic brain connectivity, through the brain compared to normocannental. While the exact relationship between white matter integrity and functional connectivity is not fully understood, congruent results between the two modalities have been reported (Damoiseaux 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 MRS137 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 (Groenras and Paus, 2006; Schneider et al., 2011; Talibas et al., 2012; Thyreau et al., 2012). We considered both brain activation and functional connectivity of the amygdala using an established seed-based correlation approach (Erik et al., 2010; Esslinger et al., 2009; Paulus et al., 2013) with the aim of delineating the role of r1625579 genotype on the neurobiological underpinnings of emotion processing. In doing so, we sought to test the hypothesis that the MRS137 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 important. The role of the MRS137 risk allele in schizophrenia and that dysconnectivity is a significant feature of schizophrenia and that dysconnectivity is important in 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 comorbid 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.

MRS

Participants were imaged using a Philips Intera Achieva 3T MR system. Functional imaging consisted of whole-brain BOLD EPI in which 40, 24 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 210 ROV.

Face processing task

During fMRI, subjects performed a face processing task designed by Groenras and Paus (2006) and adapted for the IMAGEN study (Schneider et al., 2011; Schumann et al., 2010; Talibas et al., 2012). 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 first 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 18 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 were 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 MRS analysis. If 5 subjects were excluded due to poor performance (<4 correct answers) or missing data for this follow-up task.

Genotyping

Genetic 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 in 7908HT sequence detection system (Applied Biosystems). The call rate for TaqMan genotyping was >95%. The samples were observed to be in Hardy-Weinberg Equilibrium.

Data processing and analysis

After realignment of raw EPI data (MRI section), graphical plots of estimated time series of translations and rotations were carefully inspected for excessive movement in each subject. Displacement vectors of more than 2.5 mm translation and/or >3° rotation in any direction. Overall, 1 subject exhibited rotation >3° and was excluded from further MRS analysis. 2 additional subjects were excluded due to bad quality MRS data and/or significant artifacts. Of the 81 remaining subjects, there were 1 'GG' homozygotes, 17 'CT' heterozygotes and 57 'TT' homozygotes. Due to the relative infrequency of 'GG' homozygotes, we compared subjects carrying 0 or 1 copy of the risk allele ('GG' or 'CT' GT N = 26) with homozygous risk 'TT' allele carriers ('TT' N = 51). The sample was not expected to be <28 common allele in Rijpe et al. (2011) and we used the same grouping strategy as previously used in other imaging genetics investigations of this SNP (Lett et al., 2013; 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 (spm2), 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 stereotaxic 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 8 mm filter (i.e. kernel width 2-3 times greater than the original voxel size).

Statistical analyses was performed using a standard general linear model (GLM) in SPM 8 (Friston et al., 1994), for each condition, a basis function representing 4 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 movement, condition effects at each voxel were then calculated using a t-contrast, producing a statistical parametric map of the following contrasts for 200.
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 (Midjan 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 (WM section), and right and left amygdalae were used as seed regions in two-separate connectivity analyses. Time series from the amygdala were extracted using first eigenvarias 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, and then input, 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 < 0.5 (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 vs. 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 eigenvarias 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 using 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 were produced by the analysis, and 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., 2011) and anatomical localisation of these coordinates was performed using Talairach Client 2.4.3 (Lancaster et al., 1997, 2000).

Discussion

This study investigated the functional effects of the genome-wide associated schizophrenia risk variant rs1625579 within MRRI37 on neural 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 risk allele.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age (SD)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (N = 20)</td>
<td>28.50 (8.52)</td>
</tr>
<tr>
<td>TT (N = 35)</td>
<td>27.06 (8.66)</td>
</tr>
<tr>
<td>Stable a</td>
<td>t = 623.00</td>
</tr>
<tr>
<td>p value</td>
<td>0.196</td>
</tr>
</tbody>
</table>

a. SD = standard deviation.

b. The statistical tests used are listed in the subject demographics section.

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**Table 2**

Clusters, including individual peaks, showing significantly increased functional activation during face (angry and neutral) versus control and angry versus neutral face conditions.

<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>Face</td>
<td>Right middle temporal gyrus/BA 22</td>
<td>23.44</td>
<td>-8</td>
<td>54 - 40 7</td>
</tr>
<tr>
<td>2</td>
<td>2431</td>
<td>&lt;0.001</td>
<td>Face</td>
<td>Right middle frontal gyrus/BA 6</td>
<td>18.39</td>
<td>-8</td>
<td>48 22 22</td>
</tr>
<tr>
<td>3</td>
<td>1288</td>
<td>&lt;0.001</td>
<td>Face</td>
<td>Left inferior frontal gyrus/BA 9</td>
<td>11.41</td>
<td>-8</td>
<td>-29 11 25</td>
</tr>
<tr>
<td>4</td>
<td>2409</td>
<td>&lt;0.001</td>
<td>Face</td>
<td>Left middle frontal gyrus/BA 6</td>
<td>8.64</td>
<td>-8</td>
<td>-41 2 49</td>
</tr>
<tr>
<td>5</td>
<td>676</td>
<td>&lt;0.001</td>
<td>Angry</td>
<td>Left superior frontal gyrus/BA 6</td>
<td>10.00</td>
<td>-8</td>
<td>6 14 58</td>
</tr>
<tr>
<td>6</td>
<td>385</td>
<td>&lt;0.001</td>
<td>Angry</td>
<td>Left inferior frontal gyrus/BA 8</td>
<td>5.99</td>
<td>4.08</td>
<td>9 44 61</td>
</tr>
<tr>
<td>7</td>
<td>336</td>
<td>&lt;0.001</td>
<td>Angry</td>
<td>Left superior frontal gyrus/BA 8</td>
<td>4.97</td>
<td>4.27</td>
<td>-52 29 19</td>
</tr>
<tr>
<td>8</td>
<td>412</td>
<td>&lt;0.001</td>
<td>Angry</td>
<td>Left superior frontal gyrus/BA 8</td>
<td>4.97</td>
<td>4.27</td>
<td>-52 29 19</td>
</tr>
</tbody>
</table>

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

---

*Fig. 5. 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 set at p < 0.001 uncorrected with a cluster threshold for display purposes; df = 80). Green brain regions showing decreased activation during the angry versus neutral face condition (N = 81; one-sample t-test; significance set at p < 0.001 uncorrected with a cluster threshold for display purposes; df = 80). Coloured bars represent t-values. Each 2D sagittal slice is labelled with an x-coordinate (MNI space). Clusters are rendered on the MNI958 brain template using MRCIG. (http://www.med.unc.edu/mricron-software). Additional editing of figure (e.g., changing the time-resolution) performed using MS-Paint and Paint.NET v2.0.10.*

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Emotion processing in the brain can be conceptualised as being mediated by two distinct, yet interconnected pathways/systems (Philips 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 supplemental motor cortex, 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 (Dus 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 stimuli. Healthy subjects have responded similarly to both emotional and neutral faces (Lee et al., 2008) and reported neutral faces as emotional stimuli (Ike 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) (Wexler and Bronk, 2012).

The present findings 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 results in high incidence of false negatives (Fnerlick 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...
The effects of miR-17 on fronto-amygdala functional connectivity: A preliminary study


ACKNOWLEDGMENTS

We 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 BCSParun v. 3.5-1-21 (Biocomputing Platforms Ltd, Finland).

This work was generously supported by Science Foundation Ireland (SFI grant 12IP3/359 to GD and SFI/08/R marginalised groups in schizophrenia). We also acknowledge the support of the Health Research Board (HRA/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


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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 = 0.78) 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 (Z = 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 (Z = 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 €353.9 billion; Gartisvan 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 (GWA) and copy number variation studies have identified several common and rare gene variants associated with the disorder (GTDonovan 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, and 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

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of these effects, in order to determine the extent of the genetic impact on brain connectivity.

**ALTEDED FUNCTIONAL CONNECTIVITY IN SZ**

In the early 20th century, German neurologist Karl Wernicke proposed that SZ arises from altered neural connectivity (or disconnection) rather than from abnormalities in specific parts of the brain (see Stephan et al., 2000 for a review). One hundred years later, advances in neuroimaging technology have enabled scientists to empirically consider disconnection 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, 1995; 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. Electromyelogram (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-perfrontal 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 Perogam et al. (2005). These authors examined the effects of a 5-HTTLPR/polyorphism 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 disconnection 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 (Umbrecht 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 (Keshavan 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., G72GRM3 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 interactions* (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 (Dalal and Xiao, 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 (Kalmbach 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 (Wittmann 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 (Bucholzam 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 Kalicki 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 part of the tempo-parietal function 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, thalami and cingulate gyms. 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, hippocampi-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/striatum. 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 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 by altering synaptic function and information processing (Fields, 2008). 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 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. Hakal 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), gelon (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. Mangan et al. (2008) examined the effect sizes of the 5-HTTLPR polymorphism and amygdala activation, while Miet 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.succ.edu/~faculty/becker/ and
www.lyonmorris.com/mai/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-analyses considering the relative difference in the impact of SZ risk variants 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 = 0.46–2.65) with an average effect size of 0.76 (SD = 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 (Rastelli 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 (Eslingier et al., 2009, 2011; Rastelli et al., 2011) these results were not consistent: the smallest effect size was also reported for this SNP (d = 0.46; Panhu et al., 2013).

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 NRGI SNP on WM integrity in the left ATR (Sprooten et al., 2009). Large effect sizes were also observed for all the other studies examining the impact of NRGI on WM integrity (d > 0.80). Similar effect sizes were revealed for studies investigating the ErbB4 gene, with Cohen's d for these studies ranging from 0.83 to 1.41. Both the MTIF 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.

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).

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 individuals 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.

FUNCTIONAL CONNECTIVITY STUDIES
To date, the effects on functional connectivity of a number of candidate SZ genes (DISC1, PRODH, PPP1R1B) and one gene with genome-wide significance for SZ risk (ZNF804A), each of which were found to be associated with altered functional connectivity. The mean effect size for the functional connectivity studies was large (d = 0.76), with the largest effect size reported for the impact of the rs1344706 variant in ZNF804A. The ZNF804A risk variant rs1344706 has become the focus of much interest in SZ research over the last 3 years, as GWAS and follow-up analyses have established strong evidence for a link between this variant and risk for the disorder (O'Donovan et al., 2008). While the function of the ZNF804A gene is unknown, it has been speculated to play a role in gene regulation (O'Donovan et al., 2008) and glutamate and dopamine transmission (Eslingier et al., 2009). ZNF804A represents the only SZ-implicated gene that we are aware of whose effects on functional connectivity have been investigated now on a number of occasions. Importantly, the size of effect reported for this variant has varied considerably. For example, as well as having the highest effect of the variants considered it also has the lowest effect of the variants considered (d = 0.46; Panhu et al., 2013), suggesting that establishing the true effect of any variant on functional connectivity will require investigation in multiple and adequately powered cohorts. Variants from the candidate SZ gene literature by comparison, while each showing large effects on functional connectivity (d-range: 0.73–0.87), have each only been the subject of
### 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>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>
<td></td>
</tr>
<tr>
<td></td>
<td>R. PFC – striatum, frequent haplotype carriers</td>
<td>SC</td>
<td>4.57</td>
<td>126</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. PFC – striatum, frequent haplotype carriers &gt; non-frequent haplotype carriers</td>
<td>SC</td>
<td>4.31</td>
<td>142</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Kempf et al. (2008)</td>
<td>dPFC – striatum, reference haplotype carriers &gt; protective haplotype carriers</td>
<td>SC</td>
<td>3.91*</td>
<td>103/108</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Di Giorgio et al. (2008)</td>
<td>R. hippocampus – R. dPFC, Ser/Ser &gt; Cyto carriers</td>
<td>PPI</td>
<td>3.68*</td>
<td>80</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Eisinger et al. (2008)</td>
<td>R. dPFC – L. hippocampus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.98*</td>
<td>115</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.69*</td>
<td>115</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Eisinger et al. (2011)</td>
<td>R. dPFC – L. MTG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.91*</td>
<td>115</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>&amp; R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>5.09*</td>
<td>111</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. MTG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.69*</td>
<td>111</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. MTG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.69*</td>
<td>111</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. MTG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.91*</td>
<td>111</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. hippocampus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>4.28*</td>
<td>111</td>
<td>0.98</td>
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<tr>
<td></td>
<td>R. dPFC – R. hippocampus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.34*</td>
<td>111</td>
<td>0.64</td>
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</tr>
<tr>
<td>Walker et al. (2011)</td>
<td>R. dPFC – L. inferior frontal gyrus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.77*</td>
<td>109</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. TPJ – L. inferior frontal gyrus, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.16*</td>
<td>109</td>
<td>0.73</td>
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<tr>
<td></td>
<td>L. TPJ – R. thalamus, AA &gt; CA &gt; CC</td>
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<td>3.68*</td>
<td>109</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L. TPJ – L. caudate, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.96*</td>
<td>109</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. rostral cingulate gyrus, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.37*</td>
<td>109</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. MTG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.22*</td>
<td>109</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. LG, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.63*</td>
<td>109</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. LF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.31*</td>
<td>94</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. LF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.23*</td>
<td>94</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. LF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.85*</td>
<td>94</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. HF, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.19*</td>
<td>94</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. dPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>3.34*</td>
<td>94</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – L. dPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.76*</td>
<td>94</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. dPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.35*</td>
<td>94</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. dPFC, AA &gt; CA &gt; CC</td>
<td>SC</td>
<td>2.35*</td>
<td>94</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>2.43*</td>
<td>94</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Paulus et al. (2011)</td>
<td>Controls: R. dPFC – L. HF, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>2.72*</td>
<td>96</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls: R. dPFC – L. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.86*</td>
<td>96</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls: R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>3.21*</td>
<td>96</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls: R. dPFC – L. hippocampus, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>3.34*</td>
<td>96</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls: R. dPFC – R. hippocampus, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>2.89*</td>
<td>96</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Siblings: R. dPFC – R. hippocampus, AA &gt; CA &gt; C carriers</td>
<td>SC</td>
<td>2.63*</td>
<td>83</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Siblings: R. dPFC – R. dPFC, AA &gt; C carriers</td>
<td>PPI</td>
<td>3.76*</td>
<td>83</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>2.73*</td>
<td>83</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, AA &gt; C carriers</td>
<td>PPI</td>
<td>4.01*</td>
<td>83</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>SC</td>
<td>4.68*</td>
<td>33</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, AA &gt; C carriers</td>
<td>PPI</td>
<td>5.62*</td>
<td>33</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>PP</td>
<td>3.84*</td>
<td>33</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients: R. dPFC – R. dPFC, CC &gt; CA &gt; AA</td>
<td>PPI</td>
<td>4.40*</td>
<td>33</td>
<td>1.72</td>
<td></td>
</tr>
</tbody>
</table>

* Sample size, SC: seed–connectivity, PPI: psychophysiological interaction; dPFC: dorsolateral prefrontal cortex; MTG: middle temporal gyrus; SFG: superior frontal gyrus; TPJ: temporo-parietal junction, M2: middle temporal gyrus; L2: 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.

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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>Cohe d's</th>
</tr>
</thead>
<tbody>
<tr>
<td>McIntosh et al. (2008)</td>
<td>NRG1 5'UNPHNR243177</td>
<td>Reduced FA in ALIC</td>
<td>t = 7.65*</td>
<td>43</td>
<td>0.83</td>
</tr>
<tr>
<td>Winterer et al. (2008)</td>
<td>NRG1 5'UNPHNR2271633</td>
<td>Reduced FA in MF subcortical WM</td>
<td>t = 4.67***</td>
<td>50</td>
<td>1.56</td>
</tr>
<tr>
<td>Sprooten et al. (2008)</td>
<td>NRG1 5'UNPHNR2271633</td>
<td>Reduced FA in left ATR</td>
<td>t = 5.52***</td>
<td>28</td>
<td>1.26</td>
</tr>
<tr>
<td>Wang et al. (2009)</td>
<td>NRG1 5'UNPHNR2271633</td>
<td>Reduced FA in anterior cingulum</td>
<td>F = 6.27*</td>
<td>31</td>
<td>0.96</td>
</tr>
<tr>
<td>Conrad et al. (2008)</td>
<td>EnlB4 rs707284</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 4.24***</td>
<td>34</td>
<td>1.64</td>
</tr>
<tr>
<td>EnlB4 rs708440</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 2.61***</td>
<td>50</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>EnlB4 rs239641</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 4.31***</td>
<td>50</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>EnlB4 rs239625</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 4.73***</td>
<td>50</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>G-T-G versus lower risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 3.85***</td>
<td>32</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>G-T-G versus all other</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 3.2***</td>
<td>50</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>All other versus non-risk</td>
<td>Reduced FA in temporal lobe WM</td>
<td>F = 3.66***</td>
<td>62</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Zuliani et al. (2011)</td>
<td>EnlB4 rs497328</td>
<td>Reduced FA in right ALIC</td>
<td>t = 3.48*</td>
<td>36</td>
<td>1.19</td>
</tr>
<tr>
<td>EnlB4 rs497329</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 = 6.19*</td>
<td>79</td>
<td>0.51</td>
</tr>
<tr>
<td>LPS, HPS and APS haplotypes</td>
<td>Association with mean FA in left PT 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 PT 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 = 3.60*</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 = 6.95*</td>
<td>18</td>
<td>1.29</td>
</tr>
<tr>
<td>Padreno et al. (2009)</td>
<td>5-HTTLPR</td>
<td>Increasing number of low expressing alleles – decreasing FA in left F UF</td>
<td>F = 3.03*</td>
<td>37</td>
<td>0.90</td>
</tr>
</tbody>
</table>

n, sample size; FA, fractional anisotropy; MI, medial frontal; WM, white matter; ALIC, anterior limb of internal capsule; AD, axial diffusivity; RD, radial diffusivity; ATR, anterior thalamo-radiation; U, uncinate fasciculus; GCC, genu of corpus callosum; CST, corticospinal tract; PT, pentothal; DACC, dorsal anterior cingulate cortex; UF, frontal uncinate fasciculus; *p < 0.05; **p < 0.01; ***p < 0.001; *p < 0.05 family-wise error corrected

Single studies to date (DISCH; Di Giorgio et al., 2006; PRODHI; Kempt et al., 2006; PPVR1B; Meyer-Lindenberg et al., 2007).

In 10 of the 12 fMRI 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 (Callcott et al., 2003). There are several possibilities for why PFC function is altered in SZ as reflected in these studies. For example, the "reduced neuropil hypothesis" of schizophrenia suggests that reduced PFC gray matter (observed in the absence of a concomitant change in cell numbers/mass) reflect decreased dendritic spine/axon terminal density, inefficiencies in synaptic transmission expected to result may well lead to the altered functional connectivity patterns seen here (Seiden and Goldman-Rakic, 1999).

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) and 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 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 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 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>
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</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>
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<tr>
<td>Total between</td>
<td></td>
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</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-HTTLPR 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 (Goldbry et al., 2007). The remnant 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 (Lindberg 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 (Chuang et al., 2011) and DISC1 (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 (Toft 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 Merrer-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., 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 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 (Nicolodermus 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., 2008).
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).

Integration of DT with other measures of WM such as measures of axon density and myelination can be acquired using techniques such as magnetization transfer or multicomponent relaxed echo. 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

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Altered medial prefrontal activity during dynamic face processing in schizophrenia

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Abstract

Background: Processing the emotional content of faces is recognised as a key deficit of schizophrenia, associated with poorer functional outcomes and possibly contributing to the severity of clinical symptoms such as paranoia. At the neural level, fMRI studies have reported altered limbic activity in response to facial stimuli. However, some studies may be limited by the use of cognitively demanding tasks and static facial stimuli. The current study used a face processing task involving implicit face processing and dynamic stimuli to further characterise neural activity differences in emotional brain regions in schizophrenia patients relative to healthy controls.

Methods: Functional MRI was used to examine neural activity in 25 patients with a DSM-IV diagnosis of schizophrenia and 21 age- and gender-matched healthy controls while they participated in a face processing task designed by Grosbras and Paus (2006), which involved viewing video clips of angry and neutral facial expressions, and a non-biological baseline condition.

Results: While viewing faces (angry and neutral combined versus baseline), patients showed significantly weaker deactivation of the medial prefrontal cortex, including the anterior cingulate, and decreased activation in the left cerebellum, compared to controls. Patients also showed weaker medial prefrontal deactivation while viewing the angry faces relative to the baseline condition.

Discussion: Given that the anterior cingulate plays a role in processing negative emotion, weaker deactivation of this region in patients while viewing faces may contribute to an increased perception of social threat, which may in turn contribute to paranoia. Further examination of the neurobiology of social cognition in schizophrenia using fMRI may help establish targets for treatment interventions.
1. Introduction

Social cognition is a broad construct consisting of cognitive processes that allow people to perceive, interpret and store information about themselves and others (Van Overwalle, 2009; Penn et al., 2008). Examples include recognising emotions from facial expressions or tone of voice, or thinking about the thoughts and goals of others. Deficits in social cognition have been identified in several neurodevelopmental disorders including autism (Frith, 2001), attention deficit hyperactivity disorder (ADHD) (Uekermann et al., 2010), and schizophrenia (Couture et al., 2006). In schizophrenia, social cognitive deficits are a defining feature, affecting quality of life and functional outcomes in work, relationships and independent living (Brekke et al., 2005; McGlad et al., 2008). For example, a recent meta-analysis by Fett et al., (2011) suggests that social cognition predicts more variation in social and occupational functioning than cognitive performance alone.

One aspect of social cognition that is significantly impaired in schizophrenia is processing the emotional content of faces (Li et al., 2010). For example, patients have been reported to have difficulties recognising emotions from faces (Aleman and Kahn, 2005), but are also more sensitive to negative facial expressions such as anger and fear compared to healthy controls (Mandal et al., 1998; Evans et al., 2011). Excessive threat detection from facial expressions has also been hypothesised to contribute to the development of persecutory delusions (Green and Phillips, 2004), which are associated with patient distress (Freeman et al., 2002) and predict admission to hospital (Castle et al., 1994).
At the neural level, emotional face processing activates limbic regions including the amygdala, which is important for detecting the emotional salience of a stimulus and generating an affective response, and the medial prefrontal cortex (mPFC) and anterior cingulate (ACC), which are important in expressing negative emotion and regulating other limbic regions (Etkin et al., 2011). Meta-analysis of fMRI studies by Li et al. (2010) indicates that schizophrenia patients generally show reduced activation of the bilateral amygdala in response to emotional faces compared to healthy controls, which may contribute to difficulties understanding the emotions of other people. Similarly, Hempel et al., (2003) and Habel et al., (2010) report decreased activation of the ACC in patients viewing emotional faces. However, increased activation of the amygdala and ACC have been reported in response to neutral faces in patients versus controls, which may result in patients mistakenly attaching emotional salience to non-emotional expressions (Hall et al., 2008; Habel et al., 2010).

There are two limitations, however, with studies of face processing in schizophrenia to date. One limitation is that many studies have used explicit face processing tasks, where participants must judge the emotional content of the faces presented and select an emotion from a list of two or more. Taylor et al., (2003) have previously shown that explicit emotional face processing during a task can reduce neural activity in limbic regions. Some studies have tried to overcome this limitation, for example, by instructing participants to determine the gender of faces to ensure attention to the task but also to make sure that the emotion recognition component of the task was implicit (e.g. Phillips et al., 1999). However, this type of task may also modulate limbic activity, given the widespread dysconnectivity and cognitive deficits observed in schizophrenia (Stephan et al., 2009; Meyer-Lindenberg et al., 2005); e.g. these tasks
may influence limbic responses in ways that vary with connectivity, cognitive ability and/or task difficulty (Holt et al., 2006). Therefore, implicit face processing tasks with minimal additional demands may provide a more accurate measure of neural activity during face processing.

A second limitation is that many of the previous studies of face processing in schizophrenia have used static stimuli, such as Ekman’s Pictures of Facial Affect (Ekman et al., 1975) (e.g. Holt et al., 2006). However, human faces and facial expressions are dynamic in nature, and temporal cues contribute to the recognition of facial expressions (e.g. Sato et al., 2004). Therefore, tasks that include dynamic facial expressions may more accurately reflect real world social interactions. fMRI has revealed that several brain regions, including the amygdala, are more active while viewing dynamic facial expressions compared to static images (Sato et al., 2004), and task-induced functional connectivity between the amygdala and cingulate is also increased in response to dynamic facial expressions compared to static stimuli (Foley et al., 2012). Similar to studies using static images, schizophrenia patients show poorer ability to recognise dynamic facial expressions compared to healthy controls during neuropsychological examination (Johnston et al., 2010). Also, patients have been reported to show increased limbic activation while watching dynamic fearful faces compared to controls under fMRI (Russell et al., 2007).

The purpose of the present study was to further explore and characterise activation differences between schizophrenia patients and healthy controls during face processing. We used a dynamic face processing task designed by Grosbras and Paus (2006), which
is also being used to examine the effects of cognitive remediation therapy for psychosis (Cognitive Genetics and Remediation (CogGene) Laboratory, Ireland), and in the IMAGEN project, a Europe-wide longitudinal imaging genetics project examining risk factors for mental illness in fourteen-year-old adolescents (Schumann et al., 2010). We examined neural activation in patients with schizophrenia/schizoaffective disorder and age- and gender-matched healthy controls during passive viewing of dynamic angry and neutral faces. Specifically, we tested the hypothesis that patients with schizophrenia or schizoaffective disorder would show altered limbic activity while passively viewing dynamic angry and neutral facial expressions, compared to healthy controls.

Identifying differences in these regions in patients during a task that involves both (1) implicit face processing, and (2) dynamic face stimuli is important for better understanding the neurobiological correlates of social cognitive deficits in schizophrenia and identifying targets for further treatment.
2. Methods

Sample characteristics: 39 patients with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder were recruited for the present study. All subjects were right-handed, aged between 18 and 65, had no history of substance misuse in the preceding six months, no prior injury to the head associated with a loss of consciousness of more than a few minutes and provided consent in compliance with the local ethics committee. Five patients were excluded due to excessive movement during functional imaging (>3mm translation and/or 3° rotation), seven patients were excluded due to bad quality MRI data and/or significant artefacts and two patients were excluded due to missing data for the faces follow-up task (see below), yielding a total of 25 patients.

These patients were then compared to 21 healthy controls matched for age and gender from our on-going imaging genetics study on psychosis (Rose et al., 2012a; Rose et al., 2012b; Rose et al., 2013; Mothersill et al., 2013).

Emotional face processing task: Subjects performed an emotional face processing task designed by Grosbras and Paus (2006), which has previously been shown to robustly activate several brain regions involved in face and emotion processing, such as the amygdala and medial prefrontal cortex (Grosbras and Paus, 2006; Schneider et al., 2011; Tahmasebi et al., 2012; Mothersill et al., 2013). During the task, participants watched a series of 2-5 second black-and-white video clips of faces starting from a neutral expression, and then turning into an angry expression or displaying a neutral/ambiguous expression (e.g. licking of lips). A non-biological control condition consisted of videos of black and white concentric circles expanding and contracting.
Videos were divided into 18-second blocks consisting of four to seven video clips. Overall five angry, five neutral and nine baseline blocks were presented (19 blocks in total). The total number of exposures to each condition was the same between participants. To ensure that participants had paid attention to the face videos, participants completed a face recognition task following their time in the scanner based on a still shot of faces presented in the scanner and a series of foils. Patients were excluded if they scored < three/five correct answers for this follow-up task. Healthy controls were excluded if they scored < four/five correct answers for this follow-up task.

**MRI acquisition parameters:** All participants were imaged using a Philips Intera Achieva 3T MRI scanner (Philips Medical Systems, Best, The Netherlands) with a SENSE 8-channel head coil, in the Trinity College Institute of Neuroscience. Whole-brain BOLD EPI was acquired with 40, 2.4 mm slices, 1 mm slice gap, TR = 2200 ms, TE = 30 ms, field of view = 220 x 220 mm, flip angle = 75° and spatial resolution = 3.4 x 3.4 x 2.4 mm³. Functional scanning lasted 160 TRs or ~5.87 minutes.

In addition, a T1-weighted image (180 slices, ~6 minutes) was acquired using a TFE gradient echo pulse sequence, with slice thickness of 0.9 mm, a 230 x 230 field of view, and a spatial resolution of 0.9 x 0.9 x 0.9 mm³.

**MRI data pre-processing and analysis:** Spatial pre-processing and statistical analysis of MRI data was performed using Statistical Parametric Mapping (SPM8, revision 258).
Functional images were first realigned to the mean functional image to reduce variance due to movement. The structural image was then co-registered to the mean functional image for more precise spatial normalisation. Functional images were normalised to MNI (Montreal Neurological Institute) space using the unified segmentation approach with a voxel size of $3 \times 3 \times 3 \text{ mm}^3$ (Ashburner and Friston, 2005), and subsequently smoothed using a 10 mm FWHM (full width at half maximum) isotropic Gaussian filter (i.e. a kernel width two-three times greater than the original voxel size). After pre-processing, graphical plots of estimated time series of translations and rotations were carefully inspected for excessive motion in each participant.

Statistical analysis was performed using the general linear model (GLM) (Friston et al., 1994). A boxcar function was created for each condition and convolved with a haemodynamic response function (HRF). This first-level model included these three condition regressors (i.e. angry faces, neutral faces and baseline) and 6 regressors to model head movement. A high-pass filter of 128 s was used to remove low-frequency signals (e.g. scanner drifts, physiological noise) and serial correlations in the fMRI time-series were accounted for by an autoregressive AR(1) model. Next, t-contrasts were used to model condition effects at each voxel for the following contrasts: faces (angry and neutral combined) versus baseline and angry faces versus neutral faces. We also modelled effects of the angry and neutral conditions separately (i.e. contrasts of angry faces versus baseline and neutral faces versus baseline), consistent with previous
studies of emotion recognition in schizophrenia (e.g. Habel et al., 2010; Holt et al., 2006).

Individual contrast maps were entered into a second-level random effects analysis to investigate task effects at the group level (independent t-test between groups). Results were examined at a p<0.001 (uncorrected) level and clusters were considered statistically significant at a p<0.05 level, family-wise error (FWE) corrected for multiple comparisons across the whole brain at the cluster level. Coordinates of results in MNI space were converted to Talairach space using BrainMap GingerALE 2.1 software (Eickhoff et al., 2009; Turkeltaub et al., 2012). Talairach Client 2.4.3 (Lancaster et al., 1997; Lancaster et al., 2000) was then used for anatomical localisation of these coordinates.
3. Results

**Participant demographics:** Independent t-tests were performed to compare age and years of education between groups in SPSS (19.0.0); a Pearson's chi-squared test was performed to compare gender frequencies between groups. There were no significant differences between groups for age or gender (p>0.05; see Table 1). As there were significant differences between groups for years of education, we examined the effects of this variable across our sample for all contrasts examined (multiple regression with years of education as covariate of interest). There were no significant effects of education observed on neural activation.

>> Table 1 <<

**Neural activation:** Relative to controls, patients showed weaker deactivation of the bilateral ACC and left medial frontal gyrus while viewing faces (angry and neutral combined) \((t_{(46)}=4.85; p<0.05\), corrected; see Table 2 and Figure 1). Patients also showed reduced activation of the left cerebellum relative to controls \((t_{(46)}=4.49; p<0.05\), corrected; see Table 2 and Figure 1). No significant differences were observed when the groups were compared using a contrast of angry versus neutral faces.

Examining the angry and neutral face conditions separately, patients also showed weaker deactivation of the mPFC/ACC during the angry faces versus baseline condition \((t_{(46)}=5.94; p<0.05\), corrected; see Table 3 and Figure 1), while altered mPFC activity...
was only observed at uncorrected thresholds ($p<0.001$) during the neutral faces versus baseline condition.

As an additional data quality check, in each individual the average parameter estimates of all voxels was calculated for each cluster that showed a significant activation difference between groups. Next, average parameter estimates were checked in SPSS (19.0.0) for the presence of outliers. One outlier was identified for the faces versus baseline contrast; as removal of this participant did not significantly affect results, results are reported with all participants included.

>> Table 2 <<

>> Table 3 <<

>> Figure 1 <<
4. Discussion

This study examined neural activity in patients with schizophrenia and healthy controls during a dynamic face-processing task. While passively viewing faces (regardless of emotional content), patients showed weaker deactivation of the mPFC/ACC compared to controls, and decreased left cerebellum activation. While viewing the angry faces specifically, patients also showed a pattern of weaker mPFC deactivation relative to controls.

Although schizophrenia patients show deficits in facial emotion perception, they are hypersensitive to expressions of fear and anger (Mandal et al., 1998; Evans et al., 2011). For example, Evans et al. (2011) have previously reported that schizophrenia patients show increased aversion to angry faces during an associative learning task. Our finding of weaker mPFC/ACC deactivation during angry face processing in patients compared to healthy controls supports this view, given the role of the ACC in processing negative emotion. Increased detection of social threat (or cortical reaction to anger in others) may contribute to the development of paranoia.

Notably, our finding contrasts with one previous imaging study (Habel et al., 2010) which reported reduced ACC activity in schizophrenia patients compared to controls while viewing angry faces; however, Habel et al. used an explicit emotional face processing task which the authors suggested may not be comparable to implicit tasks due to differing cognitive demands; e.g. explicit face processing tasks may modulate neural activity in limbic regions. Further research using different tasks will be required.
to elucidate specific differences between explicit and implicit face processing in schizophrenia.

An alternative interpretation of the present results is that the weaker mPFC deactivation observed in patients reflects altered function of the default mode network, a network of brain regions that are more active when a person is not engaged in a cognitive task, i.e. at rest (Raichle et al., 2001). The default mode network, which includes the mPFC, has been hypothesised to play a role in a variety of functions such as self-referential processing that may be important for social cognition (Buckner et al., 2008).

Given that default mode hyperactivity has been observed in schizophrenia patients during cognitive tasks (e.g. Garrity et al., 2007), the present finding of weaker deactivation of the mPFC in patients during face processing may reflect a reduced ability to disengage this network when attending to the faces. This may lead to impairments in accurately processing the facial stimuli as patients may not be able to devote the same cognitive resources to the task. Future studies could incorporate a behavioural component to examine if mPFC activity was correlated with reduced task accuracy or reaction time. This could be performed using task stimuli, but after the scan, in order to minimise confounding effects of explicit face processing on neural activation.

Although the causes of altered mPFC/ACC activity observed in schizophrenia are not fully understood, the aberrant function observed in these regions may partially result
from altered brain structure and cell density. For example, studies of post-mortem brains of schizophrenia patients report reduced expression of astrocyte markers in the deep layers of the ACC, suggesting a population of these cells are adversely affected in the ACC in schizophrenia patients (Katsel et al., 2011). This could lead to altered neural activity, given the suggested role of astrocytes in neuronal signalling (Navarrete et al., 2013).

Altered ACC function may also result from altered grey matter volume in this region, and reduced structural connectivity to other parts of the cortex. For example, Zhou et al., (2005) and Fujiwara et al., (2007) report decreased ACC volume, and Fujiwara et al. also report decreased white matter integrity in the cingulum (which connects the ACC to other cortical regions), in schizophrenia patients compared to healthy controls. Fujiwara and colleagues also reported that reduced ACC volume was associated with significantly impaired emotional face processing in the same sample of patients and healthy controls, further highlighting the importance of this region for social cognition.

These differences in both form and function are likely to be driven by a combination of environmental and genetic factors. For example, Lederbogen et al. (2011) used fMRI and the Montreal Imaging Stress Task (MIST) to examine neural activation in healthy volunteers with an urban or rural upbringing. In the MIST, participants perform arithmetic tasks within a limited amount of time, while also receiving negative feedback on performance by study investigators. Urban upbringing was associated with increased ACC activation during social stress, suggesting that activity in this region may be particularly sensitive to early-life environmental factors when perceiving social
threat. Early life stress may affect ACC function via increased levels of the stress hormone cortisol, high levels of which have previously been associated with reduced ACC volume in other psychiatric disorders (Treadway et al., 2009).

The ACC may be susceptible to genetic variation also. For example, Voineskos et al., (2011) report that healthy volunteers carrying two copies of the genome-wide associated schizophrenia risk variant, rs1344706, within ZNF804A, show reduced grey matter cortical thickness in this region. A priority for future studies will be to examine possible gene x environment (G x E) interactions on face processing and other measures of social cognition.

The cerebellum also plays an important role in social cognition (Van Overwalle et al., 2013), including processing negative facial emotions (Ferrucci et al., 2012). It will also be a priority of future imaging studies of schizophrenia to examine how altered activity in this region during face processing might also affect social cognitive performance.

One limitation with the current study is the heterogeneity of the patient sample, which includes patients with a diagnosis of schizophrenia or schizoaffective disorder, with different doses of anti-psychotic medication, and heterogeneous positive and negative symptom severity. Future studies examining dynamic face processing in patient subgroups, critically with larger sample sizes in each subgroup, will help determine whether patterns of neural activity observed here are consistent across these groups (e.g.
grouping schizophrenia and schizoaffective disorder separately, examining patient groups with similar medication doses and symptoms).
5. Conclusions

In conclusion, we report significantly weaker mPFC/ACC deactivation and decreased cerebellum activity in schizophrenia patients during implicit processing of dynamic facial stimuli relative to healthy controls. Further examination of the neurobiology of social cognition in schizophrenia using fMRI may help establish targets to probe effects of emerging treatments (e.g. cognitive remediation therapy), and/or help identify genetic or environmental risk factors for illness, which may help in the development of prevention strategies.
Acknowledgments

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Financial Disclosures

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

Table 1: Participant demographics

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<th>Mean age (s.d.(^a))</th>
<th>Mean years of education (s.d.(^b))</th>
<th>Gender (M:F)</th>
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<td><strong>Healthy controls (N=21)</strong></td>
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\(^a\)s.d. = standard deviation; \(^b\)Education available for 45 of 46 participants

\(^c\)t value derived from independent t-tests between groups; \(\chi^2\) value derived from Pearson’s chi-squared test with variables group and gender

Mean medication (and s.d.) in patient group (chlorpromazine equivalent in mg/day) = 377.52 (270.82)

Mean SAPS in patient group (Scale for the Assessment of Positive Symptoms (Andreasen, 1984(a))) = 9

Mean SANS in patient group (Scale for the Assessment of Negative Symptoms (Andreasen, 1984(b))) = 21

Note - SAPS/SANS scores listed above are mean scores for the schizophrenia patient sample recruited for neuroimaging as part of a psychosis research project in Trinity College, of which the 25 patients included in this fMRI study are a subset.
Table 2: Clusters, including individual peaks, showing significant activity differences between schizophrenia patients and controls during face processing (neutral and angry faces combined, relative to baseline), corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Extent (voxels)</th>
<th>p</th>
<th>Direction</th>
<th>Cluster t- value</th>
<th>Z- value</th>
<th>Peak coordinates (MNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patients &gt;</td>
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<tr>
<td>1</td>
<td>699</td>
<td>&lt;0.00</td>
<td>Right</td>
<td>4.85</td>
<td>4.32</td>
<td>6 32 4</td>
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<td></td>
<td>controls</td>
<td>anterior</td>
<td></td>
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<td></td>
<td></td>
<td>cingulate</td>
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<td></td>
<td>/ BA 24</td>
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<td></td>
<td></td>
<td>Left</td>
<td>4.71</td>
<td>4.22</td>
<td>-9 56 4</td>
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<td>Patients &lt;</td>
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<td>-39 -70 -23</td>
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<tr>
<td>Side</td>
<td>T-value</td>
<td>p-value</td>
<td>X</td>
<td>Y</td>
<td>Z</td>
<td></td>
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</tr>
<tr>
<td>Left</td>
<td>4.03</td>
<td>3.70</td>
<td>-18</td>
<td>-76</td>
<td>-35</td>
<td></td>
</tr>
<tr>
<td>cerebellum</td>
<td>m</td>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Side</th>
<th>T-value</th>
<th>p-value</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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</thead>
<tbody>
<tr>
<td>Left</td>
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<td>3.45</td>
<td>-24</td>
<td>-79</td>
<td>-23</td>
</tr>
<tr>
<td>cerebellum</td>
<td>m</td>
<td></td>
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</tbody>
</table>

*p values are FWE-corrected for multiple comparisons at the cluster level*
**Table 3:** Clusters, including individual peaks, showing significant activity differences between schizophrenia patients and controls during angry face processing compared to baseline, corrected for multiple comparisons at the cluster-level.

<table>
<thead>
<tr>
<th>Cluster Extent (voxels)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Direction</th>
<th>Cluster Direction</th>
<th>t- value</th>
<th>Z- value</th>
<th>Peak Coordinates (MNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>813</td>
<td>&lt;0.00</td>
<td>Patients &gt; Left</td>
<td>5.94</td>
<td>5.06</td>
<td>-9 56 4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>controls</td>
<td>medial</td>
<td>frontal</td>
<td>gyrus/B BA 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>5.37</td>
<td>4.68</td>
<td>12 38 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>anterior</td>
<td>cingulate</td>
<td>gyrus/ BA 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>5.02</td>
<td>4.44</td>
<td>-3 41 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>anterior</td>
<td>cingulate</td>
<td>gyrus/ BA 24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>p values are FWE-corrected for multiple comparisons at the cluster level
Figure 1: Altered neural activity in schizophrenia patients during face processing

Red-yellow: Brain regions showing altered activity during face processing in patients relative to healthy controls (N=46; independent t-test between groups; significance is set at p<0.001 uncorrected without a cluster threshold for display purposes; d.f. = 44).

Colour bars represent t-values. Each 2D sagittal slice is labelled with a MNI-coordinate. Clusters are rendered on the ‘ch256’ brain template using MRicroGL (http://www.mccauslandcenter.sc.edu/mricrogl/). Additional editing of figure (e.g. changing the size/resolution) performed using MS Paint and Paint.NET v3.5.10. Faces
= Faces (angry and neutral combined) versus baseline contrast; Angry = Angry versus baseline contrast; a.u. = arbitrary units; mPFC = medial prefrontal cortex.
Appendix E

List of conference talks/papers and Faculty of 1000 evaluations contributed to as part of this thesis, and awards won
Conference talks:

1. Effects of common and rare NOS1 (nNOS) variants on neurocognitive intermediate phenotypes for schizophrenia. 11th World Congress of Biological Psychiatry, 23rd - 27th June, 2013, Kyoto, Japan.

Conference posters:

1. Increased medial prefrontal activity during dynamic face processing in schizophrenia. Galway Neuroscience Annual Research Day, 5th December, National University of Ireland Galway, Galway, Ireland.


4. Effects of a schizophrenia-associated variant rs10503253, within the CSMD1 gene, on brain activity and functional connectivity. 7th Annual Neuroscience Ireland Meeting, 5th - 6th September, 2012, Royal College of Surgeons, Dublin, Ireland.

5. Effects of a schizophrenia-associated variant rs10503253, within the CSMD1 gene, on functional connectivity. 8th Federation of European Neuroscience Societies Forum of Neuroscience, 14th - 18th July, 2012, Barcelona, Spain.

6. Investigating the impact of psychosis risk genes on neural connectivity: A review and quantification of effects. Trinity College School of Medicine
Tercentenary Symposium, 4\textsuperscript{th} November, 2011, Trinity College Dublin, Dublin, Ireland.

7. The \textit{NOS1} Polymorphism rs6490121 is Associated with Variation in Prefrontal Function and Gray Matter Density in Healthy Individuals. Wiring the Brain conference, 12\textsuperscript{th} - 15\textsuperscript{th} April 2011, Wicklow, Ireland.

Awards:

1. 1st place, Poster Competition, Wiring the Brain conference, 12\textsuperscript{th} to 15\textsuperscript{th} April 2011, Wicklow, Ireland.

Faculty of 1000 (F1000) evaluations:

1. Recommended by Gary Donohoe and Omar Mothersill: Coupling social attention to the self forms a network for personal significance (Sui \textit{et al.}, 2013). http://f1000.com/prime/718174267

2. Recommended by Gary Donohoe and Omar Mothersill: Disordered corticolimbic interactions during affective processing in children and adolescents at risk for schizophrenia revealed by functional magnetic resonance imaging and dynamic causal modelling (Diwadkar \textit{et al.}, 2012). http://f1000.com/prime/717547820

4. Recommended by Gary Donohoe and Omar Mothersill: Abnormal medial prefrontal cortex resting-state connectivity in bipolar disorder and schizophrenia (Chai et al., 2011). http://fl000.com/13954956

5. Recommended by Gary Donohoe and Omar Mothersill: Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task (Walter et al., 2011). http://fl000.com/11515957

6. Recommended by Gary Donohoe and Omar Mothersill: Exaggerated brain activation during emotion processing in unaffected siblings of patients with schizophrenia (van Buuren et al., 2011). http://fl000.com/13393963