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The Impact of Schizophrenia Genome Wide Association Study Genes on Neurocognition and Social Cognition: A Behavioural Study.

By

April Hand Hargreaves

2013

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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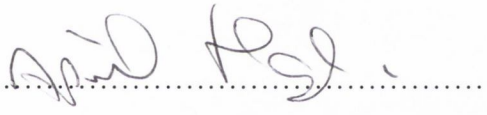

April Hand Hargreaves

Summary

Genome wide association studies (GWAS) have established numerous previously unidentified variants associated with increased risk for schizophrenia (SZ). Exactly how most of these variants exert their influence remains largely unknown. Deficits in cognition and social cognition are core, stable, debilitating symptoms of psychotic disorders including SZ, and increased genetic risk for SZ is hypothesised to be at least partly mediated via a deleterious effect on these. This thesis aims to characterise the effects of GWAS identified SZ risk variants on variation in cognition and social cognition, based on analyses of both single variants and biological pathway specific polygenic risk.

Chapter 1 outlines the evidence in literature supporting the use of neurocognitive and social cognitive variables as intermediate phenotype measures for SZ. The literature review focuses in particular on assessing the value of social cognitive variables in characterising the effects of SZ associated risk variants. It reveals a paucity of research on emotion recognition as measured by more ecologically valid dynamic (moving) stimuli. Chapter two, based on the emotion recognition task designed by Montagne (2007) looks at the processing of emotion recognition based on dynamic visual stimuli in psychosis. This is the first study to our knowledge that investigates the role of dynamic facial stimuli presented at varying intensity levels on performance accuracy on an emotion recognition task in patients with psychosis and healthy subjects. The data supports a role for the use of dynamic stimuli in this line of research and also highlights the impact of emotion intensity on correct identification.

Chapters three, four, five, and six present a series of neuropsychological and social cognition studies of GWAS identified common SZ risk vaiants. These include 1) novel SZ variants identified by the largest SZ GWAS to date (Ripke, 2011)(Chapter three), 2) the first risk variant identified by SZ GWAS – the *Zinc finger binding protein 804A (ZNF804A)* (Chapter four) and 3) polygenic risk as calculated from risk allele load in two pathways; *ZNF804A* (Chapter five) and *cell adhesion molecule (CAM)*(Chapter six). Findings support an association with

cognitive decline for a third of the SZ GWAS variants under investigation (*MIR-137, CSMD1, CNNM2, ZNF804A, HLADQA1*). Those that were associated with neurocognition were not associated with social cognition and vice versa. Analysing association with cognition in terms of polygenic risk showed that whilst *ZNF804A* pathway polygenicity explained more variation in neuropsychological performance than single variant analysis, *CAM* pathway polygene scores and individual common variants explained a comparable amount of variation.

In conclusion, this thesis provides an original contribution to the cognitive genetics of SZ in several ways. Firstly, a literature review provides support for the feasibility of using social cognitive variables as endophenotypes. Secondly, in emotion recognition tasks, the importance of dynamic images and emotional intensity are highlighted for patients with psychosis. Thirdly, the research demonstrates that the novel SZ GWAS variants of *CSMD1, HLADQA1* and *MIR-137* are associated with neurocognition, whilst *CNNM2* and *ZNF804A* are associated with social cognition. Fourthly, whilst polygenic analysis of SZ associated variables may account for more variation in neuropsychological performance than single risk variants in some pathways (*ZNF804A*), in other pathways (*CAM*) polygenic analysis explains a comparable amount of variation to single variant analysis. Taken together, these findings support the continued role of common variants in the investigation of cognitive and social deficits in SZ.

Acknowledgements

"It is good to have an end to journey toward; but it is the journey that matters, in the end."
— Ernest Hemingway

The truest joy of completion is to be able to look back over the journey and smile: smile at the highs and lows, the lessons learned, the knowledge gained; but equally importantly, smile at the memory of the teachers, friends and family who have enabled each step along that road.

I would like to express my heartfelt gratitude to Professor Gary Donohoe; my supervisor and mentor. A geographical wide advisory search (GWAS) could not have unfurled a better role model; you have been inspirational, supportive, and above all, patient. I am proud of my academic roots and hope that I, in turn, can pass on the research values and dreams that you have cultivated in me. You have bolstered me professionally and personally throughout my PhD journey and I feel very fortunate that you were there, lighting the auxiliary beacons as I clambered the many peaks and troughs. Thank you.

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I am grateful to my wonderful departmental colleagues who have provided a rich and fertile environment in which to study and explore new ideas. Omar, our early morning philosophical discussions often injected me with enough stimulation juice to buckle down and swamp myself with all things 'neuro'. Your gusto for science, passion for knowledge and penchant for forward thinking intrigues and motivates me. Sinead, your unassuming enthusiastic energy and continued intellectual camaraderie has been enjoyable, informative and motivating. Christina, Denise and Catherine, thanks for providing not only the patients you recruited, but also the breaks in between the slog. I find it impossible to picture the stone building (aka refuge, haven, escape) without you all there, smiling encouragingly.

I often ask myself how my research would have fared without the help and support of the Maguire brothers. Jim and Adam, knowing my sons were safe with you when I could not be with them freed my mind to concentrate on the science. You are my surrogate family – jewels in the night. Thank you. The chats over tea, the bottles of Jack, the moans, the laughs, the madness – being able to share it all with Mick, Lal and Ultan – I think at times it darned near saved me. To Emma (my precious friend) and Sheelagh (you mad yoke), I will never forget your encouragement. Always there, as you have been since childhood, you listened, consoled and bolstered over the countless cups of coffee we have shared and for that I am forever grateful.

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Chapter 1

Introduction

Schizophrenia (SZ) is associated with severe **cognitive** deficits that interfere significantly with daily functioning and quality of life and contribute to chronic disability and unemployment (Bilder et al, 2011). In people with SZ, cognitive impairment can be considered a core deficit, in that it is detectable from the earliest age at which children receive any formal psychological testing - as early as the age of 6 or 7 years (Bilder et al, 2006). The underlying pathology is most likely present in some form at birth. Children who go on to develop SZ are already performing at nearly a full grade equivalent below their peers by first grade, and there appears to be a period of further cognitive decline between the ages of 12 and 17—several years before the first psychotic episode (Bilder et al, 2006; Ang, 2004). This cognitive deficiency remains stable throughout the patient's clinical history, regardless of psychotic state, with scores of global cognition ranging from between 1 and 2 standard deviations below those of healthy cohorts (Heinrichs, 1998).

Initially it was hoped that new antipsychotic drug treatments might improve these deficits, but large-scale effectiveness studies suggest that antipsychotic agents bestow very limited cognitive benefit (Hill, 2010). On account of this, current research aims to to identify molecular targets relevant to cognitive dimensions rather than the traditional symptomatic dimensions of schizophrenia (Carter & Barch, 2007; Harvey, 2007). Genetics, and the development of genomic research, offers real opportunities to understand the molecular mechanisms relevant to cognition, both normal and aberrant, as well as opportunities for increasing understanding of pathophysiology and developing more effective treatments.

This chapter defines and describes the main cognitive phenotypes, both traditional (IQ, memory and attention) and social, which are investigated in schizophrenia. With high-throughput genomics it is now possible to explore novel common (and rare) risk variants for psychiatric disorders and their role in cognitive function at a genome-wide level. The outcome of this research to date is reviewed and the obvious next steps required to progress this work in a meaningful direction is discussed.

1.1 The genetics of cognition

Both schizophrenia and cognitive impairment are highly heritable - the heritability of schizophrenia is estimated at greater than .8, while the heritability of cognitive phenotypes is most often found to be near .5, regardless of whether the estimate is derived from healthy or ill groups (Sabb, 2008). Heritability (h^2) is the estimation of the proportion of individual differences in performance in a population at a given time that are due to genetic differences. It is estimated from the similarities observed in subjects varying in their level of genetic similarity (e.g. monozygotic twins share 100% of genes, dizygotic twins/siblings 50%, and half-siblings 25%). The availability of twin and population-based disease register data has confirmed the importance of heritability for many psychiatric disorders. Unfortunately such twin data has not been so readily available to enable confirmation of the heritability of cognitive deficits. Due to this practical sampling constraint, most epidemiological studies have used a family-based design to investigate cognitive deficits in healthy relatives of patients. The finding that this group has higher rates of a particular cognitive deficit than the general population is taken as suggestive evidence of heritability. Until recently, research identifying the shared components of cognitive phenotypes and schizophrenia has found them to be significantly correlated, with much of this covariance (72% to 92%) due to shared genetic effects (Toulopoulou, 2007). This overlap is disputed at present with Fowler et al (2012) suggesting that in epidemiological studies the amount of shared covariance between cognition and SZ that can be attributed to shared genetic effects is ~10%. In the case of schizophrenia, the heritability of a number of specific deficits have been confirmed by family and twin studies, including general cognitive ability, working memory, and episodic memory (Goldberg et al, 1990/1995; Cannon et al., 2000; Kremen et al, 2006; Toulopoulou et al, 2007/2010). The heritability of deficits in general cognitive ability and working memory in particular appear to overlap strongly with the heritability of illness risk in schizophrenia (Toulopoulou et al, 2007) although not to the point of suggesting an identity between the genetic architecture of schizophrenia and cognition.

1.2 Testing cognitive deficits in neuropsychiatric genetics

Cognitive neuroscience approaches to investigating both cognitive performance in the healthy population and cognitive disability associated with psychiatric disorders have included behavioral paradigms both of the neuroimaging variety (such as functional Magnetic Resonance Imaging (fMRI), and electroencephalogram recordings (EEG)), and of the traditional variety. The more traditional neuropsychological tasks include paper and pen tests (e.g. the Wechsler Intelligence Scales, the Wechsler memory scales) and computer based tasks (e.g. various versions of the Continuous Performance Task (CPT); the Cambridge automated test battery). Selection of specific cognitive functions for analysis in genetic studies has been heavily influenced by the kinds of deficits observed within specific disorders and the observed heritability of these deficits. Across psychotic disorders, deficits in executive function, memory function and attentional control have each been a particular focus for research (Donohoe et al, 2009). Clinical awareness of these impairments has increased as it has been established that such deficits are predictive of social and occupational function. While cognitive deficits are somewhat correlated with clinical symptoms (for example, negative symptoms in schizophrenia) the amount of variance shared by these variables appears to be small, and cognitive function often emerges as a separate factor from clinical symptoms in factor analysis (Donohoe & Robertson, 2003). As such, cognition in psychosis may be viewed as a discrete, complex variable – one which is likely due to both genetic and environmental variation in the development and/or maintenance of specific neural systems. Improving our understanding of the biology involved, through assaying function as close to the level of these systems as possible is a necessary next step (Meyer-Lindenberg, 2010) – a rationale that has led to an interest in identifying 'endophenotypes' for investigation of schizophrenia.

1.3 Cognitive deficits as endophenotypes

The 'endophenotype' concept in psychiatry (described by Gottesman & Gould, 2003) relates to the identification of heritable quantifiable characteristics, which may be useful targets for genetic studies as they represent some intermediate stage between genotype and clinical disorder. Endophenotypes (or intermediate phenotypes as they are also know) represent discrete aspects of behavior or brain

structure and function that mediate genetic effects on the broader phenotype (Walters & Owen, 2007). As such, the endophenotypic concept centers on disorder-associated endophenotypes being specific and representing uncomplicated phenomena; which would likely decrease the number of genes required to produce variation in these traits compared to those involved in producing a clinical disorder. This may facilitate identification of genes, but also, where specific genes for a disorder have already been identified, endophenotypes may point towards neural pathways by which individual genes contribute liability. Gottesman & Gould (2003) suggested that the potential utility of an endophenotype can be judged against a set of six criteria and there is ample evidence to support the satisfaction of these criteria in relation to the more traditional cognitive variables investigated in schizophrenia research, some of which is considered in **table 1.1** below.

Endophenotype criteria	Evidence to support the criteria
Association with illness in the population.	Up to 75% of patients with schizophrenia suffer significant cognitive impairment (O'Carroll, 2000), with many cognitive variables showing an effect size of over 10% (e.g. global verbal memory; Md=1.41, sd=0.59).
Co-segregation of the endophenotype with illness in families. (i.e. within the family, affected family members are more likely to carry the endophenotype than non-affected members).	The Consortium on the Genetics of Schizophrenia (COGS) administered a neurocognitive assessment including measures of attention, verbal memory, working memory, and a computerized neurocognitive battery to patients and families across 7 sites, and found all measures to co-segregate with illness in families (Gur, 2007).
Evidence that it is genetically mediated (i.e. heritable).	Genetic epidemiological research using family and twin studies indicates that cognitive deficits may have a substantial genetic component (Goldberg et al., 1990/1995; Cannon et al., 2000).
Evidence that the endophenotype is state-independent (i.e. present whether or not illness is active).	In the case of schizophrenia for example, cognitive deficits are present from an early stage of the disorder and often predate the emergence of clinical symptoms (Erlenmeyer-Kimling et al, 2000). They are relatively stable over time and closely related to functional outcome (Green et al, 2004). This includes deficits in general cognitive ability (Donohoe et al, 2009b) and specific deficits in working and episodic memory (Donohoe et al, 2009a) and attentional control (Bellgrove & Mattingley, 2008; Donohoe et al., 2009a).
That the endophenotype can be measured accurately and reliably.	The neuropsychological tests used to measure these cognitive variables have well-established psychometric properties that can be easily administered to large groups of varying ability.
The endophenotype is also shared by non-affected family members of the proband.	Gur (2007) report evidence that in all neurocognitive domains, cognitive deficits are experienced by family members to a greater extent than they are in the general population (though not as much as in the probands themselves).

Table 1.1: The 6 criteria used to define an endophenotype as outlined by Gottesman & Gould (2003), and some of the literature to date which supports the application of these criteria to cognition.

1.4 Social cognition and psychiatric disorder

A significant development in cognitive genetic studies of psychiatric disorders has been the increased focus on the genetics of social cognition. Social cognition is the sum of those processes that allow individuals of the same species (conspecifics) to interact with one another (Frith & Frith, 2007), specifically it refers to the set of skills that allow us to understand the thoughts and intentions of others and frequently involves the investigation of social information processing, especially its encoding, storage, retrieval, and application to social situations. Essentially it depends upon the exchange of signals, such as speech, facial expression, body posture and eye gaze (Frith & Frith, 2007). Signals such as these can be socially informative in that they tell us what someone may be feeling (Vuilleumier & Pourtois, 2007), where they are focusing their attention, and what they are intending to do (Frith & Frith, 2006). The ability to process, comprehend and act appropriately to these signals is tantamount to social success and relies on coordination of several cortical regions, e.g. dorsomedial and dorsolateral prefrontal cortices, the paracingulate cortex and the right and left temporoparietal junctions and amygdala (Mitchell, 2009). In several psychiatric disorders this ability is impaired leading to social misperceptions, unexpected reactions to and from the person, and social withdrawal (Green et al., 2005), resulting in difficulties with maintaining friendships, employment, and general community functioning (Penn et al, 2008). Alongside these clinical and outcome goals, there is increasing interest in identifying the neural basis underlying social cognitive deficits in psychiatric illness. As such, further research in this domain is viewed as highly valuable (Green et al., 2005).

There are three principal components to social cognition; perception of others, perception of self and perception of social context. When perceiving other people we use sensory information to process both verbal and nonverbal cues in order to gain an understanding of those we encounter socially. This understanding often takes the form of emotion recognition, which has been identified as a central component of emotional competence (Bänziger et al., 2009). It incorporates the reading of emotion from face, voice and body; with facial emotion recognition being the most widely studied. Perceiving other people also involves such constructs as assigning or attributing causality (attribution theory) for social

outcomes. Attribution theory is a motivational theory which looks at how the average person constructs the meaning of an event based on his /her motives to find a cause and his/her knowledge of the environment. Causality can be attributed to other people, to the situation or circumstance, or to oneself; the self being second component of social cognition. Like other people, the self is a social object that needs to be understood (Adolphs et al., 2001; Barone et al., 1997). Understanding one's social self enables not only emotional self-regulation, but also the ability to differentiate between self and others; for example, the ability to attribute mental states (beliefs, intents, desires, pretending, knowledge, etc.) to both oneself and others, with the understanding that one's own mental states are different from those of other people (Baron-Cohen et al., 2001). This ability is commonly referred to as Theory of Mind (ToM). The final component of social cognition is the understanding of social context; the social setting in which people live or in which something happens or develops. It includes the culture that the individual was educated or lives in, the people and institutions with whom they interact and the fund of social knowledge (scripts, rules, skills) that enables people to successfully manage life tasks (Kihlstrom and Cantor, 2000; Wood et al., 2003). The three social cognitive components outlined here can be broken down into 5 domains: emotion recognition, theory of mind, attribution, social perception and self-regulation. **Table 1.2** outlines the most commonly used tests for each of these domains.

Currently, social cognition is investigated both in behavioural terms and in terms of neuroanatomy. Some of the tests that fall into the former category include theory of mind tests (such as Mind in the eyes, Faux pas and Hinting task), the Hotel Task, Multiple errands task, Iowa gambling task, the Social Cognitive Skills Test (SCST), Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT), the Penn emotional recognition task, Facial Affect recognition, Attribution test and tests of self-control (see **table 1.2** for task summaries). The latter category however is most often assessed using magnetic resonance imaging (MRI) and involves performing simple social cognitive tests such as facial aspect recognition, and imaging the brain to see what areas are activated during task performance.

	TEST	DEFINITION/USAGE
Emotion Recognition	MSCEIT	Measures emotional intelligence, namely 4 areas: Perceiving Emotions, Facilitating Thought, Understanding Emotions, Managing Emotions
	Penn emotional recognition task	A computer-based test that includes 96 color photographs of facial expression of evoked—or felt—emotions: happy, sad, angry, fearful, disgusted, and non-emotional or neutral
	Facial affect recognition	Facial Emotion Identification Test (FEIT) and the Facial Emotion Discrimination Test (FEDT). Both use black and white photographs of facial emotions that are presented on DVD.
Theory of Mind	ToM	A "Theory of Mind" (often abbreviated to ToM) is a specific cognitive ability to understand others as intentional agents, that is, to interpret their minds in terms of theoretical concepts of intentional states such as *beliefs* and *desires*.
	Mind in the eyes	Measure of adult mentalising. The ability to deduce emotion from looking at photographs of people's eyes.
	Faux pas test	The faux pas test ascertained the participants' ability to identify and understand a social faux pas, and to understand the mental states of the characters (the speaker and the recipient) in a conversation with a social faux pas.
	Hinting task	Mental state reasoning, inferring. Measures ability of subject to 'read between the lines' in a social context.
Attribution	Internal personal and situational attributions questionnaire (IPSAQ)	How causality is assigned in a social context. It produces two measurements 1) a measure of self-blame and 2) a measure of the extent to which external attributions implicate other persons as opposed to situations
Social Perception	Social cognitive skills test (SCST)	Focuses on the assessment of social reasoning skills.
	Iowa gambling task	Decision making abilities. Looks at trust and the ability to analyze intentions.
Self Regulation	Self control test (implicit association test (IAT))	Measure the strength of automatic association between mental representations of objects (concepts) in memory

TABLE 1.2: Definition and usage of various social cognitive tests

In this thesis, three of the social cognitive tests listed in **table 1.2** are administered as part of the neuropsychological test battery; two ToM tasks (Reading the mind in the Eyes and The Hinting task) and an attributions test (the IPSAQ). A fourth test administered in the current research, the computerised Emotion Recognition Task (ERT) is relatively novel, being designed in 2007 by Montagne and colleagues. The ERT assesses recognition of the six basic emotions (happiness, sadness, fear, anger, surprise, disgust) using dynamic stimuli of varying intensities. As such it may be viewed as a progression of the Penn emotion recognition task, which displays these emotions in static form. Of particular interest to me was whether these social cognitive tasks could as robustly be considered endophenotypes as their more traditional cognitive counterparts.

To investigate the utility of these five measures of social cognition as psychiatric 'endophenotypes' (based on the criteria outlined above), I undertook a review of the literature up to January 2013. The Penn emotion recognition task was included due the novelty of Montagne's ERT and its resultant paucity of literature. Search terms included test name, heritability, twin, sibling, state, independent, co-segregation, brain region, and endophenotype. Secondary search terms used in the event of the primary terms yielding no results included family, trait, dependent, gene, psychiatric, schizophrenia and autism. A list of the five measures of social cognition reviewed and evidence of their feasibility as endophenotypes is presented in **table 1.3**. As with traditional neuropsychological measures, most of the evidence of heritability for each of the measures of social cognition reviewed is derived from evidence that family members of psychiatrically affected probands also show deficits at a level intermediate between cases and healthy controls. These deficits have been associated with a number of candidate risk genes for psychiatric disorders, including 5HT2A, Oxytocin, and vasopressin (Donaldson, 2008).

Although the list of research articles in **table 1.3** is not exhaustive, it demonstrates that while the four widely administered social cognitive tasks (excluding Montagne's ERT) can be viewed as good examples of endophenotypes in that they are i) associated with illness, ii) heritable (at least based on assessments in first degree relatives), iii) state independent, and iv) co-segregated with illness; it also indicates that the quantity of research supporting each criteria is not equal. In

particular, studies of co-segregation in families and studies establishing heritability (particularly twin studies) are currently lacking. This is likely due to the relative novelty of social cognition as an investigatory field, coupled with the practical difficulties involved in securing groups of probands plus their family members for the purpose of research.

Also evident from the review is that to date, due to a lack of research, Montagne's ERT satisfies only two of the four endophenotypic criteria outlined here. The fact that the Penn emotional task satisfies all four, and that Montagne's ERT may be viewed as a progression of the Penn task is promising as to its future endophenotypic status. However, more research using this particular test would be beneficial, particularly as its dynamic design and varying levels of emotional intensity are more true to real life social encounters than the still photographs depicting various emotions at full intensity which are normally employed.

The identification of useful cognitive endophenotypes, both traditional and social, aids the progression of research into the underlying genetics of psychiatric disorders. Recent advancements in genomics methods – the introduction of whole genome sequencing for example and the ensuing genome wide association studies (GWAS) - enable identification of genes that are associated with particular illnesses (such as schizophrenia). Endophenotypes can then be used to better understand gene functionality and establish disease pathophysiology. For example, by checking the illness associated gene for concomitant association with cognitive endophenotypes, it can be established whether or not the gene is exerting its influence through cognitive decline, a common facet of psychiatric illness.

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Test	First Author	N	Associated with Illness	Heritability/Present in other family members	State Independent	Cosegregates with illness
Penn emotion recognition task	Greenwood, 2007	183 nuclear families ascertained through probands with SZ	Yes	Yes		Yes
	Eack, 2010	70 first-degree relatives of SZ probands; 63 healthy controls	Yes	Yes		
	Calkins, 2010	SZ/Szaff =610, Relatives=928, healthy controls=334	Yes	Yes		Yes
	Gur, 2002	14 patients with SZ and 14 matched comparison subjects	Yes			
	Bediou, 2007	drug-naive patients with first-episode SZ (n=40), their unaffected siblings (n=30) and healthy controls (n=26).	Yes	Yes	Yes	
	Gee, 2012	20 clinically high risk adolescents and 14 age and gender matched healthy controls between 15 and 23 years old	Yes		Yes	
	Addington, 2008	86 clinical high-risk individuals, compared with 50 individuals with first-episode psychosis, 53 with multi-episode SZ and 55 non-psychiatric controls	Yes		Yes	
	Brown & Cohen, 2010	Eighty-nine individuals with psychometrically defined schizotypy and 27 controls	Yes		Yes	
Montagne's emotion recognition task (ERT)	Law-Smith, 2011	21 adolescent males with high-functioning Austism Spectrum Disorders (ASD) and 16 age and IQ matched typically developing control males	Yes			
	Poljac, 2012	500 healthy individuals			Yes	
	Poljac, 2011	Post-traumatic stress disorder patients and healthy matched controls	Yes			
Hinting Task	Janssen, 2003	43 patients with SZ or schizoaffective disorder, 41 first degree non-psychotic relatives and 43 controls from the general population.	Yes	Yes	Yes	Yes
	Thompson, 2012	30 ultra-high-risk, 40 first episode psychosis and 30 control participants	Yes		Yes	
	Bora, 2009	36 studies included 1,181 (67% male) patients with SZ and 936 (58.3% male) healthy control	Yes		Yes	
	Vermissen, 2008	40 patients with psychosis, 49 non-psychotic first-degree relatives, 41 subjects from the general population with a high level of positive psychotic experiences, 54 controls.	Yes	Yes		

Reading the Mind in the Eyes task	Losh, 2007	Forty-eight parents of individuals with autism (13 of whom were identified as 'aloof'), and 22 control parents	Yes	Yes	.	Yes
	Bora et al, 2005	Forty-three euthymic bipolar patients and 30 controls			Yes	
	de Achaval, 2010	20 SZ patients, 20 healthy age- and gender-matched individuals, 20 unaffected first-degree relatives of the SZ patients, and 20 healthy individuals matched for age and gender	Yes	Yes		
	Baron-Cohen, 2006	Twelve parents of children with Asperger's syndrome, vs. 12 sex-matched controls	Yes	Yes		
	Irani, 2006	Ten patients diagnosed with SZ, 10 of their first-degree relatives and 10 healthy controls were included	Yes	Yes		
Attribution test (IPSAQ)	Lau, 2008	Childhood(birth-12 yr) (100), School Age (6-12 yrs) (180), Adolescence (13-17yr) (200), Adulthood(18 yr plus) (300), Young Adult(18-29 yr) (320)	Yes	Yes	Yes	
	Diez-Alegría, 2006	40 acute deluded participants, 25 remitted deluded participants, 35 depressed patients and 36 normal controls	Yes		Yes	
	Humphreys, 2006	Thirty-five participants suffering from recent onset psychosis			Yes	
	Janssen, 2006	23 patients with psychosis, 36 first-degree relatives, 31 subjects with subclinical psychotic experiences and 46 normal controls.	Yes			Yes

Table 1.3: Examples from the literature of studies which demonstrate the suitability as endophenotypes of the social cognition measures used in this thesis.

1.5 Application of genomics methods – identifying illness associated genes

A principal hypothesis for explaining the genetics of common complex disorders such as schizophrenia is that large numbers of common variants of small effect work in combination to undermine healthy brain development and signaling (the common variant common disease hypothesis). These common variants generally take the form of single nucleotide polymorphisms (SNPs), which are single base-pair changes in the DNA sequence that occur with high frequency in the human genome. For the purposes of genetic studies, SNPs are typically used as markers of a genomic region, with the large majority of them having a minimal impact on biological systems. SNPs can have functional consequences, however, causing amino acid changes, changes to transcription factor binding affinity, and changes to mRNA transcript stability (Bush & Moore, 2012). SNPs are by far the most abundant form of genetic variation in the human genome, and are currently analysed through GWAS, which measures and analyzes DNA sequence variations from across the human genome in an effort to identify genetic risk factors for diseases that are common in the population. The ultimate goal of GWAS is to use genetic risk factors to make predictions about who is at risk and to identify the biological underpinnings of disease susceptibility for developing new prevention and treatment strategies (Bush & Moore, 2012). The main SZ GWAS to date include Stefansson et al (2009), Need et al (2009) and Purcell et al (2009).

Whilst GWAS have revolutionized our understanding of SZ disease genetics, they are not without limitation; particularly the significant multiple testing burden that comes from requiring large sample sizes -in the thousands or tens of thousands- to identify what are typically modest gene effects (Corvin et al., 2009). In psychiatry as with other medical specialties, this requirement has driven collaboration. The Psychiatric GWAS consortium (PGC), for example, has performed meta-analyses of GWAS data for numerous psychiatric disorders, such as schizophrenia, bipolar disorder and recurrent major depression (Psychiatric GWAS Consortium Coordinating Committee, 2009), and has recently published a GWAS on novel susceptibility loci in SZ (Ripke et al., 2011) amongst other studies. These collaborative research efforts have not only been successful at identifying illness associated genetic variants, but have also proved informative as to how these

variants interact and overlap. According to the International Schizophrenia Consortium (2009b), schizophrenia and bipolar disorder risk, for example, may involve thousands of overlapping gene variants of small effect. One such variant, the psychosis risk variant at gene *ZNF804A*, has been implicated in both schizophrenia and bipolar disorder (Williams et al., 2011) and the *CACNA1C* variant, identified in bipolar disorder has also been implicated in schizophrenia and recurrent major depression (Green et al., 2010).

GWAS platforms were designed to assay common genetic variation and SNPs with a population frequency of at least 5%. However, rare or even unique genetic variants are much more frequent in the human genome. Up until the past five years, our ability to test for involvement of this type of variation in human disease 'the rare variant common disease hypothesis' has been very limited. GWAS platforms and custom-designed microarrays using comparative genomic hybridization (CGH) have allowed investigation of one class of rare genetic variation, namely, copy number variation (CNV), which are submicroscopic deletions or duplications involving the gain or loss of entire DNA segments (e.g. from a thousand to several million bases). CNVs play an important role in susceptibility to disorders involving cognitive deficits including autism (Sebat et al., 2007), learning disability (Roubertoux & de Vries, 2011) and schizophrenia (Walsh et al., 2008). In schizophrenia, the seven most established CNVs collectively account for ~2-4% of susceptibility (reviewed Sebat et al., 2009; Hosak et al, 2012). This is substantially smaller than the 30% of SZ susceptibility purported to be explained by common variants, through the cumulative action of thousands of common alleles each with small effect (International Schizophrenia Consortium (2009a, 2009b)). In summary, it appears that SZ is most likely caused by a large number of common variants of small effect coupled with rare variants of large effects (alongside environmental factors) in different individuals. In the extreme, each clinical case of SZ could be genetically unique, reflecting an interactive combination of a wide but ultimately limited spectrum of pathological genetic variants.

1.6 GWAS, Schizophrenia and cognition

Of the SZ associated genes identified by GWAS to date, in terms of cognition it is the psychosis risk variant at gene zinc finger protein 804A (*ZNF804A*) that has received the most attention, with 11 published studies over the last three years investigating the association between cognition and a particular single nucleotide polymorphism (SNP) from this gene – rs1344706 (see chapters 4 & 5 for details of research conducted). The results of these studies are not what would typically be expected from a SZ risk gene. Amongst the healthy population the rs1344706 risk allele largely conveys impairments to cognition, both when measured by behavioural and imaging means. For example, we see a negative association between *ZNF804A* risk and performance on measures of working memory (Esslinger et al. 2011), visuomotor skills (Lencz et al. 2010) and attention (Voineskos et al. 2011, Balog et al. 2011). However, this interpretation that the risk allele has a deleterious effect on cognitive performance has been questioned by several more recent studies, particularly in relation to the patient population. First, Walters et al (2010) found and replicated evidence for better cognitive performance on working memory and episodic memory tasks - which involve the dorso-lateral prefrontal cortex (DLPFC) and hippocampal formation (HF) - in patient carriers of the risk allele. This effect was not present in controls. In a subsequent study, of a different patient group, the authors found relatively larger hippocampal volumes in risk allele carriers (Donohoe et al., 2011). Several equivocal studies have also been reported associating *ZNF804A* with preserved cognition in patients (Becker et al., 2012; Van den Bossche et al, 2012). These data suggest that the *ZNF804A* risk variant may identify a patient subgroup with relatively spared cognitive performance. On this basis, researchers began to question by what means, other than traditional measures of cognition, this psychosis risk variant might be exerting its influence. In 2011, Walter and colleagues published a study in which 109 healthy volunteers underwent functional magnetic resonance imaging (fMRI) whilst performing a theory of mind task. They showed that whilst performing this ToM task, carriers of ZNF risk demonstrated a risk allele dose effect on neural activity in the prefrontal cortex and the tempero-parietal cortex as well as brain areas involved in the mirror neuron system. Aberrant functional connectivity between frontal and tempero-parietal regions was also apparent during performance of the ToM task,

leading the authors to conclude that a dysfunction of the ToM network is associated with *ZNF804A* mediated schizophrenia risk. In order to more fully establish this association between *ZNF804A* and social cognition however, further studies are necessary.

In total, ten schizophrenia common risk variants have been identified to date and are detailed in a review by Corvin (2011) (see **table 1.4** for details). As the table demonstrates, five of these SZ loci were identified in a recent PGC GWAS by Ripke and colleagues (2011), a study which also confirmed the association of two previously identified SZ risk genes – neurogranin (*NRGN*) and tumor necrosis factor 4 (*TCF4*). Knowing that these genes are associated with SZ is only the first step in understanding their role in the pathophysiology of the illness, with more research being required into the impact these genes have on the disorder. To date, what is known about the function of many of these genes does little to explain their association with SZ. Taking the most recently identified genes from the Ripke (2011) study for example, microRNA 137 (*MIR-137*) has been associated with the suppression of tumor growth and invasion (Chen et al., 2013; Zhu et al., 2013); cyclin M2 (*CNNM2*) is implicated in the transport of magnesium (Mg^{2+}), which plays a crucial role in many biological processes, including neuronal transmission (Goytaine & Quamme, 2005; Meyer et al., 2010); the CUB and Sushi multiple domains 1 (*CSMD1*) has been associated with risk for multiple neurodevelopmental disorders (Krauss et al., 2006; Glancy et al., 2009) and is implicated in immune function via a complement regulatory role (Håvik et al, 2011); and the last two variants located at 2q32.3 and 8q21.3, which are not yet identified as being part of a known gene, have both been linked to mental retardation and speech impairment (Cocchella et al., 2010; Horn et al., 2000; Ludwig et al., 2012), with 8q21.3 also being associated with tumor suppression (Varon et al., 2002) and childhood leukemia risk (Pastorczak et al., 2011).

Approaches to elucidating the biological role of novel SZ genetic susceptibility factors, includes delineating their effects on endophenotypes. For some there is a clear a-priori reason for looking at cognitive endophenotypes like memory and attention, as with *NRGN*, for example, which plays an important role in the calcium-calmodulin signalling pathway (Hayashi, 2009) and is associated with

neuromodulation, synaptic plasticity, long-term potentiation and strengthened NMDA receptor signalling (Broadbelt et al.. 2006), as well as being abundantly expressed in areas of the brain that are important for cognitive processing (Huang et al., 2007). For others, as with *MIR-137* (mentioned above), although a definitive a-priori reason may be lacking for investigating the gene in terms of cognition, (due to a lack of previous studies) it none-the-less makes empirical sense to conduct such investigations on the basis that genes, whose function is predominantly unknown, yet are associated with an illness which strongly features cognitive decline in its phenotype, may well have a role to play in these cognitive deficits.

Confirmed common risk variants for schizophrenia

Chromosome	Variant	P-value	Odds Ratio	95% CI	Gene	Reference (for named variant and other variants at the same locus)
1p21.3	rs1625579	1.5×10^{-11}	1.12	1.09-1.16	*MIR 137*	Ripke (2011)
2p15.1	rs2312147	1.9×10^{-9}	1.09		*VRK2*	Steinberg (2011)
2q32.1	rs1344706	2.5×10^{-11}	1.1	1.07-1.14	*ZNF804A*	O'Donovan (2008)
2q32.3	rs17662626	4.65×10^{-8}	1.2	1.13-1.26		Ripke (2011)
6p21.3-p22.1*	rs2021722	2.18×10^{-12}	1.15	1.11-1.19	*HLA* region	ISC (2009), Stefansson, 2009), Shi (2009)
8p23.2	rs10503253	1.45×10^{-8}	1.16	1.11-1.21	*CSMD1*	Ripke (2011)
8q21.3	rs7004633	2.75×10^{-8}	1.1	1.07-1.14		Ripke (2011)
10q24.32*	rs7914558	2.23×10^{-8}	1.22	1.15-1.29	*CNNM2*	Ripke (2011)
11q24.2*	rs12807809	2.8×10^{-9}	1.16	1.09-1.24	*NRGN*	Ripke (2011), Stefansson (2009)
18q21.2*	rs12966547	2.35×10^{-8}	1.4	1.28-1.52	*TCF4*	Ripke (2011), ISC (2009), Stefansson (2009), Shi (2009)

Table 1.4: The main replicated risk variants identified for schizophrenia with their locations and effect sizes. The Odds Ratio (OR) is a measure of effect size. It is the ratio of the odds of the variant occuring in the group of people with disease versus the ratio in the control group. The 95% confidence interval (CI) gives the range within which the true OR lies with a 95% probability. An asterisk indicates that more than one variant has been implicated at this locus.

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1.7 Conclusion

Good cognition is integral to satisfactory everyday functioning. Without it, people encounter difficulty in carrying out everyday activities such as grocery shopping, interacting with family and friends, and maintaining employment (Schmidt, 2011). Understanding the molecular mechanisms relevant to cognition, both normal and aberrant, both traditional and social, is hoped to increase understanding of illness pathophysiology and enable the development of more effective treatments, which should in turn significantly augment a person's quality of life. Genetics, and the development of genomic research, offers real opportunities to accomplish this, and a great many advances have been made through the application of GWAS and the endophenotypic approach. The speed of this advancement has created many questions that have thus far remained unanswered in the literature.

The work described in the following chapters attempts to investigate some of these questions in detail. If the genetics of SZ is to provide enlightenment as to the cognitive decline apparent in the disorder, we must study both the influence of SZ associated genes on cognitive function, and the underlying genetic architecture of these genes. Specifically, what role do these genes play in the relatively novel field of social cognition, a deficiency of which greatly impacts the lives of patients with SZ? Which of the novel SZ associated loci, if any, impact cognition and to what extent? Do these genes have a greater impact when acting individually, or is their impact increased when they are assessed as part of a group of genes or pathway? Do genes that have been found to impact traditional cognition also impact social cognition? Or conversely, for genes, such as *ZNF804A* for which a cognitive role requires further delineation, are they in fact impacting social cognition *rather* than traditional cognition? Finally are the endophenotypes used to assess cognition adequate or are there newer, better endophenotypes that could be employed?

Chapter 2

Detecting subtle facial emotion recognition deficits in schizophrenia using dynamic stimuli of varying intensities

Abstract

Background: Facial emotion recognition has been linked to poor functional outcome in patients with Schizophrenia (SZ). Whilst a strong ecological argument has been made for the use of both dynamic facial expressions and varied emotion intensities in research, most studies to date have implemented static stimuli of a singular, 100%, intensity. To address this issue, the present study aimed to investigate accuracy of emotion recognition amongst patients with SZ and healthy subjects using dynamic facial emotion stimuli of varying intensities. To this end a novel emotion recognition task (ERT) designed by Montagne (2007) was adapted and employed.

Methods: 50 patients with psychosis and 51 healthy participants were assessed in terms of emotion recognition using dynamic stimuli of variable intensity. Results of the ERT were tested for correlation with performance in areas of cognitive ability typically found to be impaired in psychosis: including *(1) IQ* (using selected subtests from the Wechsler Adult Intelligence Scale, 3rd edition), *(2) memory* (using the Wechsler Memory Scale, 3rd edition and the spatial working memory task (SWM) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB), *(3) attention* (using the continuous performance task identical pair's version (CPT-IP), and *(4) social cognition* using the Reading the mind in the eyes task (Eyes), the Hinting task, and the Internal, personal and situational attributions questionnaire (IPSAQ).

Results: When assessing accurate recognition of the 6 emotions combined, patients performed more poorly than healthy participants across all levels of intensity. When the emotions were assessed individually, healthy participants were observed to outperform patients in terms of accurate recognition of happiness, sadness, anger and fear, but not of disgust or surprise. Accuracy of emotion recognition was correlated with cognition in both patients and healthy participants.

Discussion: In line with previous literature findings, this study provides support for emotion recognition deficits in psychotic patients. It also points to the usefulness of dynamic face stimuli when investing emotion recognition, as well as the potential impact on recognition accuracy of the emotion intensity displayed.

2.1 Introduction

Social cognition is a rapidly growing area of schizophrenia research. Deficits in social cognitive skills, including facial emotion recognition, social cue perception, Theory of Mind (ToM) and attributional style, are evident in schizophrenia and contribute unique information about functional outcome beyond that provided by neurocognitive measures such as memory and IQ (Green & Horan, 2010). In light of the substantial impact of social cognition on functional outcome, a growing number of studies have attempted to use structured behavioural training to ameliorate these deficits. A meta-analysis of these studies conducted by Kurtz & Richardson (2011) revealed that social cognitive training had a moderate to large effect on facial emotion recognition and small to moderate effects on ToM ability. No effects on social cue perception and attributional style were apparent. The ability to improve patients emotion recognition skills to such a large extent, is particularly important considering that impairments in facial emotion recognition have been linked to unemployment, need for assisted living (Sergi et al, 2006; Horton & Silverstein, 2008), decreased social competence, conversational skills and social interest (Penn et al., 1996; Ihnen et al., 1998), poor performance on social role-plays (Pinkham & Penn, 2006) and inappropriate personal appearance (Gibson et al., 2010).

The impact of facial emotion recognition deficits on functional outcome is perhaps not surprising when one considers that facial emotional expressions are the building blocks of social interaction; as noted by Charles Darwin, they convey information about the intentions and motivations of other people, which promotes efficient interpersonal behaviour that can help to maximize social outcomes ((Darwin, 1872, cited in McArthur & Baron, 1983). Decades of research have established that individuals with SZ have difficulty in reading and interpreting

facial emotion when compared with control subjects (e.g., Dougherty et al., 1974; Walker et al., 1984; Borod et al., 1993; Lee et al., 2010); impairments which have been demonstrated to be moderately stable over time (Kee et al., 1998; Addington, 1998). Of the various emotions expressed by the human race, Ekman (1992) states that six are universal; that they are cross-cultural psychological responses that evolved in response to various ancestral problems. These six basic emotions are happiness, sadness, anger, disgust, fear and surprise. They are thought to be basic in two ways (psychological and biological) in that they do not contain other emotions as parts and they are innate (Prinz, 2004). This notion of the six basic emotions remains widely accepted today, although it is not without its critics (e.g. Jack, 2012).

Of interest is how recognition of these emotions relates to performance on neurocognitive measures such as IQ and memory, particularly as people with deficits in emotion recognition often suffer deficits in neurocognition also. There have been surprisingly few studies in this field to date, although the consensus between those that exist is strong. Among healthy participants, neurocognition might be said to have a minimal impact on expression recognition. For example, Kohler et al (2000) found that there were no correlations between emotion recognition and the neurocognitive variables they administered; Kessels and colleagues (2013) found that IQ only minimally affected the perception of disgust in children but not adults; whereas Horning and colleagues (2012) found that while cognitive abilities contribute to performance on emotion recognition tasks in participants over age 45, the cognitive functions did not fully account for the older adults' impairments on expression recognition. When assessing the impact of cognition on emotion recognition amongst patients with schizophrenia, however, the opposite relationship can be seen. For example, Lee and colleagues (2009) showed that, deficits in executive function in schizophrenia are associated with poorer performance on facial emotion recognition tasks; Kohler and colleaqgues (2000) found that emotion recognition correlated with attention, verbal and spatial memory, and language abilities; whereas Sachs and colleagues (2004) found that poorer performance in emotion discrimination correlated with abstraction-flexibility, verbal memory and language processing.

Many neuropsychological tests have been developed to assess facial emotion recognition. The majority of these (e.g. the Penn 2D emotion recognition task (Gur et al., 2001), The NimStim set of facial expressions (Tottenham et al., 2009), The CANTAB Emotion recognition task (Robbins et al., 1994), the Ekman faces task (Ekman & Friesen (1976)) use still black-and-white or colour photographs displaying full intensity emotions. It has been argued that such tasks are not ecologically valid (Sato et al., 2007a; Archer et al., 1994); in everyday society the faces encountered are (1) dynamic and (2) display emotions of varying levels of intensity.

In the last decade, research has begun to focus on the dynamic aspect of face recognition with some interesting results. It was demonstrated that dynamic displays of emotion are recognized more accurately (Weyers et al., 2006), and lead to greater levels of arousal (Sato et al., 2007a), than static displays. Moreover, there is growing evidence of specialized brain systems being preferentially activated by moving faces (Peuskens et al., 2005; Thompson et al., 2007). A study by Kilts and colleagues (2003), for example, showed that during facial emotion processing, static stimuli invoke a greater response than dynamic stimuli in regions of the frontal cortex, whilst dynamic stimuli were associated with greater temporal lobe activation than static stimuli. As such, the processing of static and dynamic facial emotion may well rely upon separable brain systems. This differential biology, coupled with the differing impact of static and dynamic stimuli on functional outcome provide good arguments for the use of dynamic emotion stimuli in research.

Despite this, to my knowledge there have only been five behavioural studies to date involving patients with SZ in which facial emotion-processing accuracy to dynamic facial emotion stimuli was assessed (Archer, 1992; Russell, 2007; Johnston, 2010; Mendoza, 2011; Behere, 2011). The validity of the earliest two of these studies as measurements of patient accuracy during dynamic face emotion recognition tasks has been questioned (Johnston, 2010). Notwithstanding this however, all five studies found that patients had a decreased ability to correctly identify the emotion expressed in dynamic faces compared to controls. This deficit in emotion recognition was also apparent in relatives of patients in the

study by Mendoza (2011). In this study, whereas patients showed deficits in recognizing all types of emotion, relatives' deficits appeared specific to recognition of fear and disgust.

Most of the dynamic research in SZ to date has employed facial expressions of saturated intensity. Only one study, by Mendoza (2011), implemented a multi-intensity dynamic model. This study did not however report on the effects of intensity on emotion recognition accuracy. A few studies have used static stimuli to assess the relationship between emotion intensity and recognition. In the only case/control study conducted to date, Kohler (2003) found that patients with SZ were more impaired than healthy comparison subjects in identifying high-intensity expressions than identifying low intensity expressions, even though the former was an easier task. In patient only studies, Fullam and Dolan (2006) reported that patients who scored high for psychopathy were found to have impaired recognition of sadness at low intensity compared to those with low psychopathy, whilst Silver and colleagues (2005) found that violent schizophrenia patients were less able to assess the intensity of emotions than non-violent patients. As such, research investigating emotion intensity using static stimuli suggests that the intensity at which an emotion is expressed influences a patient's ability to correctly identify that emotion. The obvious next step in research is to examine the effect of emotion intensity on recognition performance using the more ecologically valid stimulus of dynamic emotion.

To this effect, the current study used an adaptation of the innovative dynamic Emotion Recognition Task (ERT) (Montagne et al., 2007), to measure differing intensities of 6 emotion expressions ranging from 20% to 100%. The dynamic aspect brings the experience for the participant closer to 'real-life' than would be achieved using static facial expressions, whilst the differing intensities used afford more sensitivity to subtle deficits in facial emotion processing. The task is computerised, using stimuli of the six basic facial expressions outlined by Ekman (1992). On each trial participants watch a video clip of an expression morphing from neutral to an emotion displayed at variable intensities from 20% to 100%. Six emotional labels, corresponding to the six basic universal emotions, are

displayed on the screen and the participant has to select which of these best 'fits' with the emotion on display in the video clip.

The current study is unique in that it aims to test recognition of dynamic facial expressions of various intensities in patients with schizophrenia. It also aims to establish whether a correlation exists between performance on the dynamic ERT and performance on other cognitive variables, both social and neuropsychological (e.g. memory and IQ) amongst both patients and healthy participants. It should be noted that the healthy participant group used in this study were opportunistically sampled from a larger cohort to whom a full neuropsychological test battery had been administered. The present healthy participant group comprises those who agreed to complete the further ERT test at the end of the battery and, as such, have not been controlled for age and gender. We hypothesised that (1) patients will show poorer performance on the ERT than healthy participants across all six basic emotions; (2) the intensity of the emotion displayed will impact recognition accuracy in both patients and healthy participants, with lower intensity levels leading to poorer recognition accuracy; (3) amongst patients, performance on the ERT will correlate with other social cognitive variables involving face or emotion recognition, as well as with the more traditional cognitive variables of IQ, memory and attention (as it has done in previous research (Kohler et al., 2000; Sachs et al., 2004). To test these hypotheses, the ERT was administered to 50 patients and 51 healthy subjects who had concluded a full neuropsychological battery that included memory and attention, testing for associations between diagnostic status, emotion type and emotion intensity, as well as for correlations between ERT performance and other cognitive variables.

2.2 Methods

2.2.1 Neuropsychological sample characteristics: 50 cases with psychosis (predominantly SZ or schizoaffective disorder (SZA)) and 51 healthy participants who had completed a full neuropsychological assessment battery (see **table 2.1** for participant demographics) were included in this study. Participants comprised those who were happy to partake in the ERT *after* they had completed the

neuropsychological battery. As such, the healthy participants were not recruited as matched controls for the patient group.

Cases consisted of clinically stable patients with a DSM-IV diagnosis of schizophrenia (SZ) or schizoaffective disorder (SZA) (see **table 2.1** for details) recruited from two sites in Dublin, Ireland. Inclusion criteria required that participants were clinically stable at the time of neuropsychological assessment, aged 18 to 65 years, had no history of co-morbid psychiatric disorder, no substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID) (First et al., 2002). Additional diagnostic details and clinical sample characteristics ascertained at time of interview including symptom severity (SAPS/SANS) (Andreasen 1984(a), Andreasen 1984(b)) and medication dosage are detailed elsewhere (Walters et al., 2010).

Healthy participants were recruited via both online and poster advertising. They were aged 18 to 65 years, with no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Neither they nor any first degree relative had a history of psychosis.

All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

2.2.2 Emotion recognition task (Montagne et al, 2007)

The emotion recognition task (ERT) is a computer-generated program showing 'video clips' of facial expressions of emotion at various intensities. Stimuli were developed using algorithms (Benson & Perret, 1991) which created intermediate morphed images between a neutral face (0% emotion) and full or complete expression (100% emotion). Stimuli for this test were based on videos taken of actors mimicking emotional expressions and a neutral face. Four actors (two male and two female) each posed six emotions – happiness, sadness, anger, disgust, fear and surprise. These images were used to construct video clips that incrementally increase the degree of expression by steps of ~20% from 0% to

100%, so that there were 5 video clips per actor for each emotion. Actual animation times used were (in seconds) 0.31, 0.56, 0.81, 1.06 and 1.31. This represents a shortened version of the original ERT designed by Montagne and colleagues (2007) in which 9 animation times were used, progressing in 10% increments from 20% to 100%. The shortened task was used in this study to better facilitate its administration as part of a large neuropsychological battery which also measured IQ, memory, attention and other social cognitive variables.

Administration of the task began with two practice trials which consisted of the presentation of two different faces morphing from 0% expression to 100% expression. Feedback was given to the participant regarding their response to the trial, and it was explained that for the main task the morphing of expressions would build with intensity as the task progressed, such that the first round of faces would demonstrate mini or 'micro' expressions only. Once their understanding of procedure was clear the main task was begun. The participants were shown, in a random order, the 24 video clips running from neutral to 20% expression (4 actors demonstrating 6 emotional expressions each) and continued in blocks of increments of 20% until they reached the final sequence of clips in which the neutral face morphed into a full blown expression (100%). After each trial six emotional expression labels were displayed on the screen from which participants had to select the emotion that matched the image presented. There was no time restriction for each trial, with the static image of the final intensity remaining on the screen until the forced choice is made. The total task takes approximately 15 minutes.

2.2.3 Other cognitive measures

General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition (Wechsler, 1997a), yielding a full scale, verbal and performance IQ. ***Memory*** was assessed using subtests from the Wechsler Memory Scale, 3rd edition (WMS-III) (Wechsler, 1997b), and the spatial working memory task (SWM) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB). ***Attentional control*** was assessed using the continuous performance task identical pair's version (CPT-IP)

(Cornblatt, 1988). ***Social cognition*** was assessed using 1) two theory of mind tasks; a) Reading the mind in the eyes task ((Baron-Cohen et al., 2001) and b) The Hinting task (Corcoran et al., 1995), and 2) an attributions task called the Internal, personal and situational attributions questionnaire (IPSAQ) (Kinderman and Bentall, 1996), which yields two bias scores; a) externalising bias (EB), which indicates a propensity to attribute positive events to oneself rather than to other people and b) a personalising bias (PB), which indicates a propensity to attribute negative events to other people rather than to situational factors.

2.2.4 Design

The study used a mixed-factor design, one between subjects factor (group) with two levels (patients vs. healthy participants), and two within subjects factors; facial emotion type – with six levels (happiness, sadness, anger, disgust, fear, surprise); and intensity of emotion – 5 levels (the 'full-blown' emotion was viewed at 1.31 seconds after the trial began. The other 4 intensity levels were viewed at 0.31, 0.56, 0.81 and 1.06 seconds respectively).

2.2.5 Statistical analysis

All analyses were conducted using SPSS 17 (SPSS 2008).

Associations between diagnosis and 1) emotion identification, 2) emotion intensity and 3) reaction times were tested using 2-way ANOVA analyses. In each analysis status as patient or healthy participant was entered as the independent variable.

Associations between correct emotion identification and level of emotion intensity were tested using mixed 2-way ANOVA repeated measures.

Mixed 3-way ANOVA repeated measure was used to assess the interaction between diagnosis, emotion and intensity level.

Correlation of emotion recognition accuracy and other cognitive variables was assessed using Pearson's r test of correlation for cognitive variables of parametric distribution, and Spearman's rho test of correlation for cognitive variables of non-parametric distribution.

2.3 Results

2.3.1 Demographics and clinical sample characteristics:

Demographic and clinical characteristics for patients and healthy participants, expressed in terms of means and standard deviations appear in **table 2.1**. Because patients and healthy participants were included opportunistically from the larger genetics sample, and hence were not matched for age and gender, it was important to assess the impact of these variables on emotion recognition accuracy. Comparing patients with healthy subjects, there was a significant difference in mean scores for age, years of education, IQ and gender between groups, such that patients were older ($F=32.7$, $p<0.001$), had less years of education ($F=9.23$, $p<0.05$), had a lower full scale IQ ($F=50.1$, $p<0.001$), and comprised significantly more males ($F=7.74$, $p<.05$).

Table 2.1: Participant demographics. SZ=schizophrenia; SZA=schizoaffective disorder; BP=bipolar disorder type 1; MDD=major depression with psychotic presentation; SD=standard deviation

	Patients with Psychosis N=50	Healthy participants N=51
Psychosis subtype		
SZ	N= 36	
SZA	N= 4	
BP	N=7	
MDD	N=3	
Gender (ratio; M:F)	2.1:1	1:1.4
Age (years; mean (SD))	44.64(11.37)	31.9(11.01)
Age at onset (years; mean(SD))	39(11.3)	n/a
Chlorpromazine equivalent (mg/day; mean(SD))	297.6(148.3)	n/a
Cognition: full scale IQ (mean (SD))	91.6(17.2)	115.49(15)

2.3.2 Impact of age at time of assessment and gender on correct emotion identification:

Using a 2-way MANOVA, with age at time of assessment entered as the fixed variable and correct response scores to emotion identification entered as the dependent variables, age was assessed for impact on 1) number of correct responses scored overall, 2) number of correct responses scored for each emotion individually and 3) speed of response.

Analysing the patient and healthy groups separately, there was no significant association between age at time of assessment and number of correct responses scored overall (patients: F=1.08, p=.439; healthy participants: F=.87, p=.639), nor was there a significant association between age at time of assessment and any of the six emotional variables tested. Age at time of assessment was, however, associated with speed of response. In the healthy group the association was found to be due to a single outlier (F=2.8, p=.007). This outlier took an average of 10.78 seconds to respond to each emotional stimulus presented, whereas the mean average response amongst the remainder of the healthy participant group was 3.8 seconds (minimum = 2.46, maximum = 6.2). Removing the outlier from the analysis revealed no association between age and reaction time (F= .53, p= .93). In the patient group, the association persisted despite removal of the four outliers in the analysis (F=2.62, p=.014). These four outliers scored a mean reaction time of 13.7, 17.5, 17.5 and 24.3 seconds respectively, whereas the mean average response amongst the remainder of the patient group was 5.7 seconds (minimum = 2.46, maximum = 8.21).

Comparing males to females, gender was not associated with 1) number of correct responses scored over all, 2) number of correct responses scored for each emotion individually or 3) speed of response. This was true for both patients and healthy participants.

As such, although patients and healthy participants were not matched for age and gender, these variables were not significantly associated with variation in emotion recognition as measured by the ERT used here. For this reason, age and gender were not entered as covariates in the subsequent analyses, apart from any analysis investigating reaction time, for which age at time of assessment was entered as a covariate.

2.3.3 Patients verses healthy participants

Emotion: For total number of correct trials, which included correct responses for all emotions at all levels of intensity, there was a main effect of group (F=54.2, p<.001), with patients with psychosis performing less accurately than healthy participants (mean total correct trials: patients = 52, SD = 11.5; healthy participants = 67.7, SD = 9.8). In analysing each emotion separately for overall

correct response (the number of correct response for each emotion across all intensity levels), patients can be seen to perform less well than healthy participants for all six emotions (see **table 2.2**).

Table 2.2: Association between status as patient verses healthy participant and correct responses for each of the six emotions.

Emotion	F	p
happiness	7.46	.007
sadness	11.33	.001
anger	33.88	.000
disgust	24.29	.000
fear	7.224	.008
surprise	7.6	.007

Intensity: In considering total correct response for all 6 emotions combined, healthy participants outperformed patients across all levels of intensity (table 2.3). As such, the difference between the two groups in terms of emotion recognition accuracy persisted across all levels of intensity analysed.

Table 2.3: Association between status as patient verses healthy participant and number of correct responses at each of the 5 levels of emotion intensity.

Animation time in seconds	F	p
0.31	23.57	<.001
0.56	51.3	<.001
0.81	36.5	<.001
1.06	36.4	<.001
1.31	24.5	<.001

Reaction time: Healthy participants outperformed patients in speed of response across all trials. This was also true at each individual intensity level, apart from 0.31 and 0.81 for which there was no significant difference in reaction time between the two groups (**table 2.4**).

Table 2.4: Association between status as patient verses healthy participant and reaction time of response to task across all levels of emotional intensity.

Reaction time across intensities	F	p
Overall	10.38	.002
0.31	3.4	.067
0.56	5.36	.023
0.81	.468	.496
1.06	9.35	.003
1.31	5.89	.017

2.3.4 Emotions

Mean responses: Across diagnostic categories, amongst the six emotions analysed, happiness was identified more accurately than all other emotion expressions (mean correct response out of 20: patients = 15.15; healthy participants = 17.06), whilst fear was identified less accurately than all the rest (mean correct response out of 20: patients = 3.98; healthy participants = 5.67). **Table 2.5** describes the means and standard deviations of correct responses to each of the six emotions analysed.

Table 2.5: Means and standard deviations of correct responses to each of the six emotions analysed.

	Patients				**Healthy participants**			
Emotion	Min. correct	Max. correct	mean	SD	Min. correct	Max. correct	mean	SD
anger	3	18	9.7	3.85	5	20	13.98	3.54
sadness	0	14	5.14	3.69	1	15	7.41	3.07
happiness	1	20	15.15	3.85	4	20	17.06	3.18
disgust	1	18	9.16	4.31	2	19	13.2	3.88
fear	0	13	3.98	3.03	0	16	5.67	3.26
surprise	3	15	8.22	2.83	3	17	9.92	3.35

Intensity:

3-way-Anova: Group x Emotion x intensity

Using general linear models repeated measures, a three way interaction was calculated looking at diagnosis by the number of correct responses for each emotion by intensity level. **Figure 2.1** demonstrates this 3-way interaction in two

separate plots (for patients and healthy participants). A significant within subjects effect was detected (F=2.21, p=.006), showing that the interaction between diagnosis and number of correctly identified emotions varied across differing levels of intensity.

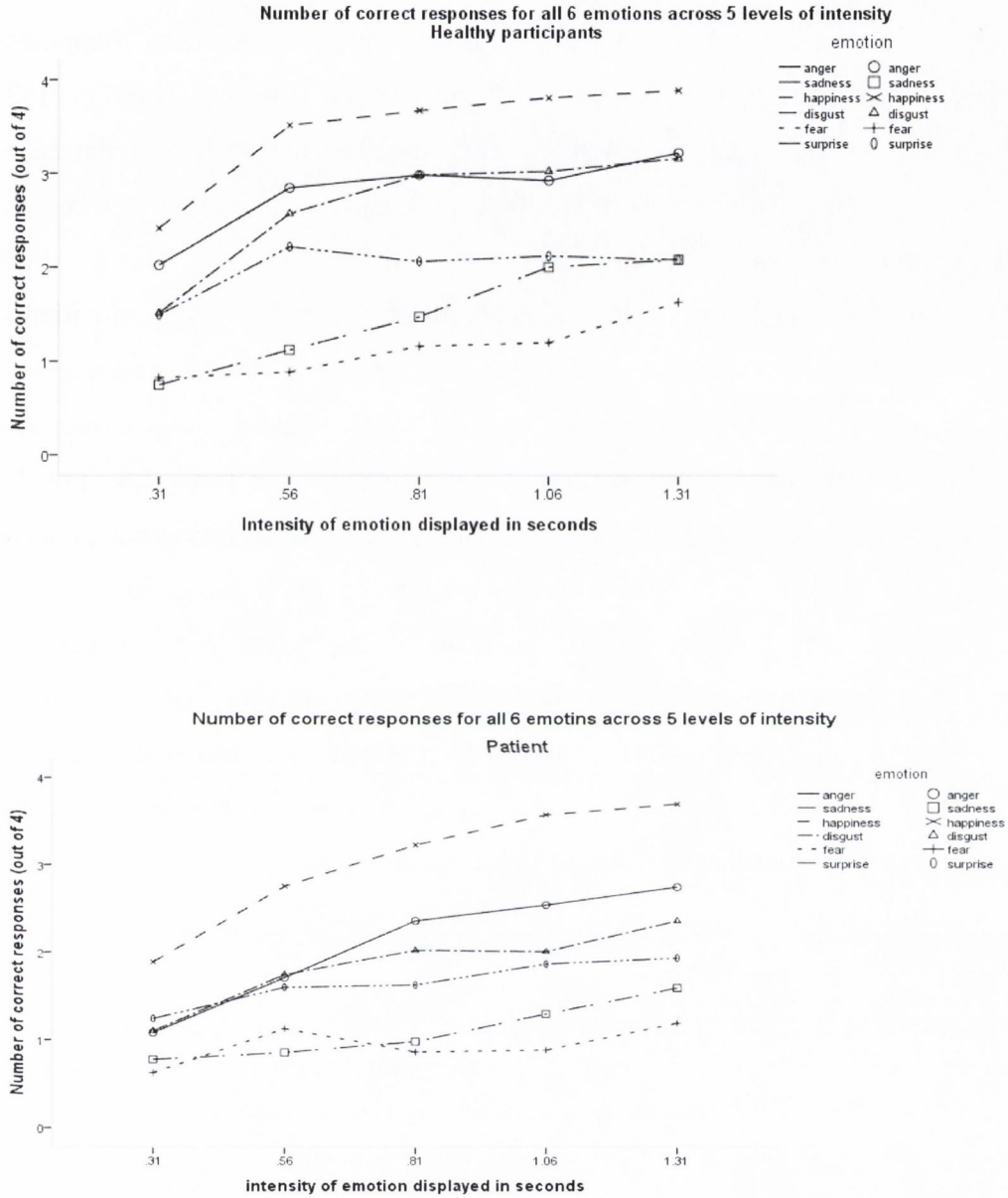


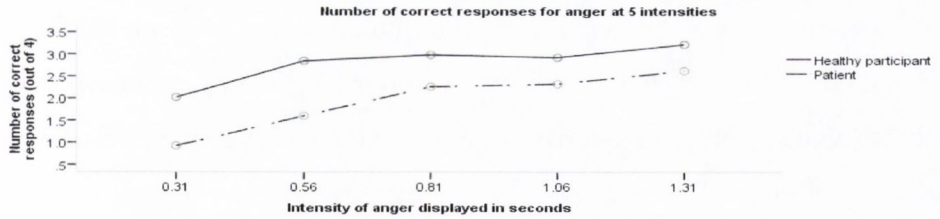
Figure 2.1: Total correct trials of six basic emotions across differing intensity levels in both healthy participants and patients.

In order to assess each emotion independently for differences between patients and healthy participants across levels of intensity, a series of two way interactions between these two variables was conducted using general linear models repeated measure. Results are depicted in **figure 2.2**. A significant interaction between status as patient or healthy participant and emotional intensity can be seen for the emotions of anger ($F=3.08$, $p=.016$), sadness ($F=2.78$, $p=.027$), happiness ($F=4.17$, $p=.003$) and fear ($F=3.06$, $p=.017$), but not for disgust ($F=1.09$, $p=.357$) or surprise ($F=1.01$, $p=.396$), where patients can be seen to follow the same pattern of accuracy across levels of emotion intensity. In examining the direction of this interaction effect, a series of t-tests was performed to ascertain at which intensity level (high or low) healthy subjects differed significantly from patients. High levels of intensity are those at which the expression is viewed at an intensity level above 80%, whereas low levels of intensity are those at which the expression is viewed at an intensity of below 20%. Results are shown in **table 2.6**. For the emotions of fear and sadness, patients perform similarly to healthy participants at low levels of intensity, but are significantly poorer at emotion recognition at high intensity levels. In contrast, for the emotion of happiness, patients perform similarly to healthy subjects at high levels of intensity, but much poorer at lower intensity levels. For the emotion of anger, whilst patients performed significantly poorer than the healthy group at both high and low levels of emotion intensity, they perform most poorly at the lower intensity levels.

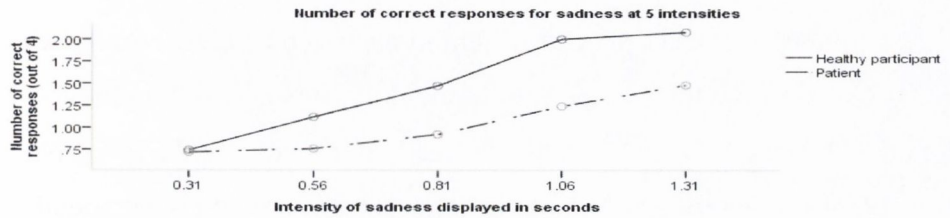
Table 2.6: Results of t-tests comparing patients to healthy participants on accuracy of emotion recognition at both low and high levels of intensity for each of the four emotions for which a significant interaction between status as patient or healthy participant and emotional intensity was observed.

Emotion	Low intensity	High Intensity
Anger	$t=5.7$; $p=<.0001$	$t=2.88$; $p=.005$
Sadness	$t=.155$; $p=.877$	$t=3.45$; $p=.001$
Happiness	$t=4.11$; $p=<.0001$	$t=1.52$; $p=.131$
Fear	$t=1.41$; $p=.164$	$t=2.6$; $p=.011$

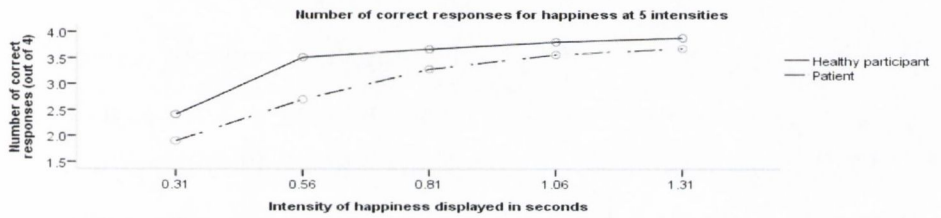
Anger - Within subject effects: F=3.08, p=.024



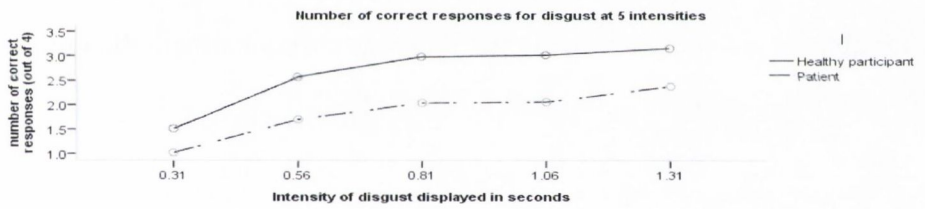
Sadness - Within subject effects: F=2.78, p=.027



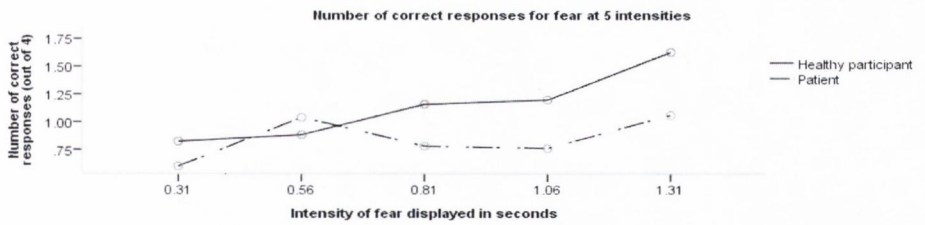
Happiness - Within subject effects: F=4.17, p=.003



Disgust - Within subject effects: F=1.09, p=.357



Fear - Within subject effects: F=3.06, p=.017



Surprise - Within subject effects: F=1.01, p=.396

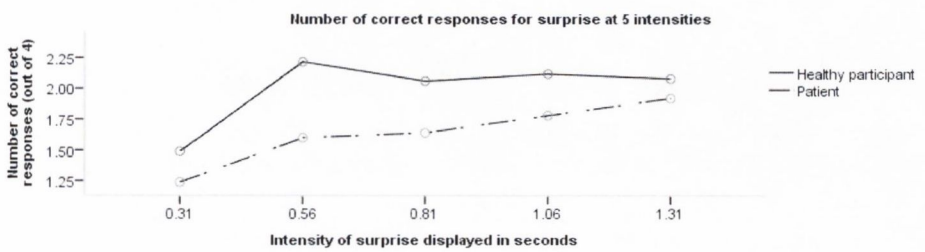


Figure 2.2: Accuracy of emotion recognition for both patients and healthy participants, across 5 levels of intensity, for each of the 6 basic emotions.

2.3.5 Correlation with cognition:

Most cognitive variables were parametrically distributed and thus warranted the use of the Pearson's r correlation coefficient when assessing their correlation with overall correct score for emotion identification. For the cognitive variables of non-parametric distribution (WTAR, Faces 1, CPT D'Prime, Eyes and Hinting) the Spearman's rho correlation coefficient was used.

Patients: The number of correctly identified emotions was positively correlated with all measures of IQ, and most measures of memory. It was also correlated with attention and the social cognition task of Reading the Mind in the Eyes. No correlation was apparent between correctly identified emotions and other social cognitive measures (see **table 2.7**).

Healthy participants: In contrast to the patient group, the number of correctly identified emotions was positively correlated with only one measurement of IQ (performance IQ). In terms of memory, total emotion recognition scores were correlated only with the Faces task. There was no correlation between correctly identified emotions and attention, and (as with the patient group) only one social cognitive variable - Reading the mind in the Eyes was correlated with the number of correctly identified emotions (see **table 2.7**).

Table 2.7: Correlation between the number of correctly identified emotions overall and cognition. Most correlations were conducted using Pearson's r, apart from those starred (*) which used Spearman's rho on account of the cognitive variables being non-parametric in distribution.

		Number of correctly identified emotions			
	Cognitive	Patients		Healthy participants	
	Variable	Correlation	Significance	Correlation	Significance
IQ	WTAR*	.609	**.000**	.177	.218
	Verbal IQ	.481	**.001**	.195	.175
	Performance IQ	.366	**.014**	.303	**.034**
	Full scale IQ	.482	**.001**	.26	.071
Memory	LNS	.367	**.013**	.232	.109
	Logical memory 1	.239	.114	.2	.183
	Logical memory 2	.129	.398	.241	.106
	Faces 1*	.373	**.018**	.497	**.001**
	Faces2	.346	**.029**	.395	**.012**
	SWM errors	.477	**.006**	-.015	.929
	SWM strategy	.141	.442	.164	.331
Attention	CPT d'Prime 2 digit*	.4	.065	.378	.052
	CPT d'Prime 3 digit*	.638	**.002**	.142	.48
	CPT d'Prime 4 digit*	.583	**.007**	.379	.062
Social	Eyes*	.445	**.003**	.285	**.05**
Cognition	Hinting task*	.263	.096	.058	.72
	IPSAQ EB	.205	-.115	-.143	.331
	IPSAQ PB	.182	.459	.033	.824

2.4 Discussion

The purpose of the present study was to investigate whether patients with SZ (n=50) were impaired relative to their healthy counterparts (n=51) at accurately identifying emotions in a novel dynamic emotion recognition task developed by Montagne (2007). We further wished to investigate the impact of emotion intensity on correct emotion identification in both patient and healthy participant groups. Finally we sought to ascertain whether any correlation existed between performance on a dynamic emotion recognition task and performance on other cognitive variables, both social and neuropsychological. Findings show that (1) patients perform less well than healthy subjects in the correct identification of all six emotions analysed (happiness, sadness, anger, surprise, fear and disgust); (2) in considering total correct response for all 6 emotions combined, healthy participants outperformed patients across all five levels of intensity; (3) healthy participants outperformed patients in speed of response across all trials and at each individual intensity level apart from 0.31 and 0.81 seconds for which there was no significant difference in reaction time between the two groups; (4) across diagnostic categories, happiness was identified more accurately than all other emotions, whilst fear was identified least accurately; (5) a significant interaction between status as patient or healthy participant and emotion intensity can be seen for the emotions of anger, sadness, happiness and fear, but not for disgust or surprise. Specifically, for the emotions of fear and sadness, patients performed similarly to healthy subjects at low levels of intensity, but were significantly poorer at emotion recognition at high intensity levels. In contrast, for the emotion of happiness, patients performed similarly to healthy participants at high levels of intensity, but much poorer at lower intensity levels. For the emotion of anger, whilst patients performed significantly poorer than healthy subjects at both high and low levels of emotion intensity, they performed most poorly at the lower intensity levels; (6) whilst performance on the ERT was positively correlated with the expected cognitive variables involving faces, emotion recognition and performance IQ for healthy subjects, it was further positively correlated with variables of premorbid, verbal and full scale IQ, as well as working memory and attentional control in the patient group.

It is not surprising that the current study found patients to be deficient in emotion recognition accuracy compared to healthy individuals, as this is the finding in the vast majority of studies conducted on emotion recognition in SZ (Kohler, 2010). The finding that happiness is recognised most easily, whilst fear is recognised least easily is also uncontroversial. Emotion recognition studies have shown consistently that happiness is the most easily identified emotion (Kohler et al., 2004; Kirouac et al., 1983; Kirita et al., 1995; Matsumoto et al., 2000; Tracy & Robbins, 2008), approaching 100% accuracy, even at low levels of intensity (Hess et al., 1997). The ranking of the remaining 6 basic emotions has varied across studies and differences may be partly due to test designs, as these studies have varied in types and intensities of emotions. For the emotion of fear, while it was reported as being ranked as the third most easily recognisable out of the 6 basic emotions in a study by Erwin and colleagues (1992), a more recent study by Kohler and colleagues (2003), showed that fear was one of the least accurately identified emotions amongst patients with SZ; a finding that was supported by Tracy and Robbins (2008) in their study of healthy participants.

Of notable interest in the current study is the finding that for some emotions (fear and sadness), patients performed similarly to healthy participants at low levels of intensity, but were significantly poorer at emotion recognition at high intensity levels (group 1), whereas for other emotions (happiness and anger), the opposite pattern is the case (group 2). Looking at these two groups of emotions, it is apparent that the former two are those which were the least accurately recognised overall, whilst the latter two were the most accurately recognised throughout the study. Indeed the two emotions for which there was no difference in accurate emotion recognition between patients and healthy subjects across intensities (surprise and disgust – group 3), were correctly identified at a rate that lay intermediate between these two groups. Examining this in further depth, for group 1 emotions, no participant correctly identified more than 2/4 emotions at any of the intensity levels, with zero correct being the mean score for both emotions at the lowest level of intensity. In contrast to this, group 2 saw emotions being correctly identified an average of 4/4 times for the high intensity levels, and not falling below a mean of 2/4 for the low levels of intensity. Therefore, while this study provides strong support for the impact of intensity on emotion recognition

in patients, in order to accurately establish the direction of intensity effect, a greater number of trials at each intensity level would be advantageous.

A further point of interest in relation to the study's findings is that, amongst patients, performance on the ERT correlated generally with cognition; in areas of IQ, memory, attention and social cognition. This correlation existed with far fewer cognitive variables amongst the healthy group – predominantly with tasks that involve faces and emotion recognition. The patient finding corroborates previous research (e.g. Kohler et al., 2000; Sachs et al., 2004; Lee et al., 2009), which found that amongst patients with SZ better emotion recognition performance was correlated with better abstraction-flexibility, verbal and spatial memory and language processing; all of which relates to frontal and temporal functioning. The correlations might therefore suggest that impairment in frontotemporal functions extends to limbic regions related to emotional processing. These findings lend support to the notion that difficulties in emotion recognition are associated in schizophrenia with key cognitive deficits. Indeed, it may be the case, as Pomarol-Clotet (2010) suggests, that without the generalised cognitive decline experienced by patients with SZ, there would be no emotion recognition deficits at all.

The finding amongst the healthy group of fewer cognitive variables being correlated with emotion recognition performance also reflects previous research findings. Kohler and colleagues (2000) found that there were no correlations amongst healthy subjects between emotion recognition and the neurocognitive variables they administered, although those variables did not include the Faces task or the Reading the mind in the Eyes task that were found to be positively correlated with emotion recognition in the present study. Of course, it is not surprising that the current study found these two tasks were correlated with facial emotion recognition in both patients and healthy participants, considering that all three tasks involve faces or emotions or both. It may be considered slightly surprising that, contrary to research to date, we also found a correlation amongst the healthy group between performance on the ERT and performance IQ. A possible explanation for this lies in the fact that performance IQ, like emotion recognition, involves non-verbal abilities, and perhaps it is this similarity that

forms the basis for the correlation. Further research into this area would be desirable.

Although sample size is not a limitation of the current study, which involves a greater number of patients and healthy participants than most comparative research in the literature, the composition of the patient group might be considered problematic. Whilst all of the patients involved suffer with psychosis, only 36 out of the 50 patients have been diagnosed with SZ. That leaves 14 patients, 7 of which have BP, 4 have SZA and 3 have MDD. Research by Chen and colleagues (2012) found that patients with SZ and SZA responded differently to the emotions of happiness and fear, with patients with SZA not showing the emotion recognition deficits of those with SZ. A review article by Kohler (2004) concludes that patients with BP disorder are not as impaired as their SZ counterparts at accurately recognising emotions, whilst patients suffering from depression were impaired when viewing happy faces only, although these patients did not suffer with psychotic symptoms as did the patients with depression in the current study. As such, including patients with SZA, BP and MDD may have weakened any findings obtained. Ultimately, it would have been better to limit the patient group to individuals with SZ only; a task for future research.
Regarding the healthy participant group, although age and gender did not impact emotion recognition accuracy in the present study, an age and gender matched control group would have enhanced the generalizability of the results. The healthy participants were recruited purely on the basis of availability and willingness to partake in the ERT. Future studies will benefit from more careful matching of samples on these criteria.

In conclusion, this study is the first to our knowledge to investigate the role of dynamic facial stimuli at varying intensity levels in performance accuracy on an emotion recognition task in patients with psychosis and healthy subjects. The data supports a role for the use of dynamic stimuli in this line of research and also highlights the impact of emotion intensity on correct identification. Further research with larger sample sizes would hopefully prove informative as to whether it is the low or high emotion intensities that prove most problematic for psychotic patients.

Chapter 3

Seven schizophrenia loci recently identified by the psychiatric genetics consortium and their impact on the neuropsychology of both patients and controls.

Abstract

Background: In identifying genetic risk factors for schizophrenia (SZ), genome wide association studies (GWAS) have facilitated discovery of variants whose roles in illness etiology or pathophysiology were neither known nor easily predictable. In the largest schizophrenia GWAS to date (Ripke et al., 2011), which comprised a 'mega-analysis' of over 51,000 cases and controls, seven novel variants were identified as meeting genome wide significance, five of which are new (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33) and two of which have been previously implicated (6p21.32-p22.1 and 18q21.2; 2). Of these seven loci, most had no obvious functional relationship to disease etiology.

Methods: In order to better understand their role in SZ illness, we examined each locus in turn for association with the neuropsychological variables commonly impacted in SZ. This included traditional cognitive variables such as IQ, memory and attention and social cognitive variables including tests of theory of mind (ToM) and attributional style.

Results: Of the seven loci investigated, only three demonstrated a significant association with neurocognition. *MIR-137* was associated with episodic memory, working memory and attentional control, *CSMD1* was associated with general cognitive ability and episodic memory, and *CNNM2* was associated with the social cognition variable of attributional style.

Discussion: Three out of seven novel SZ associated variants identified by Ripke and colleagues (2011) were found to contribute to illness risk via a mechanism that is likely to at least partly involve cognition and social cognition, whereas the remaining four are likely to increase SZ risk by factors other than cognition.

3.1 Introduction

The Psychiatric Genetics Consortium (PGC) recently examined the role of common genetic variation in schizophrenia in a GWAS of substantial size (Ripke et al., 2011). The study is comprised of two stages; a stage 1 discovery sample of 21,856 individuals of European ancestry and a stage 2 replication sample of 29,839 independent subjects. The combined stage 1 and 2 analysis yielded genome-wide significant associations with SZ for seven loci, five of which are new (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33) and two of which have been previously implicated (6p21.32-p22.1 and 18q21.2) (see **table 3.1**).

The strongest new finding ($P = 1.6 \times 10^{-11}$) was with rs1625579 within an intron of a putative primary transcript for *MIR-137* (microRNA 137), a known regulator of neuronal development. Four other SZ loci achieving genome-wide significance in the PGC study contain predicted targets of *MIR-137* (*TCF4*, *CACNA1C*, *CSMD1* and *C10orf26*), leading the authors to conclude that *MIR-137*-mediated dysregulation may represent a novel etiologic mechanism in schizophrenia. As such, understanding the neural mechanisms affected by of *MIR-137* is likely to be imortant to understanding the biology of this disease.

GWAS identified single nucleotide polymorphisms (SNPs) are associated with the illness in the absence of an a-priori functional role. For some genes, an obvious role within the pathophysiology of the disease is apparent, while for others a functional role is not immediately evident. As liability for SZ is hypothesized to be at least partially mediated by the influence exerted by susceptibility genes on cognitive performance, we investigated all seven genes identified in the recent PGC GWAS analysis for associations with cognition. In terms of cognition, of the seven loci identified in the combined stage 1 and 2 analysis of the PGC GWAS, four (*MIR-137, NRGN, CSMD1* and *CNNM2*) were previously associated with variation in cognition, while for the remaining three it was unclear what role they might play in SZ etiology. The following paragraphs describe each of these genes in more detail.

Table 3.1: Details of the seven SZ loci identified by Ripke and colleagues (2011) and analysed in the current investigation for association with neurocognitive variables commonly identified as deficient in SZ. P value given is that for the combined principal and replication GWAS. * indicates the risk allele. SNP = single nucleotide polymorphism.

	Locus	Alleles	SNP	p	OR (95% CI)	Gene name	Distance (kb)
Novel loci	1p21.3	G T*	rs1625579	1.59×10^{-11}	1.12 (1.09–1.16)	*MicroRNA-137 (MIR-137)*	Intragenic
	8p23.2	A* C	rs10503253	4.14×10^{-8}	1.11 (1.07–1.15)	*CUB and Sushi multiple domains 1 (CSMD1)*	Intragenic
	10q24.32	A G*	rs10748835	1.07×10^{-3}	1.10 (1.07–1.13)	*Cyclin M2 (CNNM2)*	Intragenic
	2q32.3	A* G	rs17662626	4.65×10^{-8}	1.20 (1.13–1.26)	*PCGEM1*	343
	8q21.3	A G*	rs7004633	2.75×10^{-8}	1.10 (1.07–1.14)	*MMP16*	421
Previously identified loci	11q24.2	T* C	rs12807809	2.18×10^{-12}	1.15 (1.11–1.19)	*Neurogranin (NRGN)*	Intragenic
	18q21.2	A* G	rs4309482	1.05×10^{-6}	1.23 (1.14–1.31	*Tumour necrosis factor 4 (TCF4)*	Intragenic

3.1.1 NRGN: Neurogranin *(NRGN)* plays an important role in the calcium-calmodulin signalling pathway (Stefansson et al., 2009). Ca^{2+} induced oxidation of *NRGN* leads to Calmodulin's activation of CaMKinase II, which is associated with strengthened NMDA receptor signalling. This cellular role in neuroplasticity, together with the evidence that *NRGN* is abundantly expressed in areas of the brain that are important for cognitive processing, particularly CA1 pyramidal neurons in the hippocampus (Huang et al., 2007), has led to the suggestion that *NRGN* may be important in the neurobiology of learning and memory. This role in memory and learning is supported by evidence of severe deficits in hippocampus-dependent tasks in neurogranin knock-out mice (Pak et al., 2000). Based on this evidence we hypothesised that *NRGN*'s role in increasing risk for schizophrenia might be associated with variation in neuropsychological performance. Specifically, we expected that the risk 'T' allele at rs12807809 would be associated with poorer performance on measures of memory and cognition.

3.1.2 CSMD1: The CUB and Sushi multiple domains-1 gene *(CSMD1)* has been shown to be primarily synthesized in the developing central nervous system and epithelial tissues (Kraus et al., 2006). It is enriched in the nerve growth cone, suggesting that it may be an important regulator of complement activation and inflammation in the developing CNS. The SNP under investigation in the present study, rs10503253, is located in a large intron of *CSMD1*. It is of unknown function, although like its parent gene, it too has been linked to the inflammatory process. SNP expression analyses using both the mRNA by SNP Browser and the SNPExpress database (http://www.sph.umich.edu/csg/liang/asthma/; http://people.genome.duke.edu/~dg48/SNPExpress/) identified the risk 'A' allele at rs10503253 as having a *trans*-acting effect that reduced expression of the *beta-carotene oxygenase 2 (BCO2)* gene on chromosome 11. Variants in *BCO2* are strongly associated with plasma levels of interleukin 18 (IL18), a proinflammatory cytokine involved in immune function (He et al., 2010). In a recent study of 4000 gene variants related to immune function, variants within *CSMD1* and *CSMD2* showed the strongest association with SZ risk (Glancy et al., 2009). Genes designated as being relevant to immunity have been hypothesized as relevant to schizophrenia pathophysiology either directly via a role in immune function (Brown & Susser, 2002), or via a role in synaptic refinement and

neuronal connectivity (Schatz, 2009). Both synaptic refinement and neuronal connectivity are important processes in cognition (West & Greenberg, 2011; Thivierge & Marcus, 2007), which is suggestive of a potential role for *CSMD1* in the cognitive decline apparent in patients with SZ. In support of this, *CSMD1* has also been previously associated with risk for multiple neurodevelopmental disorders, including SZ, epilepsy, speech delay, and learning difficulties (Ripke et al., 2011; Håvik, 2011; Glancy, 2009).

3.1.3 CNNM2: Cyclin M2 *(CNNM2)* is implicated in the transport of magnesium (Mg^{2+}), which plays a crucial role in many biological processes, including neuronal transmission. Despite the extensive evidence for unique mammalian Mg^{2+} transporters, *CNNM2* is one of only a few proteins biochemically identified as fulfilling this role (Goytain & Quamme, 2005). Sequence variation within *CNNM2* increases hypertension risk *and* alters serum Mg^{2+} blood levels, a risk factor for a variety of vascular diseases (Meyer, 2010). Furthermore, *CNNM2* mutations are associated with hypomagnesaemia risk (Stuiver et al, 2011). Hypomagnesaemia is an electrolyte disturbance in which there is an abnormally low level of magnesium in the blood (*Dorland's Medical Dictionary).* Deficiency of magnesium causes such symptoms as weakness, muscle cramps and cardiac arrhythmia, but it has also been linked to confusion, depression (Cundy & Mackay, 2011), psychosis (Ang, 1995) and hallucinations (Bosch-Barrera, 2010). Recently a study by Guran (2011) found that in children with familial hypomagnesaemia, common psychiatric diagnoses were Attention Deficit Hyperactivity Disorder, borderline intelligence, mild mental retardation and speech disorders. Furthermore, Parent-rated Child Behavior Checklist and Child Health Questionnaire mean scores indicated a deficit in psychosocial well-being. It thus seems plausible that the association between SZ risk and the newly identified SZ GWAS gene *CNNM2* may have its roots in the cognitive decline, both traditional and social, that is so often apparent in patients with SZ.

3.1.4 MIR-137: MicroRNA 137 *(MIR-137)* has been implicated in the regulation of adult neurogenesis (Silber et al., 2008; Szulwach et al., 2010) and neuronal maturation (Smrt et al., 2010). Variations at *MIR-137* might thus contribute to

brain development abnormalities in SZ through either or both of these mechanisms. Although the associated 'T' allele at the *MIR-137* SNP rs1625579 has a modest overall effect on SZ risk (OR=1.12), it is of interest as it may implicate a particular molecular risk mechanism. MicroRNAs (miRNAs) are small noncoding RNAs that play a regulatory role in cellular processes, including brain functioning, by regulating the function of potentially hundreds of genes through RNA interference. In the PGC analysis, SNPs mapping to 301 high-confidence predicted gene targets of *MIR-137* were more likely to be associated with schizophrenia than would be expected by chance. Furthermore, of the loci with genome-wide significant support, four (*TCF4*, *CSMD1*, *CACNA1C*, which was identified as reaching genome wide significance in a separate joint analysis with bipolar disorder, and *C10orf26*, which was identified as reaching genome-wide significance in stage 1 of the analysis) have predicted *MIR-137* target sites. The fact that *MIR-137* is the most strongly associated SZ SNP in the PGC (2011) analysis, coupled with associations in multiple predicted *MIR-137* target sites and the known role of *MIR-137* in neuronal maturation and function together suggest an intriguing new insight into the pathogenesis of SZ.

Recent research into *MIR-137* by Green and colleagues (2012) reports that *MIR-137* risk allele carriers were more likely to belong to a SZ subtype characterized by cognitive deficits than they were to a cognitively spared SZ subtype, but only in combination with higher severity of negative symptoms. These findings provide the first evidence for association of *MIR-137* with cognitive decline, a finding which is consistent with the emerging role of microRNAs in the regulation of proteins responsible for neural development and function.

3.1.5 TCF4: Common SNPs in the transcription factor 4 (*TCF4*) gene (including rs4309430) are associated with SZ, conferring a small increase in risk. *TCF4* is highly expressed in the brain, where it plays a role in neurodevelopment (Navarette et al., 2013). The Ca(2+) sensor protein calmodulin interacts with the DNA binding domain of *TCF4*, inhibiting transcriptional activation. It is also the target of microRNAs, including *MIR-137*, which is implicated in SZ (Ripke et al., 2011). RS4309430 is in linkage disequilibrium with common variants within

putative DNA regulatory elements, suggesting that regulation of expression may underlie association with SZ (Navarette et al., 2013).

3.1.6 MMP16: Matrix metalloproteinase-16 gene (*MMP16*) encodes an enzyme called matrix metalloproteinase 16 (Takino et al., 1995). Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. How this relates to SZ etiology remains unclear.

3.1.7 PCGEM1: Prostate-specific transcript 1 (non-protein coding), also known as *PCGEM1*, is a long non-coding RNA gene located on chromosome 2q32. It is over-expressed in prostate cancer (Srikantan, 2000). As with *MMP16*, it is unclear how this relates to SZ etiology.

3.1.8 The present study:

The purpose of the present study was to investigate the association between each of these seven variants and cognition by looking at the impact of the risk alleles on cognition in patients with SZ and their healthy counterparts. We hypothesised that possessing the risk allele would be deleterious to cognitive functioning in the patient group.

To test this hypothesis, 400 patients with a diagnosis of either SZ or schizoaffective disorder (SZA) and 171 controls were assessed in areas of cognitive ability typically found to be impaired in schizophrenia (SZ): including *(1) IQ* (using selected subtests from the Wechsler Adult Intelligence Scale, 3rd edition), *(2) memory* (using the Wechsler Memory Scale, 3rd edition and the Paired Associate Learning (PAL) and spatial working memory task (SWM) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB), *(3) attention* (using the continuous performance task identical pair's version (CPT-IP) and the Intradimensional-extradimensional shift task (IDED) and, where the literature suggested a potential link, (4) *social cognition* (using the Internal, Personal, and Situational Attributions Questionnaire (IPSAQ) (Kinderman & Bentall, 1996) and two theory of mind tasks; The Hinting Task (Corcoran et al., 1995) and the Reading the Mind in the Eyes Task (Baron-Cohen et al., 2001)).

Using these measures of neuropsychological function, the neurocognitive effects of the seven SZ associated variants were investigated in vivo by comparing carriers and non-carriers of the risk alleles.

3.2 Methods

3.2.1 Participants: Patients and controls who had completed a full neuropsychological assessment battery and for whom full genome wide data was available were analyzed.

Cases consisted of clinically stable patients with a DSM-IV diagnosis of SZ or schizoaffective disorder (SZA) recruited from five sites across Ireland. Inclusion criteria required that participants were aged 18 to 65 years, had no history of co-morbid psychiatric disorder, substance abuse in the preceding six months, or prior head injury with loss of consciousness or a history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID; First et al., 2002). Additional diagnostic details and clinical sample characteristics ascertained at time of interview including symptom severity (SAPS/SANS: Andreasen, 1984(a); Andreasen, 1984(b)) and medication dosage are detailed elsewhere (Walters et al., 2010).

Healthy participants were recruited via both online and poster advertising. They were aged 18 to 65 years, with no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Neither they nor any first degree relative had a history of psychosis.

All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

It should be noted that the number of participants varies from one neuropsychological test to another. This is due to some tests being administered to a greater number of participants based on a) availability of participants, b) time

point at which a test was introduced to the battery and c) acquiescence of participant to complete the test.

3.2.2. Variants: Variants analysed were taken from those identified in the Ripke (2011) paper as being associated with SZ. It should be noted that three SNPs analysed in the current study are not the actual SNP given in the Ripke study (those for *NRGN, TCF4* and *CNNM2*). Reasons for the SNP substitution are given in the supplementary section at the end of the chapter.

3.2.3 Procedure:

General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III; Wechsler, 1997a), yielding a full scale, verbal and performance IQ. Verbal and spatial ***episodic memory*** was assessed using the logical memory and faces subtests from the Wechsler Memory Scale, 3rd edition (WMS-III; Wechsler, 1997b), the Paired Associate Learning (PAL) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB). Verbal and spatial ***working memory*** was assessed using the WMS-III letter number sequencing task and the spatial working memory task from the Cambridge Automated Neuropsychological Test Battery (CANTAB SWM; Robbins et al., 1994). ***Attentional control*** was assessed using the continuous performance task (CPT), identical pair's version (CPT-IP, Cornblatt et al., 1988) and the the Intradimensional-extradimensional shift task (IDED; CANTAB). ***Social cognition*** was assessed (for those variants for which the literature suggested a potential link between the variant and social functioning) using 1) two theory of mind tasks; a) Reading the mind in the eyes task ((Baron-Cohen et al., 2001) and b) The Hinting task (Corcoran et al., 1995), and 2) an attributions task called the Internal, personal and situational attributions questionnaire (IPSAQ) (Kinderman & Bentall, 1996), which yields two bias scores; a) externalising bias (EB), which indicates a propensity to attribute positive events to oneself rather than to other people and b) a personalising bias (PB), which indicates a propensity to attribute negative events to other people rather than to situational factors.

***Genotyping*:** Genetics analysis was carried out using DNA extracted from blood or saliva samples. Each SNP was genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium ($p>0.05$). Along with these samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for each SNP for quality control purposes and were found to be concordant with available HapMap data for each SNP.

3.2.4 Data Analysis:

Neuropsychological data analysis Association between the variables and the phenotypes of general cognitive function, episodic memory, working memory, attentional control and social cognition was tested using a general factorial design in SPSS version 17 (SPSS 2008). For variants whose minor allele resulted in small/underpowered homozygous group sizes, genotypes were grouped into homozygous risk versus heterozygous non-risk for the purpose of analysis (*MIR-137* – which had with only 26 individuals in the GG group compared with 231 and 547 in the TG and TT groups respectively). In a series of ANCOVAs, scores for each neuropsychological subtest were entered as the dependent variables, with age and gender included as covariates as appropriate. Genotype and diagnosis (cases versus controls) were entered as fixed effects.

3.3 Results

3.3.1 *MIR-137*

MIR-137 and the sample's demographic and clinical characteristics Demographic and clinical characteristics by rs1625579 genotype (TT, GT, GG) for patients and healthy participants appear in **table 3.2**. For all demographic variables, means and standard deviations were computed using SPSS 2008. There was no association between the risk SNP and diagnosis, age at time of assessment, gender, or medication dosage in chlorpromazine equivalents.

Table 3.2: rs1625579 SNP genotype groups and demographic variables.
Clpz = chlorpromazine.

	GG (n=26) Mean(SD)	GT (n=231) Mean (SD)	TT (n=547) Mean (SD)	F	P
Age at onset	21.17(7.07)	24.23(8.29)	24.03(8.48)	1.388	.25
Illness duration (yrs)	14.92(15.4)	19.99(12.27)	19.96(12.3)	1.85	.158
Clpz equivalent (mg)	412(412.8)	506.8(449.3)	468.8(411.8)	.73	.483
Gender (% male)	84%	62.7%	66.2%	4.68	.096

The effects of MIR-137 on cognition

Cognitive analysis by *MIR-137* Genotype is presented in **table 3.3**.

Apart from tests of social cognition, patients performed below controls on all cognitive tests administered ($p<0.0001$). *MIR-137* genotype was not associated with differences in IQ, either as a main effect or as an interaction effect with case-control status. Analysis of the two allele groups (TT, GT/GG) identified association between homozygous risk 'T' allele carriers and 1) verbal working memory (Letter-number sequencing; $F=1.94$, $p= .014$), 2) verbal episodic memory (Logical Memory, Immediate recall; $F=1.535$, $p=0.023$) and 3) extradimensional set shifting (as measured by the IDED-ED scores) attentional control phenotypes; $F=5.16$, $p=0.047$). In each case carrying the risk allele was associated with poorer performance; there was no interaction effect with case/control status.

For the purposes of both interest and continuity of methodology with the previous SNPs investigated in this chapter, the analysis was repeated using a three group design (TT versus GT versus GG). This confirmed the verbal episodic memory (Logical Memory, Immediate; $F=1.16$, $p=0.048$) and extradimensional set shifting attentional control (IDED; $F=3.23$, $p=0.04$) associations found in the two group design analysis. The risk allele was consistently associated with worse performance and there was no interaction effect with case/control status

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Table 3.3: Cognitive analysis by *MIR-137* Genotype

Cognitive function	Test or Subscale	sample	n	Mean (SD)	Mean (SD)	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p
				GG/GT	TT						
IQ	Full Scale IQ	Patients	275	93.31(17.82)	90.63(17.19)						
		Controls	133	124.33(12.2)	121.82(15.4)	293.1	<.0001	2.384	.123	.410	.522
	WTAR	Patients	275	96.45(11.93)	97.32(11.32)						
		Controls	133	110.06(3.73)	109.55(5.34)	.012	<.0001	.012	.461	0	.985
	Verbal IQ	Patients	275	94.06(16.02)	93.03(17.51)						
		Controls	133	125.79(13.8)	123.7(14.99)	282.1	<.0001	.817	.367	.079	.778
	Performance IQ	Patients	275	93.75(20.8)	89.71(18.59)						
		Controls	133	119.33(13.5)	116.03(19.3)	173.4	<.0001	3.04	.051	.099	.753
Working Memory	LN sequence	Patients	370	8.162(3.548)	7.51(3.24)						
		Controls	167	13.38(2.91)	12.78(3.34)	.014	<.0001	1.940	**.014**	.005	.956
	SWM errors	Patients	366	-.80(1.32)	-1.02(1.3)						
		Controls	159	.237(.716)	.281(.823)	96.98	<.0001	.628	.149	1.186	.277
Episodic Memory	Logical Memory Immediate	Patients	379	7.01(3.52)	6.18(3.31)						
		Controls	167	12.75(2.54)	12.72(2.95)	346.2	<.0001	1.535	**.023**	1.72	.19
	Logical Memory Delayed	Patients	379	7.46(3.19)	7.21(3.21)						
		Controls	167	13.29(2.4)	13.34(2.65)	369.1	<.0001	.035	.244	.354	.552
	PAL Std score	Patients	291	-2.65(3.84)	-2.77(3.77)						
		Controls	123	.1592(1.2)	.2654(1.08)	53.89	<.0001	.001	.297	.092	.762
	Faces immediate	Patients	355	8.69(2.83)	8.76(2.77)						
		Controls	168	11.14(2.64)	11.54(2.88)	85.1	<.0001	.221	.639	.031	.861
	Faces delayed	Patients	355	9.2(2.86)	9.34(2.93)						
		Controls	168	11(2.46)	11.63(2.84)	46.5	<.0001	2.054	.152	.845	.358
Vigilant attention	CPT-IP 3 letters	Patients	74	1.85(1.01)	2.02(.973)	----	----			----	----
		Controls	0					1.457	.229		
	IDED errors 8 shapes	Patients	322	10.85(10.65)	12.93(10.97)	17.85	<.0001	5.16	**.047**	.042	.838
		Controls	157	7.05(7.95)	9.39(10.15)						
	IDED errors 6 shapes	Patients	322	1.05(2.85)	.812(1.49)	2.53	.112	.204	.652	2.738	.099
		Controls	157	.381(.652)	.814(2.3)						
Social Cognition	Hinting Task	Patients	78	16.51(2.68)	16.1(2.23)	3.417	.067	.492	.12	.121	.729
		Controls	50	17.11(1.13)	16.96(1.61)						
	Eyes in the mind	Patients	78	22.03(6.69)	23.24(139.4)	.137	.712	.435	.511	.399	.529
		Controls	50	26.05(4.53)	26.03(4.5)						
	IPSAQ EB	Patients	253	1.06(3.84)	.85(3.48)	.286	.593	.054	.816	.348	.556
		Controls	131	.773(3.71)	1.05(3.98)						
	IPSAQ PB	Patients	253	.487(.27)	.498(.289)	2.475	.116	.871	.626	.401	.146
		Controls	131	.41(.35)	.627(.248)						

3.3.2 *CNNM2*

CNNM2 and sample demographic and clinical characteristics

Demographic and clinical characteristics for patients and healthy participants appear in **table 3.4**. For all demographic variables, means and standard deviations were computed using SPSS 2008. There was no difference in genotype frequency between patients and controls and no genotype-related variability in gender ratio, age or years of education in any of the samples. Moreover, in patients, rs7914558 was not associated with age of onset or medication (i.e. chlorpromazine equivalents). Comparing patients with healthy controls, there was a significant difference in age and years of education between groups, such that patients were older ($F=4.86$, $p<0.05$) and had less years of education ($F=105.6$, $p<0.001$).

The effects of CNNM2 on cognition

Cognitive analysis by *CNNM2* Genotype is presented in **table 3.5**.

Traditional cognition: Patients performed worse than controls on all measures of general cognitive function ($p\leq0.001$). While a statistically significant finding was evident for the episodic memory task - Faces delayed recall, closer examination shows that the significance lies with the heterozygous allele. As such we concluded that there was no genotype-related variability on indices of IQ, memory or attentional control.

Social cognition: Regarding ToM processes, Hinting task performance was unaffected by group, yet patients performed significantly worse on the Reading the mind in the eyes task ($F=8.11$, $p<0.05$). Performance on ToM measures was unaffected by genotype.

IPSAQ cognitive bias scores were differentially impacted by group and genotype. For example, PB was significantly lower in patients than controls ($F=6.12$; $p<0.05$) but was unaffected by genotype. Conversely, EB varied according to genotype ($F=5.46$, $p=0.004$) but not group (**figure 3.1**). This effect was due to the linear impact of genotype on EB ($F=10.14$, $p<0.001$), such that 'GG' individuals scored highest on this subscale, followed by 'AG' and then 'AA' individuals($p<0.05$).

Table 3.4: Participant demographics. Clpz = chlorpromazine; s.d = standard deviation.

	Total Sample		*CNNM2/rs7914558*						*Comparison*
			GG		*AG*		*AA*		
	N=560		*N=89*		*N=258*		*N=213*		
	Patients N=400	Controls N=160	Patients N=66	Controls N=23	Patients N=174	Controls N=84	Patients N=160	Controls N=53	
Gender (F:M)	111:288	76: 84	22: 44	12: 11	49: 125	43: 41	41: 119	21:32	ns
Age in years (mean(s.d.))	41.45 (12.25)	37.14 (12.70)	39.96 (10.45)	39.61 (14.14)	42.52 (11.86)	37.13 (12.33)	42.32 (11.79)	36.12 (13.02)	Control< Patient $F_{=}4.86, p<0.05$
Years of Education (mean (s.d.))	13.09 (2.55)	15.98 (2.42)	13.86 (2.47)	16.13 (2.63)	12.95 (2.45)	16.17 (2.60)	13.11 (2.71)	15.61 (2.00)	Control > Patient $F=105.61, p<0.001$
Age at onset (mean (s.d.))	22.79 (7.32)	n/a	21.57 (5.52)	n/a	23.28 (8.22)	n/a	21.87 (6.03	n/a	ns
Clpz Equivalents-mg/day (mean (s.d.))	582.84 (543.76)	n/a	549.22 (478.55)	n/a	570.17 (511.79)	n/a	597.11 (618.99)	n/a	ns

Table 3.5: Cognitive analysis by *CNNM2* Genotype

Cognitive function	Test or Subscale	sample	n	Mean (SD) AA	Mean (SD) AG	Mean(SD) GG	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p	F simple effect	p
IQ	Full Scale IQ	Patients	305	94.6(20.5)	88.9(16.98)	90.2(17.7)								
		Controls	158	119.7(13.4)	122.7(13.6)	121.8(16.8)	275.3	<.0001	.246	.435	1.835	.161	2.24	.108
	Verbal IQ	Patients	280	94.5(18.9)	92.1(15.2)	93.7(17.9)								
		Controls	133	122.2(12.1)	123.5(13.6)	126.6(16.8)	250.4	<.0001	.662	.589	.472	.624	.492	.612
	Performance IQ	Patients	285	94.9(21.7)	89.2(19.5)	90.3(17.6)								
		Controls	133	115.4(18.9)	119.6(16.4)	115.3(18.9)	146.9	<.0001	.71	.492	2.118	.122	1.83	.162
Working Memory	LN sequence	Patients	373	8.26(3.69)	7.6(3.3)	7.4(3.25)								
		Controls	156	13.6(2.89)	12.8(3.08)	12.4(3.4)	220.1	<.0001	2.42	.09	.129	.879	1.378	.253
	SWM errors	Patients	369	-.96(1.56)	-.86(1.26)	-1.06(1.25)								
		Controls	151	.403(.72)	.169(.813)	.266(.742)	90.4	<.0001	.268	.266	.807	.447	.887	.413
Episodic Memory	Logical Memory	Patients	385	6.9(3.89)	6.3(3.27)	6.1(3.360								
	Immediate	Controls	156	13.1(2.8)	12.6(2.89)	12.5(2.75)	309.2	<.0001	.986	.129	.054	.948	1.294	.275
	Logical Memory	Patients	382	8(3.42)	7.1(3.1)	7.03(3.2)								
	Delayed	Controls	156	13.4(2.74)	13.2(2.7)	13.3(2.3)	311.6	<.0001	.864	.182	.449	.638	2.121	.121
	PAL Std score	Patients	362	-2.5(4.23)	-2.6(3.5)	-2.9(3.9)								
		Controls	150	-.14(1.67)	.22(1.29)	.36(.62)	58.1	<.0001	.043	.235	.516	.597	.373	.689
	Faces immediate	Patients	361	8.5(2.46)	9.07(2.9)	8.4(2.6)								
		Controls	157	11.4(2.82)	11.7(2.9)	11.1(2.4)	77.1	<.0001	2.29	.102	.085	.919	2.416	.091
	Faces delayed	Patients	358	8.9(2.58)	9.6(3.1)	9.1(2.8)								
		Controls	157	11.1(2.98)	11.7(2.74)	10.9(2.39)	41.32	<.0001	3.09	**.046**	.038	.962	2.138	.119
Attentional Control	CPT_IP (3 letters)	Patients	261	1.91(1.01)	1.93(.97)	1.98(.995)	---	---	.091	.913	---	---	.091	.913
		Controls	---	---	---	---								
	IDED errors (8 shapes)	Patients	324	11.6(11.2)	12.4(10.7)	12.5(10.9)	11.9	.001	.006	.872	.355	.701	.255	.775
		Controls	149	9.4(10.6)	8.9(9.3)	8(9.07)								
	IDED errors (6 shapes)	Patients	327	.94(1.8)	.77(1.51)	1.01(2.52)	.307	.58	.914	.402	1.24	.287	.499	.608
		Controls	148	1.25(2.42)	.67(2.3)	.41(.64)								
Social Cognition	Hinting Task	Patients	276	15.6(3.3)	15.5(3.5)	15(3.41)	15.8	<.0001	1.58	.063	.055	.946	.889	.412
		Controls	132	17.2(1.4)	16.8(1.7)	16.3(2.1)								
	Eyes	Patients	151	25(4.7)	38(116.6)	24.3(4.8)	.022	.883	.254	.776	.148	.862	.575	.564
		Controls	50	28.4(3.04)	26.3(4.1)	24.5(5.02)								
	IPSAQ EB	Patients	256	1.6(2.9)	1.17(3.7)	.26(2.8)	.167	.683	5.72	**.004**	1.53	.217	2.2	.113
		Controls	131	3.2(3.8)	.78(4.25)	.38(3.6)								
	IPSAQ PB	Patients	256	.48(.26)	.48(.29)	.51(.27)	2.17	.115	2.17	.82	2.27	.104	.485	.616
		Controls	131	.61(.21)	.64(.25)	.41(13.2)								

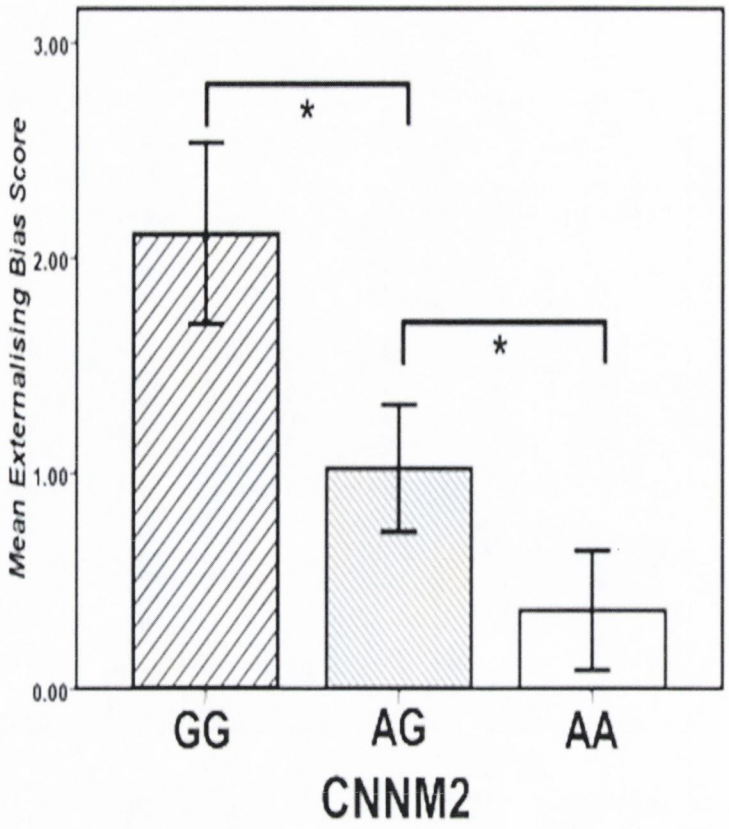

Figure 3.1: Main effect of *CNNM2*/rs7914558 genotype on IPSAQ EB score.
Note: Error bars show +/- 1 standard error. * $p < 0.05$

3.3.3 *CSMD1*

CSMD1 genotype and clinical characteristics

Associations between clinical characteristics and genotype appear in **tables 3.6 & 3.7**, with means and standard deviations computed using (SPSS 2008). In summary, no differences were observed between genotypes for age at onset, medication dosage, duration of illness in years or clinical symptomatology as measured using both (1) the Bipolar Affective Disorder Dimension Scale (BADDS), which is a dimensional scale for rating lifetime psychopathology in Bipolar spectrum disorders and (2) the Operational Criteria Checklist (OPCRIT), which generates diagnoses according to 12 operational diagnostic systems (e.g. DSM-III, DSM-III-R, Research Diagnostic Criteria, ICD-10).

Table 3.6: *CSMD1* rs10503253 & Other Clinically Relevant Variables (all non-significantly related to genotype, all p>.05). Std Dev = standard deviation. Std Error = standard error.

		N	Mean	Std Dev.	Std. Error	Minimum	Maximum
Age at onset	CC	439	23.9	8.242	0.393	8	66
	AC	247	24.79	9.055	0.576	9	70
	AA	31	22.03	6.113	1.098	15	40
	Total	717	24.12	8.466	0.316	8	70
Chlorpromazine Equivalent	CC	378	486.74	442.47	22.758	0	2600
	AC	197	485.22	398.84	28.416	0	2100
	AA	27	367.88	340.83	65.593	0	1650
	Total	602	480.91	424.63	17.307	0	2600
Duration Of Illness in Years	CC	430	19.9	11.88	0.5727	0.04	56.55
	AC	241	19.64	13.24	0.8526	0.25	71.45
	AA	30	16.82	12.72	2.323	0.51	44.24
	Total	701	19.687	12.4	0.4681	0.04	71.45

Table 3.7: RS10503253 alleles (*CSMD1*) and Clinical Symptomatology

		N	Mean	St Dev	95% Confidence Interval for Mean Lower Bound	Upper Bound	F	Sig.
BADDS Mania Dimension Score	CC	493	31.08	31.03	28.34	33.83	0.076	0.927
	AC	275	31.46	31.41	27.73	35.19		
	AA	36	29.33	29.35	19.4	39.27		
BADDS Depression Dimension Score	CC	493	41.46	40.88	37.84	45.08	0.041	0.96
	AC	275	41.89	41.19	37	46.78		
	AA	36	39.86	42.3	25.55	54.17		
BADDS Psychotic Dimension Score	CC	493	39.06	28.84	36.51	41.61	0.436	0.647
	AC	275	38.84	26.69	35.67	42.01		
	AA	36	43.47	33.6	32.1	54.84		
BADDS Incongruence Dimension Score	CC	493	59.39	30.21	56.71	62.06	0.087	0.917
	AC	275	58.83	29.99	55.27	62.39		
	AA	36	60.92	32.03	50.08	71.76		
Opcrit Manic Factor	CC	488	0.0819	1.031	-0.0098	0.1736	0.321	0.726
	AC	272	0.0393	1.019	-0.0823	0.161		
	AA	36	-0.0358	1.064	-0.396	0.3243		
Opcrit Depression Factor	CC	488	-0.0096	1.001	-0.0994	0.0802	0.316	0.729
	AC	272	-0.0199	0.996	-0.1389	0.0991		
	AA	36	0.1207	0.926	-0.1929	0.4342		
Opcrit Positive Symptoms Factor	CC	488	0.0234	0.991	-0.0648	0.1115	0.06	0.942
	AC	272	0.048	0.979	-0.0689	0.1649		
	AA	36	0.0512	1.072	-0.3116	0.414		
Opcrit Disorganisation Factor	CC	488	0.0438	1.011	-0.0461	0.1338	0.114	0.893
	AC	272	0.0802	0.992	-0.0382	0.1987		
	AA	36	0.0623	1.161	-0.3306	0.4553		
Opcrit Negative Factor	CC	488	0.0142	1.003	-0.075	0.1035	1.052	0.35
	AC	272	0.0881	0.99	-0.0301	0.2063		
	AA	36	-0.1436	1.114	-0.5206	0.2334		

CSMD1 & Neuropsychological performance

A main effect of the risk 'A' allele was found on measures of both IQ and memory (see **table 3.8**). Specifically, homozygous carriers of the 'A' allele performed below other genotype groups in both verbal IQ and verbal episodic memory. A genotype by diagnosis interaction was observed for verbal working memory in the absence of a genotype main effect ($F=5.55$, $p=0.004$). Follow-up analysis (taking cases and controls separately) revealed an association with genotype in controls ($F=5.47$, $p=0.005$) but not cases.

Table 3.8: Difference in neuropsychological performance associated with rs10503253 in patients and controls

Test or Subscale	sample	n	Mean (SD)	Mean (SD)	Mean(SD)	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect\ Genotype}$	P	$F_{Interaction\ effect}$	p
			CC	AC	AA						
Verbal IQ	Patients	378	92.59(19.7)	91.04(17.8)	85.6(14.89)	115.2	<.0001	3.95	**0.02**	1.009	0.365
	Controls	168	122.2(15.6)	124.2(15.69)	108.5(10.4)						
Performance IQ	Patients	378	89.35(18.2)	88.5(18.8)	87.5(12.6)	116.1	<.0001	0.455	0.635	0.222	0.801
	Controls	168	118.05(18.7)	115.5(18.9)	117.3(17.9)						
Full Scale IQ	Patients	378	90.5(18.27)	88.9(17.4)	85.4(13.1)	160.9	<.0001	0.996	0.37	0.118	0.888
	Controls	168	121.6(16.07)	122.2(15.88)	116.7(8.9)						
Letter-number sequencing	Patients	367	7.85(3.38)	7.5(3.29)	6.78(3.55)	152.5	<.0001	1.31	0.27	5.55	**0.004**
	Controls	171	12.19(3.05)	13.2(3.05)	15.3(3.34)						
CANTAB SWM errors	Patients	351	-.91(1.3)	-1.1(1.3)	-.41(1.42)	34.83	<.0001	1.08	0.12	1.23	0.291
	Controls	159	.19(.79)	.31(.76)	-.39(.587)						
Logical Memory Immediate	Patients	370	6.5(3.48)	6.2(3.36)	5.2(3.13)	145.4	<.0001	2.71	0.068	0.117	0.889
	Controls	171	12.9(3)	12.8(2.48)	11.3(2.9)						
Logical Memory Delayed	Patients	370	7.4(3.23)	6.8(3.33)	6.2(3.06)	129.3	<.0001	3.23	**0.04**	1.785	0.169
	Controls	171	13.2(2.99)	13.6(2.4)	11.1(3.21)						
CANTAB PAL	Patients	359	-2.61(3.85)	-3.21(3.77)	-1.65(3.07)	24.9	<.0001	0.433	0.649	0.255	0.775
	Controls	144	.239(1.11)	.15(1.25)	.19(1.72)						
CPT_IP (3 Letters)	Patients	256	2.02(.96)	1.8(1.01)	2.5(.923)	-	-	2.56	0.08	-	-
	Controls	-	-	-	-						

3.3.4 *TCF4*

Results of cognitive analysis: For the *TCF4* SNP, rs4309430, a single association between genotype and Letter-number sequencing – a working memory test – was found. On closer inspection of patient and control means and standard deviations however (see **table 3.9**), it is clear that no note-worthy difference exists. As such it was concluded that rs4309430 is *not* associated with cognition.

3.3.5 *NRGN*

Results of cognitive analysis: No association was found between rs12807809 and cognition (**see table 3.10**). This suggests that *NRGN* effects as a genetic risk factor for SZ are independent of cognition.

3.3.6 *MMP16*

Results of cognitive analysis: rs7004633 was found to be associated with visual recognition memory as measured by the WMS-III Faces task, both immediate and delayed recall. Inspection of patient and control means and standard deviations (see **table 3.11**), suggests that genotype affected performance in the patient group only, such that risk allele carriers perform better than non-risk carriers in recall of faces. As this observation was specific to cases, for only one measure, and no other associations were observed, this association may well represent a false positive. By comparison with other common variants analyzed here, this variant is unlikely to strongly impact cognition.

3.3.7 *PCGEM1*

Results of cognitive analysis: rs17662626 was only found to be associated with attributional style (a measurement of social cognition); specifically with externalizing bias from the Internal personal and situational attributions questionnaire (IPSAQ). No other associations were observed with this variant. In the case of the association with externalizing bias, the significant difference observed was not related to genotype dosage; the association observed lay between the heterozygous risk and homozygous non-risk groups amongst patients with SZ (see **table 3.12**). As with MMP16, the previously reported increased risk for SZ associated with rs17662626 is unlikely to occur via an effect on cognition.

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Table 3.9: Cognitive analysis by rs4309430 (*TCF4*) genotype. A is the risk allele.

Cognitive function	Test or Subscale	sample	n	Mean (SD) GG	Mean (SD) AG	Mean(SD) AA	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p	$F_{simple\ effect}$	p
IQ	Full Scale IQ	Patients	306	89.65(19.3)	89.27(18.1)	92.1(17.2)								
		Controls	162	121.09(11.9)	122.1(16.5)	122.6(13.6)	325.5	<.0001	.744	.476	.054	.948	.451	.597
	Verbal IQ	Patients	281	93.71(16.27)	91.63(17.08)	94.8(17.33)								
		Controls	134	126.2(11.23)	122.5(16.05)	125.8(13.8)	275.1	<.0001	2.02	.134	.118	.889	.146	.732
	Performance IQ	Patients	286	90.3(16.8)	89.6(20.4)	92.1(18.3)								
		Controls	134	115.4(13.8)	118.2(18.9)	117.4(17.8)	163.7	<.0001	.405	.667	.088	.916	.217	.655
Working Memory	LN sequence	Patients	374	7.7(3.48)	7.69(3.36)	7.6(3.2)								
		Controls	160	13.2(2.9)	12.8(3.7)	12.8(3.7)	241.3	<.0001	.158	**.036**	.189	.828	.025	.975
	SWM errors	Patients	370	-.88(1.22)	-.92(1.2)	-1.03(1.4)								
		Controls	155	.49(.712)	.237(.71)	.09(.87)	99.4	<.0001	1.36	.321	.356	.701	.365	.694
Episodic Memory	Logical Memory Immediate	Patients	386	6.4(3.67)	6.29(3.23)	6.4(3.57)								
		Controls	160	12.1(2.74)	12.8(2.68)	12.8(3.1)	377.1	<.0001	3.05	.56	.758	.469	.076	.927
	Logical Memory Delayed	Patients	383	7.3(3.59)	7.1(2.99)	7.26(3.39)								
		Controls	160	12.7(2.68)	13.3(2.36)	13.7(2.85)	340.3	<.0001	.605	.48	.954	.386	.057	.945
	PAL Std score	Patients	364	-2.7(3.35)	-3.04(4.24)	-2.4(3.28)								
		Controls	154	.38(.494)	.14(1.05)	.23(1.59)	69.1	<.0001	.469	.568	.401	.67	1.102	.333
	Faces Immdiate	Patients	362	8.5(2.59)	8.9(2.88)	8.4(2.6)								
		Controls	161	11.2(3.04)	11.5(2.91)	11.7(2.46)	84.46	<.0001	.6	.549	.619	.539	1.227	.28
	Faces Delayed	Patients	359	9.6(2.9)	9.1(2.88)	9.2(2.94)								
		Controls	161	11.6(3.3)	11.2(2.6)	11.4(2.46)	45.2	<.0001	.63	.533	.076	.927	.668	.513
Attentional Control	CPT_IP 3 letters	Patients	261	2.04(1.03)	1.86(.92)	2.05(1.04)								
		Controls	---	---	---	----	---	---	1.12	.328	---	---	1.119	.328
	IDED 8 shapes	Patients	325	11.7(10.7)	12.5(11.3)	12.2(10.48)								
		Controls	153	9.7(10.6)	8.4(9.4)	8.4(9.11)	13.5	<.0001	.084	.919	.28	.756	.07	.93
	IDED 6 shapes	Patients	328	1.4(3.7)	.7(1.32)	.91(1.8)								
		Controls	152	.58(.56)	.82(2.46)	.5(1.48)	3.35	.068	.656	.668	1.94	.145	2.68	.07
Social Cognition	Hinting Task	Patients	277	14.9(2.57)	15.4(3.65)	15.3(3.65)								
		Controls	133	16.8(1.37)	16.5(1.97)	16.9(1.8)	19.38	<.0001	.183	.238	.66	.517	.355	.702
	Eyes in the mind	Patients	151	25(4.2)	23(5.1)	42.9(132.6)								
		Controls	50	26(4.6)	26(4.5)	25(4.5)	.08	.773	.35	.703	.19	.82	.89	.413
	IPSAQ EB	Patients	257	.95(2.49)	.92(3.99)	.91(3.3)								
		Controls	132	.95(3.48)	.81(3.41)	1.2(4.7)	.07	.789	.07	.854	.036	.965	.012	.988
	IPSAQ PB	Patients	257	.46(2.87)	.52(2.8)	.47(.27)								
		Controls	132	.61(.201)	.61(.23)	.41(.36)	.764	.383	1.99	.251	1.75	.174	.921	.399

Table 3.10: Cognitive analysis by rs12807809 (*NRGN*) Genotype. T is the risk allele.

Cognitive function	Test or Subscale	sample	n	Mean (SD) CC	Mean (SD) CT	Mean(SD) TT	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p	F simple effect	p
IQ	Full Scale IQ	Patients	279	93.67(19.3)	90.24(16.4)	91.3(17.4)								
		Controls	130	123.7(13.5)	121.6(13.9)	122.9(14.9)	63.1	<.0001	.598	.55	.088	.916	.208	.812
	Verbal IQ	Patients	279	92.1(13.87)	92.8(17.2)	92.4(17.42)								
		Controls	130	132(10.58)	123(13.78)	124.4(15.2)	76.27	<.0001	.374	.688	.425	.654	.044	.957
	Performance IQ	Patients	279	96.5(23.85)	89.41(18.2)	91.1(19.6)								
		Controls	130	111.7(13.2)	117.7(13.3)	118.2(17.9)	26.2	<.0001	.665	.515	.653	.521	.486	.616
Working Memory	LN sequence	Patients	364	6(2.08)	7.6(3.44)	7.78(3.34)								
		Controls	146	13(3)	12.6(2.8)	12.9(3.35)	48.76	<.0001	.81	.446	.369	.691	1.69	.186
	SWM Errors	Patients	364	-.99(1.06)	-1.07(1.42)	-.98(1.31)								
		Controls	146	.003(.554)	.24(.79)	.238(.769)	16.48	<.0001	.173	.841	.138	.871	.294	.745
Episodic Memory	Logical Memory Immediate	Patients	340	6.5(2.77)	5.98(3.55)	6.61(3.4)								
		Controls	146	13(3.46)	12.65(2.8)	12.8(2.45)	69.5	<.0001	.75	.473	.174	.84	1.12	.327
	Logical Memory Delayed	Patients	340	7.2(2.37)	6.89(3.3)	7.4(3.14)								
		Controls	146	13.66(3.05)	13.2(2.9)	13.3(2.5)	72.1	<.0001	.697	.499	.13	.879	.96	.384
	PAL Std Score	Patients	340	-3.08(3.4)	-2.24(5.13)	-2.36(5.06)								
		Controls	146	.486(.377)	.173(1.51)	.228(1.05)	7.44	.007	.02	.98	.076	.927	.11	.896
	Faces Immdiate	Patients	340	9.12(2.03)	8.42(2.68)	8.85(2.83)								
		Controls	146	10.33(1.15)	11.6(2.9)	11.63(2.78)	13.66	<.0001	.316	.729	.643	.526	.845	.43
	Faces Delayed	Patients	340	10.12(2.58)	9.4(2.96)	9.3(2.9)								
		Controls	146	11(2)	11.25(2.67)	11.45(2.67)	5.59	.018	.045	.956	.258	.773	.257	.773
Attentional Control	CPT_IP 3 letters	Patients	248	1.9(.99)	1.96(.97)	1.99(.97)								
		Controls	0	-	-	-	---	---	.011	.916	---	---	.011	.916
	IDED 8 shapes	Patients	322	11.72(9.97)	11.46(10.7)	12.45(10.8)								
		Controls	144	7.54(8.49)	7.86(9.14)	9.1(9.56)	16.22	<.0001	.713	.39	.074	.786	.173	.892
	IDED std score	Patients	322	-1.34(1.09)	-1.34(1.37)	-1.4(1.48)								
		Controls	144	-.08(.96)	-.06(.93)	-.14(1.08)	68.29	.0001	.963	.327	.23	.879	.131	.878
Social Cognition	Hinting Task	Patients	274	15.14(2.9)	15.14(3.48)	15.4(3.7)								
		Controls	129	15.75(3.3)	16.55(1.7)	16.88(1.8)	2.27	.133	.512	.6	.166	.847	.105	.901
	Eyes in the mind	Patients	128	25(2.91)	22.2(2.8)	21.67(3.2)								
		Controls	124	29(2.94)	26.01(2.7)	26.07(3.36)	5.68	.012	1.49	.228	.247	.675	1.46	.345
	IPSAQ EB	Patients	245	2(4.08)	.912(2.95)	.919(3.85)								
		Controls	128	.75(4.34)	.421(3.88)	1.29(3.89)	.018	.893	.269	.764	.443	.642	.29	.748
	IPSAQ PB	Patients	245	.634(.28)	.478(.266)	.52(.265)								
		Controls	128	.68(.22)	.71(.24)	.624(.256)	.285	.594	2.06	.128	2.08	.126	1.21	.3

Table 3.11: Cognitive analysis by rs7004633 (*MMP16*) genotype. G is the risk allele.

Cognitive function	Test or Subscale	sample	n	Mean (SD) AA	Mean (SD) AG	Mean(SD) GG	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p	$F_{simple\ effect}$	p
IQ	Full Scale IQ	Patients	303	90.3(18.02)	89.5(18.4)	98.5(12.6)								
		Controls	158	122.1(15.3)	122.5(13.5)	115.8(16.2)	46.1	<.0001	.019	.981	.63	.533	.569	.567
	Verbal IQ	Patients	278	93.6(17.57)	91.6(16.4)	96.3(8.8)								
		Controls	131	124.3(15.4)	125.7(12.6)	109.6(10.7)	42.46	<.0001	.511	.6	1.41	.245	1.481	.229
	Performance IQ	Patients	283	90.4(19.4)	90(18.9)	101.3(16.9)								
		Controls	131	116.7(18.4)	119.1(16.5)	120(26.15)	33.23	<.0001	.638	.529	.17	.843	.835	.435
Working Memory	LN sequence	Patients	293	7.8(3.38)	7.3(3.2)	10.16(3.18)								
		Controls	156	12.59(3.13)	13.6(3.4)	14.33(3.21)	40.2	<.0001	1.72	.18	2.06	.128	2.21	.112
	SWM errors	Patients	443	-1.03(1.41)	-.79(1.22)	-.24(.92)								
		Controls	291	.222(.69)	.205(.957)	1.17(.595)	18.3	<.0001	2.37	.168	.557	.573	1.79	.168
Episodic Memory	Logical Memory Immediate	Patients	310	6.37(3.41)	5.9(3.51)	9(1.89)								
		Controls	156	12.7(2.99)	12.4(2.3)	12(2.64)	41.68	<.0001	1.01	.32	1.226	.295	2.422	.09
	Logical Memory Delayed	Patients	307	7.3(3.17)	6.7(3.43)	9.6(1.5)								
		Controls	156	13.4(2.62)	12.9(2.58)	12(2.64)	47.1	<.0001	1.85	.241	1.85	.157	2.698	.069
	PAL Std score	Patients	292	-2.9(3.9)	-2.5(3.67)	-.91(3.02)								
		Controls	151	19(1.34)	.236(.57)	.53(.27)	8.69	.003	.717	.167	.433	.649	1.17	.31
	Faces Immdiate	Patients	285	8.4(2.59)	9.45(3.12)	11(2.82)								
		Controls	157	11.6(2.8)	11.12(2.7)	12.3(4.04)	7.559	.006	1.52	.219	3.266	**.039**	5.97	**.003**
	Faces Delayed	Patients	282	9.2(2.8)	9.7(3.1)	12.3(2.5)								
		Controls	157	11.5(2.77)	10.8(2.43)	12(2)	1.88	.171	1.62	.198	2.258	.106	3.584	**.029**
Attentional Control	CPT_IP 3 leters	Patients	258	1.96(.996)	1.88(.947)	2.8(.936)								
		Controls	---	---	---	---	---	---	2.38	.095	---	---	2.381	.095
	IDED errors (8 shapes)	Patients	253	15.04(10.4)	17.08(9.15)	10.8(7.85)								
		Controls	150	9.15(9.63)	7.36(8.89)	11(15.58)	5.92	.015	.082	.573	1.47	.229	1.37	.256
	IDED errors (6 shapes)	Patients	256	.86(1.63)	.85(1.41)	.4(.89)								
		Controls	149	.75(2.33)	.39(.679)	1(0)	.018	.894	.394	.674	.648	.523	.238	.788
Social Cognition	Hinting Task	Patients	274	15.3(3.5)	15.16(3.39)	17.3(1.96)								
		Controls	130	16.6(1.84)	16.8(1.8)	16.6(2.51)	1.219	.27	.418	.417	.576	.563	1.1	.333
	Eyes in the mind	Patients	85	23(5.39)	26.5(2.3)	29(2.1)								
		Controls	50	26.2(4.7)	25.4(3.8)	29(2.4)	1.29	.257	.946	.911	1.93	.166	3.37	.07
	IPSAQ EB	Patients	254	.81(3.77)	1.38(3.19)	-.66(1.75)	.132	.717	.782	.458	.186	.831	.929	.396
		Controls	129	.88(3.82)	.88(3.89)	-.5(.7)								
	IPSAQ PB	Patients	254	.49(.278)	.51(.288)	.257(.296)	.007	.934	.382	.683	.362	.697	2.064	.129
		Controls	129	.33(9.13)	.58(.234)	.815(.261)								

Table 3.12: Cognitive analysis by rs17662626 (*PCGEM1*) genotype. A is the risk allele.

Cognitive function	Test or Subscale	sample	n	Mean(SD) GG	Mean (SD) AG	Mean(SD) AA	$F_{Case\ v\ Controls}$	p	$F_{Main\ effect}$	p	$F_{Interaction\ effect}$	p	$F_{simple\ effect}$	p
IQ	Full Scale IQ	Patients	379	100.5(14.8	89.1(18)	89.9(17.)								
		Controls	187	116(-)	120.4(15)	121.3(15.6)	14.9	<.0001	.21	.81	.25	.776	.46	.619
	Verbal IQ	Patients	379	97.(2.12)	89.3(19.8)	92.2(18.8)								
		Controls	187	108(-)	122.1(15.6)	121.4(15.5)	11.17	<.0001	.192	.826	.626	.53	.628	.534
	Performance IQ	Patients	379	104(33.9)	90.5(17.6)	88.7(18.3)								
		Controls	187	124(-)	115.9(19.2)	117.3(18.2)	12.2	<.0001	.681	.506	.35	.7	.987	.374
Working Memory	LN sequence	Patients	368	9.5(3.5)	7.7(3.1)	7.6(3.39)								
		Controls	190	12(-)	12.3(2.21)	12.8(3.38)	9.3	<.0001	.212	.816	.458	.633	.314	.731
	SWM errors	Patients	339	-.08(.827)	-.89(1.09)	-.98(1.38)								
		Controls	170	1.5(-)	.18(.92)	.25(.74)	7.47	<.0001	1.22	.39	.174	.84	.539	.584
Episodic Memory	Logical Memory Immediate	Patients	371	7(1.4)	6(3.39)	6.4(3.45)								
		Controls	189	14(-)	12.1(3.1)	12.9(2.66)	22.77	<.0001	1.116	.792	.109	.897	.341	.712
	Logical Memory Delayed	Patients	371	7.5(17)	6.8(3.52)	7.2(3.23)								
		Controls	189	14(-)	12.9(3.1)	13.29(2.68)	21.6	<.0001	.427	.956	.008	.992	.326	.722
	PAL Std score	Patients	359	.075(.58)	-2.7(3.58)	-2.8(3.8)								
		Controls	161	.7(-)	.39(.55)	.16(1.24)	2.457	.118	.387	.302	.214	.808	.533	.587
	Faces Immdiate	Patients	307	11.5(2.12)	8.78(2.99)	8.85(2.7)								
		Controls	168	8(-)	11.19(2.48)	11.6(2.8)	.07	.791	.25	.779	1.8	.16	.771	.463
	Faces Delayed	Patients	307	11.5(.71)	9.2(2.9)	9.3(2.9)								
		Controls	168	11(-)	11.4(2.7)	11.4(2.7)	.954	.329	.097	.907	.311	.73	.539	.584
Attentional Control	CPT_IP 3 letters	Patients	258	1.56(.51)	2(1.03)	1.9(.98)	---	---	.149	.862	---	---	.149	.862
		Controls	-											
	IDED 8 shapes	Patients	321	26(0)	13.2(11.5)	12.07(10.7)								
		Controls	157	8(-)	7.9(9.9)	9.1(9.7)	5.06	.025	.321	.31	.96	.384	1.61	.187
	IDED 6 shapes	Patients	321	0(0)	.91(1.7)	.88(2.08)								
		Controls	157	0(-)	.64(2.05)	.64(2.05)	.043	.836	.197	.821	.007	.993	.192	.826
Social Cognition	Hinting Task	Patients	78	-	15.3(1.7)	16.4(2.48)								
		Controls	50		16.6(1.8)	17.1(1.32)	3.8	.052	2.84	.634	.37	.54	1.75	.189
	Eyes in the mind	Patients	78	-	22.8(6.8)	22.9(5.7)								
		Controls	50		25.6(4.3)	26.2(4.5)	5.1	.024	.228	.634	.062	.8	.075	.784
	IPSAQ EB	Patients	253	-.5(2.12)	2.25(4.4)	.73(3.4)								
		Controls	131	-	.228(3.8)	1.03(3.7)	2.97	.047	.715	.49	4.68	**.031**	3.43	**.034**
	IPSAQ PB	Patients	253	.447(.01)	.4516(.29)	-.19(8.4)	.093	.76	.124	.884	.39	.53	.561	.571
		Controls	131	-	.59(.139)	.51(.279)								

3.4 Discussion

The purpose of the present study was to investigate whether seven common variants identified in a recent PGC SZ GWAS analysis (Ripke et al., 2011) were associated with variation in cognition in 400 patients and 171 healthy controls. In order to better understand their role in SZ illness, we examined each locus in turn for association with the neuropsychological variables commonly impacted in SZ. This included traditional cognitive variables such as IQ, memory and attention and social cognitive variables including tests of theory of mind and attributional style.

Based on these analyses we observed that three out of the seven loci were associated with either neurocognition or social cognition such that 1) the *MIR-137* risk 'T' allele carriers were associated with poorer performance on tests of verbal working memory, verbal episodic memory and attentional control, 2) with *CNNM2* a linear impact of genotype on externalizing bias scores (as measured by the IPSAQ) was found with homozygous risk 'GG' individuals scoring highest on this subscale, followed by 'AG' and then 'AA' individuals, 3) a main effect of the *CSMD1* risk 'A' allele was found on measures of both verbal IQ and verbal memory.

3.4.1 MIR-137

The variant rs1625579 located within *MIR-137* is one of five novel variants to have shown genome wide significance in the largest schizophrenia GWAS to date (Ripke et al., 2011). In this study we investigated the effects of the identified risk 'T' allele at rs1625579 on behavioral measures of brain function. We found that when analysing the data using a two group design (homozygous risk versus homo and heterozygous non-risk) the risk allele was associated with deleterious effects in neuropsychological performance in terms of both memory and attention; with homozygous carriers of the risk 'T' allele performing below other genotype groups in verbal episodic memory, verbal working memory and attentional control. Repeating the analysis using a three group design confirmed the verbal episodic memory and attentional control findings.

This association between *MIR-137* and cognitive functioning, particularly memory, has been supported by two other studies. In the first study, Willemsen and colleagues (2011) showed that dosage effects of *MIR-137* are associated with 1p21.3 microdeletions and may therefore contribute to intellectual disability in patients with deletions harboring

this miRNA. The study showed that patients with intellectual disability displayed a significantly decreased expression of both precursor and mature *MIR-137* levels, as well as a significantly increased expression of validated downstream targets. The study also demonstrated significant enrichment of *MIR-137* at the synapses of cortical and hippocampal neurons, suggesting a role of *MIR-137* in regulating local synaptic protein synthesis machinery. This would suggest that a local effect at the synapse might be responsible for the intellectual disability evident in the patients. In the second study, an as yet unpublished functional magnetic imaging study by van Erp from the University of Irving, California, individuals with a *MIR-137* TT risk genotype showed significantly higher dorsolateral prefrontal cortex (DLPFC) activations than those with a GG/GT genotype during a working memory task. They conclude that the *MIR-137* TT SZ risk genotype is associated with the SZ risk phenotype DLPFC hyperactivation commonly considered a measure of brain inefficiency.

While an association between *MIR-137* and cognitive functioning has been suggested by these two studies, ours is the first study that demonstrates a simple association between *MIR-137* and cognition. Green and colleagues (2012) have shown that *MIR-137* risk allele carriers are more likely to belong to a SZ subtype characterized by cognitive deficits, but only in combination with higher severity of negative symptoms. Here we show that the association can exist independently of clinical symptomology. *MIR-137* has been associated with both SZ and bipolar disorder (Ripke et al., 2011). These two psychiatric illnesses are related in both genetic underpinnings (Lichtenstein et al., 2009) and cognitive functioning (Green, 2006) and are most often distinguished in terms of symptomology. Perhaps it is this overlap in cognitive decline between the two disorders that is impacted by *MIR-137*. Exactly how *MIR-137* exerts this influence is, however, unclear. It may exert it, as the aforementioned studies suggest, through impacting a local effect at the synapse or through DLPFC hyperactivation. Whatever the mechanism, it seems apparent that it is a pathway of *MIR-137* targeted genes that act together to produce the functional outcome. As demonstrated in the recent PGC study (Ripke et al., 2011), *MIR-137* has 301 high-confidence predicted gene target sites. SNPs mapping to these target sites were enriched for association signals with SZ compared with other genes of similar size or genetic marker density in the genome. Excluding the gene itself and the major histocompatibility complex region (MHC), 4 of 9 associated loci in a combined meta-analysis of SZ and bipolar GWAS data had predicted *MIR-137*

target sites, ie *TCF4, CACNA1C, CSMD1* and *C10orf26*. Further questions raised by this study, are whether having a greater burden of common risk variants from this gene network is associated with increased illness risk, the extent of this risk, and whether this maps a molecular subtype of psychosis characterized by cognitive deficits.

3.4.2 CNNM2

The variant rs7914558 located within *CNNM2* is one of five novel variants to have shown genome wide significance in the largest schizophrenia GWAS to date (Ripke et al.., 2011). In this study we investigated the effects of the identified risk 'A' allele at rs7914558 on behavioral measures of brain function. We found that in both patients and healthy participants the risk 'A' allele was associated with variability in social cognitive function, in the absence of genotype-related deficits in general cognitive function that are typically altered in SZ. Specifically, carriers of the risk allele exhibited a *reduced* 'self-serving' bias – i.e. the adaptive tendency to attribute more positive than negative events to oneself.

Differences in attribution style are commonly reported in psychiatric conditions. In patients with paranoia, attributional style is characterized by the tendency to attribute negative events to external, global and stable factors and in depression attributional style is defined by a tendency to consistently attribute negative events internally (Bentall et al., 1994). This notion is further supported by Jolley and colleagues (2006), who found that depression and grandiosity in SZ symptomology were significantly associated with attributional style. Individuals who exhibited grandiosity had high externalizing bias scores, while individuals with depression had low externalizing bias scores. Our observations show that carriers of the *CNNM2* risk 'A' allele had low externalising bias scores. This suggests that they made fewer positive and more negative internal attributions for negative events, i.e. in a manner similar to attributional profiles in depression (Candido & Romney, 1990). This finding is interesting as *CNNM2* mutations are associated with hypomagnesaemia risk (Stuiver et al., 2011), which causes abnormally low level of magnesium in the blood – a deficiency that has been linked to depression (Cundy & Mackay, 2011). Perhaps then *CNNM2* contributes to SZ risk by lowering blood magnesium levels leaving one prone to depressive like symptoms.

To date, aside from a role in magnesium transportation, little is known regarding the functional pathways of the *CNNM2*/rs7914558 SNP considered here, either in general or with regards to SZ specifically. Certainly, our understanding of how or why this particular variant contributes to social cognitive processes involved in attributional style will be significantly enhanced by the elucidation of the functional biology of this particular variant. As such, further research into this gene is warranted.

3.4.3 CSMD1

The variant rs10504253 located within *CSMD1* is one of five novel variants to have shown genome wide significance in the largest schizophrenia GWAS to date (Ripke et al., 2011). In this study we investigated the effects of the identified risk 'A' allele at rs10504253 on behavioral measures of brain function. We found that the risk allele was associated with deleterious effects in neuropsychological performance in terms of both IQ and memory; with homozygous carriers of the 'A' allele performing below other genotype groups in verbal IQ, verbal episodic memory and verbal working memory.

The fact that the variables associated with *CSMD1* SZ risk in the present study represent a diversity of cognitive ability (from episodic memory to working memory to IQ) would suggest that it is overall cognitive functioning that is impacted by the *CSMD1* SNP rs10504253. In line with this, both working memory and episodic memory have been shown to be closely correlated with overall cognitive functioning. Deficits in working memory have previously been correlated with general cognitive decline; being characterized as the common factor in Spearman's 'g', a measure of general cognitive function derived from the statistical correlation between cognitive tasks (Kane et al, 2005). Indeed there is a large overlap between working memory and psychometric tests of 'g' (Plomin & Spinath, 2002), suggesting that the two terms may be interchangeable. This is supported by evidence from previous gene-cognition studies, in which variants associated with intelligence have also been associated with variation in working memory and vice versa (e.g. *DTNBP1*, Karlsgodt et al., 2011; *NOS1*, Donohoe et al., 2009b). The association between *CSMD1* and behavioral measures of IQ and working memory is consistent with this view, even if in the present study this existed amongst control participants only. Similarly, episodic memory, while not overlapping to the same extent with intelligence as working memory, is correlated with general cognitive ability (Ruiz et al., 2007).

The significant associations between *CSMD1* and cognition seen in the current study, and the interpretation of these findings as reflecting a broader role for *CSMD1* in neurocognitive function, is supported by (1) its previous association with neurodevelopmental disorders such as epilepsy, speech delay, and learning difficulties (Glancy et al., 2009; Schimizu et al., 2003), and (2) the association between rs10504253 and SZ (Ripke et al., 2011). This is further substantiated by a recent Alzheimer's/mild cognitive impairment CNV study where *CSMD1* was one of six gene loci at which case-only CNVs were identified (Swaminathan et al., 2011). Finally, a recent genome-wide study of cognition (Cirulli et al., 2010) in healthy controls identified a separate *CSMD1* variant association (rs2616984, positioned > 30KB from rs10503253), suggesting that additional *CSMD1* variants may also, be relevant to cognition.

3.5 Conclusion

In conclusion, while four out of the seven SZ associated SNPs identified in a recent PGC study (Ripke et al, 2011) were not associated with cognition, the present study provides evidence that three novel genome-wide associated risk variants for SZ – *MIR-137* (rs1625579), *CSMD1* (rs10504253) and *CNNM2* (rs7914558) - may contribute to illness risk via a role in neurocognitive or social cognitive processing.

3.6 Supplementals

Single nucleotide polymorphism (SNP) substitution: It should be noted that three SNPs analysed in the current study are not the actual SNP given in the Ripke (2011) paper (see **table 3.13**). The reasons for the substitutions are as follows:

TCF4: PGC report data for SNP rs12966547 which is in complete linkage disequilibrium (LD) (r2 = 1, HapMap CEU) with rs4309482 (our SNP). The A allele of rs4309482 is on the same haplotype as the risk G allele of rs12966547. The reason we typed rs4309482 is that it was reported as the SNP of interest at this gene in a preliminary analysis by the PGC – this changed in the final analysis.

NRGN: PGC report data for SNP rs548181 which is in complete LD (r2 = 1, HapMap CEU) with rs12807809 (SNP typed by us). Also, rs12807809 was originally reported in

a GWAS headed by Stefansson (2009). The reason we typed rs12807809 was because of the Stefansson paper, predating the PGC analysis.

CNNM2: PGC report data for SNP rs7914558 which is in complete LD (r2 = 1, HapMap CEU) with rs10748835 (our SNP), which is actually located on a gene called *AS3MT*. rs7914558 was not available on the genotyping Chip we used and so instead we typed rs10748835 on account of the two SNPs being in LD.

Table S3.1: SNP substitution between the PGC analysis and the current study

SNP from Ripke paper	SNP assessed in the current study	Gene name
rs548181	rs 12807809	*NRGN*
rs17512836	rs4309482	*TCF4*
rs7914558	rs10748835	*CNNM2*

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Chapter 4

ZNF804A and social cognition in patients with schizophrenia and healthy controls

Abstract

Background: *ZNF804A* rs1344706 was the first genetic risk variant to achieve genome wide significance for psychosis. A number of studies have since shown an association between *ZNF804A* and variation in neurocognitive functioning. To date, the results of these studies deviate from what would typically be expected from a schizophrenia (SZ) risk gene, with the literature repeatedly pointing to more preserved cognitive function in patients who carry the risk allele. A recent study by Walter and colleagues (2011) however, found *ZNF804A* to be associated with poorer social cognitive function in a functional magnetic resonance imaging (fMRI) theory of mind study in healthy controls. The present study tested whether the same risk allele at *ZNF804A* was associated with variation on behavioural measures of social cognition in both healthy controls and patients with schizophrenia.

Methods: 418 patients with SZ and 200 controls were assessed in areas of social cognitive ability typically found to be impaired in schizophrenia (SZ): including Faces immediate and delayed recall (Wechsler, 1997b), the Internal, Personal, and Situational Attributions Questionnaire (IPSAQ) (Kinderman & Bentall, 1996) and two theory of mind tasks; The Hinting Task (Corcoran et al., 1995) and the Reading the Mind in the Eyes Task (Baron-Cohen et al., 2001).

Results: The rs1344706 risk 'A' allele was associated with a significantly higher personalising bias score in healthy participants (F=3.105; P=.046) but not patients. Personalising bias reflects the attribution of negative events to other people as opposed to situations or circumstance and has been previously associated with paranoia (Combs et al., 2007). No significant association was found between rs1344706 and either of the two theory of mind tasks.

Discussion: Consistent with previous fMRI findings in healthy controls we conclude that the *ZNF804A* SZ risk variant rs1344706 may influence some behaviourally measured aspects of social cognition.

4.1 Introduction

Schizophrenia has consistently shown high heritability (~80%) and elevated sibling recurrence (Sullivan et al., 2003) and has a lifetime risk of approximately 1% (Prince et al., 2007). Despite this, identifying the genetic variations responsible for SZ has proved challenging (Sanders et al., 2008). For several years, genome wide association studies (GWAS) have been at the forefront of identifying candidate genes for complex genetic disorders such as schizophrenia. One of the first genetic variants to achieve genome wide significance for psychosis was a single-nucleotide-polymorphism (SNP) rs1344706 located within the zinc finger binding protein 804A (*ZNF804A*). Since then association of this SNP with SZ has been demonstrated in several other studies (e.g. Shi, 2009; Stefansson, 2009; Riley, 2010; Steinberg, 2011; Zhang, 2011) as well as in a meta-analysis by Williams and colleagues (2011), who demonstrated an association between *ZNF804A* and both schizophrenia (OR 1.10; P=2.5 x 10-11) and schizophrenia and bipolar disorder combined (OR 1.11; P=4.1 x 10-13) at a level far in excess of typical thresholds for GWAS identified variants. The SNP in question, rs1344706, is located in an intron of *ZNF804A* on chromosome 2q32.1 and is known to be brain expressed. *ZNF804A* is predicted to encode a protein with a C2H2 zinc finger domain. This suggests a role in the regulation of gene expression through DNA and/or RNA binding (Donohoe et al., 2010). It has further been associated with brain activity and structure (Esslinger et al., 2009; Donohoe et al., 2011), and in a recent study by Hill and colleagues (2012) which assessed the effects of its knockdown on the cellular transcriptome, it has been linked to cell adhesion molecules suggesting a role in neural migration, neurite outgrowth and synapse formation, which are commonly hypothesized to be aberrant in schizophrenia.

In terms of illness symptomatology, *ZNF804A* has been repeatedly linked to cognitive deficits, albeit in an unexpected manner, with the results of cognitive studies deviating from what would typically be expected from a SZ risk gene. Emerging from the literature is the message that risk allele carriers from the healthy population are likely to demonstrate reduced cognitive functioning in areas of attention (Voineskos et al., 2011; Balog et al., 2011), working memory (Esslinger et al., 2011) and visuomotor skills (Lencz et al., 2010), whilst risk allele carriers in the patient population are likely to exhibit a relatively preserved cognition. Walters and colleagues (2010) report that in large independent Irish and German samples, patient carriers of the risk allele (but not

controls) showed relatively preserved cognitive functioning in the areas of both episodic and working memory, with the homozygous risk 'A' carriers significantly outperforming the non-risk carriers. They further observed that the association with SZ risk strengthened when patients with lower general cognitive ability were removed from the analysis. This led to the conclusion that the risk allele at *ZNF804A* is not so much associated with better cognitive performance as with less impaired cognitive performance, delineating an illness susceptibility pathway that is independent of an effect on cognition. Indeed, this cognitively preserved phenotype can be seen in several other studies in relation to *ZNF804A* risk. Becker and colleagues (2012) found that the risk SNP rs1344706 was significantly associated with an improved performance in 6 language relevant tasks; Van Den Bossche and colleagues (2012) showed an association of higher rs1344706 risk allele load with improved performance on the cognitive domain of processing speed; and Donohoe and colleagues (2011) showed that in patients with SZ, the risk allele at rs1344706 is associated with relatively intact grey matter volumes compared to non-risk carriers, notably in brain regions associated with memory function (superior temporal gyrus, hippocampus). These data were interpreted to suggest relative cortical intactness associated with the genotype rather than that the genotype bestowed a cortical advantage (as it was not seen in healthy controls), again consistent with the idea of a phenotype characterized by less impaired cognitive deficits.

Due to the mounting evidence that *ZNF804A* is delineating a cognitively spared patient sub-population, researchers began to turn their attention to fields outside of traditional cognition in the hope of discovering by what means this psychosis risk gene is exerting its influence. Two studies emerged, supporting the view that *ZNF804A* may mediate illness risk via an influence on social, rather than traditional, cognition. Esslinger and colleageus (2011) reported that *ZNF804A* phenotype was associated with an altered pattern of connectivity between several regions including the dorsolateral prefrontal cortex, hippocampus and amygdala, all of which are known to be involved in social processing. A subsequent study from the same group (Walter et al., 2011) showed that healthy risk carriers performing a theory of mind (ToM) task exhibited a significant risk allele dose effect on neural activity in the medial prefrontal cortex and left temporoparietal cortex. This suggests that the impact of the previously reported altered connectivity between areas such as frontal and temporoparietal regions may well be indicative of difficulties in processing socially relevant cues. This study also showed

that parts of the human anologue of the mirror neuron system (the left inferior parietal cortex and left inferior frontal cortex) were also significantly affected, further supporting the idea that *ZNF804A* genotype might be particularly relevant for processing social information.

While these two studies support a role for *ZNF804A* in social cognition, it is noteworthy that both concentrate on healthy participants and not patients with SZ. As such, the purpose of the present study was to investigate the behavioural effects of the identified risk 'A' allele at *ZNF804A* rs1344706 on both patients and controls using four different social cognitive tasks: (1) Faces 1 & 2 (Wechsler Memory Scale-III) - included due to the close relationship between facial identification and social cognition, (2) the Internal, Personal, and Situational Attributions Questionnaire (IPSAQ) (Kinderman & Bentall, 1996), and two ToM tasks; (3) The Hinting Task (Corcoran et al., 1995) and (4) the Reading the Eyes in the Mind Task (Baron-Cohen et al., 2001).

4.2 Methods

4.2.1 Neuropsychological sample characteristics: 418 cases with SZ or schizoaffective disorder (SZA) and 200 healthy participants who had completed a full neuropsychological assessment battery and for whom full genome wide data was available were analysed (see **table 4.1** for participant demographics).

Cases consisted of clinically stable patients with a DSM-IV diagnosis of schizophrenia (SZ) or schizoaffective disorder (SZA) (see **table 4.1** for details) recruited from five sites across Ireland. Inclusion criteria required that participants were clinically stable at the time of neuropsychological assessment, aged 18 to 65 years, had no history of co-morbid psychiatric disorder, no substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID) (First et al., 2002). Additional diagnostic details and clinical sample characteristics ascertained at time of interview including symptom severity (SAPS/SANS) (Andreasen, 1984(a); Andreasen, 1984(b)) and medication dosage are detailed elsewhere (Walters et al., 2010).

Healthy participants were recruited via both online and poster advertising. They were aged 18 to 65 years, with no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Neither they nor any first degree relative had a history of psychosis.

All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

4.2.2 Cognitive assessment

All participants completed a neuropsychological assessment battery involving four social cognitive measures designed to target the social cognitive deficits typically reported in SZ – namely deficits in face recognition, theory of mind and attributional style. To this end four social cognitive tasks were administered; (1) Faces, immediate and delayed recall (Wechsler, 1997b), (2) the Hinting Task (Corcoran et al., 1995), (3) the Reading the mind in the eyes task (Eyes) (Baron-Cohen et al., 2001) and (4) the Internal, personal and situational attributions questionnaire (IPSAQ) (Kinderman & Bentall, 1996).

Details of tests used are as follows:

Faces I: (Immediate recall) This test, generally used as a measure of episodic memory, assesses the examinee's facial processing and visual memory abilities. We chose to use the test as an alternative measure of social cognition due to its fundamental social content (human faces) and analysis of social memory, which according to Kihlstrom & Park (2002), can be assessed through studying lists of social content such as faces, personality traits or sentences describing social behaviours. In Faces 1 the examinee is shown a series of photographs of faces, one at a time, and asked to remember each one. The examinee is then shown a second series of photographs of faces, one at a time, and asked to identify each face as either one that s/he was asked to remember or a new one.

Faces II: (Delayed recall) Twenty to thirty minutes after the administration of Faces I, the examinee is presented with a series of photographs of faces, one at a time. The examinee is asked to say "yes" if the face is one that s/he was asked to remember in

Faces I or "no" if it is not. This test assesses the examinee's facial processing and social memory abilities after a time lapse.

Hinting task: This is a ToM test which assesses mental state reasoning. During the test ten short vignettes which describe a social interaction between two characters are read aloud to the participant, after which the participant is required to make inferences about the intent behind a hint dropped by one of the characters. If the examinee does not initially correctly identify the hint, a second related hint is dropped in order to facilitate recognition.

Reading the mind in the eyes task: This is a ToM task which assesses mental state decoding. It measures the ability of a person to determine what another person is thinking or feeling at a precise period in time from looking at their eyes. The examinee is presented with a series of 36 black and white photographs of adult male and female eyes. These are presented one at a time. At each corner of the photograph is an emotive word (such as' flirtatious') which might possibly describe what the person in the photograph is thinking or feeling at the time the picture is taken. One of these words is the correct answer.

Internal, personal and situational attributions questionnaire: This pen and paper questionnaire is an assessment of causal locus. The test consists of 32 scenarios, 16 positive and 16 negative, such as "A friend gave you a lift home". The examinee must decide what they feel might be the primary reason for the scenario taking place and, based on their decision, decide whether that is something to do with themselves, something to do with the person or other people involved, or something to do with the situation or circumstance at hand. Six main outcome scores are obtained: Internal positive, external positive, situational positive, internal personal negative, external personal negative and situational negative. From these six scores, two biases are calculated: an externalising bias and a personalising bias. Externalising bias (EB) is calculated by subtracting the number of internal attributions for negative events from the number of internal attributions for positive events. A positive EB score (one greater than 1) therefore indicates a strong self-serving bias where one blames oneself less for negative events than for positive events. Personalising bias (PB) indicates the proportion of external attributions for negative events which are personal (other people) as opposed to situational (events/circumstance) and is calculated by dividing the number

of personal attributions by the sum of both personal and situational attributions for negative events. A PB score of greater than 0.5 therefore represents a greater tendency to blame other people rather than situations for negative events (Kinderman & Bentall, 1996).

4.2.3 Genotyping:

Genetics analysis for patients was based on DNA extracted from blood, and for controls using DNA extracted from saliva samples obtained using Oragene DNA self-collection kits (DNA Genotek; Ontario, Canada). The *ZNF804A* SNP rs1344706 was genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium ($p > 0.05$). Several HapMap CEU DNA samples (n=90 (http://www.hapmap.org)) and duplicates (n=60) were genotyped for rs1344706 for quality control purposes. All the genotypes were found to be concordant with either the available online HapMap data or each other for this SNP.

4.2.4 Statistical Analysis:

Association between *ZNF804A* rs1344706 and the four phenotypes of Faces, Hinting Task, Reading the eyes in the mind task and IPSAQ, was tested using a general factorial design in a statistical software programme (SPSS 16, 2008). In the original GWAS (O'Donovan et al, 2008) there was no difference in genotypic verses allelic models; because there was no evidence on which to test a specific dominant or recessive model and as sample sizes allowed, the analysis was based on a comparison of all 3 genotype groups. *ZNF804A* genotype (AA vs AC vs CC) and diagnosis (cases v controls) were entered as fixed effects. In a series of ANCOVAs, scores for each neuropsychological subtest were entered as dependent variables, with age and gender included as covariates where appropriate.

4.5 Results

4.5.1 The effects of ZNF804A on demographic and clinical measures

Demographic and clinical characteristics for patients and healthy participants appear in **table 4.1**. For all demographic variables, means and standard deviations were computed using SPSS, 2008. There was no difference in genotype frequency between patients and controls and no genotype-related variability in gender ratio, age, years of education, or IQ in any of the samples. Moreover, in patients, rs1344706 was not associated with age of onset or medication (i.e. chlorpromazine equivalents). On clinical measures of positive and negative symptoms (SAPS/SANS), no genotypic differences were detectable for the factors of depression, positive symptoms, negative symptoms, disorganisation symptoms or mania. Comparing patients with healthy controls, there was a significant difference in age, years of education, and IQ between groups, such that patients were older ($F=10.52$, $p<0.05$), had less years of education ($F=252.5$, $p<0.001$), and had a significantly lower full scale IQ ($F=450.39$, $p<.001$).

4.5.2 The effects of ZNF804A pathway risk allele load on social cognition.

The *ZNF804A* pathway was associated with the social cognition measure of interpersonal attributional bias. F and p values for each of the 4 social cognitive tasks administered are given in **table 4.2**. A significant interaction effect between *ZNF804A* and case or control status revealed associations with personalising bias from the IPSAQ in controls but not patients; $F = 3.068$, $P = .048$. Tukey post hoc analysis within the control group revealed that both the AA and AC genotypes were associated with a significantly higher personalising bias score than the CC genotype ($P = .015$ and $P = .012$ respectively). This is illustrated in **figure 4.1** below.

Table 4.1: Participant demographics. Clpz = chlorpromazine.

Neurocognition	*Total Sample* *N=618*		*ZNF804A/rs1344706* *CC* *N=79*		*AC* *N=272*		*AA* *N=267*		*Comparison*
	Patients N=418	Controls N=200	Patients n=51	Controls n=28	Patients n=184	Controls n=88	Patients n=183	Controls n=84	
-Gender (F:M)	98:93	188:417	13:38	17:9	44:139	43:43	57:124	38:41	ns
-Age (years; mean(s.d.))	40.64 (12.29)	37.16 (12.65)	40.38 (12.02)	35.96 (12.97)	40.98 (11.76)	37.51 (13.04)	41.56 (12.92)	37.18 (12.25)	controls<patients F=10.52, p<.05
-Years of Education (mean (s.d.))	13.13 (2.14)	16.07 (2.09)	13.46 (1.12)	16.07 (2.78)	13.58 (1.07)	15.87 (1.95)	13.61 (1.29)	16.28 (1.98)	controls>patients F=252.5, p=.001
-Age at onset (years; mean (s.d.))	22.92 (7.25)	na	23.95 (6.86)	na	22.71 (7.09)	na	22.62 7.64	na	ns
-Clpz Equivalents (mg/day; mean (s.d.))	573.58 (529.2)	na	567.72 (383.87)	na	609.02 (526.23)	na	576.02 (526.23)	na	ns
SAPS/SANS:									
-Manic (mean (SD))	-.17(.92)		-.04(1.1)		-.16(.89)		-.19(.96)		ns
-Depression (mean (SD))	.16(1.05)		.12(1.06)		.12(1.06)		.28(1.11)		ns
-Positive (mean (SD))	.26(1.47)	na	.124(1)	na	-.02(.95)	na	.075(1.1)	na	ns
-Disorganised (mean(SD))	-.21(.8)		-.49(.66)		-.21(.79)		-.17(.75)		ns
-Negative (mean (SD))	.83(2.24)		.084(.83)		.36(.96)		.41(.89)		ns
-Cognition: full scale IQ (mean (SD))	90.36 (17.8)	122.18 (15.4)	89.6 (19.2)	126.15 (14.1)	89.1 (17.1)	120.87 (14.76)	91.6 (18.7)	122.2 (16.37)	controls>patients F=450.39,p<.001

Table 4.2: Social cognitive analysis by ZNF804A genotype

Cognitive function	Subscale	sample	n	Mean (SD) CC	Mean (SD) AC	Mean (SD) AA	F Case v Controls	p	F Main effect	p	F Interaction effect	p	F_{simple} effect patients	p	F_{simple} effect controls	p
Social Cognition	Faces	Patients	390	9.09(3.09)	8.8(2.8)	8.8(2.6)										
	(immediate recall)	Controls	180	11.2(2.9)	11.2(3.05)	11.8(2.7)	58.46	<.0001	.846	.43	.887	.41	.131	.87	1.076	.343
	Faces	Patients	390	9.04(2.49)	9.31(2.9)	9.4(22.9)										
	(delayed recall)	Controls	180	11.8(3.02)	11.07(2.8)	11.5(2.4)	46.27	<.0001	.549	.578	.771	.463	.295	.74	.906	.406
	Hinting Task	Patients	341	15.7(3.1)	15.4(3.3)	15.4(3.2)										
		Controls	127	16.8(1.8)	16.6(1.9)	16.7(1.7)	11.15	.001	.112	.894	.006	.994	.131	.87	.121	.886
	Eyes	Patients	207	23.6(5.5)	24(4.5)	24(5.1)										
		Controls	48	23.8(4.4)	27(3.1)	25(5.6)	2.3	.13	.92	.4	.886	.414	.19	.827	2.62	.084
	IPSAQ	Patients	313	1.1(3.2)	1.2(3.2)	1.2(3.8)										
	(externalising bias)	Controls	126	.57(4.04)	.52(4.22)	1.5(3.3)	1.16	.281	.735	.48	.925	.397	.02	.981	1.001	.371
	IPSAQ	Patients	313	.52(.27)	.52(.29)	.49(.28)										
	(personalising bias)	Control	126	.47(.2)	.64(.22)	.64(.24)	3.34	.068	2.059	.129	3.068	**.048**	.369	.692	4.798	**.01**

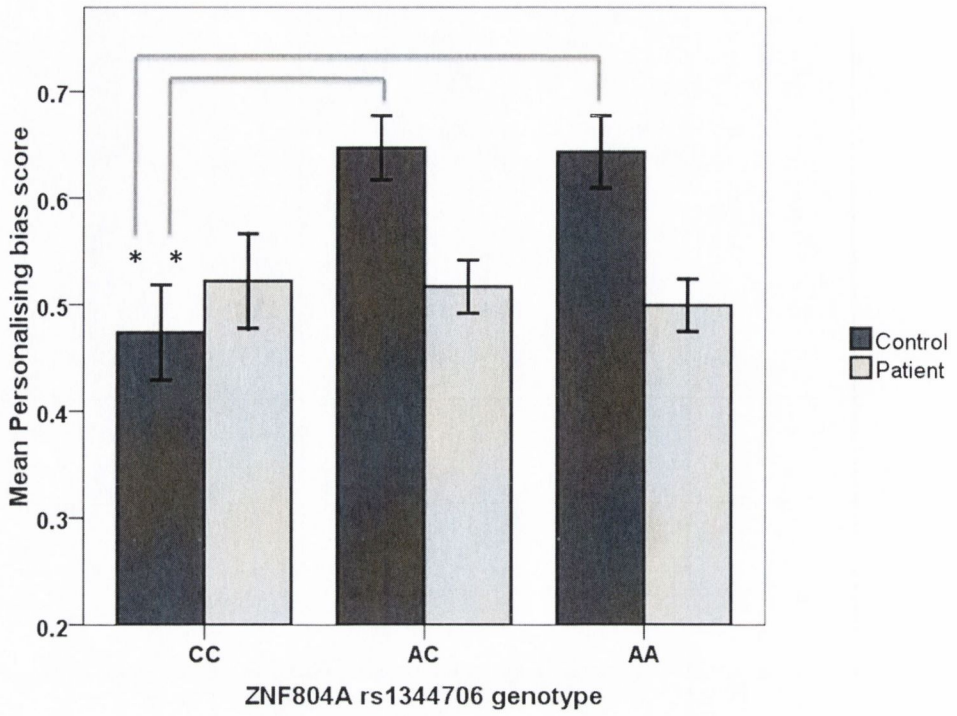

Figure 4.1: Mean scores (and standard deviations) for IPSAQ personalizing bias (PB) grouped by genotype. Carriers of 1 and 2 copies of the 'A' risk allele at rs1344706 show significantly increased personalizing bias scores compared to non-risk carriers in healthy controls but not in patients. * $p \leq .01$.

4.6 Discussion

The purpose of the present study was to investigate whether the identified risk 'A' allele at the SZ-associated SNP rs1344706 was associated with variation in social cognition in 418 cases and 200 controls using four different social cognitive tasks (Faces 1 & 2 (Wechsler Memory Scale-III), The Hinting Task (Corcoran et al., 1995), Eyes (Baron-Cohen et al., 2001), and the IPSAQ (Kinderman & Bentall, 1996)). For the personalising bias score of the IPSAQ, a significant interaction effect between *ZNF804A* and *case or control status* was revealed in the control but not the patient group. This association was dose independent, showing an increased risk of displaying the bias in risk allele carriers (AC/AA). No effects of *ZNF804A* were observed for any of the other three social cognitive tasks. To our knowledge, this is the first study to identify an attributional association with the rs1344706 SNP, a finding which supports the growing hypothesis that the *ZNF804A* gene influences social cognition.

Findings from the current study are consistent with earlier findings from the Walter and colleagues (2011) imaging study, in that in both studies the *ZNF804A* risk allele was associated (and in the same direction) with altered responsiveness in social cognition in healthy participants. The two studies differed, however, in that we observed this association for attributional style but not for ToM. ToM is the ability to attribute mental states—beliefs, intents, desires etc.—to oneself and others and to understand that others have mental states that are different from one's own, whereas attributional style relates to how one explains the cause of events. However, in order for a person to attribute blame to another individual, (an action evident in those with a personalising bias) it must first be recognised that that person has different intentions and desires to one's own. Thus, the concepts of attributional bias and ToM are intrinsically connected, and it is perhaps this recognition of others as being mentally different from the self that is impacted by *ZNF804A*.

The most intriguing aspect of our PB finding is that it applies to healthy participants only and not to patients, which is inconsistent with the hypothesis that illness risk is mediated via effects on social cognition. Support for that hypothesis would necessitate a similar effect in patients to that observed in healthy participants. This prompts the question of how a well established common variant associated with increased risk for SZ seems to confer social cognitive risk to the non-patient group whilst leaving the

patient group unaffected? It may be possible that subtle effects on social attribution are overshadowed by clinical symptoms in the patient group, particularly as the attributional bias associated with *ZNF804A* (PB) is closely linked to the clinical symptoms associated with the same gene in previous research. Personalising bias has been associated with paranoid ideation (Bentall & Penn, 2001) anger (Fornells-Ambrojo & Garety 2009) learned helplessness (Kinderman et al., 1996) and psychosis (Coon, 2008), whilst the *ZNF804A* SNP under investigation (rs1344706) has previously been found to be associated with such clinical variables as the BADDS mania factor (Cummings et al., 2010) and the SANS depression factor (Walters et al., 2010). Mania is associated with paranoid ideation, anger and psychosis, whilst depression is associated with learned helplessness. However, as noted by to Donohoe and colleagues (2010), *ZNF804A* genotype only explains ~1% of variance in these symptoms, which is not suggestive of a clinically identifiable phenotype associated with this risk variant. Further studies will be required to answer this question more fully.

Another factor to consider in attempting to understand why *ZNF804A* risk impacts social cognition in healthy participants but not patients, is that subtle effects on social cognition might be particularly difficult to observe behaviourally in patients given a background of general cognitive decline. Such background cognitive 'noise' inevitably increases difficulties with detecting more subtle gene-specific changes in patients, and may explain the apparent difference in effects observed in healthy participants. Alternatively, the rs1344706 risk allele may be impacting social cognition in a manner similar to its impact on more traditional cognitive measures (such as attention and memory), in effect conferring less impairment to the patient group. It is perhaps to be expected that not all identified SZ susceptibility genes will have detrimental effects on cognitive abilities in patients with SZ. The schizophrenia syndrome is heterogeneous in nature, and approximately 20-25% of patients with schizophrenia are cognitively intact (Goldstein et al., 2005). Indeed, other susceptibility genes besides *ZNF804A* have demonstrated a preserved cognition with recent studies of *PPP1R1B*, encoding *DARPP-32*, and *CHI3L1* finding that the schizophrenia-associated risk alleles at both gene loci were associated with a relatively spared performance (Yang et al., 2008; Meyer-Lindenberg et al., 2007).

In conclusion, the current study sought to elucidate the phenotypic effects of an identified risk allele on indices of social cognition. Carrying the risk allele at rs1344706 was associated with variation in performance in the PB score of the IPSAQ, with risk carriers in the control group demonstrating a greater likelihood of attributing blame to other people rather than to situation or circumstance. The results of this study concur with those by Esslinger and colleagues (2011) and Walter and colleagues (2011), supporting the idea that this risk variant is impacting negatively on social cognitive processes, although the mechanism by which this occurs remains unclear. As such, *ZNF804A* may be viewed as a social cognition gene, impacting on how people view the mentality of those around them. Of particular interest is the counterintuitive finding of impaired social cognitive functioning amongst healthy participants, but not patients, which supports the approach of subgrouping patients with schizophrenia to better understand molecular and biological processes, as has been done for other complex genetic diseases such as breast cancer (Garcia-Closas et al., 2008). These findings suggest that individuals with less compromised cognitive functioning may show a different pattern of association or may even be a genetically distinct group worthy of further study in genetic association studies. Either way, these data highlight the need for caution in extrapolating from findings based solely on healthy participant data to neural mechanisms of illness in patients, at least when considering the effects of *ZNF804A*.

Chapter 5

The impact of the *ZNF804A* pathway on cognition in patients with psychosis and healthy controls

Abstract

Background: *ZNF804A* rs1344706 is the first genetic risk variant to achieve genome wide significance for psychosis. Recent studies show an association between *ZNF804A* and variation in neurocognitive functioning in psychotic patients. Using the *ZNF* pathway curated by Hill and colleagues (2012) from all genes whose expression is affected by *ZNF804A* knockout, we tested whether *ZNF* pathway risk allele load scores correlated with poorer neuropsychological function. We further tested whether pathway level analysis would explain a greater percentage of the variance in neuropsychological performance than is explained by individual risk alleles.

Method: 424 patients with psychosis and 89 controls were assessed in areas of cognitive ability typically found to be impaired in schizophrenia (SZ): including *(1) IQ* (using selected subtests from the Wechsler Adult Intelligence Scale, 3rd edition), *(2) memory* (using the Wechsler Memory Scale, 3rd edition and the Paired Associate Learning (PAL) and spatial working memory task (SWM) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB), *(3) attention* (using the continuous performance task identical pair's version (CPT-IP), the Intradimensional-extradimensional shift task (IDED) and the sustained attention to response task (SART)) and *(4) social cognition* using the Reading the mind in the eyes task (Eyes) and the Internal, personal and situational attributions questionnaire (IPSAQ).

Results: Increased risk allele load on the *ZNF* pathway was associated with poorer performance amongst both patients with psychosis and healthy subjects in IQ, memory, attention and social cognition, explaining ~3-5% of variation on these scores in patients and ~5-9% of variation on these scores in healthy participants.

Discussion: These data support a role for the *ZNF* pathway in IQ, memory, attention and social cognition. Specifically, increased risk allele load was associated with poorer cognitive performance, in both patients and healthy participants. This is in contrast with previous literature findings in which the *ZNF804A* SNP rs1344706 has been associated with relatively intact cognitive functioning. We discuss whether this is because the pathway is acting in a manner independent of the rs1344706 SNP, or for other reasons.

5.1 Introduction

For several years genome wide association studies (GWAS) have been at the forefront of identifying candidate genes for complex genetic disorders such as psychosis. One of the first genetic variants to achieve genome wide significance for psychosis was a single-nucleotide-polymorphism (SNP) rs1344706 located within the zinc finger binding protein 804A (*ZNF804A*). Several independent replication studies (Esslinger et al., 2009; Donohoe et al., 2011) have supported the association between schizophrenia and the risk allele of this SNP, and in a meta-analysis by Williams and colleagues (2011b), the association was shown to greatly exceed accepted levels of genome wide significance ($P = 2.5 \times 10^{-11}$). *ZNF804A*, which is expressed in the brain, is predicted to encode a protein with a C2H2 zinc finger domain. This suggests a role in the regulation of gene expression through DNA and/or RNA binding (Donohoe et al., 2010) It has been reported to show association with brain activity and structure (Esslinger et al., 2009; Donohoe et al,. 2011), and in a recent study by Hill and colleagues (2012) which assessed the effects of its knockdown on the cellular transcriptome, it has been linked to cell adhesion molecules suggesting a role in neural migration, neurite outgrowth and synapse formation, which are commonly hypothesized to be aberrant in schizophrenia.

5.1.1 ZNF804A and Traditional cognition

Several studies have linked *ZNF804A* to cognition, based on imaging studies, traditional neuropsychological measures (e.g. Becker et al., 2012; Esslinger et al., 2011; Walters et al., 2010) and measures of social cognition such as theory of mind and attributional style (Walter et al., 2011; Hargreaves et al., 2012). To date, the results of these studies deviate from what would typically be expected from a SZ risk gene in that whilst the rs1344706 risk allele appears to convey impairments in cognition to the healthy population, (an impairment suggested by both behavioural and imaging studies, with such imaging findings as decreased connectivity in cognition-associated brain areas) (5/6 studies, **see table 5.1**), the literature repeatedly points to a more preserved cognition in the patient population (4/6 studies, see **table 5.1**).

Amongst the control-only studies we see a negative association between *ZNF804A* risk and the cognitive variables of attention (Voineskos et al., 2011b; Balog et al., 2011), working memory (Esslinger et al., 2011) and visuomotor skills (Lencz et al., 2010). Amongst the patient group, however, studies point to an unexpected, apparently protective, role of *ZNF804A* risk. Walters and colleagues (2010) report that in large independent Irish and German samples, patient carriers of the risk allele (but not controls) showed relatively preserved cognitive functioning in the areas of both episodic and working memory, with the homozygous risk 'A' carriers significantly outperforming the non-risk carriers. They further observed that the association with SZ risk strengthened when patients with lower general cognitive ability were removed from the analysis. This led to the conclusion that the risk allele at *ZNF804A* is not so much associated with better cognitive performance as with less impaired cognitive performance, delineating an illness susceptibility pathway that is independent of an effect on cognition. Indeed, this cognitively preserved phenotype can be seen in several other studies in relation to *ZNF804A* risk. Becker and colleagues (2012) found that the risk SNP rs1344706 was significantly associated with an improved performance in 6 language relevant tasks, whereas the non-risk allele was associated with a decrease in cognitive performance on these same measures; Van Den Bossche and colleagues (2012) showed an association of higher rs1344706 risk allele load with improved performance on the cognitive domain of processing speed; and Donohoe and colleagues (2011) showed that in patients with SZ, the risk allele at rs1344706 is associated with relatively intact grey matter volumes compared to non-risk carriers, notably in brain regions associated with memory function (superior temporal gyrus, hippocampus). These data were interpreted to suggest relative cortical intactness associated with the genotype rather than that the genotype bestowed a cortical advantage (as it was not seen in healthy controls), again consistent with the idea of a phenotype characterized by less impaired cognitive deficits.

Table 5.1: Summary of the literature showing associations between rs1344706 risk A allele and cognition in both patient and control populations.

	Study	N	Behavioural/ imaging based study	Cognitive measure	Preserved/ Impaired cognition	Outcome Rs1344706 risk A allele associated with:
Patient population	Walters 2010	548 Patients 1637 Controls	Behavioural	Episodic memory Working memory	Preserved	Increased episodic and working memory in patients, but not controls
	Becker 2012	927 Dyslexia 1096 Controls	Behavioural	Language relevant cognitive processes	Preserved	Increased performance in 6 language relevant traits. Non-risk allele associated with decreased performance in these language relevant traits.
	Van Den Bossche 2012	89 Patients	Behavioural	Processing speed	Preserved	Increased performance on processing speed
	Chen 2012	570 Patients 448 Controls	Behavioural	IQ Memory Attention	Preserved /impaired	High IQ patients demonstrated impaired memory and attention Low IQ patients demonstrated intact memory and attention
	Hashimoto 2010	113 Patients 184 Controls	Behavioural	Visual memory	Impaired	Decreased performance in a visual memory task
Control population	Esslinger 2011	111 Controls	Imaging	Working memory	Impaired	Decreased interhemispheric prefrontal connectivity
	Voineskos 2011	62 Controls	Imaging	Attention	Impaired	Decreased attentional control
	Balog 2011	200 Controls	Behavioural	Attention	Impaired	Increased attentional reaction time when conflicting information present
	Lencz 2010	169 Controls	Behavioural	Visuomotor: Trails A	Impaired	Increased reaction time on a visuomotor performance task.
	Paulus 2011	94 Controls	Imaging	Working memory	Preserved	Increased functional connectivity between dorsolateral prefrontal cortex (DLPFC) and hippocampal formation (HF)
	Thurin 2012	208 Controls	Imaging	Cognitive control – response inhibition & interference monitoring and suppression	Preserved	Increased coupling between anterior cingulate cortex (ACC) and DLPFC during interference monitoring and suppression task

5.1.2 ZNF804A and Social cognition

Due to the mounting evidence that *ZNF804A* is delineating a cognitively spared patient sub-population, researchers began to turn their attention to fields outside of traditional cognition in the hope of discovering by what means this psychosis risk gene is exerting its influence. One of the first studies to support an alternative route for *ZNF804A* action was by Walter and colleagues (2011) in which 109 healthy volunteers underwent functional magnetic resonance imaging (fMRI) whilst performing a theory of mind task (ToM); ToM being a measure of social cognition which demonstrates the ability to infer the full range of action causing mental states (Baron-Cohen et al., 2001). They showed that whilst performing this ToM task, carriers of ZNF risk demonstrated a risk allele dose effect on neural activity in the prefrontal cortex and the temporoparietal cortex as well as brain areas involved in the mirror neuron system. Aberrant functional connectivity between frontal and temporoparietal regions was also apparent during performance of the ToM task, leading the authors to conclude that a dysfunction of the ToM network is associated with *ZNF804A* mediated SZ risk.

This association between social cognition and *ZNF804A* was further supported by the results of my own behavioral study (Hargreaves et al., 2012; see chapter 4), which looked at the relationship between the SZ risk SNP rs1344706 and social cognition in both patients and healthy controls. Findings demonstrated an association in controls (but not patients) between *ZNF804A* risk and personalizing bias - a social cognitive measurement of the Internal, personal and situational attributions questionnaire (IPSAQ), which suggests a tendency amongst risk allele carriers to attribute negative events to other people rather than to themselves – a tendency which has been linked to paranoid ideation, anger and learned helplessness (Bentall & Penn, 2001); social cognitive concepts common in patients with psychosis. A third control-led study by Esslinger and colleagues (2011) also showed an association between *ZNF804A* risk and social cognition, as measured using an emotion recognition task, whereby increased *ZNF* risk was correlated with decreased interhemispheric prefrontal activity during task performance. Taken together these data suggest that *ZNF804A* is associated with impaired social cognition in control subjects. It is difficult to say whether or not the SZ gene might also confer a disadvantage to the patient population as thus far only one study (Hargreaves et al., 2012) has included patients in the analysis.

5.1.3 A ZNF804A pathway

As such, the literature to date demonstrates a relatively cohesive picture of the involvement of *ZNF804A* in both neurocognition and social cognition in control subjects. Yet one primary question remains: If *ZNF804A* confers risk of psychosis, why is it that the risk-allele carrying patient population appears relatively unscathed cognitively? Perhaps the answer lies in the impact of *ZNF804A* on other cognition related genes. A recent study by Hill and colleagues (2012) looked at the impact of *ZNF804A* knockdown on the genome by manipulating the expression of *ZNF804A* in neural progenitor cells derived from human cortical neuroepithelium and assessing its effects on the cellular transcriptome. They found that gene ontology analysis of differentially expressed genes indicated a significant effect of *ZNF804A* knockdown on the expression of genes involved in cell adhesion, suggesting a role for *ZNF804A* in processes such as neural migration, neurite outgrowth and synapse formation – all processes known to be important for the development of a working cognition. In total 134 genes were impacted by *ZNF804A* knockdown in the Hill (2012) study (see supplementals **table S5.1** for details). This form of pathway analysis may confer certain advantages over the study of individual variants. Apart from being robust to the influence that differences in linkage disequilbrium (LD) (for example, between study populations or SNP arrays) may have on identification of associated variants (O'Dushlaine et al., 2011), pathway analysis may empower us to better delineate the effects of genetic variants by investigating the effects on phenotypes (e.g. cognitive decline) of genes curated according to their biological function (Lesnick et al., 2007). In such a polygene approach, the individual contribution of each gene to the development of a polygenic disease/trait can be small or even unnoticeable. Genetically, complex diseases/traits depend on the joint contribution of a large number of independent or interacting polymorphic genes (Lvovs et al., 2012), many of which fall below the accepted levels of genome-wide significance. With this in mind, the current study chose to investigate the effects of combined risk allele load within the *ZNF804A* pathway, taken from Hill's (2012) knockdown of *ZNF804A*, on both neurocognition and social cognition with the expectation that it would be more informative with regard to the gene's impact on cognition than individual SNP analysis, particularly in the patient group.

5.1.4 The present study

The purpose of the present study was to investigate the effects of common variants within the *ZNF804A* pathway on neuropsychological function in patients with psychosis. A secondary preliminary investigation (preliminary due to limited numbers) on healthy subjects was also conducted to this effect. We hypothesised both that an additive effect of risk allele load from genetic variants located within the *ZNF804A* pathway would account for more of the variance in patients' neuropsychological function than is explained by single GWAS identified risk variants and that any association between *ZNF804A* polygenic risk and cognition would be led by combined polygenicity and not individual SNPs from within the pathway itself. To test these hypotheses we based our analysis on recent data from the PGC schizophrenia GWAS (Ripke et al., 2011). Selecting all gene variants located within the *ZNF804A* pathway, as identified in a previous study by Hill and colleagues (2012), we gave each patient a *ZNF804A* pathway polygenic risk allele score based on the number of risk alleles they carried. We then determined the amount of variation in patients' neuropsychological function explained by these *ZNF804A* scores. To determine the relative strength of this pathway based analysis over the analysis of individual variant effects on cognition, we compared the amount of variance explained at the pathway level to the amount of variance explained by individual SNPs in a previous meta-analysis conducted by our group (Rose & Donohoe, 2012). Finally, as individual *ZNF804A* SNPs (such as rs1344706) have been linked with cognition in the literature, we chose to examine the hypothesis that it is polygenicity (rather than individual SNPs from within the pathway) that accounts for any observable association between *ZNF804A* risk allele load and cognition. We did this by deleting all *ZNF804A* SNPs (27 in total) from the pathway, recomputing risk allele scores, and redetermining the amount of variation in neuropsychological function explained by these scores.

5.2 Methods

5.2.1 Neuropsychological sample characteristics: 424 cases and 89 healthy participants who had completed a full neuropsychological assessment battery and for whom full genome wide data was available were analysed.

Cases consisted of clinically stable patients with a DSM-IV diagnosis of schizophrenia (SZ), schizoaffective disorder (SZA), bipolar disorder (BP), major depressive disorder with psychotic features (MDD) or psychosis not otherwise specified (PNOS) (see **table 5.2** for details) recruited from five sites across Ireland. Inclusion criteria required that participants were clinically stable at the time of neuropsychological assessment, aged 18 to 65 years, had no history of co-morbid psychiatric disorder, no substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID) (First et al., 2002). Due to the number of patients whose diagnosis of psychosis fell outside of SZ and SZA disorder, we based our analysis on both (1) a narrow definition of SZ and SZA and (2) a broad definition of psychosis which encompassed all those meeting the criteria for psychosis. Additional diagnostic details and clinical sample characteristics ascertained at time of interview including symptom severity (SAPS/SANS) (Andreasen, 1984(a); Andreasen, 1984(b)) and medication dosage are detailed elsewhere (Walters et al., 2010).

Healthy participants were recruited via both online and poster advertising. They were aged 18 to 65 years, with no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Neither they nor any first degree relative had a history of psychosis.

All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

5.2.2 Cognitive assessment

All participants completed a full neuropsychological assessment battery designed to target the cognitive deficits typically reported in SZ – namely deficits in general cognitive function, memory function, working memory, attentional control and social cognition. Where possible both a verbal and a visuo-spatial measure of each construct were included.

General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition (Wechsler, 1997a), yielding a full scale, verbal and performance IQ. ***Episodic memory*** was assessed using the logical memory subtest from the Wechsler Memory Scale, 3rd edition (WMS-III) (Wechsler, 1997b). ***Working memory*** was assessed using the spatial working memory task (SWM) from the Cambridge Automated Neuropsychological Test Battery (CANTAB) (Robbins et al., 1994) and letter number sequencing (LNS) from WMS-III. ***Attentional control*** was assessed using the continuous performance task identical pair's version (CPT-IP) (Cornblatt et al. 1988), the Intradimensional-extradimensional shift task (IDED) (CANTAB) and the sustained attention to response task (SART) (Robertson, 1994). ***Social cognition*** was assessed using the Reading the mind in the eyes task (Baron-Cohen et al., 2001) and the Internal, personal and situational attributions questionnaire (IPSAQ) (Kinderman & Bentall, 1996), which yields two bias scores; 1) externalising bias (EB), which indicates a propensity to attribute positive events to oneself rather than to other people and (2) a personalising bias (PB), which indicates a propensity to attribute negative events to other people rather than to situational factors.

5.2.3 Genotyping

Genetic analysis was conducted on DNA extracted from blood. SNP data for these samples was available from a recent genome-wide association study using the Affymetrix SNP Array 6.0 (ISGC/WTCCC2, 2012).

5.2.4 Calculating risk allele load

Polygenic scores for variants located within the *ZNF* pathway were calculated in four steps. Firstly, all available SNPs within 20Kb of genes in the *ZNF* pathway were identified. *ZNF* pathway genes were identified based on data from the Hill et al paper

(2012). Secondly, alleles within these SNPs were identified as risk or non-risk using data from the PGC SZ GWAS analysis according to three different thresholds: $P<10^{-5}$, $P<.05$, $P<.5$. These arbitrary threshold cut-off points for determining risk followed thresholds used in previous polygenic analysis (Purcell et al,. 2009). Thirdly, to account for difference between variants in the size of the association with illness, each risk allele was weighted as the log10 of the effect size described in the PGC dataset (W_{SNP}= log10 (OR_{PGC})). Finally, a risk-score for each individual was calculated based on the number of risk alleles they carried at each of the three p-value thresholds using the equation: Score(p<threshold) =$\Sigma j(S_{SNP})/(j-m)$, where j = number of SNPs at P<threshold and m= number of SNPs with missing genotypes. A risk score for each of the *ZNF* SNPs was calculated as ($S_{SNP} = W_{SNP}$ * Risk Allele Count).

5.2.5 Statistical analysis

Associations between *ZNF* pathway risk allele load and the phenotypes of IQ, episodic memory, working memory, attention and social cognition were tested in a series of multiple regression analyses implemented in SPSS 17 (SPSS 2008). In each case, scores for each neuropsychological subtest were entered as dependent variables, where appropriate age and gender were entered on the first step of the analysis as effects of no interest, followed by *ZNF* pathway risk allele load on the second step. Effects sizes for all significant effects were calculated using Cohen's d in (ClinTools 2005) to enable comparison to individual SZ risk SNPs already reported (Rose & Donohoe, 2012).

In order to test our second hypothesis that any association between *ZNF804A* polygenic risk and cognition would be led by combined polygenicity and not individual *ZNF804A* SNPs (regardless of strength of association with psychosis), we deleted from the *ZNF804A* pathway all *ZNF804A* SNPs (27 in total). *ZNF804A* risk allele load scores were then recalculated and retested for association with neuropsychological function in the absence of these SNPs. From this second analysis we hoped to determine whether the impact of the *ZNF804A* pathway on cognition was predominantly led by either polygenic risk, or individual SNPs.

5.3 Results

5.3.1 The effects of ZNF804A pathway risk allele load on demographic and clinical measures

Demographic and clinical characteristics for patients and healthy participants appear in **table 5.2**. For all participants demographic variables, means and standard deviations were computed using (SPSS 2008). The mean, number of observations and the standard deviation of the broad psychosis group were then compared to both the narrow psychosis group and to the healthy group using an online t-test calculator: http://www.quantitativeskills.com/sisa/statistics/t-test.htm. In summary, no differences were observed between the narrow psychosis group (SZ and SZA) and broad psychosis group (all patients with psychosis) in terms of age, gender, age at onset, or general cognitive ability as indexed by full scale IQ. On clinical measures of positive and negative symptoms (SAPS/SANS), no differences were detectable between the two groups for the factors of depression, positive symptoms, negative symptoms or disorganisation. Differences were however observed for the 'mania' factor, with the broad psychosis group scoring significantly higher on the manic scale than the narrow psychosis group. No differences were observed in medication dosage as measured by chlorpromazine equivalents. Comparing patients with healthy participants, the patient group contained more males than the healthy group, was significantly older at the time of assessment and had a significantly lower full scale IQ.

Table 5.2: Participant demographics

	Patients		Healthy participants
	Psychosis narrow N=340	Psychosis broad N=424	N=89
Psychosis subtype			
SZ	N= 282	N= 282	N/A
SZA	N= 58	N= 58	
BP		N= 61	
MDD		N= 11	
PNOS		N= 12	
Gender (ratio; M:F)	2.6:1	2.2:1	1.4:1
Age (years; mean (SD))	41.3(12.2)	41.3(12.4)	36.27(12.8)
Age at onset (years; mean(SD))	22.8(7.2)	23.2(7.5)	N/A
Chlorpromazine equivalent (mg/day; mean(SD))	589.8(562.4)	555.5(540.7)	N/A
SAPS/SANS:			N/A
Manic (mean (SD))	-.18(.95)	.04(1.09)	
Depression (mean (SD))	.16(1.07)	.23(1.06)	
Positive (mean (SD))	-.02(.99)	-.12(.95)	
Disorganised (mean (SD))	-.22(.76)	-.31(.78)	
Negative (mean (SD))	.39(.9)	.32(.87)	
Cognition: full scale IQ (mean (SD))	.89.6(17.8)	90.3(18.3)	124.6(13.3)

5.3.2 The effects of ZNF804A pathway risk allele load on cognition in patients.

ZNF804A pathway risk allele load scores were associated with measurements of both neurocognition and social cognition. F, r^2, and P-values for each of the 4 cognitive domains of IQ, memory, attention and social cognition by *ZNF804A* pathway risk allele load are given in **table 5.3**. Variation in performance on those tasks that was significantly explained by polygenic risk was of moderate effect size, typically lying between 3 and 5 % (see **table 5.4** for details) – up to 2% greater than what would be expected for any individual SNP as calculated by Rose and Donohoe (2012).

***Neurocognition*:** Possessing a higher *ZNF804A* risk allele load score was predictive of poorer performance on measurements of IQ, episodic memory and attention across diagnostic categories. Specifically these measurements included performance IQ, full scale IQ, Faces (immediate recall) and CPT-IP (4-digit paradigm).

***Social cognition*:** Of the three social cognitive tasks used, the IPSAQ demonstrated significant association with polygenic *ZNF804A* risk. IPSAQ scoring enables the computation of two bias scores: (1) externalising bias (EB), which indicates a propensity to attribute positive events to oneself rather than to other people and (2) a personalising bias (PB), which indicates a propensity to attribute negative events to other people rather than to situational factors. Within the *ZNF804A* pathway, while patients with narrow sense psychosis (SZ and SZA) who have greater polygenic risk demonstrate a decreased EB score, patients with broad sense psychosis who possess a large risk allele load demonstrate both a decreased externalising bias and an increased personalising bias. This would suggest that patients with psychosis have a tendency to attribute causality (both positive and negative) to other people rather than to themselves or situations, whereas patients on the schizophrenia spectrum are more likely to attribute only positive causality externally. This is interesting as attributing negative events externally has been linked to paranoid ideation, a feature of mania, and mania was the only factor from the SAPS and SANS which significantly differed between the two psychosis groups.

5.3.3 The effects of ZNF804A pathway risk allele load on cognition in healthy volunteers - both when measured independently and when incorporated into patient analyses

ZNF804A pathway risk allele load scores were associated with measurements of both neurocognition and social cognition. F, r^2, and P-values for each of the 4 cognitive domains of IQ, memory, attention and social cognition by *ZNF804A* pathway risk allele load are given in **table 5.5**. Variation in task performance explained by polygenic risk differs depending on the exclusion/inclusion of the patient group. When healthy participants are analysed independently, variation in task performance is of moderate effect size, typically lying between 5 and 9% (up to 6% greater than what would be expected for individual SNPs as calculated by Rose and Donohoe (2012). On the inclusion of the patient group, this effect size drops to between 2 and 4% (see **table 5.6** for details).

Table 5.3: Patient analyses: *ZNF804A* pathway polygenic score regression analysis for each neuropsychological variable. ZNF risk alleles included were thresholded at (a) p=10x-5 (b) p=.05 (c) p=.5

	Neuropsych	All patients with psychosis			SZ and SZA		
		$10x^{-5}$ F(r2)p	.05 F(r2)p	.5 F(r2)p	$10x^{-5}$ F(r2)p	.05 F(r2)p	.5 F(r2)p
IQ	WTAR	.363 (.001) .547	1.13(.003).288	.071 (.000) .79	1.25 (.004) .256	1.8 (.006) .179	.003(.000) .956
	Verbal IQ	2.14 (.011) .12	2.23(.011).108	2.13 (.011) .12	2.28(.014) .104	2.44(.016).089	2.25(.014) .107
	Performance IQ	**5.18 (.026) .006**	**4.02 (.02) .019**	**3.71(.019).025**	**5.02 (.032) .007**	**4.8 (.031) .009**	**4.4 (.028) .013**
	Full scale IQ	**3.5 (.018) .031**	2.82(.014).061	**3.1 (.016) .049**	**3.67 (.024) .026**	**3.56 (.023) .03**	**3.7 (.024) .024**
MEMORY	Logical memory Immediate recall	1.1 (.003) .29	2.9 (.008) .085	1.04(.003).308	1.25 (.004) .264	1.3 (.004) .255	.113(.000) .737
	Logical memory Delayed recall	.851 (.002) .357	1.69(.004 .193	.555(.001).457	.768 (.003) .382	.73 (.002) .393	.174(.001) .677
	Faces Immediate recall	**3.65 (.034) .013**	**3.99(.037).008**	**4.53(.041).004**	**3.86 (.045) .01**	**3.89 (.045) .01**	**4.36(.051) .005**
	Faces Delayed recall	.389 (.001) .533	.351(.001).554	.648(.002 .409	.251 (.001) .617	.004 (.00) .95	.389(.002) .533
	LNS	1.18 (.003) .278	.003(.000).957	.535(.001).465	1.246 (.004) .265	.001(.000 .975	.153(.001) .696
	SWM errors	.039 (.000) .843	.972(.000).325	.12 (.000) .729	.274 (.001) .601	.429(.002).513	.000(.000) .994
	SWM strategy	1.34 (.004) .247	4 (.011) .046	1.78(.005).183	2.88 (.01) .09	3.17(.011).076	.643(.002) .423
ATTENTION	SART total correct response	.022 (.000) .881	.002(.000).962	1.22(.004).271	1.19 (.005) .276	.000(.000).986	.266(.001) .606
	SART reaction time	1.05 (.004) .306	.018(.000).894	2.23(.008).136	.06 (.000) .807	.796(.003).373	.406(.002) .525
	IDED 8 blocks	2.04 (.013) .132	2 (.012) .137	2.27 (.014) .11	.000 (.000) .997	.241(.001).624	1.1 (.004) .293
	CPT d'Prime 2 digit	.074 (.000) .785	.16 (.001) .68	.97 (.003) .325	.72 (.003) .397	.05 (.000) .824	.706(.003) .402
	CPT d'Prime 4 digit	**4.35 (.032) .014**	**4.46(.033).012**	**3.83(.029).023**	**3.12 (.029) .046**	**3.68(.034).027**	**3.13(.029) .046**
SOCIAL	Eyes	.096 (.001) .757	1.14 (.016) .28	1.36(.019).246	.259 (.004) .613	1.18(.017).281	1.79(.026) .185
	IPSAQ EB	**5.958(.044) .003**	**5.78(.043).003**	**2.44(.019).089**	**5.27 (.05) .006**	**4.33(.041).014**	2.1 (.021) .122
	IPSAQ PB	**3.078(.023) .048**	**3.31(.025).038**	**3.18(.024).043**	.185 (.001) .668	.28 (.001) .597	1.42(.007) .234

Table 5.4: Effect sizes for significant neuropsychological variables within the *ZNF* pathway polygene analysis in patients.

Threshold for risk allele inclusion	Neuropsychological variable	Adjusted r square	R square change	Cohen's d
Patients (all psychosis)				**μ = .337**
$10x^{-5}$	Performance IQ	.021	.012	.326
	Full scale IQ	.013	.004	.268
	Faces immediate recall	.025	.001	.377
	CPT d'Prime (4-digit)	.025	.004	.366
	IPSAQ externalising bias	.034	.025	.417
	IPSAQ personalising bias	.016	.000	.31
.05	Performance IQ	.015	.007	.289
	Faces immediate recall	.027	.004	.389
	CPT d'Prime (4-digit)	.026	.004	.37
	IPSAQ externalising bias	.036	.026	.423
	IPSAQ personalising bias	.017	.002	.32
.5	Performance IQ	.014	.005	.277
	Full scale IQ	.01	.002	.251
	Faces immediate recall	.032	.008	.415
	CPT d'Prime (4-digit)	.021	.000	.343
	IPSAQ externalising bias	.011	.002	.275
	IPSAQ personalising bias	.017	.001	.314
Patients only (SZ + SZA)				**μ = .376**
$10x^{-5}$	Performance IQ	.026	.009	.36
	Full scale IQ	.017	.001	.311
	Faces immediate recall	.033	.000	.434
	CPT d'Prime (4-digit)	.02	.000	.345
	IPSAQ externalising bias	.04	.03	.457
.05	Performance IQ	.024	.008	.355
	Full scale IQ	.016	.001	.305
	Faces immediate recall	.034	.001	.436
	CPT d'Prime (4-digit)	.025	.005	.374
	IPSAQ externalising bias	.032	.021	.415
.5	Performance IQ	.022	.005	.341
	Full scale IQ	.018	.002	.316
	Faces immediate recall	.039	.006	.462
	CPT d'Prime (4-digit)	.02	.000	.347

***Neurocognition*:** In the healthy participant only group, possessing a higher *ZNF804A* risk allele load score was predictive of an increased performance on measurements of IQ, and a decreased performance on measurements of memory and attention. Specifically these measurements included premorbid IQ, verbal IQ, full scale IQ, Faces (immediate recall), LNS, SWM, SART reaction time and omission errors, and IDED (8-block paradigm).

When patients and healthy participants are analysed together, possessing a higher *ZNF804A* risk allele load score was predictive of a decreased performance on measurements of IQ, memory and attention. Specifically these measurements included performance IQ, logical memory (immediate recall), logical memory (delayed recall), Faces (immediate recall), LNS, SWM and SART reaction time.

***Social cognition*:** In the healthy participant only group *ZNF804A* risk allele load is associated with an increased performance on the ToM task, Reading the mind in the eyes. Analysing healthy participants and patients together revealed an association between the *ZNF804A* pathway and measurements of the IPSAQ, demonstrating a decreased externalising bias and an increased personalising bias. This would suggest a tendency to attribute causality (both positive and negative) to other people rather than to themselves or situations.

5.3.4 The impact of ZNF804A SNPs on the association between neurocognitive variables and ZNF804A risk allele load in both patients and healthy participants.

Individual *ZNF804A* SNPs, such as rs1344706, have been shown in previous research to be associated with neuropsychological variation. We wished to determine how much of the cognitive variation associated with *ZNF804A* risk allele load in the present study was attributable to these same individual SNPs and how much was due to the combined effect of multiple genes within the *ZNF804A* pathway. As such, we re-ran our pathway analysis to exclude all variants at the *ZNF804A* gene locus (this excluded 27 SNPS). We found that without the *ZNF804A* gene, the *ZNF804A* pathway continued to explain a significant amount of variation in IQ and attention (but not memory or social cognition) in the healthy participant group (see **table 5.8**). However, the *ZNF804A* pathway was no longer significantly associated with IQ, memory, attentional control or social cognition either in the broad or narrow psychosis patient groups (see **table 5.7**).

Table 5.5: All analyses involving the healthy participant group: *ZNF804A* pathway polygenic score regression analysis for each neuropsychological variable. *ZNF* risk alleles included were thresholded at (a) p=10x-5 (b) p=.05 (c) p=.5

		Healthy participants (HP) only			HP plus all patients with psychosis		
	Neuropsych	$10x^{-5}$ F(r2) p	.05 F(r2)p	.5 F(r2)p	$10x^{-5}$ F(r2)p	.05 F(r2)p	.5 F(r2)p
I Q	WTAR	**3.25(.072).044**	.837 (.01) .363	.86 (.011) .356	.493(.001).483	.594 (.001) .441	.932 (.002) .335
	Verbal IQ	**3.57(.079) .032**	**3.2 (.071) .045**	2.8 (.063) .064	.04 (.000) .841	.106 (.000) .745	1.02 (.002) .312
	Performance IQ	.226(.003) .636	1.2 (.014) .274	.159(.002).691	**3.95(.008).047**	**4.14 (.009) .042**	**4.92 (.01) .027**
	Full scale IQ	**4.03(.089) .021**	2.5 (.057) .087	2.5 (.057) .085	1.19(.003 .274	.745 (.002) .389	2.9 (.006) .088
M E M O R Y	Logical memory immediate recall	2.45(.055) .092	2.06(.046).134	1.98(.045).143	**3.01 (.013) .05**	**3.74 (.016) .024**	**4.25 (.018) .015**
	Logical memory delayed recall	1.54(.018) .217	.012(.000).913	.051(.001).822	**4.87 (.02) .008**	**5.08 (.021) .007**	**5.46 (.023) .005**
	Faces immediate recall	**4.58(.052) .035**	2.75(.031).101	1.45(.017).231	**5.52(.027).004**	**6.43 (.031) .002**	**7.85 (.038) .000**
	Faces delayed recall	2.42(.028) .124	.085(.001).772	.014(.000).905	.096(.000).756	.352 (.001) .553	1.75 (.004) .186
	LNS	**3.9 (.046) .049**	**5.8 (.065) .017**	**6.6 (.073) .012**	2.23(.005).136	1.52 (.003) .217	**6.9 (.015) .009**
	SWM between errors	1.55(.018) .215	**7.4 (.08) .008**	**5.6 (.063) .019**	.344(.001).558	3.5 (.008) .061	2.9 (.007) .089
	SWM strategy	2.3 (.026) .132	**6.5 (.072) .012**	**12.2(.125).001**	2.55(.006).111	**7.87 (.018) .005**	**6.5 (.015) .011**
A T T E N T I O N	SART total correct response	2.3 (.029) .32	3.39(.042).669	1.5 (.019) .218	.149(.000).699	.039 (.000) .843	.307 (.001) .58
	SART reaction time	**6.38(.142) .003**	**6.34(.142).003**	**6.39(.142).003**	**3.82 (.02) .023**	2.79 (.015) .062	3 (.016) .051
	IDED 8 blocks	**5.57(.173) .002**	**4.4 (.142) .006**	**4.4 (.141) .006**	2.51(.012).082	2.52 (.012) .082	2.6 (.013) .075
S O C I A L	Eyes	**4.5 (.103) .014**	**5.17(.116).008**	**6.5 (.141) .002**	1.58 (.01) .21	3.02 (.02) .084	.866 (.006) .354
	IPSAQ EB	.05(.001).83	.845(.01) .361	2.58 (.03) .112	**5.8(.03).003**	**4.37(.025).013**	**3.13 (.018) .045**
	IPSAQ PB	.706(.008) .403	.336(.004).564	.095(.001).759	**5.15(.029).006**	**5.06 (.029) .007**	**5.13 (.029) .006**

Table 5.6: Effect sizes for significant neuropsychological variables within the *ZNF* pathway polygene analysis in all analyses involving the healthy group.

Threshold for risk allele inclusion	Neuropsychological variable	Adjusted r square	R square change	Cohen's d
Healthy participants				**μ = .675**
$10x^{-5}$	WTAR	.05	.002	.556
	Verbal IQ	.057	.000	.588
	Full scale IQ	.067	.002	.624
	Faces immediate recall	.04	.052	.576
	LNS	.034	.046	.438
	SART reaction time	.12	.001	.814
	IDED 8 blocks	.142	.000	.915
	Eyes	.08	.019	.675
.05	Verbal IQ	.066	.009	.619
	LNS	.063	.074	.565
	SWM between errors	.066	.077	.576
	SWM strategy	.068	.079	.585
	SART reaction time	.119	.001	.812
	IDED 8 blocks	.142	.001	.915
	Eyes	.093	.032	.723
.5	LNS	.06	.071	.554
	SWM between errors	.054	.065	.53
	SWM strategy	.113	.124	.75
	SART reaction time	.12	.002	.814
	IDED 8 blocks	.147	.005	.928
	Eyes	.066	.005	.624
Healthy participants plus patients with psychosis				**μ = .288**
$10x^{-5}$	Performance IQ	.006	.008	.183
	Logical Memory immediate recall	.008	.003	.225
	Logical Memory delayed recall	.016	.003	.289
	Faces immediate recall	.022	.005	.33
	SART reaction time	.015	.005	.287
	IPSAQ externalising bias	.027	.015	.368
	IPSAQ personalising bias	.024	.001	.347
.05	Performance IQ	.007	.009	.187
	Logical Memory immediate recall	.011	.006	.25
	Logical Memory delayed recall	.017	.004	.295
	Faces immediate recall	.026	.009	.358
	SWM Strategy	.016	.018	.268
	IPSAQ externalising bias	.019	.007	.32
	IPSAQ personalising bias	.023	.001	.343
.5	Performance IQ	.008	.01	.203
	Logical Memory immediate recall	.014	.008	.268
	Logical Memory immediate recall	.019	.005	.31
	Faces immediate recall	.033	.016	.395
	LNS	.013	.015	.245
	SWM Strategy	.013	.015	.245
	IPSAQ externalising bias	.012	.000	.27
	IPSAQ personalising bias	.023	.001	.347

Table 5.7: Patients: regression analysis and Cohen's d calculated for the *ZNF804A* pathway minus the *ZNF804A* gene. Cognitive variables analysed are those which were significantly associated with the intact *ZNF804A* pathway.

Neuropsych variable	All patients with psychosis			Patients with SZ or SZA		
	$10x^{-5}$ F(r2)p Cohen's d	.05 F(r2)p Cohen's d	.5 F(r2)p Cohen's d	$10x^{-5}$ F(r2)p Cohen's d	.05 F(r2)p Cohen's d	.5 F(r2)p Cohen's d
IQ:						
Performance	.232(.001).63 .06	.114(.000).74 0	.226(.001).64 .06	.032(.000).86 0	.03(.000).86 0	.028(.009).87 .19
Full scale	.314(.001).58 .06	.016(.000).89 0	.303(.001).58 .06	.067(.000).79 0	.072(.000).79 0	.022(.000).88 0
MEMORY:						
Faces (immediate recall)	.914(.003).34 .108	.908(.003).34 .108	.227(.001).63 .06	.777(.003).38 .002	.647(.002).42 .088	.33(.001).57 .06
ATTENTION:						
CPT (4 digits)	.108(.000).74 0	.022(.009).88 .19	.477(.002).49 .08	2.79(.013).09 .229	2.19(.01).14 .2	.336(.016).07 .254
SOCIAL						
IPSAQ EB	.065(.000).79 0	.008(.000).93 0	.006(.000).94 0	.003(.000).96 0	.038(.0000.85 0	.161(.001).69 .06
IPSAQ PB	1.23(.005).27 .14	.931(.004).34 .126	.529(.002).47 .08	1.81(.009).18 .191	1.04(.005).31 .14	.658(.003).42 .108

Table 5.8: Healthy participants: regression analysis and Cohen's d calculated for the *ZNF804A* pathway minus the *ZNF804A* gene. Cognitive variables analysed are those which were significantly associated with the intact *ZNF804A* pathway.

	Healthy participants					
Neuropsychological Variable	**$10x^{-5}$**		**.05**		**.5**	
	F(r2)p	Cohen's d	F(r2)p	Cohen's d	F(r2)p	Cohen's d
IQ:						
Premorbid	4.2(.09).018	.629	6.36(.096).014	.65	4.16(.089).019	.624
Verbal	3.67(.08).03	.61	3.71(.081).029	.592	3.63(.079).031	.585
Full scale	4.26(.092).017	.63	4.08(.089).02	.624	4.04(.088).021	.619
MEMORY:						
Faces (immediate recall)	.095(.001).759	.06	.631(.007).429	.166	.085(.001).771	.06
LNS	.000(.000).999	0	.447(.005).506	.14	.002(.000).961	0
ATTENTION:						
SART reaction time	6.495(.143).002	.82	6.49(.143).002	.816	6.59(.145).002	.824
IDED (8 blocks)	6.29(.133).003	.78	6.54(.138).002	.799	6.19(.131).003	.776
SOCIAL						
Eyes	2.75(.077).107	.57	2.116(.06).155	.505	3.24(.089).081	.624

5.4 Discussion

In this study we used *ZNF804A* pathway risk allele scores, derived from the PGC schizophrenia GWAS dataset, to investigate whether increased numbers of *ZNF804A* pathway risk alleles correlated with poorer neuropsychological function amongst 424 patients with either narrow sense or broad sense psychosis. We also conducted a preliminary investigation on 89 healthy subjects in order to determine the impact of the *ZNF804A* pathway on cognition in a healthy population. The amount of variance explained by the *ZNF804A* pathway was further compared to that explained by individual SNPs as calculated in the Rose and Donohoe (2012) meta-analysis. Based on these analyses we found that (1) polygenic scores for the *ZNF804A* pathway explained a significant proportion of variance in neuropsychological function, irrespective of the risk threshold used; (2) *ZNF804A* risk allele load impacted cognition in the patient population differently to the healthy population, with more cognitive variables being associated with polygenic risk amongst the healthy group; (3) social cognition was impacted differently in the healthy group compared to the patient group, both in terms of the social cognitive variable concerned and in terms of the direction of association; (4) the proportion of variance explained by the polygenic analysis exceeded that calculated for significant gene-cognition associations in schizophrenia to date (based on a recent meta-analysis conducted within our group; Rose & Donohoe, 2012); and (5) removing all *ZNF804A* SNPs from polygene analysis also removed the association between *ZNF804A* polygenic risk and cognition amongst patients, but did not impact the association with IQ and attention in the healthy population.

Cognitively, amongst patients, higher polygenic risk scores within the *ZNF804A* pathway correlated with poorer IQ, memory, attention and social cognition. This finding is consistent across diagnostic categories. In the preliminary control investigation, *ZNF804A* pathway risk is seen to correlate with poorer memory and attention alongside an improvement on measurements of IQ and ToM. These *ZNF804A* pathway findings are in contrast with previously published individual *ZNF804A* SNP studies which generally report a preserved cognition amongst patients and an impaired cognition in healthy participants. Possible explanations for this discrepancy in findings include (1) the *ZNF804A* pathway used in this research is that curated by Hill and colleagues (2012) and encompasses all

downstream genes affected by *ZNF804A* knockdown. These include genes related to cell adhesion, regulation of growth and response to organic substance (amongst others), which are known to effect cognition (Soronen et al., 2010; Baker et al,. 2012; Bartha et al., 2002). As such viewing cognition via the impact *ZNF804A* risk allele load intrinsically involves the impact of these various other genes, which may be acting on cognition in a manner different to the GWAS identified *ZNF804A* risk SNP rs1344706; (2) Hill and colleagues (2012) performed their experiments using clinical grade human neural progenitor cells. Although this cell line is likely to be a suitable candidate for investigating the role of *ZNF804A* at the pathway level, information on the cell types that normally express this gene, let alone those most relevant to its role in psychosis aetiology, is still currently unknown. It is possible that *ZNF804A* impacts cognition *in-vivo* in a different manner to how it impacts cognition *in-vitro,* thus explaining the discrepancy between individual SNP (*in-vivo*) and pathway analysis (*in-vitro*) findings. It can sometimes be very challenging to extrapolate from the results of *in vitro* work back to the biology of the intact organism. Investigators doing *in vitro* work must be careful to avoid over-interpretation of their results, which can sometimes lead to erroneous conclusions (Rothman, 2002).

Of particular interest was the finding that removing the *ZNF804A* gene from the *ZNF804A* pathway removed all associations between pathway polygenic risk and cognition in the patient group, but did not impact the association in healthy participants. A possible explanation for this is that in the patient group, the *ZNF804A* gene strongly impacts neuropsychological function, whereas in a healthy population, cognition is more greatly impacted by genes downstream of *ZNF804A*. Perhaps this might explain why the literature to date finds that *ZNF804A* impacts patients and healthy volunteers in different ways. Further investigation of this with a larger healthy volunteer sample would prove useful.

A specific criticism of the single variant approach in studying both illness phenotypes and intermediate phenotypes (including cognition) is that the function of single variants cannot be understood in isolation from their biological pathway and individually are unlikely to contribute significantly to our understanding of pathology. The explanatory power of polygenetic analysis for explaining risk for

schizophrenia as estimated by Purcell and colleagues (2009) would appear to support this view. In this context, a central hypothesis in our analysis was that variance in neuropsychological deficits would be better modeled and explained by a polygenic risk score than by individual SNP genotypes. This hypothesis was supported by current study findings. The amount of variance explained by individual SNPs in a previous meta-analysis conducted by our group (Rose & Donohoe, 2012) was ~3%. In the current study this variance increased in patients by ~2%, and in controls by about ~5%, arguing for the benefits of a pathway based approach when studying the impact of psychosis genetics.

In relation to explaining how much cognitive variability can be attributed to the *ZNF804A* pathway, of particular interest is the increased variance (alongside an increase in the quantity of cognitive variables associated with *ZNF804A* polygenic risk) in the control group compared to the patient group, as is contrary to expectation. Afterall, *ZNF804A* is a psychosis risk gene. Due to the preliminary nature of the healthy participant investigation, it is possible that these differences can be accounted for by the smaller number of healthy volunteers involved in the study (89 controls verses 424 patients). However, what cannot be overlooked is the many other studies which showed a correlation between *ZNF804A* and cognition in a healthy population (see **table 5.1**) and as such a replication of the current study involving larger numbers of healthy volunteers would prove useful.

The use of polygene risk scores is relatively new in psychiatric genetics, particularly in the use of these scores to examine a target pathway, and as such a number of methodological issues require highlighting, both at a polygene level and at the level of the pathway. Polygenically, following in the footsteps of previous literature (Purcell et al., 2009) we chose to look at three risk groups whilst conducting analysis: those whose scores derived from all SNPs with a p-value of less than 10^{-5}, .05 and .5. Other cut-off thresholds might have been considered, and the appropriateness of one or other of these has not been well characterized. In fact, a cut-off-based approach treats association signals qualitatively rather than quantitatively; whether such dichotomization of markers as significant or non-significant either reduces signal or introduces bias is unclear (Jia et al., 2011). A further issue to consider is the degree of genetic overlap

between cognitive performance and psychosis. Purcell and colleagues (2009) suggested that ~30-40% of genetic risk was attributable to common variant polygenicity and that the amount of variation in risk explained by independent samples was much lower. The question is whether one would expect SZ polygenic scores to influence psychotic traits such as impaired cognition, or whether cognition is influenced by a separate group of genes. Toulopoulou and colleagues (2007) infers a high degree of overlap between cognitive deficits and SZ in his twin genetic modeling study. However, recent genetic epidemiological evidence by Fowler and colleagues (2012) has suggested that this may not be quite as high when based on non-biased samples. In the context of these findings, when modeling cognitive performance in psychosis, polygenic risk scores may therefore introduce as much noise as signal into the analysis (i.e. variation neither specific to cognition, nor to the target patient population). On account of this, the variation in neurocognition explained by the individual SNP, although failing to capture much of the genetic variation associated with cognition, may not carry the same burden of non-relevant genetic information.

There are also certain limitations to consider at the level of the pathway. The data by Hill and colleagues (2012) does not indicate whether altered expression of any given gene on the *ZNF804A* pathway reflects direct actions of *ZNF804A* on that gene or secondary consequences. Like primary changes, secondary changes in gene expression could be part of the disease pathway or be aetiologically neutral. They may reflect compensatory mechanisms, or pathway overlap (Ramanan et al., 2012), and as such the impact of the ZNF804A pathway apparent in the present study, might possibly be the impact of two or more pathways combined, which in turn might account for the greater effect sizes seen. Related to this issue is the difficulty in determining which of the SNPs used to calculate the polygene score correlates to the true SNPs/genes that polygenically determines risk. It is likely that not 100% of the pathway is informative and that annotation error could lead to the inclusion of genes that are not pathway specific.

5.5 Conclusion

In conclusion, this study is the first to our knowledge to investigate the role of the *ZNF804A* pathway in the cognitive decline commonly evident amongst both psychotic patients and healthy volunteers. The data supports a role for the *ZNF804A* pathway in IQ, memory, attention and social cognition. The study also demonstrates that in both patient and healthy groups, the variance in cognition attributable to whole *ZNF804A* pathway analysis is greater than that attributable to individual SNPs; a finding which supports the continued investigation of pathway analysis in cognitive decline.

Supplementals

Supplementary Table S5.1. Genes showing differential expression ($P < 0.05$), in the same direction relative to the negative control, in association with both HSS150612 and HSS150613 *ZNF804A* siRNA conditions.

A2M	C1ORF104	CLUAP1	ELAVL1	GREM1	LOC100129905
ACPL2	C1ORF54	CNNM2	ELF4	GSTTP2	LOC100132805
ACTG2	C1ORF91	COL8A2	EML2	GUSB	LOC100133019
ADA	CAPRIN2	COLEC12	F2R	GUSBL1	LOC144438
ANTXR1	CCDC66	CPSF2	FAM107A	H6PD	LOC400836
APBB3	CCL2	CROP	FAM176A	HMGCR	LOC441126
APBB3	CCNJL	CRYAB	FAM46A	HS.143018	LOC643911
ARHGAP19	CD151	CTDSPL	FBLN7	HS.158923	LOC646808
ASB8	CD83	CTNND1	FHDC1	HS.444999	LOC648366
ATP10B	CD83	CYTH2	FRZB	HS.561493	LOC728128
ATP1B1	CDCA4	DCTN2	FSTL1	HS.572219	LOC728969
BEX1	CDKN1A	EIF4A2	GAR1	IKBKAP	LOC729082
BID	CES2	EIF4G1	GNG7	LAMA4	LOC729234
C14ORF43	CHCHD7	ELAC1	GPR19	LOC100129758	LOC730074
LUC7L2	NELL2	RASD1	STAC	UBXN4	LRCH4
MAP7	NOTCH2	RGS16	STARD7	UQCC	
MAPK8IP2	OSBPL10	RPRD2	STMN3	VGF	
MCM3	OSCP1	RUNDC2C	SUGT1	ZBED4	
MOXD1	PARP2	SCARNA9	SULT1A3	ZBTB4	
MRC2	PARVA	SEPT13	SYTL2	ZC3H7A	
MTERFD1	PCDH7	SEZ6	TAF15	ZFAND6	
MTHFS	PDZD4	SFRP4	TAF9L	ZFC3H1	
MUTYH	PELI2	SFRS18	TDP1	ZIK1	
NBPF1	PMP22	SKIV2L	TMEM154	ZNF131	
NCAM1	PPP4R1	SLC4A7	TNK2	ZNF177	
NCAPG2	PRSS35	SNORA7B	TRMT61A	ZNF439	
NDRG3	QSOX2	SPAG16	TXN	ZNF473	
NDUFS1	RAB11FIP2	SSPN	UBE3C		

Chapter 6

The impact of the *CAM* pathway on cognition in patients with psychosis and healthy participants

Abstract

Background: *Cell adhesion molecule* (*CAM*) biology has been repeatedly linked to psychosis and, independently, to neurocognitive function, which is known to be aberrantly effected in psychotic patients. Here we tested, using *CAM* pathway risk allele scores derived from the PGC schizophrenia GWAS dataset, whether *CAM* pathway polygenic risk scores correlated with poorer neuropsychological function. We also investigated the variation in neuropsychological performance explained by common SNP variants in four of the *CAM* pathway genes most strongly associated with SZ in the PGC analyses. Any of these SNPs that showed association with neurocognition was then removed from the *CAM* pathway whose polygenic risk scores were then retested for correlation with cognition.

Method: 424 patients with psychosis were assessed in areas of cognitive ability typically found to be impaired in schizophrenia (SZ): including IQ (using selected subtests from the Wechsler Adult Intelligence Scale, 3rd edition), memory (using the logical memory subtest of the Wechsler Memory Scale, 3rd edition and the Paired Associate Learning (PAL) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB), and attention (using the continuous performance task identical pair's version (CPT-IP) and the sustained attention to response task (SART)).

Results: Increased risk load on the *CAM* pathway was significantly associated with variation in premorbid IQ, memory and attention, explaining ~3% of variation on these measures. Specifically, increased risk allele load was associated with poorer performance in memory and attention. Of the four individual *CAM* pathway SNPs considered, only one (*HLADQA1*) was associated with neurocognitive variation, where it explained a comparable amount of variation to that explained by the *CAM* polygene score. Removal of this gene from the polygene analysis impacted the association between *CAM* pathway risk allele load and attentional control.

Discussion: These data support a role for the *CAM* pathway in memory formation and attention, both at the level of pathway analysis and at the individual SNP level. The potential reasons that the *CAM* pathway polygene scores explained a comparable amount of variation to SNP level analysis are discussed in terms of factors related to both the *CAM* pathway and the polygene score approach.

6.1 Introduction

It has been more than two decades since the involvement of cell adhesion molecules (*CAMs*) in the pathophysiology of schizophrenia (SZ) was first hypothesised (Lyons et al, 1988; Vawter, 2000). Research since then has shown that genes encoding *CAMs* play an important role in neurodevelopmental processes including axonal and dendritic growth and brain segmentation (e.g. *CDH4*; (Wang et al., 2009) cell-cell binding (e.g. *CDH7*; (Soronen et al., 2010) and synapse formation (e.g. *neurexin*; (Dean et al., 2003). Disruption of several *CAM* genes has been reported in patients with psychosis, including *de novo* copy number variants (CNVs) in *neurexin-1* (Kirov et al., 2009; Rujescu et al., 2009), *neuroligan-2* (Sun et al., 2011) and several others (Kirov et al., 2012), each of which has been associated with increased illness risk. In addition, we have previously observed that the *CAM* pathway, out of 212 neurodevelopmentaly relevant pathways considered, was significantly enriched for common risk variants for psychosis (O'Dushlaine et al., 2011).

Alongside their association with psychosis, genes encoding *CAMs* have been shown to impact cognitive function. Neuronal cell adhesion molecule (*NCAM*) has been found to be associated with memory consolidation in both rats and hippocampal neuron cultures (Cambon et al., 2004). Contactin-associated protein-like 2 (*CNTNAP2*), which encodes a member of the neurexin family and has been implicated in SZ, is also associated with mental retardation (Friedman et al., 2008). More recently, Soronen and colleagues (2010), found that the gene cadherin 7 (*CDH7*) is associated with variation in performance on measures of working memory and visual attention in patients with bipolar disorder. Yet despite the evidence demonstrating that *CAMs* influence cognition, the mechanism by which this occurs remains unknown. Of particular interest is whether the influence

of *CAMs* on both cognition and psychosis risk occurs via the same biological pathway.

Analysis of variants within biologically defined pathways may offer several advantages over the study of individual rare and common variants, despite the subjective manner by which they are often curated. Whereas the function of GWAS identified individual SNPs is often unclear, pathway analysis involves the study of genes encoding proteins that are functionally related in terms of their biological role (Yaspan & Veatch, 2011). It is informed by description of function that groups individual proteins and their encoding genes; a functional grouping which empowers us to better delineate their effects on phenotypes such as illness and quantitative traits (Lesnick et al., 2007). Pathway analysis also has the advantage of being robust to the influence that differences in linkage disequilbrium (LD) (for example, between study populations or SNP arrays) may have on identification of associated variants (O'Dushlaine et al., 2011).

The purpose of the present study was to look at the *CAM* pathway, as curated from the KEGG database, in order to investigate the effects of common variants within this pathway on neuropsychological function in patients with psychosis. We hypothesised that an additive effect of risk allele load from genetic variants located within the *CAM* pathway would account for a significant percentage of the variation in neuropsychological function in patients. To test this hypothesis we based our analysis on recent data from the PGC schizophrenia GWAS (Ripke et al., 2011). Selecting all gene variants located within the *CAM* pathway, each patient received a *CAM* pathway polygenic risk score based on the number of risk alleles they carried. We then determined the amount of variation in patients' neuropsychological function explained by *CAM* polygene risk scores. We compared the amount of variance explained in this polygene analysis to the amount of variance explained by individual variants from four genes within the *CAM* pathway that were most strongly associated with SZ risk in the PGC analysis - *CDH4, HLADQA1, NRXN1* and *CNTNAP2*. Finally, for any of the four SNPs that were associated with the same cognitive variables as *CAM* pathway polygenic risk, the gene from which they were taken was deleted from the *CAM* pathway and the initial analysis rerun in order to determine whether the impact of the *CAM* pathway on cognition was predominantly led by (1) polygenic risk, or

(2) individual highly associated genes. Our hypotheses were that substantially more of the variation in patients' neuropsychological performance would be explained by the *CAM* pathway polygenic risk score than by individual risk variants within the pathway and that any association between *CAM* polygenic risk and cognition would be led by combined polygenicity and not individual SNPs from within the pathway itself.

6.2 Methods

6.2.1 Neuropsychological sample characteristics: 424 cases who had completed a full neuropsychological assessment battery and for whom full genome wide data was available were analysed. Cases consisted of clinically stable patients with a DSM-IV diagnosis of schizophrenia (SZ), schizoaffective disorder (SZA), bipolar disorder (BP), major depressive disorder with psychotic features (MDD) or psychosis not otherwise specified (PNOS) (see **table 6.1** for details) recruited from five sites across Ireland. Inclusion criteria required that participants were clinically stable at the time of neuropsychological assessment, aged 18 to 65 years, had no history of co-morbid psychiatric disorder, no substance abuse in the preceding six months, no prior head injury with loss of consciousness and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID) (First, Spitzer, Gibbon, & Williams, 2002). Due to the number of patients whose diagnosis of psychosis fell outside of SZ and SZA disorder, we based our analysis on both (1) a narrow definition of SZ and SZA and (2) a broad definition of psychosis which encompassed all those meeting the criteria for psychosis. Additional diagnostic details and clinical sample characteristics ascertained at time of interview include medication dosage and symptom severity. This was calculated based on a factor analysis of Operational Criteria Checklist for Psychotic Illness (OPCRIT) (McGuffin et al., 1991), as previously described for this sample (Cummings et al., 2013). All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

6.2.2 Cognitive assessment: All patients completed a full neuropsychological assessment battery designed to target the cognitive deficits typically reported in SZ – namely deficits in general cognitive function, memory function, working memory and attentional control. Where possible both a verbal and a visuo-spatial measure of each construct were included.

General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition (Wechsler, 1997a), yielding a full scale, verbal and performance IQ. Verbal and visual ***episodic memory*** were assessed using the logical memory subtest from the Wechsler Memory Scale, 3rd edition (WMS-III)(Wechsler, 1997b) and the Paired Associate Learning (PAL) task from the Cambridge Automated Neuropsychological Test Battery (CANTAB) (Robbins et al., 1994). ***Attentional control*** was assessed using the continuous performance task identical pair's version (CPT-IP) (Cornblatt et al, 1988) and the sustained attention to response task (SART) (Robertson et al, 1997).

6.2.3 Genotyping: Genetic analysis was conducted on DNA extracted from blood. SNP data for these samples was available from a recent genome-wide association study using the Affymetrix SNP Array 6.0 (ISGC/WTCCC2, 2012).

6.2.4 Calculating risk allele load: Polygenic scores for variants located within the CAM pathway were calculated in four steps. Firstly, all available SNPs within 20Kb of genes in the CAM pathway were identified. CAM pathway genes were identified based on data from the KEGG database as previously described by us (O'Dushlaine et al., 2011). A total of 132 genes were identified, five of which could not be tagged with SNPs using the above criteria. Secondly, alleles within these SNPs were identified as risk or non-risk using data from the PGC SZ

GWAS analysis according to three different thresholds: $P<10^{-5}$, P<.05, P<.5. These arbitrary threshold cut-off points for determining risk followed thresholds used in previous polygenic analysis (Purcell et al., 2009). Thirdly, to account for difference between variants in the size of the association with illness, each risk allele was weighted as the log10 of the effect size described in the PGC dataset (W_{SNP}= log10 (OR_{PGC})). Finally, a risk-score for each individual was calculated based on the number of weighted risk alleles they carried at each of the three p-value thresholds using the equation: Score(p<threshold) = $\Sigma j(S_{SNP})/(j-m)$, where j = number of SNPs at P<threshold and m= number of SNPs with missing genotypes. A risk score for each of the CAM SNPs was calculated as (S_{SNP} = W_{SNP} * Risk Allele Count).

6.2.5 Statistical analysis: Associations between *CAM* pathway risk allele score and the phenotypes of IQ, episodic memory and attention were tested in a series multiple regression analyses implemented in SPSS 17 (SPSS, 2008). In each case, scores for each neuropsychological subtest were entered as dependent variables, where appropriate age and gender were entered on the first step of the analysis as effects of no interest, followed by *CAM* pathway risk allele load on the second step. Exactly the same approach was taken in the analysis of the single variants, with the risk genotype score (0, 1, or 2 alleles) in each case replacing the polygenic score as the independent variable. Finally, effects sizes for all significant effects were calculated using Cohen's d in (ClinTools, 2005) to enable comparison to individual SZ risk SNPs already reported (Rose & Donohoe, 2012).

As we hypothesized that polygene risk scores would better explain variance in neuropsychological function than would be explained by single SNP analyses, we planned to follow up any significant neurocognitive findings from the *CAM* polygene analysis by characterizing the effects of single SNPs within *CAM* on neurocognition. To do this, we selected the most strongly associated single SNPs within each of the four genes most strongly associated with SZ risk in the PGC analysis (outside of variants within the *MHC* region, given the issues of high LD between these variables). These were: *HLADQA1* (rs9272105), *CDH4* (rs2427104), *NRXN1* (rs1919972) and *CNTNAP2* (rs1548743).

In order to test our second hypothesis that any association between *CAM* polygenic risk and cognition would be led by combined polygenicity and not individual *CAM* SNPs (regardless of strength of association with psychosis), we deleted from the *CAM* pathway any gene whose SNPs were associated with the same cognitive variables as *CAM* pathway polygenic risk. *CAM* risk allele load scores were then recalculated and retested for association with neuropsychological function in the absence of these genes. From this second analysis we hoped to determine whether the impact of the *CAM* pathway on cognition was predominantly led by either polygenic risk, or individual genes.

6.3 Results

6.3.1 The effects of CAM pathway risk allele load on demographic and clinical measures

Demographic and clinical characteristics for all patients appear in **table 6.1**. For all demographic variables, means and standard deviations were computed using SPSS (2008). The mean, number of observations and the standard deviation of the two psychosis groups (broad and narrow) were then compared using an online t-test calculator: http://www.quantitativeskills.com/sisa/statistics/t-test.htm. In summary, no differences were observed between the narrow psychosis group (SZ and SZA) and broad psychosis group (all patients with psychosis) in terms of age, gender, age at onset, or general cognitive ability as indexed by full scale IQ. On clinical measures of positive and negative symptoms (SAPS/SANS), no differences were detectable between the two groups for the factors of depression, positive symptoms, negative symptoms or disorganisation. Differences were however observed for on the 'mania' factor, with the broad psychosis group scoring significantly higher on the manic scale than the narrow psychosis group. No differences were observed in medication dosage as measured by chlorpromazine equivalents.

Table 6.1: Patient demographics

	Psychosis narrow N=340	Psychosis broad N=424
Psychosis subtype		
SZ	N= 282	N= 282
SZA	N= 58	N= 58
BP		N= 61
MDD		N= 11
PNOS		N= 12
Gender (ratio; M:F)	2.6:1	2.2:1
Age (years; mean (SD))	41.3(12.2)	41.3(12.4)
Age at onset (years; mean(SD))	22.8(7.2)	23.2(7.5)
Chlorpromazine equivalent (mg/day; mean(SD))	589.8(562.4)	555.5(540.7)
SAPS/SANS:		
Manic (mean (SD))	-0.18(0.95)	0.04(1.09)
Depression (mean (SD))	0.16(1.07)	0.23(1.06)
Positive (mean (SD))	-0.02(0.99)	-0.12(0.95)
Disorganised (mean (SD))	-0.22(0.76)	-0.31(0.78)
Negative (mean (SD))	0.39(0.90)	0.32(0.87)
Cognition: full scale IQ (mean (SD))	89.6(17.8)	90.3(18.3)

6.3.2 The effects of CAM pathway risk allele load on cognition.

The R^2 and *F*-test p-values from the regression analyses of each of the 3 cognitive domains of IQ, memory and attention by *CAM* pathway risk allele load are presented in **table 6.2**. Across both narrow sense and broad sense psychosis groups, higher polygenic risk scores within the *CAM* pathway were statistically significantly correlated with memory function (as measured by the CANTAB paired associate learning test) and sustained attention (as measured by the SART). For the broad diagnoses groups, *CAM* pathway risk allele load was also correlated with poorer verbal episodic memory function as measured by WMS-III logical memory task. In the narrow psychosis group, this effect was observed at trend level only, possibly because of the smaller sample included. By comparison, the narrow psychosis group showed a nominal association between higher *CAM* pathway risk polygene scores and preserved premorbid IQ, whereas this was only observed at trend level in the broad psychosis group. Furthermore this trend level association was only observed for SNPs associated with illness risk below p x10-5, whereas the effects on memory and attention were observed for SNP

thresholded at p x10-5, p=.05 and p=.5. Finally, the amount of variance in cognitive performance explained by *CAM* pathway polygenic risk scores ranged between 1-3%, with the highest percentage of variance explained for the SART attentional control task. Effect sizes for these significant findings ranged from 0.23 to 0.37 with a mean effect size of 0.29 (see **table 6.3**).

Table 6.2: *CAM* pathway polygenic score regression analysis for each neuropsychological variable. *CAM* risk alleles included were thresholded at (a) p=$10x^{-5}$ (b) p=.05 (c) p=.5

Neuropsych variable	All patients with psychosis			Patients with SZ or SZA		
	$10x^{-5}$ r2 (p)	.05 r2 (p)	.5 r2 (p)	$10x^{-5}$ r2 (p)	.05 r2 (p)	.5 r2 (p)
Premorbid IQ	.008(.07)	.005(.17)	.005(.168)	**.013(.045)**	.006(.178)	.004(.239)
Verbal IQ	.000(.854)	.000(.675)	.001(.63)	.000(.944)	.001(.633)	.000(.693)
Performance IQ	.000(.954)	.000(.807)	.003(.309)	.000(.776)	.000(.777)	.005(.201)
Full scale IQ	.000(.985)	.000(.713)	.002(.419)	.000(.791)	.000(.715)	.002(.403)
Logical memory 1	.002(.407)	.004(.206)	.005(.176)	.000(.768)	.005(.24)	.006(.181)
Logical memory 2	.008(.079)	**.013(.023)**	**.014(.021)**	.003(.36)	.011(.063)	.013(.051)
PAL total errors	**.011(.044)**	**.021(.005)**	**.032(.001)**	.004(.27)	.01(.083)	**.021(.013)**
SART reaction time	.006(.201)	**.026(.005)**	**.024(.008)**	.009(.141)	**.029(.009)**	**.027(.011)**
CPT d'Prime 2 digit	.004(.304)	.000(.957)	.000(.94)	.012(.103)	.002(.549)	.001(.57)
CPT d'Prime 3 digit	.000(.798)	.007(.16)	.004(.296)	.004(.346)	.001(.69)	.000(.76)

Table 6.3: Effect sizes for each significant association between CAM pathway risk allele load and cognition.

Threshold for risk allele inclusion	Neuropsych variable	Adjusted r square	R square change	Cohen's d
All Patients with Psychosis				
$10x^{-5}$	PAL total errors	.008	.011	.225
.05	PAL total errors	.018	.021	.297
	SART overall reaction time	.023	.026	.32
	Logical memory 2	.011	.013	.233
.5	PAL total errors	.03	.032	.365
	SART overall reaction time	.021	.024	.315
	Logical memory 2	.011	.014	.235
Patients with SZ + SZA only				
$10x^{-5}$	WTAR	.009	.013	.225
.05	SART overall reaction time	.025	.029	.347
.5	SART overall reaction time	.023	.027	.336
	PAL total errors	.018	.021	.297

6.3.3 Cognitive analysis of SZ associated individual SNPs within the CAM pathway genes, HLADQA1, CDH4, NRXN1 and CNTNAP2

We next tested whether variance in neurocognitive function explained by *CAM* pathway scores could be explained on the basis of any individual SNPs within the *CAM* pathway. To do this, we investigated whether variation in performance on cognitive measures implicated in the pathway analysis was also associated with single SNPs within each of the four genes most strongly associated with SZ risk in the PGC analysis – rs9272105 within *HLADQA1*, rs2427104 within *CDH4*, rs1819972 within *NRXN1* and rs1548743 within *CNTNAP2* (see **table 6.4**). Based on these regression analyses, only the *HLADQA1* SNP was associated with variation in neurocognitive performance; on pre-morbid IQ in the narrow diagnosis group (WTAR: r^2=0.021; f=6.25; p=0.013), and attentional control in both the narrow and broad diagnosis groups (SART reaction time: SZ/SZA group: r^2=0.36; F=6.25; p=0.01; broad diagnosis group: r^2=0.28; f=4.0; p=0.019). No association was observed with memory function. None of the other variants within *CDH4, NRXN1,* or *CNTNAP2* were associated with any of these three neurocognitive measures. Similarly, a combined regression analysis including all four SNPs failed to explain a significant amount of variation on any of the three target neurocognitive variables.

Table 6.4: Regression analysis for SNPs within *HLADQA1*, *NRXN1*, *CNTNAP2* and *CDH4*

	Neuropsych variable	r square	Adjusted r square	F	p
HLA-DQA1 **rs9272105**	**All patients with psychosis**				
	Premorbid IQ	0.01	0.007	3.7	0.055
	Logical memory 2	0.035	-0.002	0.44	0.508
	SART Reaction time	0.028	0.021	4.0	0.019
	PAL Total errors	0.003	0.001	0.898	0.344
	Patients with SZ + SZA only				
	Premorbid IQ	0.021	0.017	6.25	0.013
	Logical memory 2	.009	.005	2.38	0.123
	SART Reaction time	0.036	0.027	3.98	0.02
	PAL Total errors	0	-0.003	0.083	0.773
NRXN1 **rs1819972**	**All patients with psychosis**				
	Premorbid IQ	0	-0.002	0	0.996
	Logical memory 2	0.032	-0.002	0.386	0.535
	SART Reaction time	0.012	0.008	3.538	0.061
	PAL Total	0.008	0.005	2.819	0.094
	Patients with SZ + SZA only				
	Premorbid IQ	0	-0.003	0	0.983
	Logical memory 2	.003	-0.001	.831	0.363
	SART RT	0.012	0.008	2.81	0.095
	PAL Total errors	0.011	0.007	3.1	0.079
CNTNAP2 **rs1548743**	**All patients with psychosis**				
	Premorbid IQ	0	-0.002	0.188	0.664
	Logical memory 2	0.01	-0.003	0.038	0.845
	SART Reaction time	0	-0.003	0.066	0.797
	PAL Total errors	0.006	0.004	2.43	0.119
	Patients with SZ + SZA only				
	Premorbid IQ	0.001	-0.002	0.271	0.603
	Logical memory 2	0.03	0.001	0.324	0.57
	SART Reaction time	0	-0.004	0.005	0.946
	PAL Total	0.008	0.005	2.4	0.122
CDH4 **rs2427104**	**All patients with psychosis**				
	Premorbid IQ	0	-0.002	0.154	0.695
	Logical memory 2	0.042	0.002	0.656	0.419
	SART Reaction time	0.001	-0.002	0.311	0.577
	PAL Total errors	0	-0.002	0.113	0.737
	Patients with SZ + SZA only				
	Premorbid IQ	0.001	-0.002	0.411	0.522
	Logical memory 2	0.085	0.007	2.19	0.14
	SART Reaction time	0.003	-0.001	0.785	0.377
	PAL Total errors	0	-0.003	0.055	0.815

6.3.4 The impact of the HLADQA1 gene on the association between neurocognitive variables and CAM risk allele load.

Due to the significant contribution of the *HLADQA1* SNP in explaining variation in neuropsychological functioning, we re-ran our *CAM* pathway polygenic regression analysis to exclude all variants at this gene locus (this excluded 3 SNPS). Specifically we wanted to determine whether the polygenic impact of *CAM* on cognition was led by (1) the *HLADQA1* gene, which was reported as being strongly associated with SZ risk in the PGC analysis (Ripke et al., 2011), or (2) the combined effect of multiple *CAM* genes.

Using the re-calculated *CAM* pathway polygenic risk allele load scores we reran the initial regression analysis. Results of these regression analyses (presented in **table 6.5**) indicate 1) that the *HLADQA1* gene may impact SZ to a greater extent than it does a more broad psychosis phenotype and 2) that *HLADQA1* is associated with deficits in attention, rather than memory.

Table 6.5: Recomputation of the regression analyses for the CAM pathway polygeneic risk scores without 3 SNPs mapped to the *HLADQA1* gene.

	Neuropsych variable	All patients with psychosis			Patients with SZ or SZA		
		$10x^{-5}$	0.05	0.5	$10x^{-5}$	0.05	0.5
		r2(p)	r2(p)	r2(p)	r2(p)	r2(p)	r2(p)
IQ	Premorbid IQ	**.013(.038)**	.008(.107)	.007(.129)	.014(.066)	.008(.15)	.005(.274)
Memory	Logical memory 2	.005(.23)	.007(.129)	.004(.244)	.001(.649)	.005(.284)	.002(.479)
	PAL total errors	.011(.062)	**.017(.019)**	**.021(.01)**	.004(.306)	.01(.112)	.014(.065)
Attention	SART reaction time	.003(.424)	.004(.32)	.005(.28)	.001(.743)	.002(.593)	.002(.568)

6.4 Discussion

In this study we used *CAM* pathway risk allele load derived from the PGC schizophrenia GWAS dataset to investigate whether increased number of *CAM* pathway risk alleles correlated with poorer neuropsychological function amongst 424 patients with either narrow sense or broad sense psychosis. We further investigated whether variation in neuropsychological performance explained by *CAM* pathway polygenic risk was also evident when analysis was restricted to highly-associated individual risk SNPs from the *CAM* pathway (*HLADQA1, CDH4, NRXN1* and *CNTNAP2*). Finally we deleted from the *CAM* pathway the one individual risk gene – *HLADQA1* - which was significantly associated with the same cognitive variables explained by *CAM* polygenic risk, and retested the pathway for association with neuropsychological function in the absence of this gene. Based on these analyses we found that (1) polygenic scores for the *CAM* pathway explained a significant proportion of variance in neuropsychological function, irrespective of the risk threshold used; (2) only one risk SNP – *HLADQA1* – was individually associated with variation in neuropsychological function; (3) the proportion of variance explained by the polygenic analysis versus the most strongly associated individual SNP analysis was comparable (~3%); and (4) removal of this gene from the polygene analysis also removed the association between *CAM* pathway risk allele load and attentional control across diagnostic categories, although the association with memory and premorbid IQ in the broad psychosis group was maintained.

Cognitively, higher polygenic risk scores within the *CAM* pathway correlated with more preserved premorbid IQ, poorer memory function, and poorer attentional control. When results for the broad psychosis group and the narrow psychosis group are compared, results were generally analogous across samples, with the somewhat greater significance in the broad psychosis group most likely due to the larger sample size available for this group (n=424 versus n=342). Furthermore, the variance in neuropsychological performance explained was not strongly influenced by the polygenic risk threshold set ($p=10x^{-5}$ versus p=.05 versus p=.5). This is interesting as it is contrary to the finding by Ripke and colleagues (2012) in which for the overall SZ phenotype, variance explained does increase as p value threshold increases, indicating that "less-associated" SNPs may be contributing to risk. As this is not what we found in the current study it may suggest that cognition is not under

polygenic control. Yet the data collectively suggests that across psychoses diagnoses, carriers of higher numbers of *CAM* pathway risk alleles are more likely to show intact pre-morbid IQ but greater (current) deficits in memory and attention.

An important rationale for using a pathway led approach over an individual SNP approach when studying both illness phenotypes and intermediate phenotypes (including cognition), is that the function of single variants are less likely to be understood in isolation from their biological pathway and individually are unlikely to contribute significantly to our understanding of pathology. This view is supported by Purcell and colleagues (2009), who estimated the explanatory power of polygenic analysis for explaining risk for schizophrenia to be at least 30%. As such, a core hypothesis in our analysis was that variance in neuropsychological deficits would be better modeled and explained by a polygenic risk score than by individual SNP genotypes. When single SNPs within each of four of the top most strongly associated *CAM* pathway genes were considered, only one of these – *HLADQA1* - was significantly associated with neurocognitive performance. For this SNP, however, the variance explained by the *CAM* pathway polygenic risk analysis was actually comparable to that explained by *HLADQ1A* (both ~2-3% of variation in neurocognitive performance).

One possible explanation for the finding that *CAM* pathway risk allele load is not more strongly predictive of neurocognitive performance is that the *CAM* pathway may be less significantly associated with deficits in neuropsychological function than other gene pathways. For example, the pathway of genes regulated by the *MIR-137* genome-wide variant may have a larger impact on cognition, especially as the *MIR-137* variant is associated with poorer cognitive performance (Green et al., 2012). Yet, the *CAM* pathway has previously been implicated in a variety of neurocognitive processes, including memory, and as such remains a likely candidate for association with cognition amongst patients with psychosis. Consistent with this, an association with variation on neurocognitively-associated phenotypes has been reported for three of the four genes selected: *CDH4* has been associated with total brain volume (Seshadri et al., 2007), *NRXN1* with white matter volume (Voineskos et al., 2011), and *CNTNAP2* with language processing (Kos et al., 2012). Finally, effects sizes for the neurocognitive effects of *HLADQA1*, which fell in the small to medium size category [0.27 to 0.33] according to Cohen's criteria were broadly similar to those previously calculated for significant gene-cognition associations in schizophrenia to date (the average effect size reported across studies was 0.31 in the meta-analysis by (Rose & Donohoe, 2012)).

In speculating about why a polygenic approach does not necessarily represent a major advance over the study of individual SNPs in modeling cognitive deficits in psychosis, the degree of genetic overlap between cognitive performance and both schizophrenia in particular and psychosis in general may be important. Purcell and colleageus (2012) suggested that ~30-40% of genetic risk was attributable to common variant polygenicity and that the amount of variation in risk explained by independent samples was much lower. The question is whether one would expect SZ risk genes to influence psychotic traits such as impaired cognition, or whether cognition is influenced by a separate group of genes. Toulopoulou and colleagues (2007) infers a high degree of overlap between cognitive deficits and SZ in his twin genetic modeling study. However, recent genetic epidemiological evidence by Fowler and colleagues (2012) has suggested that this may not be quite as high when based on non-biased samples. In the context of these findings, when modeling cognitive performance in psychosis, polygenic risk scores may therefore introduce as much noise as signal into the analysis (i.e. variation neither specific to cognition, nor to the target patient population). It is also worth noting that not 100% of the pathway might be informative and that annotation error could lead to the inclusion of genes that detract from the signal. On account of this, the variation in neurocognition explained by the individual SNP, although failing to capture much of the genetic variation associated with cognition, may not carry the same burden of non-relevant genetic information.

A further question we had in relation to the study findings was whether the significant associations between *CAM* risk allele load and the cognitive variables of memory and attention were specific to psychosis or whether they could be replicated in healthy participants. Unfortunately we did not have an adequately sized comparison control sample to test this. However, a post hoc analysis looking at the impact of *CAM* risk allele load on cognition in 88 healthy individuals was carried out, using identical methodology to that used in the patient group, which allowed conduction of a preliminary investigation to this effect (see Supplementals for information on the healthy participant group including demographics and results). The *CAM* pathway was shown to impact the healthy volunteers on one of the two cognitive variables associated with patients, namely attention. Furthermore, in a combined analysis of healthy participants and patients, both the associated cognitive variables of memory and attention remained significant. Only the association with premorbid IQ became non-significant (see **table S6.2**, supplementals). These data suggest that the *CAM*

pathway polygene risk score affects neuropsychological function in healthy participants in a manner similar to patients. This similarity changes however when we investigate the association between cognition and the four individual SNPs (*HLADQA1, CDH4, NRXN1* and *CNTNAP2*) in a combined population of patients plus healthy volunteers. Here we found that all four SNPs were significantly associated with episodic memory and attentional control where only one of the SNPs had been associated with cognition in the patient only group – *HLADQA1* (**table S6.3**). Removing *HLADQA1* from the *CAM* pathway yielded the same outcome as for the patient only group, namely we found no change in association between *CAM* pathway risk allele load and cognition (see **table S6.4**). Confirmation of the true impact of *CAM* risk allele load on healthy participants will require analysis with an adequately powered healthy volunteer sample.

Because the use of polygene risk scores – either as total scores or as scores for individual pathways – is relatively new in psychiatric genetics, a number of methodological issues require highlighting. Firstly, we curated our pathway from 32 SNPs which were located within 20Kb of genes implicated in the *CAM* pathway. This is four times the gene boundary limits of those used in O' Dushlaine's (2011) paper, and thus runs the risk of including SNPs which were not specifically *CAM* related (Jia et al., 2011). It might prove more useful to narrow the gene boundary limit to as little as the 5Kb used by O'Dushlaine and colleagues, despite the smaller number of pathway SNPs this would thus involve. Secondly, following the approach suggested in previous studies (Purcell et al., 2009) we chose to look at three polygenic risk cut-offs whilst conducting analysis: those whose scores derived from all SNPs with a p-value of less than 10^{-5}, .05 and .5 in the PGC GWAS. Other cut-off threshold might have been considered, and the appropriateness of one or other of these has not been well characterized. In Fact, a cut-off-based approach treats association signals qualitatively rather than quantitatively; whether such dichotomization of markers as significant or non-significant either reduces signal or introduces bias is unclear (Jia et al., 2011).

6.5 Conclusion

In conclusion, this study is the first to our knowledge to investigate the role of the *CAM* pathway in the cognitive decline commonly evident amongst psychotic patients. The data supports a role for the *CAM* pathway in memory formation and attention related reaction time. It also shows that one of the four psychosis associated *CAM* genes – *HLADQA1*– is associated with variation in neurocognitive performance in psychotic patients. Finally, while demonstrating the feasibility of pathway specific polygenic risk analysis to studying an intermediate phenotype for psychosis, this study suggests that single gene analysis may not be as poor at capturing the effects of genetics risk for schizophrenia as previously hypothesised.

Supplementary material – Healthy Participants

Healthy participants

Data on 88 healthy subjects was investigated in a preliminary analysis to test whether their inclusion altered patient findings and whether patient findings were likely to be replicated in a healthy population. Healthy participants, who were recruited on the basis of responses to local media advertisements, were only included if they were aged between 18 and 65 years and satisfied, based on clinical interview, the criteria of having no history of major mental health problems, intellectual disability or acquired brain injury, and no history of substance misuse in the preceding six months based on self-report. Healthy participants were also excluded from the study if they reported having a 1st degree relative with a history of psychosis or were not of Irish ancestry (i.e. four grandparents born in Ireland). All provided written informed consent and all assessments were conducted in accordance with the relevant ethics committees' approval.

Methodology

Genetic analysis for healthy subjects was conducted on DNA extracted from saliva, obtained using Oragene DNA self-collection kits (DNA Genotek; Ontario, Canada). All other methodology is identical to that used for the patient group.

Results

Differences were observed between patients and healthy volunteers in terms of age and gender, with healthy volunteers being younger and more predominantly masculine (see **table S6.1**).

Table S6.1: Participant Demographics

	Healthy participants (HP) N =88	Patients		Comparison between HP and psychosis broad
		Psychosis narrow N=340	Psychosis broad N=424	
Gender (ratio; M:F)	1.4:1	2.6:1	2.2:1	Males: HP < Patients F=4.07; p=.044
Age (years; mean (SD))	36.27(12.76)	41.3(12.2)	41.3(12.4)	HP < Patients F=18.38; p=.000

The effects of CAM pathway risk allele load on cognition.

F, r^2, and p-values from the regression analyses of each of the 3 cognitive domains of IQ, memory and attention by *CAM* pathway risk allele load are given in **Table S6.2**. When healthy participants were considered alone, higher polygenic risk scores within the *CAM* pathway were significantly associated with increased errors of omission in the attentional task, the SART. When healthy participants were examined in conjunction with patients with SZ, SART remained impacted by *CAM* risk allele load, although the SART variable switched to that of reaction time. When healthy participants were analysed alongside all patients with psychosis, both SART reaction time and episodic memory were significantly associated with *CAM* polygenic risk. The amount of variance in cognitive performance explained by *CAM* pathway polygenic risk scores ranged between 1-4%, with the highest percentage of variance explained for attentional reaction time as measured by the SART.

Cognitive analysis of SZ associated individual SNPs within the CAM pathway genes CDH4, HLADQA1, NRXN1 and CNTNAP2

We were interested in seeing whether combining healthy participant data with patient data would influence the impact of the four SZ risk SNPs on cognition – rs2427104 within *CDH4*, rs9272105 within *HLADQA1*, rs1819972 within *NRXN1* and rs1548743 within *CNTNAP2*. We discovered that when the healthy population was included in the analysis, all four SNPs

were found to be significantly associated with both memory (logical memory, delayed recall) and attention (SART reaction time). Effect sizes for these significant findings ranged from .25 to .38 (see **table S6.3**).

The impact of the HLADQA1 gene on the association between neurocognitive variables and CAM risk allele load.

In the patient only individual SNP analysis only *HLADQA1* was associated with variation in neuropsychological function. As such its importance to *CAM* pathway functionality was further investigated by removing it from the pathway and retesting for association between *CAM* risk allele load and cognition. As these findings were rather surprising (with only the cognitive variable of memory maintaining association with *CAM* polygenic risk), we wanted to see whether *HLADQA1* had the same impact on the *CAM* pathway when healthy participants were added to the equation. The removal of *HLADQA1* did not affect the pathway's impact on cognition for the healthy participant group, with the attentional variable of the SART remaining associated. When the healthy and patient groups are combined, the absence of the *HLADQA1* gene from the *CAM* pathway has no impact on the association between *CAM* polygenic risk and memory, but does get rid of the association with attentional control (see **table S6.3**).

TABLE S6.2: p values for regression analysis of each neuropsychological variable considered as explained by *CAM* pathway risk alleles. *CAM* risk alleles included are thresholded at (1) p=10x-5 (2) p=.05 (3) p=.5

	Neuropsych variable	Healthy Participants (HP) Only			HP &All Patients with psychosis			HP & SZ & SZA		
		$10x^{-5}$ r2 (p)	.05 r2 (p)	.5 r2 (p)	$10x^{-5}$ r2 (p)	.05 r2 (p)	.5 r2 (p)	$10x^{-5}$ r2 (p)	.05 r2 (p)	.5 r2 (p)
IQ	WTAR	.087(.218)	.074(.574)	.076(.459)	.002(.373)	.003(.202)	.001(.409)	.002(.353)	.005(.152)	.001(.539)
	Verbal IQ	.079(.942)	.079(.896)	.079(.936)	.001(.462)	0(.867)	0(.769)	.001(.57)	0(.957)	0(.993)
	Performance IQ	.046(.895)	.046(.774)	.047(.712)	.001(.486)	.001(.49)	.001(.536)	.001(.631)	0(.925)	0(.744)
	Full scale IQ	.088(.685)	.068(.592)	.065(.956)	.001(.473)	0(.764)	0(.644)	.001(.651)	0(.899)	0(.903)
MEMORY	Logical memory1	.002(.688)	.01(.349)	.006(.467)	.014(.135)	.012(.265)	.009(.18)	.014(.314)	.013(.496)	.013(.403)
	Logical memory2	0(.956)	.026(.137)	.007(.455)	.008(.056)	.006(.097)	.007(.077)	.003(.242)	.005(.167)	.005(.144)
	PAL standard score	0(.956)	.002(.71)	.001(.817)	**.007(.084)**	**.015(.011)**	**.02(.003)**	.005(.163)	.008(.089)	**.012(.036)**
ATTENTION	SART Reaction time	.157(.232)	.14(.165)	.14(.603)	.008(.111)	**.024(.005)**	.01(.067)	.012(.072)	**.028(.006)**	.011(.088)
	SART total comission error	.01(.37)	1.3(.017).253	.76(.01).386	.31(.001).581	.07(0).998	.03(0).87	.068(.0).794	.06(.0).805	.001(.61)
	SART total omission errors	.024(.167)	**.061(.027)**	.014(.289)	.002(.387)	.002(.451)	.007(.113)	.0(.744)	.007(.153)	.003(.336)
	CPT d'Prime 2 digit	n/a	n/a	n/a	.003(.324)	0(.328)	.001(.698)	.012(.103)	.005(.296)	.002(.519)
	CPT d'Prime 3 digit	n/a	n/a	n/a	.008(.758)	.016(.674)	.016(.735)	.025(.352)	.023(.508)	.022(.882)

Table S6.3: Summary of regression analysis, including all patients plus healthy participants, showing significant and trend-like findings alongside effect size for each neuropsychological variable that showed a significant association with the *CAM* pathway.

Neuropsych variable	r	r square	Adjusted r square	F	p	Coehen's d
rs9272105 (HLADQA1)						
Logical memory 2	.145	.021	.017	4.69	**.01**	.293
PAL total errors	.053	.003	.000	1.165	.281	.106
SART reaction time	.185	.034	.029	6.14	**.002**	.377
rs1819972 (NRXN1)						
Logical memory 2	.142	.02	.016	4.8	**.009**	.287
PAL total errors	.072	.005	.003	2.35	.126	.144
SART reaction time	.149	.022	.017	4.24	**.015**	.301
rs1548743 (CNTNAP2)						
Logical memory 2	.137	.019	.015	4.59	**.011**	.277
PAL total errors	.056	.003	.001	1,41	.235	.112
SART reaction time	.122	.015	.01	2.89	.057	.246
rs2427104 (CDH4)						
Logical memory 2	.141	.02	.016	4.81	**.009**	.285
PAL total errors	.024	.001	-.002	.266	.606	.048
SART reaction time	.13	.017	.012	3.25	**.04**	.262

Table S6.4: Recomputation of the regression analyses for the *CAM* pathway polygeneic risk scores without the *HLADQA1*.

Neuropsych variable	Healthy Participants (HP) Only			HP & All Patients with psychosis			HP & SZ & SZA		
	$10x^{-5}$ r2 (p) Cohen's d	.05 r2 (p) Cohen's d	.5 r2 (p) Cohen's d	$10x^{-5}$ r2 (p) Cohen's d	.05 r2 (p) Cohen's d	.5 r2 (p) Cohen's d	$10x^{-5}$ r2 (p) Cohen's d	.05 r2 (p) Cohen's d	.5 r2 (p) Cohen's d
MEMORY:									
Logical memory 2	.016(.25) .251	.01(.368) .196	.011(.329) .213	**.016(.007) .251**	**.016(.006) .248**	**.012(.019) .23**	**.01(.045) .213**	**.013(.025) .23**	**.009(.058) .18**
PAL total errors	.001(.818) .05	.001(.82) .05	.000(.95) .014	**.011(.044) .213**	**.016(.008) .248**	**.018(.005) .268**	.006(.142) .149	**.011(.048) .213**	**.014(.033) .235**
ATTENTION:									
SART Reaction time	.005(.28) .144	.8(63) 0	0(.887) 0	0(.994) 0	0(.836) 0	.001(.674) .245	0(.735) 0	.000(.857) 0	0(.999) 0
SART total omission errors	.013(.311) .23	.025(.16) .32	.023(.18) .31	.002(.447) .078	.003(.304) .11	.004(.251) .12	.002(.462) .084	.003(.308) .12	.005(.202) .144

Table S6.5: Genes which make up the *CAM* pathway used in this study.

ALCAM	CNTN1	SELL	HLA-DRA
CD6	NRCAM	MADCAM1	HLA-DRB1
SPN	NLGN1	VCAM1	HLA-DRB3
SIGLEC1	NLGN3	ITGB7	HLA-DRB4
PTPRC	NLGN2	ITGA9	HLA-DRB5
CD22	NRXN3	ITGA4	CD86
CD8A	NRXN1	CD99	CD80
CD8B	NRXN2	PECAM1	CD28
ITGB1	ITGB8	CD58	HLA-A
ITGA8	ITGAV	CD2	HLA-B
ITGB2	SDC1	ICAM3	HLA-C
ITGAL	SDC2	ICAM1	HLA-E
CD40	SDC4	ICAM2	HLA-F
NEO1	SDC3	JAM2	HLA-G
CDH15	PTPRF	JAM3	CLDN16
CDH4	NEGR1	ITGAM	CLDN4
CDH3	CADM3	F11R	CLDN3
ITGA6	CADM1	CD40LG	CLDN7
PVRL2	CDH2	CDH5	CLDN23
CDH1	NCAM1	ESAM	CLDN19
CLDN11	NCAM2	PDCD1LG2	CLDN14
VCAN	L1CAM	CD274	CLDN15
MAG	PVRL1	PDCD1	CLDN17
MPZ	PVR	HLA-DMA	CLDN20
MPZL1	CD226	HLA-DMB	CLDN18
PVRL3	SELE	HLA-DOA	CLDN22
CNTNAP2	GLG1	HLA-DOB	CLDN5
CNTNAP1	SELP	HLA-DPA1	CLDN10
PTPRM	SELPLG	HLA-DPB1	CLDN8
CNTN2	CD34	HLA-DQA1	CLDN6
ICOSLG	ICOS	HLA-DQA2	CLDN2
CTLA4	CD276	HLA-DQB1	CLDN1
OCLN	CD4	HLA-DQB2	CLDN9

Chapter 7

Discussion

This thesis has sought to investigate how cognition is impacted by genetic risk variants identified by schizophrenia (SZ) genome wide association studies (GWAS). SZ is associated with deficits in cognition, operationalised in terms of both traditional (memory, IQ, attention) and social (emotion recognition, attributional style, theory of mind) constructs, that interfere significantly with daily functioning and quality of life, contributing to chronic disability, unemployment and difficulties in interpersonal relationships (Bilder et al., 2011). Both SZ and cognition are highly heritable (Saab et al., 2008), and overlap both genetically and phenotypically suggesting that at least some of the genes that are associated with SZ risk are also associated with cognition. This thesis aimed to characterise this relationship, and explore in particular whether this effect was similar or different using traditional neurocognitive constructs, or social cognition constructs or both.

The research literature on the genetics of cognition in SZ and psychosis has a long history (Goldberg et al., 1990/1995; Cannon et al., 2000; Kremen et al., 2006; Toulopoulou et al., 2007/2010). This dissertation contributes to this literature by 1) investigating novel SZ genetic variants for association with cognition, 2) investigating SZ genetic variants, both novel and established, for association with social cognition and 3) determining whether substantially more of the variation in patients' neuropsychological performance would be explained by common variants acting in a polygenic manner within a pathway than by individual risk variants acting in isolation.

The overarching research question that guided the individual studies that make up this dissertation has been: "Do SZ GWAS-identified genes impact neuropsychology and/or social psychology, and if so in what manner do they exert their influence?"

In order to investigate this question, cognitive endophenotypes that are well validated in the field of neuropsychology for the assessment of memory, attention, IQ and social cognition were used. The 'endophenotype' concept in psychiatry (Gottesman & Gould, 2003) relates to the identification of heritable quantifiable characteristics, which may be useful targets for genetic studies as they represent some intermediate stage between genotype and clinical disorder. As a point of departure from the main question, I probed further into one of these endophenotypes in Chapter 2. One of the primary social cognitive tasks used throughout this thesis is a mental state decoding task called Reading the mind in the eyes (Eyes) (Baron-

Cohen et al., 2001), which utilises static images of faces depicting various emotions/ thoughts. The literature suggests that the use of static faces may not be ecologically valid and that dynamic images of varying intensities would better represent real life social encounters (Sato et al., 2007; Trautmann et al., 2009). This thesis sought to explore this question in greater detail, looking at the two aspects of dynamics and intensity, and how they impact emotion recognition in patients with SZ. ERT accuracy was also analysed for correlation with neurocognition.

The remaining chapters assess how SZ GWAS single nucleotide polymorphisms (SNPs) impact cognition. Chapters 3 and 4 investigate this in relation to individual SZ GWAS SNPs, whilst Chapters 5 and 6 look at two pathways of SNPs and whether SNPs acting together have a greater impact on cognition than SNPs acting in isolation. In Chapter 3, the seven loci identified in the most recent and largest SZ GWAS to date (Ripke et al., 2011) are analysed for association with neurocognition and social cognition. Chapter 4 focuses on the first genetic risk variant to achieve genome wide significance for psychosis – *ZNF804A* – and whether, considering evidence in the literature pointing to a cognitively spared *ZNF804A* phenotype (Donohoe et al., 2011), it might be exerting its influence via a role in social cognitive functioning. Chapter 5, in light of the interesting findings from Chapter 4, investigates *ZNF804A* in terms of pathway analysis; looking at whether *ZNF804A* pathway risk allele load scores correlate with poorer neuropsychological function and whether pathway level analysis would explain a greater percentage of the variance in neuropsychological performance than is explained by individual risk alleles. Chapter 6, following on from Chapter 5, repeats this analysis with another SZ associated pathway – the cell adhesion molecule (CAM) pathway – for the purpose of discovering whether the *ZNF804A* findings are unique or whether they might exist in other psychosis related genes.

A series of questions addressed by this thesis, alongside evidence drawn from various chapters in relation to each will first be discussed in detail, followed by some strengths, weaknesses and suggestions for future research. Finally, all conclusions are summarized by answering the overarching research question.

7.1 Assessing social cognition using static versus dynamic stimuli

The Primary findings of this study were that:

- Patients perform less well than healthy subjects in the correct identification of all six emotions analysed (happiness, sadness, anger, surprise, fear and disgust).
- In considering total correct response for all 6 emotions combined, healthy participants outperformed patients across all five levels of intensity.
- Across diagnostic categories, happiness was identified more accurately than all other emotions, whilst fear was identified least accurately.
- For the emotions of fear and sadness, patients performed similarly to healthy subjects at low levels of intensity, but were significantly poorer at emotion recognition at high intensity levels. In contrast, for the emotion of happiness and anger, patients performed most poorly compared to healthy participants at lower intensity levels.
- Performance on the ERT was positively correlated with neurocognitive performance.

Discussion:

In Chapter 1 (literature review), I considered the empirical evidence for whether social cognitive tasks (such as were employed in this thesis) can be considered true endophenotypes for social functioning. Three tests of social cognition are used throughout the thesis; The Hinting task, the Eyes task and the Internal, personal and situational attributions questionnaire (IPSAQ). All three tasks could be identified from the literature as meeting endophenotypic criteria, and as such were good candidates for use in the thesis. Also apparent from the literature however, in relation to the Eyes task, was that the static, single intensity emotional images used were considered by some not to be ecologically valid (Back et al., 2007). It was suggested that a dynamic, multi intensity model would better represent real life social encounters. To this effect a novel emotion recognition task (ERT) was designed by Montagne and colleagues in 2007. From my literature review however, this task satisfied only two endophenotypic criteria. It appeared that the primary reason for failing to satisfy all four criteria was paucity of research. As such I wished to investigate the ERT in more detail.

Interestingly, although the study supports a role for the use of dynamic stimuli in this line of research, results gleaned from dynamic expressions are similar to those previously reported for non-moving faces. As with dynamic stimuli, static emotion recognition literature has found that healthy participants outperform patients in correct identification of emotions (Kohler et al., 2010; Goghari & Sponheim, 2012; Comparelli et al., 2013), that happiness is universally the easiest emotion to detect (Kohler et al., 2004; Kirouac & Doré, 1983; Kirita & Endo, 1995; Matsumoto et al., 2000; Tracy & Robbins, 2008) and that fear is often one of the

most difficult (Kohler et al., 2003; Tracy & Robbins, 2008). Where the impact of static and dynamic emotion imagery differs is in accuracy of recognition. Compared to static displays, dynamic stimuli are associated with increased performance in emotion identification (Weyers et al., 2006), greater levels of arousal (Sato & Yoshikawa, 2007a) and increased spontaneous mimicry (Sato & Yoshikawa, 2007b). From this one can conclude that static images of emotion are more difficult to read than dynamic imagery. In light of the static emotion recognition task used throughout this thesis – The Reading the mind in the eyes task (Eyes; Baron-Cohen et al., 2001) – this would suggest that emotion recognition deficits recorded using this task may not be quite so severe in real life situations. In all other facets of emotion perception however, the Eyes task would be as useful and informative as a similar dynamic model.

Perhaps the most interesting outcome of the ERT study in this thesis is the impact of intensity on emotion recognition accuracy. Results show that while patients outperform healthy participants in accuracy of emotion identification at all five intensity levels employed, and while accuracy levels for all emotions increased across diagnosis as the intensity level rose, differences between the emotions were apparent with regards to at which intensity level (high or low) healthy subjects differed significantly from patients. Specifically, for the emotions of fear and sadness, patients perform similarly to healthy participants at low levels of intensity, but are significantly poorer at emotion recognition at high intensity levels. In contrast, for the emotions of happiness and anger, the greatest difference in performance between patients and healthy participants can be seen at low levels of intensity. It should be noted that the former two emotions are those which were the least accurately recognised overall, whilst the latter two were the most accurately recognised throughout the study, and as such a potential 'floor' effect cannot be ignored when assessing the results. However, until the study is replicated, the findings warn of a need for caution when assessing how well a patient cohort is performing in relation to their healthy counterparts. Most emotion recognition tasks, including the Eyes task, employ images taken at 100% intensity. According to the ERT findings, this would suggest that although patients will still be seen as performing more poorly than their healthy counterparts, the extent of the difference for the emotions of happiness and anger may not be fully actualised.

A final research finding from the ERT study shows that amongst patients, performance on the ERT correlated generally with cognition; in areas of IQ, memory, attention and social

cognition. This correlation existed with far fewer cognitive variables amongst the healthy group – predominantly with tasks that involve faces and emotion recognition. The patient finding corroborates previous research (e.g. Kohler et al., 2000; Sachs et al., 2004; Lee et al., 2009), which found that amongst patients with SZ better emotion recognition performance was correlated with better abstraction-flexibility, verbal and spatial memory and language processing; all of which relates to frontal and temporal functioning. These findings lend support to the notion that emotion recognition and neurocognition are related, but also raise the question of whether this changes how social cognition research should be conducted. Currently social cognition and neurocognition are investigated separately at a behavioural level, yet the ERT findings and emotion recognition research suggests that where the social cognitive construct of emotion perception is concerned, there may be a large degree of overlap between the two (Lee et al., 2009; Bozikas et al., 2004; Brune, 2005; Kohler et al., 2000). In terms of SZ research, patients perform at a similar level across measures of affect perception, face recognition and neurocognition (e.g. Kerr & Neale, 1993; Salem et al., 1996; Johnston et al., 2001; Penn et al., 2000; Lee et al., 2009; Bozikas et al., 2004; Brune, 2005; Kohler et al., 2000). It may thus be more appropriate to acknowledge a three tier system of cognition -social cognition, neurocognition and an intermediary level between the two.

7.2 Can Social cognition and neurocognition be considered independent constructs?

Primary findings:

- Performance on the ERT (a social cognition measurement) was positively correlated with neurocognitive performance
- *ZNF804A* and *CNNM2* impact social cognition but not neurocognition.
- *CSMD1* and *MIR-137* impact neurocognition but not social cognition.

Discussion:

On the back of the ERT findings that a correlation exists between emotion recognition and general neurocognitive functioning, one might question whether a generalized performance deficit across domains can account for findings of social cognitive deficits in SZ or whether there is a specific deficit in social cognition that is independent of neurocognition. As previously discussed, a great deal of the research in support of a generalized deficit is based on research into emotion perception, showing that individuals with schizophrenia perform at a similar level across measures of affect perception, face recognition and neurocognition (e.g. Kerr & Neale, 1993; Salem et al., 1996; Johnston et al., 2001; Penn et al., 2000; Lee et al.,

2009; Bozikas et al., 2004; Brune, 2005; Kohler et al., 2000). Indeed it is difficult to find research which shows that measures of social cognition and neurocognition are significantly associated with one another outside of emotion perception. Research does however indicate that neurocognition and social cognition correlate in the medium-range (Bryson et al., 1997; Penn et al., 1993), that social cognition appears to mediate the relationship between neurocognition and functioning (Addington et al., 2006; Bell et al., 2009; Schmidt et al., 2011; Sergi et al., 2006) and that neurocognition may be a necessary but not sufficient condition for social cognition (Ostrom, 1984; Penn et al., 1997; Fanning et al., 2012).

The findings in this thesis, which look at the impact of SZ associated genetics on neurocognition and social cognition, do not support this view of a generalized cognitive deficit in SZ however. In this thesis, study results consistently indicate that neurocognition and social cognition are impacted by separate genetic underpinnings; genes that are associated with neurocognition are not associated with social cognition and vice versa. This is quite a novel way of looking at the social cognition/neurocognition debate and one that deserves serious consideration. Is it possible that certain genetic variants impact *only* neurocognition, others impact *only* social cognition and perhaps still others impact both? To date the genetics of social cognition in SZ has focused predominantly on four genes – *oxytocin, vasopressin, dopamine* and *serotonin*. All four have been shown to be associated with social cognitive deficits (Rodrigues et al., 2009; Tost et al., 2010; Guastella et al., 2010; Rilling et al., 2011; Garcia-Garcia et al., 2010; Heinz et al., 2005; Skuse, 2006). In terms of neurocognition, while *serotonin* and *dopamine*, and to a lesser extent *vasopressin*, have been associated with cognitive variables such as memory and attention (Sumyoshi & Higuchi, 2013; Zilles et al., 2012; Jentsch et al., 2003), *oxytocin* has remained almost exclusively linked to social cognition. More recently the SZ associated gene *ZNF804A* has been associated with a cognitively preserved SZ phenotype (Walters et al., 2010; Becker et al., 2012), whilst being shown to impact social cognition (Esslinger et al., 2011; Walter et al, 2011). The research in Chapter 4 of this thesis corroborates these findings. As a preliminary investigation into *ZNF804A* we found that it is not associated with the cognitive variables of IQ, memory or attention (data not shown in the chapter). *ZNF804A* is however associated with social cognition in our study, via an impact on attributional bias and causality assignment. Attributional style was also impacted by a second gene under investigation in Chapter three – *CNNM2*. As with *ZNF804A* this was again in the absence of any association with neurocognitive deficits. On the flip side, the three genes we found to be associated with

neurocognition (*CSMD1, HLADQA1* and *MIR-137*) were not associated with social cognition. Taken together, these findings support the notion of a (at least partially) differentiated genetic architecture for social cognition and neurocognition.

This view is further supported by investigations into the neural substrates underlying neurocognition and social cognition, which suggests there are two brain systems involved in emotion processing: 1) a dorsal system (fusiform gyrus, superior temporal sulcus, amygdala, prefrontal cortices), which is involved in the allocation of planning, concentration and effortful control and 2) a ventral system (ventral anterior cingulate gyrus, ventral prefrontal cortex, amygdala), which is involved in emotion identification (King et al., 2006; Bozikas et al., 2004; Phillips et al., 2003). There is also evidence to support the presence of a separate "social cognitive neural circuit," incorporating the amygdala, fusiform gyrus, superior temporal sulcus and prefrontal cortices (Blakemore & Frith, 2004; Phillips et al., 2003; Adolphs, 2001; Pinkham et al., 2003; Lee et al., 2004). The amygdala, common to all three of these neural circuits, has been particularly associated with responses to emotional stimuli (Mukherjee et al., 2012; Adolphs & Tranel, 2003). As such, certain neural structures demonstrate increased activation during social cognitive tasks compared to neurocognitive processing, again supporting a distinction between the two constructs. It is plausible that separate genes might differentially impact the functioning of the three different neural circuits, or indeed of particular neural structures such as the amygdala, leading to some genes impacting neurocognition, some impacting social cognition and some impacting both.

Thinking in terms of neurocognition and social cognition each having their own biological/ genetic underpinnings has important implications for understanding illness risk and gene x environment interactions. Recently a group led by Andreas Meyer-Lindenberg published research looking at the impact of urban living and upbringing on participants' reaction to social stress using functional magnetic resonance imaging (fMRI) (Lederbogen et al., 2011). They discovered that current city living was associated with increased amygdala activity, whereas urban upbringing affected the perigenual anterior cingulate cortex, a key region for the regulation of amygdala activity, negative effect and stress. As such their results identify distinct neural mechanisms for an environmental risk factor, which is shown to impact social stress processing. Most likely the social stress encountered is a result of the environment (urban living) coupled with the impact of the genetics underpinning amygdala and perigenual

anterior cingulate cortex functioning. Understanding the genetics thus has important ramifications for understanding illness risk.

7.3 The impact of schizophrenia GWAS genes on neurocognition.

Primary findings:

- Out of twelve individual SZ GWAS genes investigated in the thesis, only three were found to be associated with neurocognition.

Discussion:

When researchers first began to study the genetics behind schizophrenia, a candidate gene approach was employed which focused on associations between genetic variation within pre-specified genes of interest and phenotypes or disease states (Kwon & Goate, 2000). Candidate genes are most often selected for study based on *a priori* knowledge of the gene's biological functional impact on the trait or disease in question, with the rationale being that certain mutations will directly impact the function of the gene in question, and lead to the phenotype or disease state being investigated (Zhu & Zhao, 2007). A great many of the SZ candidate genes studied were found to be significantly associated with neurocognition. For example in a recent study by Greenwood and colleagues (2012), out of 94 SZ candidate genes chosen for investigation based on hypotheses regarding biological systems of relevance to schizophrenia, as well as an extensive review of published linkage, association, gene expression, brain imaging, and model organism studies, close to half (38) were found to be associated with neurocognition.

This is in stark contrast to the findings of the research in this thesis (which utilises a GWAS approach rather than a candidate gene approach) for which only a quarter of the genes investigated (3 out of 12) were found to be associated with neurocognition. The National Institute of Health (NIH) describes GWAS as "any study of genetic variation across the entire human genome that is designed to identify genetic associations with observable traits or the presence of a disease or condition." A GWAS study is typically one in which 100,000 or more SNP markers are tested in individual DNA samples, to produce a "high density" genomic profile. With GWAS, no a-priori functional rationale for the SNP exists. It is perhaps not surprising then that fewer genes investigated via GWAS are found to be associated with neurocognition than those investigated via the candidate gene approach (for which certain a-priori reasons for investigation will include cognition related areas such as glutamate). What *is* interesting, however, is that even without prior rationale for associating

the genes with SZ, some genes are emerging that are *still* related to cognition. *CSMD1*, *MIR-137* and *HLADQA1* from the present thesis are examples of these. Examples from other studies would include *MET*, *complexin 2* (Valiente et al., 2011) and *NOS1* (Donohoe et al., 2009b). What this tells us is that SZ genes are likely to increase risk for schizophrenia by effects additional to and independent of cognitive functioning.

7.4 Pathway analysis: does it explain more variation in cognitive performance than individual SNP analysis?

Primary findings:

- Increased risk allele load on the *ZNF804A* pathway was associated with poorer performance amongst both patients with psychosis and healthy subjects in IQ, memory, attention and social cognition, explaining ~3-5% of variation on these scores in patients and ~5-9% of variation on these scores in healthy participants, exceeding that calculated for significant gene-cognition associations in schizophrenia to date (which is ~3%). Removing all *ZNF804A* SNPs from polygene analysis also removed the association between *ZNF804A* polygenic risk and cognition amongst patients, but did not impact the association with IQ and attention in the healthy population.

- Increased risk load on the *CAM* pathway was significantly associated with variation in premorbid IQ, memory and attention, explaining ~3% of variation on these measures. Specifically, increased risk allele load was associated with poorer performance in memory and attention. Of the four individual *CAM* pathway SNPs considered, only one (*HLADQA1*) was associated with neurocognitive variation, where it explained a comparable amount of variation to that explained by the *CAM* polygene score. Removal of this gene from the polygene analysis impacted the association between *CAM* pathway risk allele load and attentional control.

Discussion:

It was hypothesised that the impact of polygenic risk on cognitive performance would be greater than the impact of individual SNPs. While this was true for the *ZNF804A* pathway, it was not the case for the *CAM* pathway, in which the proportion of variance explained by the polygenic analysis versus the most strongly associated individual SNP analysis was comparable (~3%). This in turn is comparable to the variance calculated for significant gene-

cognition associations in schizophrenia to date (based on a recent meta-analysis conducted within our group; Rose and Donohoe, 2012).

One possible explanation for the finding that *CAM* pathway risk allele load is not more strongly predictive of neurocognitive performance is that the *CAM* pathway may be less significantly associated with deficits in neuropsychological function than other gene pathways. For example, the pathway of genes regulated by the *MIR-137* genome-wide variant may have a larger impact on cognition, especially as the *MIR-137* variant is associated with poorer cognitive performance (Green et al., 2012). Yet, the *CAM* pathway has previously been implicated in a variety of neurocognitive processes, including memory, and as such remains a likely candidate for association with cognition amongst patients with psychosis.

An alternative explanation for why a polygenic approach does not necessarily represent a major advance over the study of individual SNPs in modeling cognitive deficits in psychosis is the degree of genetic overlap between cognitive performance and both schizophrenia in particular and psychosis in general. Purcell and colleageus (2012) suggested that ~30-40% of genetic risk was attributable to common variant polygenicity and that the amount of variation in risk explained by independent samples was much lower. The question is whether one would expect SZ risk genes to influence psychotic traits such as impaired cognition, or whether cognition is influenced by a separate group of genes. Toulopoulou et al (2007) infers a high degree of overlap between cognitive deficits and SZ in his twin genetic modelling study. However, recent genetic epidemiological evidence by Fowler and colleagues (2012) has suggested that this may not be quite as high when based on non-biased samples. In the context of these findings, when modelling cognitive performance in psychosis, polygenic risk scores may therefore introduce as much noise as signal into the analysis (i.e. variation neither specific to cognition, nor to the target patient population). It is also worth noting that not 100% of the pathway might be informative and that annotation error could lead to the inclusion of genes that detract from the signal. On account of this, the variation in neurocognition explained by the individual SNP, although failing to capture much of the genetic variation associated with cognition, may not carry the same burden of non-relevant genetic information.

7.5 Strengths, limitations and future directions

7.5.1 Strengths:

7.5.1.1 The impact of genetics on Social cognition

Of particular interest amongst the research results in this thesis is that social deficits and social cognition are so specifically influenced by variants associated with SZ. Showing that this association exists, and indeed that it exists outside of any additional neurocognitive association, makes a noteworthy contribution to the current literature on the genetics of social cognition. Research had previously found an association between *ZNF804A* genotype and brain activation in areas associated with social cognition as measured using fMRI, with and without the inclusion of a social cognitive Theory of Mind task (Esslinger et al., 2011; Walter et al., 2011). As such being able to support these findings of an association between *ZNF804A* and social cognition in my own behavioural study was both satisfying and expected. Less expected was the discovery of an association between *CNNM2* and social cognition as such an association has never before been demonstrated. Being able to advance the literature with the addition of this social cognition associated gene is an exciting progression in understanding the genetics behind social cognitive processes. Social cognition, it seems, is not simply a by-product of more traditional cognitive processes, nor indeed an artefact of environmental influences. It is an independent construct in its own right, with a specialised underlying genetic architecture. This is one of the primary findings of this thesis. As such, including social cognitive tasks in the neuropsychological investigation of future GWAS genes may prove equally informative, with potentially more social cognitive genes being discovered. With social cognition having such an enormous impact on an individual's quality of life, effecting relationships, employment, and general community functioning (Penn et al., 2008), expanding our understanding of its underpinnings is viewed as highly valuable (Green et al., 2005).

7.5.1.2 Independence of social cognition and neurocognition at a genetic level

As previously discussed, whilst there is an overlap between neurocognition and social cognition in terms of deficits experienced by patients at a behavioural level, the research in this thesis suggests an independence of construct at a genetic level. The genes that impacted social cognition (*ZNF804A* and *CNNM2*) did not impact neurocognition, and the genes that impacted neurocognition (*MIR-137, HLADQA1* and *CSMD1*) did not impact social cognition.

To date, this has not been demonstrated so clearly in the literature and as such is an important observation for the field of SZ genetics and pathophysiology. It reaffirms that SZ is a polygenic disorder, with different genes playing different roles in the difficulties and deficits experienced by patients. This has implications for the debate on whether SZ is a unitary disorder, or whether it is a spectrum of illnesses (Maser & Akiskal, 2002), each varied and unique depending on which genetic mutations it encompasses, and goes some way to explaining the genetic overlap between psychiatric illnesses such as SZ, bipolar disorder and autism (Carroll & Owen, 2009).

7.5.1.3 The importance of the genetic pathway to cognitive deficits in SZ

Prior to the research in this thesis, it was hypothesised that genes acting in unison as a functional pathway would explain more of the variance in cognitive performance in SZ than SNPs acting in isolation (Lesnick et al., 2007; McIntosh et al., 2013). Interestingly, from the two studies conducted here, one might conclude that whilst polygenic risk *does* contribute to cognitive deficits, it does not necessarily do so at a greater level than individual GWAS SNPs. Specifically, while in Chapter 5 we saw that the proportion of variance explained by the *ZNF804A* polygenic analysis exceeded that calculated for significant gene-cognition associations in schizophrenia to date (based on a recent meta-analysis conducted within our group; Rose and Donohoe, 2012), in Chapter 6 the proportion of variance explained by the polygenic analysis versus the most strongly associated individual SNP analysis was comparable (~3%). These findings enhance our understanding of the role of polygenicity, and indeed of individual SNPs, in the cognitive decline inherent in SZ, in that it suggests that whilst pathway analysis is informative, single gene analysis may not be as poor at capturing the effects of genetics risk for schizophrenia as previously hypothesised. This is supportive of the continued use of GWAS in the investigation of the genetic underpinnings of psychiatric disorders.

7.5.1.4 Methodology

One of the overriding strengths of the research in this thesis is the use of a tried and tested, reproducible methodology. Employing validated endophenotypes to investigate the impact of illness associated genes on cognition is scientifically recognised and comprises a simple, flexible approach to understanding psychiatric genetics. This methodology been employed successfully by our own group over the last decade, with many papers and articles in peer

reviewed journals (e.g. (Donohoe et al., 2007a; Donohoe et al., 2007b, Walters et al., 2010). The research in this thesis contributes to this work through a) an increased sample size b) an increased availability and use of social cognitive tasks and c) further study of the social cognitive endophenotype.

7.5.2 Limitations:

7.5.2.1 Drawing conclusions about the non-associated GWAS SNPs investigated

Sample size is a major determinant of statistical power, which is a measure of the probability that a study will detect a real difference in the data (Taborski, 2010). A low sample size raises the chances of gaining a false negative result; that is, low sample sizes make it more likely to erroneously claim the association between variables is insignificant. As such, larger samples are preferable, particularly when looking for small effects. As complex cognitive functions are generally thought to be under the influence of multiple genes of small effect, having an appropriately large sample size is important. In GWAS, which are generally aimed at finding very small effects, lots of samples are required to confirm such small differences with statistical confidence. Indeed it is customary to see sample sizes in the order of several thousand participants in order to detect an association between the gene and the illness/ variable under question. Gaining samples of this magnitude is impractical in human behavioural studies, and it is more common to encounter sample sizes in the order of several hundred. This is true of the research throughout this thesis, where an average sample size of four hundred patients and two hundred healthy participants detected significant associations between specific genes and cognitive performance. While this leads to a confidence in the validity of the association detected, the main issue with regard to underpowered studies is that no conclusion can be drawn about the relationship between variables where no significant effect is detected. In order to assess whether the average sample size used throughout this thesis leaves the research underpowered, I conducted post-hoc power calculations on several of the ANOVAs employed, including emotion accuracy and intensity calculations in the ERT and measurements of difference in performance across diagnoses with IQ, memory, attention and social cognition tasks. The majority of tests were found to have >80% power. Notable exceptions to this were the externalising and personalising bias scores of the IPSAQ, which demonstrated a power of 30% and 50% respectively. As such, for negative findings in these measurements of social cognition, while it is possible that they are not being impacted in the study it is also possible that a real effect might not be detectable

with the sample size used. Therefore, an open mind should be kept with regard to the negative attributional style results presented in this thesis.

7.5.2.2 The ERT – validity as an endophenotype and the lack of a control group.

Sample size also proved problematic in relation to the emotion recognition task discussed in Chapter two. While it was the first study to look at the impact of both dynamic stimuli and various intensity levels on emotion recognition accuracy, the small sample size precluded the investigation of this task at a genetic level and hence it could not be included in the cognitive battery when investigating the impact of the various GWAS genes under scrutiny. Not being able to include the task in these studies meant it was impossible to attempt to satisfy the two unsatisfied endophenotypic criteria identified for this task in chapter one – both of which require genetic input (heritability and co-segregation with illness). As the task proved to be informative in relation to emotion recognition deficits in SZ and has an advantage over Baron-Cohen's 'Eyes' task in that it is more ecologically valid, it would be beneficial to secure its place as a valid endophenotype. Future work, with greater sample sizes, might well accomplish this.

A major limitation of the ERT study was the non-equivalence of the control condition arising from the opportunistic nature of the study. This task was administered at the end of a large neuropsychological battery, to participants who acquiesced to doing an additional test. As such, not only did it result in the small sample size described above, but it meant that the healthy participant group were not appropriately age and gender matched to the patient group so as to be utilised as controls. An adequate control group would have strengthened comparison findings between patients and healthy participants and led to the drawing of more robust conclusions and an enhanced generalizability of results.

7.6 Conclusion

The overarching research question that guided the individual studies that make up this dissertation has been: "Do SZ GWAS genes impact neuropsychology and/or social psychology, and if so in what manner do they exert their influence?"

Of the 12 SZ GWAS genes investigated in this thesis, three were found to be associated with neurocognition (*MIR-137, HLADQA1* and *CSMD1*) and two were found to be associated with

social cognition (*ZNF804A* and *CNNM2*). It was also demonstrated, using the *ZNF804A* and *CAM* pathways, that not only are single genes associated with the cognitive deficits associated with SZ, but whole pathways are too. It can thus be concluded that some SZ GWAS genes do indeed exert their influence by playing a role in the cognitive deficits, both neurocognitive and social cognitive, experienced by patients with SZ. They exert this influence by acting both independently (as SNPs) and polygenically (as pathways). Other SZ GWAS genes do not impact cognition, and as such, must have some other role to play in the pathophysiology of the illness. This has implications for understanding the genetic underpinnings of SZ, a disorder which comprises neurocognitive and social cognitive deficiencies, alongside positive and negative symptoms such as hallucinations and anhedonia. If a majority of the common variants found to be associated with SZ are not impacting neurocognition and social cognition (in this thesis less than half the genes studied impacted cognition), then one must conclude that they are impacting the other facets of the disorder. This lends support to the view that SZ is an illness comprised of multiple gene mutations. What the research findings in this thesis suggest is that these mutations can be quite specific in terms of which aspect of the illness they impact.

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APPENDICES

Appendix A

Ethics approval, Information letter and Consent form for the study.

Dan Lynch, Joint Research Ethics Committee Secretariat.
Telephone : 4142860. Fax : 4143371 Email: dan.lynch@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
THE NATIONAL CHILDREN'S HOSPITAL

TALLAGHT, DUBLIN 24, IRELAND
TELEPHONE +353 1 4142000

Dr. Aiden Corvin, MRCPsych,
Dept of Psychiatry,
Trinity Centre for Health Sciences,
St. James's Hospital,
James's Street,
Dublin 8.

5th September, 2002.

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

Daniel R. Lynch,
Senior Executive Officer

THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
THE NATIONAL CHILDREN'S HOSPITAL

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Dan Lynch, Secretary, SJH / AMNCH Research Ethics Committee .
Telephone : 4142860. Fax : 4142371. Email: dan.lynch@amnch.ie

Professor Michael Gill
Department of Psychiatry
Trinity Centre for Health Sciences
St. James's Hospital
James's Street
Dublin 8.

June 7th 2005

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.

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School Administrator
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Prof M Gill
Psychiatry
Trinity Centre
SJH D 8

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

pp Noelle Costelloe

Dr. Orla Sheils
Chairperson
Faculty of Health Sciences Ethics Committee

St. Patrick's University Hospital

Please reply to:
St. Patrick's University Hospital, P.O. Box 136, James's St., Dublin 8.
Tel: 01 249 3200 Fax: 01 249 3417
www.stpatrickshosp.com

Professor James V. Lucey
M.D. Ph.D. F.R.C.P.I. F.R.C.Psych.
Medical Director
Medical Council 00646

CONFIDENTIAL

30th September 2009

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

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

Dear Dr. Donohoe,

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

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

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

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

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

With very best wishes.
Yours sincerely,

JAMES V. LUCEY MD.,Ph.D.,FRCPI.,FRCPsych.,
SECRETARY TO RESEARCH ETHICS COMMITTEE

St. Edmundsbury,
Lucan, Co. Dublin.
Tel: 01 621 8200 Fax: 01 628 2979

INFORMATION SHEET AND INFORMED CONSENT FORM

1. Study background and purpose: Our research group is involved in identifying and studying genes which may increase the risk of major psychiatric disorders (e.g. schizophrenia). While a number of genes have been identified as "risk" genes for mental illness, their precise function remains unclear. One theory is that these genes may be influencing cognitive processes such as attention, concentration, and memory. Understanding the potential role of these genes in attention and memory may help us understand their role in mental ill-health. They may also be important for helping us understand the genetic components of normal brain function in healthy individuals.
The purpose of this study is to investigate whether genes identified as potentially important in schizophrenia may also be influencing memory and attention in healthy individuals. For this purpose we are collecting data on a range of neuropsychological measures of memory and attention. We are also collecting genetic information (DNA) obtained from a saliva sample. This will allow us to study whether there is a relationship between neuropsychological scores and individual genes of interest to our mental health research in healthy controls.

2. Why am I being asked to take part in this study? You are being asked to participate in this study because you are over 18 and have no history of epilepsy, head injury or psychotic illness.

3. What will happen if I agree to take part in this study? If you agree to be involved you will complete a neuropsychological assessment (this involves tests of concentration and memory function). This assessment typically takes 2.5 hrs. In addition you will be asked to give a saliva sample. All information about you used in the study will be kept in accordance with the Data protection act. This means that all information will be stored anonymously and coded. All information will be treated as confidential. None of your personal data (data which could identify you) will be shared with any third party.

4. Benefits: Your participation in this study will have no direct benefit to you but will be used to identify genes and understand how these genes relate to psychotic conditions such as Schizophrenia or Bipolar Disorder. This may have important diagnostic and therapeutic implications in the future. No individual genetic result will be available from this study, to you or to anyone else, and the study does not involve screening for genetic diseases. If you wish, we will keep you informed about the progress of the study in general.

5. Risks: There are no risks associated with this study

6. Exclusion from participation: You can be excluded from participation if you are under 18 or if the researcher thinks that this would be in your best interest. Genetic studies aim to investigate specific populations, for this reason individuals of non-Irish nationality may be excluded from the study.

7. **Voluntary participation**: The decision to participate is yours alone and you should not feel under any pressure to do so. A decision not to be involved in this study will in no way affect the treatment you receive now or in the future. You may withdraw your consent to participation at anytime. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study.

8. Will I benefit financially from this study? You will *not* benefit financially from this study.

9. Permission: This study has hospital Research Ethics Committee approval.

10. Further information: If you agree to take part you will be asked to sign the Informed Consent Form. By signing the Informed Consent Form you will not in any way waive your legal rights.
Thank you in advance for considering participation in this study. Your help is very much appreciated; this research could not be conducted without the support and co-operation of many people such as yourself. If you have any questions about this research, the study staff will be more than happy to answer them. Their contact details are given below:

Contact Details:
April Hargreaves, Dr. Gary Donohoe
Neuropsychiatric Genetics Group
Trinity Centre for Health Sciences
St James' Hospital
Dublin 8
Tel. 01-8962465

PARTICIPANT CONSENT FORM

Title of Study: **Resource for Psychiatric Genomics, Ireland**
Name Researcher: **April Hargreaves, Dr. Gary Donohoe.**

Contact details: **Dept. of Psychiatry**
Trinity Centre for Health Sciences
St. James's Hospital
Dublin 8.
Phone: 01-8962465
Mobile: 0833675807

1. I have read the attached information sheet on the above project dated _____ and have been given a copy to keep. The information has been fully explained to me and I have had an opportunity to ask questions about the project and understand why the research is being done and any foreseeable risks or consequences involved. I also understand that no guarantee can be given about the possible results.

2. I agree to give a saliva sample for research in this study. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time.

3. I may withdraw at any time without my medical treatment or legal rights being affected. If I withdraw my consent I understand that my sample will be destroyed unless I otherwise authorise.

4. I agree that the sample I have given and the information given by me can be stored and looked after by Trinity College Dublin. I understand that any genetic information obtained will not be made available to me.

5. I understand that researchers other than the above mentioned academic researchers, may use my genetic material. This may include pharmaceutical companies seeking new treatments for psychosis.

6. I understand that future research using the sample I give may include genetic research aimed at understanding the genetic influences in disease but that such test will not be of predictive/clinical value and that the results of these investigations are unlikely to have any implications for me personally.

7. I understand that I will not benefit financially in any way if this research leads to the development of new treatments or medical tests.

8. I have been told that all medical information/data pertaining to me will be protected by the principles of confidentiality and both the national and EU data protection legislation. I have further been told that this also applies to all medical information/data pertaining to me that are utilised in any non-EU state.

9. I know how to contact the research team if I need to.

Name of participant (BLOCK CAPITALS)	Date	Signature
Name of rearcher (BLOCK CAPITALS)	Date	Signature
Name of withess (BLOCK CAPITALS) In case of patient without capacity	Date	Signature

Appendix B

Neuropsychological tasks administered

Assessment of cognition

General cognitive functioning (IQ) IQ is measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III).

Episodic memory Verbal episodic memory is assessed using the logical memory subtest from the Wechsler Memory Scale, 3rd edition (WMS-III). Visual memory is assessed using the WMS-III Faces subtest.

Working memory Verbal working memory is measured using the WMS-III Letter Number Sequencing task [2]. Spatial working memory is assessed using the Spatial Working Memory Task from the Cambridge Automated Neuropsychological Test Battery (CANTAB SWM).

Attentional control Attentional control is assessed using the Continuous performance task, identical pairs version (CPT-IP). Accuracy was calculated in terms of the signal detection index d', providing a response sensitivity index to the target stimuli.

Social cognition Social cognition is assessed using the IPSAQ (Internal personal and situational attributes questionnaire; Kinderman & Bentall, 1996), the Mind in the Eyes task (Baron-Cohen et al, 2001), the hinting task (Corcoran, 2003) and a dynamic facial affect recognition task (as developed by Montagne et al, 2007).

Table A: Neuropsychological tasks in order of administration and estimation of time taken to complete each task. Patients were tested in two sittings, with research nurses having administered the following tasks during clinical assessment: Vocabulary, Eyes, Logical memory 1, Logical Memory 2 and Letter number sequencing. The second round of testing was broken as often as required by the patient with stops for tea, cigarettes and fresh air. Healthy controls were tested on two sittings where possible, again with breaks as required for tea, cigarettes and fresh air.

Test	Estimated completion time in minutes
WTAR	5
Letter-number sequencing	8
Vocabulary	10
Blocks	10
Faces 1	7
Logical memory 1	10
Spatial working memory	8
Paired associate learning	10
Faces 2	5
Logical memory 2	7
Computerised emotion recognition	10
Matrix	10
Reading the mind in the eyes	10
Hinting	7
Similarities	7
IPSAQ	10
Continuous performance task	10
	Total testing time = 2 hrs 40 minutes

Neuropsychological Tasks

Wechsler test of adult reading: Enables the measurement of pre-morbid (pre-injury) intellectual functioning for individuals ages 16 to 89 years. The test is composed of a list of 50 words that have atypical grapheme to phoneme translations. The intent in using words with irregular pronunciations is to minimize the current ability of the client to apply standard pronunciation rules and assess previous learning of the word. The rationale for using a reading test as a measure of pre-morbid functioning is that unlike many intellectual and memory abilities, reading recognition is relatively stable in the presence of cognitive decline associated with normal aging or brain injury. The examinee is asked to read the 50 words aloud starting at number one and progressing slowly through the list to number 50. Items are scored 1 for a correct response and 0 for an incorrect response. The criterion for discontinuation is 12 consecutive scores of 0.

Vocabulary: This pen and paper subtest is a measure of verbal intelligence, measuring word knowledge, verbal concept formation, and fund of knowledge. A list of 23-26 words are read aloud, one at a time, to the participant who is instructed to give oral definitions for the words presented. If the examinee's response in unclear or too vague to be readily scored a neutral enquiry such as "explain what you mean" or "tell me more about it" may be made. The task is discontinued after six consecutive scores of 0. Responses are then awarded a score of 2, 1 or 0 depending on the accuracy of the answer.

Similarities: This pen and paper subtest is a measure of verbal intelligence, measuring verbal reasoning and concept formation. The examinee is orally presented two words that represent common objects or concepts. The examinee is asked to state how the two objects or concepts are alike. If the response in unclear or ambiguous or is followed by a (Q) in the sample responses, a neutral enquiry such as "explain what you mean" or "tell me more about it" may be made. The task is discontinued after four consecutive scores of 0. Responses are awarded a score of either 1 or 0 depending on the accuracy of the answer.

Block design: This subtest is a measure of visuospatial and motor skills. The examinee is asked to replicate models or pictures of two-colour designs with blocks. The designs progress in difficulty from simple two-block designs to more complex nine-block designs. Each block has two white sides, two red sides, and two half-red and half-white sides. The task is timed, with sixty seconds allotted for the smaller designs and 120 seconds allocated to the larger

designs. The task is discontinued after three consecutive scores of 0. Scoring is based on time taken to accurately complete the task.

Matrix reasoning: This subtest is a measure of non-verbal abstract problem solving and is composed of four types of nonverbal reasoning tasks: pattern completion, classification, analogy, and serial reasoning. The examinee looks at a matrix from which a section is missing and identifies one of five response options that completes the matrix. The task is discontinued after four consecutive scores of 0, or four scores of 0 in five consecutive items. Responses are awarded a score of either 1 or 0 depending on the accuracy of the answer.

Logical memory I (Immediate recall): This pen and paper tests for episodic memory performance. The examinee listens to two different stories read aloud by the examiner, and immediately after hearing each story is asked to retell it from memory. The examinee is scored on the accuracy of his/her retelling of the stories.

Experiment sequence

- Introduction phase
- Reading of story one by examiner
- Recounting of story one by examinee
- Reading of story two by examiner
- Recounting of story two by examinee
- Re-reading of story two by examiner
- Re-recounting of story two by examinee

Logical memory II (Delayed recall): Twenty to thirty minutes after the administration of Logical memory I, the examinee is asked to retell the stories A and B. The stories are not reread to the examinee. Scoring is identical to that for Logical memory I

Faces I: (Immediate recall) This test assesses the examinee's facial processing and visual memory abilities. The examinee is shown a series of photographs of faces, one at a time, and asked to remember each one. The examinee is then shown a second series of photographs of faces, one at a time, and asked to identify each face as either one that he/she was asked to remember or a new one. One point is scored for each correct response.

Faces II: (Delayed recall) Twenty to thirty minutes after the administration of Faces I, the examinee is presented with a series of photographs of faces, one at a time. The examinee is asked to say "yes" if the face is one that he or she was asked to remember in Faces I or "no" if it is not. One point is scored for each correct response. This test assesses the examinee's facial processing and visual memory abilities after a time lapse.

Letter-number sequencing: This paper and pen task assesses the examinee's working memory, which is their ability to hold an item in short term memory while simultaneously manipulating it. The examinee is read a combination of numbers and letters and asked to repeat them, saying the numbers first in ascending order and then the letters in alphabetical order. Each item consists of three trials and each trial is a different combination of numbers and letters. The task is discontinued after a score of 0 in all three trials of an item. Responses are awarded either a 1 or a 0 depending on the accuracy of the answer.

Paired-associate learning: This computerized test assesses visual memory and new learning. Boxes are displayed on the screen and are opened in a randomized order. One or more of them will contain a pattern. The patterns are then displayed in the middle of the screen, one at a time, and the participant must touch the box where the pattern was originally located. If the participant makes an error the patterns are re-presented to remind the participant of their locations. The difficulty level increases through the test. In clinical mode (which is used in this research) the number of patterns increases from one to eight, which challenges even very able participants. Four main outcome measures are collected: total raw score, total standard score, total errors raw score and total errors standard score.

Spatial Working Memory: The CANTAB Spatial Working Memory test assesses a person's ability to retain spatial information and manipulate remembered items in working memory. The test is considered a sensitive measure of frontal lobe and executive dysfunction. This is a self-ordered task which also assesses heuristic strategy. A number of colored squares are shown to the participant on a screen. The aim of the test is that by touching the boxes and using a process of elimination you will find one blue 'token' in each of a number of boxes. These blue tokens are used to fill an empty column. The number of boxes is gradually increased until the participant is searching for a total of eight boxes. The color and position of boxes used are changed from trial to trial to discourage use of stereotyped search strategies. There are 24 outcome measures including errors, touching boxes that have been found to be empty, revisiting boxes already found to contain a token, a measure of strategy, and latency

measures. Of these, four main outcome measures are collected: between errors raw score, between errors standard score, strategy raw score and strategy standard score.

Continual performance task – Identical Pairs version:

The Continuous Performance Test, Identical Pairs version (CPT-IP) is a variant of the CPT that has been designed to be more challenging cognitively than most other CPT tasks. The CPT-IP requires a subject to respond whenever two identical stimuli appear in a row within a sequence of 150 rapidly flashed trials.

There are 30 such target pairs in every CPT-IP condition and an equal number of "catch" trials, in which two successive stimuli are very similar, but not identical. The remaining 90 trials in each condition are randomly organized and are not in any way similar. The task is considered to be a reliable test of attention. This version of the CPT-IP involves a comparison between three types of stimuli, 2-digit numbers, 3-digit numbers and 4-digit numbers, with each increase in digit size requiring a great deal more attention control to perform.

Experiment Sequence

- Introduction Phase
- Practice trial: full speed 2-digit demonstration with feedback
- 2-digit trial sequence – 150 trials of which 20% are targets
- 3 digit trial sequence – 150 trials of which 20% are targets
- 4 digit trial sequence – 150 trials of which 20% are targets

Response Mode

The subject responds, via a finger press on the left mouse key, when the number sequence on screen is identical to the last one that appeared.

Experimenter responds via the keyboard (spacebar) to cue introduction and progress the demonstration screen by screen until the practice begins.

In between each trial phase the examinee has the opportunity to rest until they feel able to continue.

Scoring: 2,3 and 4 Digits

Hits (a correct response when two stimuli in a row are identical and the subject makes a finger press to the second stimulus of the identical pair)

False alarms (where the subject makes an incorrect finger press response to the second

stimulus of a pair in which the two stimuli are similar but not identical to each other, e.g. differ by one digit)

Random errors (where the subject makes an incorrect finger press response to a second trial that is not similar to the previous one, i.e., has no features in common)

Intra-dimensional/extra-dimensional set-shifting task (IDED):

The IDED is a computerized task in which subjects are required to learn a series of discriminations in which one of two stimuli is correct utilizing feedback provided by the computer. They are told that there is a rule they can learn in order to find the correct stimulus each time, but that this rule will change once it is apparent that they have understood the currently correct rule.The test consists of 9 stages of increasing difficulty (see Fig. 1). After 6 consecutive correct choices at one stage, the test automatically proceeds to the next stage. If the criterion of 6 correct choices is not reached within 50 trials, the test is discontinued. At the first stage, the simple discrimination (SD) stage, subjects have to choose one of two stimuli of the same dimension (e.g. from the dimension "line"). At the following stage, the simple reversal (SR) stage, the contingencies change and the previously correct line element becomes incorrect. At the third and for the hypothesis of this study most critical stage, the compound discrimination (C_D) stage, distracting elements are introduced: each of the two line elements is now paired side by side with a shape element. Hence, a second stimulus dimension "shape" is introduced. In order to receive correct feedback, subjects have to stick with the previously correct line element and ignore the shape stimuli. At the fourth (CD) stage, the line element is superimposed on the shape element to form a more contiguous stimulus. Again, the stimulus containing the previously correct line element remains the correct choice. At the fifth stage, the compound discrimination reversal (CDR) stage, the stimulus containing the previously incorrect line element becomes the correct choice. At the sixth, the intra-dimensional shift (IDS) stage, new exemplars of the two dimensions "line" and "shape" are introduced. In order to receive correct feedback, subjects have to continue to ignore the shape elements and base their choices solely on the stimuli from the dimension "line". At the seventh stage, the intra-dimensional shift reversal (IDR) stage, the previously incorrect line element becomes the correct choice. Finally, at the eighth stage, the extra-dimensional shift (EDS) stage, again with new exemplars from each dimension, subjects have to shift attention between the different stimuli dimensions and instead of choosing one of the stimuli from the dimension "line" to choose one of the two "shape" stimuli. The ninth and last shift, the extra-dimensional shift reversal (EDR) stage, requires subjects to shift their

attention to the previously incorrect shape stimulus. Performance indices of the IDED task commonly are Attrition rate, Number of Errors, Number of Trials and Mean Latency per stage.

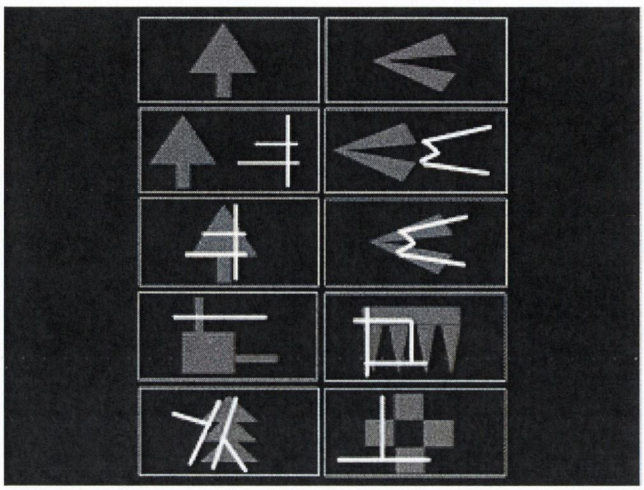

Fig. A. Schematic of the IDED task (without reversal stages SR, CDR, IDR and EDR).

The Sustained Attention to Response Task (SART):

The SART is a computer test of sustained attention. A total of 225 single digits (25 of each of the 9 digits) were presented visually to participants over a 4.3-min period. Each digit was presented for 250 ms, followed by a 900-ms mask. Participants responded with a key press to each digit, except in 25 occasions when the digit 3 appeared and they had to withhold a response. Participants were asked to give equal importance to accuracy and speed in performing the task. The digits were presented in one of five randomly allocated font sizes to enhance the demands for processing the numerical value, rather than simply setting for a search template for some peripheral feature of the no-response target. These font sizes were 48 point, 72 point, 94 point, 100 point and 120 point, respectively, corresponding to a height varying between 12 and 29mm. Each session was preceded by a practice period consisting of 18 presentations of digits, two of which were targets.

Hinting task: This is a theory mind test which assesses mental state reasoning. During the test ten short vignettes which describe a social interaction between two characters are read aloud to the participant, after which the participant is required to make inferences about the intent behind a hint dropped by one of the characters. If the examinee does not initially

correctly identify the hint, a second related hint is dropped in order to facilitate recognition. Performance is scored 0 if the hint was failed to be detected, 1 if a second hint was required or if the examinee was almost, but not quite correct, and 2 if the examinee obtained the correct answer on first attempt.

Reading the mind in the eyes task: This is a theory of mind task which assesses mental state decoding. It measures the ability of a person to determine what another person is thinking or feeling at a precise period in time from looking at their eyes. The examinee is presented with a series of 36 black and white photographs of adult male and female eyes. These are presented one at a time. At each corner of the photograph is an emotive word (such as' flirtatious') which might possibly describe what the person in the photograph is thinking or feeling at the time the picture is taken. One of these words is the correct answer. The examinee is scored either 0 or 1 depending on the accuracy of the answer given.

Internal, personal and situational attributions questionnaire: This pen and paper questionnaire is an assessment of causal locus. The test consists of 32 scenarios, 16 positive and 16 negative, such as "A friend gave you a lift home". The examinee must decide what they feel might be the primary reason for the scenario taking place and, based on their decision, decide whether that is something to do with themselves, something to do with the person or other people involved, or something to do with the situation or circumstance at hand. Six main outcome scores are obtained: Internal positive, external positive, situational positive, internal personal negative, external personal negative and situational negative. From these six scores, two biases are calculated: an externalising bias (the tendency to attribute positive events to oneself rather than to others or situations) and a personalising bias (the tendency to attribute negative events to other people rather than to situations). The biases are scored as follows: a score of over 1 on the externalising bias scale indicates the presence of an externalising bias. A score of between 0.5 and 1 on the personalising bias scale indicates the presence of a personalising bias.

Computerised emotional recognition task: This is a computer based task showing video clips of varying intensities of emotional expression on faces. Stimuli were based on colour pictures from actors mimicking emotion expressions and neutral faces. There were four actors (two male and two female) who each posed 6 emotions (anger, disgust, happiness, sadness, surprise, fear). These images were used to construct video clips which incrementally

increase the degree of expression by 20% steps from 20% to 100% (with 0% being the neutral face).

Experiment sequence

- Introduction phase
- Practice trial – 4 facial emotions shown from 0% to 100% intensity.
- 24 video clips running from neutral to 20% intensity (each actor displaying each of the emotions)
- 24 video clips running from neutral to 40% intensity (each actor displaying each of the emotions)
- 24 video clips running from neutral to 60% intensity (each actor displaying each of the emotions)
- 24 video clips running from neutral to 80% intensity (each actor displaying each of the emotions)
- 24 video clips running from neutral to 100% intensity (each actor displaying each of the emotions)

After each trial the subject must choose from one of the six emotion expression labels displayed on the screen, with the static image of the final intensity shown remaining on the screen until the forced choice is made. There was no time restriction for each trial.

Appendix C

Publications arising from this thesis.

List of publications arising from this thesis

1. Donohoe, G., Walters, J., **Hargreaves, A.**, Rose, E.J., Morris, D.W., Fahey, C., Bellini, S., Cummins, E., Giegling, I., Hartmann, A.M., Möller, H.J., Muglia, P., Owen, M.J., Gill, M., O'Donovan, M.C., Tropea, D., Rujescu, D., Corvin, A. (2013) Neuropsychological Effects Of The CSMD1 Genome-Wide Associated Schizophrenia Risk Variant rs10503253. *Genes Brain Behav.* Jan 15.

2. Cummings, E., Donohoe, G., **Hargreaves, A.**, Moore, S., Fahey, C., Dinan, T.G., McDonald, C., O'Callaghan, E., O'Neill, F.A., Waddington, J.L., Murphy, K.C., Morris, D.W., Gill, M., Corvin, A. (2013) Mood congruent psychotic symptoms and specific cognitive deficits in carriers of the novel schizophrenia risk variant at MIR-137. *Neurosci Lett.* Jan 4;532:33-8. doi: 10.1016/j.neulet.2012.08.065. Epub 2012 Sep 13.

3. Corvin, A., Donohoe, G., **Hargreaves, A.**, Gallagher, L., Gill, M. (2012) The cognitive genetics of neuropsychiatric disorders. *Current topics in behavioral neurosciences* 02/12:579-613.

4. **Hargreaves, A.**, Morris, D.W., Rose, E., Fahey, C., Moore, S., Cummings, E., Tropea, D., Gill, M., Corvin, A., Donohoe, G. (2012) ZNF804A and social cognition in patients with schizophrenia and healthy controls. *Mol Psychiatry.* Feb;17(2):118-9.

5. Donohoe, G., Walters, J., Morris, D.W., Da Costa, A., Rose, E., **Hargreaves, A.**, Maher, K., Hayes, E., Giegling, I., Hartmann, A.M., Möller, H.J., Muglia, P., Moskvina, V., Owen, M.J., O'Donovan, M.C., Gill, M., Corvin, A., Rujescu, D. (2011) A neuropsychological investigation of the genome wide associated schizophrenia risk variant NRGN rs12807809. *Schizophr Res.* Feb;125(2-3):304-6.

MANUSCRIPTS IN PROGRESS

6. Nicodemus, K.*, **Hargreaves, A.***, Morris, D., Anney, R. The Psychiatric Genomics Consortium, The Wellcome Trust Case Control Consortium, Gill M, Corvin A, Donohoe G. Increased variation in working memory explained by epistasis versus polygene scores in the ZNF804A pathway. *Molecular psychiatry.* *These authors contributed equally to this work.

7. Rose, E.J., **Hargreaves, A.**, Morris, D., Fahey, C., Tropea, D., Spoletini, I., Adriano, F., Bernardini, S., Caltagirone, C., Bossù, P., Spalletta, G., Gill, M., Corvin, A., Donohoe, G. *The novel psychosis risk variant CNNM2 rs7914558: Effects on social cognition.* Biological psychiatry.

Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

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Neuropsychological effects of the *CSMD1* genome-wide associated schizophrenia risk variant rs10503253

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The single-nucleotide polymorphism (SNP) rs10503253, located within the *CUB* and *Sushi multiple domains-1* (*CSMD1*) gene on 8p23.2, was recently identified as genome-wide significant for schizophrenia (SZ), but is of unknown function. We investigated the neurocognitive effects of this *CSMD1* variant *in vivo* in patients and healthy participants using behavioral and imaging measures of brain structure and function. We compared carriers and non-carriers of the risk 'A' allele on measures of neuropsychological performance typically impaired in SZ (general cognitive ability, episodic and working memory and attentional control) in independent samples of Irish patients ($n = 387$) and controls ($n = 171$) and German patients (205) and controls ($n = 533$). Across these groups, the risk 'A' allele at *CSMD1* was associated with deleterious effects across a number of neurocognitive phenotypes. Specifically, the risk allele was associated with poorer performance on neuropsychological measures of general cognitive ability and memory function but not attentional control. These effects, while significant, were subtle, and varied between samples. Consistent with previous evidence suggesting that *CSMD1* may be involved in brain mechanisms related to memory and learning, these data appear to reflect the deleterious effects of the identified 'A' risk allele on neurocognitive function, possibly as part of the mechanism by which *CSMD1* is associated with SZ risk.

Keywords: Attention, CMSD1, cognition, gene, GWAS, IQ, memory, psychosis, schizophrenia, working memory

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Kraepelin's (1887) classification of schizophrenia (SZ) as a 'dementia praecox' was informed by his observation that cognitive decline was a primary cause of disability in patients. Deficits in general cognitive and memory function represent core stable trait-like features that often predate the emergence of symptoms such as delusions and hallucinations, which tend to fluctuate over time (Lewandowski *et al.* 2011). The changes in brain morphology (such as reduced gray matter volume) and neural activity (such as reduced prefrontal activations) that underlie cognitive dysfunction in SZ appear to share genetic risk with disease status, and may be part of the neural mechanism by which risk is mediated (Meyer-Lindenberg 2010). Characterizing the functional effects of novel and poorly understood genetic variants on these 'intermediate' phenotypes has provided important insights into the neural mechanisms by which these variants increase risk for disease (Meyer-Lindenberg 2010).

In identifying genetic risk factors for SZ, genome-wide association studies (GWAS) have facilitated discovery of variants whose roles in illness etiology or pathophysiology are neither known nor easily predicted. In the largest SZ GWAS to date, seven novel variants were identified as meeting genome-wide significance, five of which are new (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33) and two of which have been previously implicated (6p21.32-p22.1 and 18q21.2; Ripke *et al.* 2011). Among these, the SNP rs10503253 located within the *CUB* and *Sushi multiple domains-1* (*CSMD1*) gene on 8p23.2 is particularly noteworthy given previous evidence of its association with risk for multiple neurodevelopmental disorders (Glancy *et al.* 2009; Havik *et al.* 2011; Shimizu *et al.* 2003). *CSMD2* (but not to our knowledge *CSMD3*) have also been associated with SZ risk (Havik *et al.* 2011) and related neurocognitive phenotypes (Stein *et al.* 2010). While the precise mechanism remains unclear, *CSMD1* has been implicated in immune function via a complement regulatory role (Kraus *et al.* 2006). Whether or how this role influences brain structure and function is unclear.

In this study, we sought to investigate the effects of the *CSMD1* SZ risk 'A' allele at rs10503253 on neuropsychological function. We measured neuropsychological functioning

in four independent samples of Irish patients, Irish controls, German patients and German controls, targeting those functions most robustly associated with neuropsychological deficits in SZ – general cognitive ability (IQ), working memory function, episodic memory function and attentional control. Across these samples, we tested the hypothesis that the risk 'A' allele would be associated with deficits in neuropsychological function.

Methods

Irish patient and control samples

The Irish neuropsychological sample consisted of 378 cases and 171 controls. Cases consisted of clinically stable patients with a diagnostic and statistical manual (DSM-IV) diagnosis of SZ or schizoaffective disorder recruited from five sites across Ireland. Inclusion criteria required that participants were aged 18–65 years, had no history of comorbid psychiatric disorder, substance abuse in the preceding 6 months or prior head injury with loss of consciousness or a history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis I Diagnoses (SCID; First *et al.* 1995). Additional diagnostic and clinical information ascertained at time of interview, including symptom severity (SAPS/SANS; Andreasen 1984a,b) and medication dosage are detailed elsewhere (Walters *et al.* 2010).

The healthy control sample was recruited on the basis of response to local media advertisements. Control participants were included if they were aged between 18 and 65 and satisfied, based on clinical interview, the criteria of having no history of major mental health problems, intellectual disability or acquired brain injury and no history of substance misuse in the preceding 6 months based on self-report. Control participants were also excluded from the study if they reported having a first-degree relative with a history of psychosis. All patient and control assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All patients and controls were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

German patient and control samples

The German neuropsychological sample consisted of 205 clinically stable patients with a DSM-IV diagnosis of SZ and 533 healthy controls, all of whom were genotyped as part of a previous study (O'Donovan *et al.* 2008). Patients were ascertained from mental health services in the Munich area and all participants provided written informed consent. Inclusion criteria were a diagnosis of SZ (over 6 month symptom duration) and being between the ages of 18 and 65. Exclusion criteria included a history of head injury or neurological diseases. Detailed medical and psychiatric histories were collected, including a clinical interview using the SCID to evaluate lifetime axis I and II diagnoses (First *et al.* 1990). Four physicians and one psychologist rated the SCID interviews and all measurements were double rated by a senior researcher. In cases, 68% were of strict German descent (all four grandparents born in Germany); the remaining 32% were German Caucasian.

Healthy control participants of German descent were randomly selected from the general population of Munich, Germany, and contacted by mail. Control patients for this study were only included if aged between 18 and 65. To exclude subjects with central neurological diseases and psychotic disorders or subjects who had first-degree relatives with psychotic disorders, several screenings were conducted before the volunteers were enrolled in the study. First, subjects who responded were initially screened by phone for the absence of neuropsychiatric disorders. Second, detailed medical and psychiatric histories were assessed for both themselves and their first-degree relatives using a semi-structured interview. Third, if no exclusion criteria were fulfilled, they were invited to a comprehensive interview including the Structured Clinical Interview for DSM-IV (SCID I and SCID II; First *et al.* 1990, 1995) to validate the absence of any lifetime psychotic disorder. Additionally, the Family History Assessment Module (Rice *et al.* 1995) was conducted to exclude psychotic disorders among their first-degree relatives. A neurological examination was also conducted to exclude subjects with current central nervous system impairment. In the case of volunteers older than 60 years, the Mini Mental Status Test (Folstein *et al.* 1975) was performed to exclude subjects with possible cognitive impairment.

Cognitive assessment

This study was designed so that identical, or near identical, tests of the phenotypes of general cognitive function (IQ), episodic memory, working memory and attention were used for both the Irish discovery samples and the German replication samples. Only one verbal and one visuo-spatial measure for each cognitive function were tested in the Irish samples.

General cognitive functioning (IQ) was measured in the Irish sample using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, third edition (WAIS-III; Wechsler 1997a,b), yielding a full scale, verbal and performance IQ. For the German sample, IQ was indexed by the German version of the WAIS revised third edition (Tewes 1991) using all available 11 verbal/performance subtests (vocabulary, comprehension, information, digit span, arithmetic, similarities, block design, picture completion, picture arrangement, object assembly and digit symbol coding). Verbal and visual *episodic memory* was assessed in the Irish samples using the logical memory and faces subtests respectively from the Wechsler Memory Scale, third edition (WMS-III) (Wechsler 1997) and in the German sample using the logical memory and visual memory score from the German version of the WMS revised (Wechsler 1987). Verbal and spatial *working memory* was assessed in the Irish samples using the WMS-III letter number sequencing task and the spatial working memory task from the Cambridge Automated Neuropsychological Test Battery (CANTAB SWM; Robbins *et al.* 1994). In the German samples, working memory was measured using the composite Digit Span and Spatial Span score from the WMS-R and the N-back task (Callicott *et al.* 1999). *Attentional control* was assessed in the Irish sample using the continuous performance task (CPT) identical pair's version (CPT-IP) (Keilp *et al.* 1997) and in the German sample using the CPT 3–7 version (Nuechterlein *et al.* 1991).

Genotyping

The *CSMD1* SNP rs10503253 was genotyped from saliva using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems, Carlsbad, CA, USA). The call rate for the Taqman genotyping was >95% and the samples were in Hardy–Weinberg equilibrium ($P > 0.05$). Along with these samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for rs10503253 for quality control purposes and were found to be concordant with available HapMap data for this SNP.

Data analysis

Association between *CSMD1* and measures of general cognitive function, episodic memory, working memory and attentional control was tested using a general factorial design in SPSS version 18 (SPSS Inc. Chicago, IL, USA). *CSMD1* genotype (CC vs. AC vs. AA) and diagnosis (cases vs. controls) were entered as fixed effects. In a series of analyses of covariance (ANCOVAS), scores for each neuropsychological subtest were entered as the dependent variables, with age and gender included as covariates as appropriate. Significant interaction effects were further explored by examining simple effects in cases and controls.

Results

Across Irish and German neuropsychological datasets we found a main effect of the risk 'A' allele on measures of both

IQ and memory, in the absence of statistically significant differences in clinical symptoms (see Tables 1& 2 and S1). These effects varied between samples, however, in terms of both the specific task implicated in the Irish/German samples and in terms of whether the effect was seen in both patients and controls or patients only.

Specifically, Irish homozygous carriers of the 'A' allele performed below other genotype groups in both verbal IQ and verbal episodic memory. In the German sample, the homozygous carriers of the 'A' allele performed below other genotype groups on performance IQ and spatial working memory. While this effects on spatial working memory was observed as a main effect across patients and controls, follow-up analyses of performance IQ indicated that *CSMD1*'s effect was driven mainly by patients ($F_{2,206} = 4.69$; $P = 0.01$), in the absence of a significant association in the control group when considered alone ($P > 0.05$).

Additional diagnostic group × genotype interactions were also observed for both Irish and German samples. In the Irish samples, a genotype by diagnosis interaction was observed for verbal working memory in the absence of a genotype main effect ($F_{2,534} = 5.55$, $P = 0.004$). Follow-up analysis (taking cases and controls separately) revealed an association with genotype in controls ($F_{2,169} = 5.47$, $P = 0.005$) but not in cases; however, the number of AA controls was small ($n = 9$) and was likely to represent a false positive. In the German samples, a genotype by diagnosis interaction was observed for full scale IQ ($F_{2,735} = 3.418$, $P = 0.03$) in the absence of a genotype main effect. Follow-up analysis indicated a trend level association in cases ($F_{2,206} = 2.35$; $P = 0.09$), but no association in controls ($P > 0.05$).

Discussion

This study investigated the effects of the genome-wide associated SZ risk 'A' allele at rs10504253 located within *CSMD1* on neuropsychological measures of general cognitive ability, episodic and working memory and attentional control in four independent samples. Across these samples, the risk 'A' allele was associated with deleterious effects in neuropsychological performance. While these neuropsychological effects were consistently found to be deleterious, these effects varied considerably between groups. Rather than suggesting functionally specific effects, the pattern of associations observed varied between groups in terms of both those aspects of intelligence and memory implicated.

The risk 'A' allele showed subtle effects on cognitive performance that were not task specific and not consistent between groups. Specifically, *CSMD1* was associated with poorer *verbal* intelligence and verbal episodic memory in the Irish sample and with poorer *performance (visuo-spatial)* intelligence and spatial working memory in the German sample. In our previous gene-cognition studies, we found greater identity between these samples in the cognitive functions and modalities implicated (Donohoe *et al.* 2009; Walters *et al.* 2010), despite differences in sample ascertainment and individual tests (and editions of tests). While differences in the effects of *CMSD1* genotype between cases and controls were also observed, such differences have frequently been noted in neuropsychological studies, including in previous analysis of the samples reported here (Walters *et al.* 2010). Whether the variation in associations observed across samples observed here is a consequence of *CMSD1* having more subtle cognitive effects that are more sensitive to ascertainment differences remains to be determined. Similarly, it is also uncertain whether the interactions observed between genotype and diagnosis might reflect *CSMD1*'s effects being conditional on the presence, absence or burden of additional SZ risk variants. In any event, these observed effects across a range of neurocognitive function suggest a more general influence of this *CSMD1* variant across cognitive functions and modalities, rather than a specific effect on any one aspect of cognition.

This suggestion that *CSMD1* has a general rather than specific effect on cognition is consistent with the broad role in neurodevelopment suggested by the range of neurodevelopmental illness phenotypes already associated with this gene (Glancy *et al.* 2009; Havik *et al.* 2011; Shimizu *et al.* 2003). These associations include epilepsy, speech delay and learning difficulties (Glancy *et al.* 2009; Kraus *et al.* 2006; Shimizu *et al.* 2003), and in the psychiatric GWAS consortium (PGC) analysis association between the variant investigated here and SZ (Ripke *et al.* 2011). In addition, a recent Alzheimer's/mild cognitive impairment copy number variant (CNV) study where *CSMD1* was one of six gene loci at which case-only CNVs were identified (Swaminathan *et al.* 2011). Finally, a recent genome-wide study of cognition (Cirulli *et al.* 2010) in healthy controls identified a separate *CSMD1* variant association (rs2616984, positioned >30 kb from rs10503253), suggesting that additional *CSMD1* variants may also be relevant to cognition.

CSMD1 and cognition: possible molecular mechanisms

It is as yet unclear whether or how the effects of *CSMD1* genotype on cognitive function observed here might be associated with an increased risk for SZ. In speculating about the mechanism by which *CSMD1* genotype increases risk for illness, and for the cognitive deficits observed here, in a recent study of 4000 gene variants related to immune function, variants within *CSMD1* and *CSMD2* showed the strongest association with SZ risk (Havik *et al.* 2011). Genes designated as being relevant to immunity have been hypothesized as relevant to SZ pathophysiology either directly via a role in immune function (Brown & Susser 2002), or via a role in synaptic refinement and neuronal connectivity (Shatz 2009). Whether *CSMD1*'s association with SZ risk and variation in cognition is related to a role in immune function remains to be determined.

Regarding the specific SNP tested here, rs10503253 is located in a large intron of *CSMD1* at a site with no obvious function. SNP expression analyses using both the mRNA by SNP Browser and the SNPExpress database (http://www.sph.umich.edu/csg/liang/asthma/; http://people.genome.duke.edu/~dg48/SNPExpress/) identified the risk 'A' allele at rs10503253 as having a *trans*-acting effect that reduced expression of the beta-carotene

Table 1: Difference in neuropsychological performance associated with rs10503253 in Irish patients and controls

Irish samples Cognitive function	Test or subscale	Sample	*n*	Mean (SD) CC	Mean (SD) AC	Mean (SD) AA	$F_{case\ vs.\ controls}$	*P*	$F_{main\ effect\ genotype}$	*P*	$F_{interaction\ effect}$	*P*
IQ	Verbal IQ	Patients	378	92.59 (19.7)	91.04 (17.8)	85.6 (14.89)	115.2	<0.0001	**3.95**	**0.02**	1.009	0.365
		Controls	168	122.2 (15.6)	124.2 (15.69)	108.5 (10.4)						
	Performance IQ	Patients	378	89.35 (18.2)	88.5 (18.8)	87.5 (12.6)	116.1	<0.0001	0.455	0.635	0.222	0.801
		Controls	168	118.05 (18.7)	115.5 (18.9)	117.3 (17.9)						
	Full scale IQ	Patients	378	90.5 (18.27)	88.9 (17.4)	85.4 (13.1)	160.9	<0.0001	0.996	0.37	0.118	0.888
		Controls	168	121.6 (16.07)	122.2 (15.88)	116.7 (8.9)						
Working memory	Letter number (LN) sequence	Patients	367	7.85 (3.38)	7.5 (3.29)	6.78 (3.55)	152.5	<0.0001	1.31	0.27	**5.55**	**0.004**
		Controls	171	12.19 (3.05)	13.2 (3.05)	15.3 (3.34)						
	CANTAB SWM errors	Patients	351	−0.91 (1.3)	−1.1 (1.3)	−0.41 (1.42)	34.83	<0.0001	1.08	0.12	1.23	0.291
		Controls	159	0.19 (0.79)	0.31 (0.76)	−0.39 (0.587)						
Episodic memory	Logical memory immediate	Patients	370	6.5 (3.48)	6.2 (3.36)	5.2 (3.13)	145.4	<0.0001	2.71	0.068	0.117	0.889
		Controls	171	12.9 (3)	12.8 (2.48)	11.3 (2.9)						
	Logical memory delayed	Patients	370	7.4 (3.23)	6.8 (3.33)	6.2 (3.06)	129.3	<0.0001	**3.23**	**0.041**	1.785	0.169
		Controls	171	13.2 (2.99)	13.6 (2.4)	11.1 (3.21)						
	CANTAB PAL	Patients	359	−2.61 (3.85)	−3.21 (3.77)	−1.65 (3.07)	24.9	<0.0001	0.433	0.649	0.255	0.775
		Controls	144	0.239 (1.11)	0.15 (1.25)	0.19 (1.72)						
Attentional control	CPT_IP (three letters)	Patients	256	2.02 (0.96)	1.8 (1.01)	2.5 (0.923)	—	—	2.56	0.08	—	—
		Controls	—	—	—	—						

'A' is the risk allele.

Table 2: Difference in neuropsychological performance associated with rs10503253 in German patients and controls

Test or subscale	Sample	*n*	Mean (SD) CC	Mean (SD) AC	Mean (SD) AA	$F_{case\ vs.\ controls}$	*P*	$F_{main\ effect}$	*P*	$F_{interaction\ effect}$	*P*
Verbal IQ	Cases	205	102.2 (16.5)	106.6 (15.9)	98.4 (18.3)	26.667	<0.0001	1.298	0.274	1.59	0.205
	Controls	533	113.0 (13.6)	112.2 (13.6)	111.9 (15.8)						
Performance	Cases	205	95.8 (17.6)	101.1 (15.2)	81.1 (13.7)	102.72	<0.0001	**3.312**	**0.037**	**6.708**	**0.001**
	Controls	533	113.4 (14.0)	112.2 (13.7)	115.3 (16.0)						
Full scale IQ	Cases	205	100 (18.1)	105.5 (16.8)	90.3 (17.8)	66.12	<0.0001	2.727	0.066	**3.418**	**0.033**
	Controls	533	115.4 (13.9)	114.5 (13.8)	114.7 (17.8)						
WAIS digit span	Cases	205	13.6 (3.8)	14.5 (4.0)	13.6 (4.1)	3.736	0.054	0.376	0.687	0.78	0.459
	Controls	533	14.7 (3.8)	14.5 (4.0)	14.6(4.4)						
WMS spatial span	Cases	202	15.1 (3.4)	15.8 (3.3)	12.8 (3.1)	53.12	<0.0001	**4.204**	**0.015**	0.344	0.709
	Controls	341	17.2 (3.3)	17.5 (3.4)	16.0 (4.1)						
WMS logical memory I	Cases	203	24.7 (8.6)	24.2 (9.6)	19.8 (8.7)	58.7	<0.0001	1.544	0.215	0.615	0.541
	Controls	342	31.2 (6.8)	30.2 (6.2)	29.9 (6.4)						
WMS logical memory II	Cases	203	27.2 (10.3)	26.8 (11.3)	23.9 (7.0)	53.2	<0.0001	0.329	0.72	0.391	0.677
	Controls	342	34.5 (7.4)	33.8 (7.4)	34.8 (5.8)						
CPT (three to seven version)	Cases	199	4.16 (0.92)	4.24 (0.73)	3.65 (1.19)	60.767	<0.0001	1.534	0.217	1.88	0.154
	Controls	343	4.78 (0.56)	4.74 (0.57)	4.77 (0.56)						

'A' is the risk allele.

oxygenase 2 (*BCO2*) gene on chromosome 11 ($P = 6 \times 10^{-7}$), a findings which has since been replicated (Dixon *et al.* 2007; Heinzen *et al.* 2008). Variants in BCO2 are strongly associated with plasma levels of interleukin 18, a proinflammatory cytokine involved in immune function (He *et al.* 2010). On the basis of this evidence we tested the effects of the *CSMD1* variant rs10503253 on expression of both the CSMD1 protein and the BCO2 protein in human serum samples from Irish patients who participated in our neuropsychological studies (Appendix S1). We failed to find any evidence supporting this variant's role in influencing the expression of either protein. Finally, in terms of previously reported SNPs within *CSMD1* implicated in SZ or cognition, none were in strong LD with the rs10503253 investigated here (Havik *et al.* 2011; Cirulli *et al.* 2010; Shi *et al.* 2009; max $r^2 < 0.2$).

Conclusion

While the precise causal mechanism remains unknown, our investigation of the recently identified *CSMD1* SZ variant rs10503253 suggests its potential relevance to neural functioning. In the context of previous association studies of other neurodevelopmental disorders, these data suggest the possible deleterious effects of the 'A' risk allele on neurocognitive function, and may represent at least part of the mechanism by which *CSMD1*-associated risk for SZ is mediated. Variation in neuropsychological effects across samples makes these inferences tentative, however, and further study of this variant will be required before more definite conclusions can be drawn.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1: rs10503253 alleles (*CSMD1*) and Clinical Symptomatology.

Appendix S1: Supplemental methods & results for immunostaining.

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Mood congruent psychotic symptoms and specific cognitive deficits in carriers of the novel schizophrenia risk variant at MIR-137

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HIGHLIGHTS

- We investigated the clinical symptom profiles of carriers of the schizophrenia mir137 risk allele.
- The sample included 821 patients with schizophrenia, schizoaffective disorder and bipolar I disorder.
- Risk allele carriers had lower scores for positive symptoms and less psychosis incongruity.
- On neurocognitive testing in a subset, there were more cognitive deficits in risk allele carriers.

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ABSTRACT

Objective: The Schizophrenia Psychiatric Genome-wide Association (GWAS) Consortium recently reported on five novel schizophrenia susceptibility loci. The most significant finding mapped to a microRNA, MIR-137, which may be involved in regulating the function of other schizophrenia and bipolar disorder susceptibility genes.
Method: We genotyped 821 patients with confirmed DSM-IV diagnoses of schizophrenia, bipolar affective disorder I and schizoaffective disorder for the risk SNP (rs1625579) and investigated the clinical profiles of risk allele carriers using a within-case design. We also assessed neurocognitive performance in a subset of cases ($n = 399$) and controls ($n = 171$).
Results: Carriers of the risk allele had lower scores for an OPCRIT-derived positive symptom factor ($p = 0.04$) and lower scores on a lifetime measure of psychosis incongruity ($p = 0.017$). Risk allele carriers also had more cognitive deficits involving episodic memory and attentional control.
Conclusion: This is the first evidence that the MIR-137 risk variant may be associated with a specific subgroup of psychosis patients. Although the effect of this single SNP was not clinically relevant, investigation of the impact of carrying multiple risk SNPs in the MIR-137 regulatory network on diagnosis and illness profile may be warranted.

1. Introduction

The Schizophrenia Psychiatric Genome-Wide Association (GWAS) Consortium recently reported on the largest molecular genetic investigation of schizophrenia to date [9]. The study, a meta-analysis of GWAS data, included 9394 cases and 12,462 controls; top loci were than evaluated in a replication sample of 8442 cases and 21,397 controls. This confirmed two previously identified risk loci and identified five novel loci, of which the most significant finding mapped to a single nucleotide polymorphism (SNP) (rs1625579; $p = 1.6 \times 10^{-11}$) within the precursor for microRNA 137 (MIR-137), a known regulator of neuronal development [9]. The odds ratio for this risk allele was found to be 1.12. The study adds to a growing list of common and rare genetic risk variants being implicated in schizophrenia susceptibility, although most of the population variance in risk is yet to be explained [22].

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A key question, which has both diagnostic and therapeutic implications, is whether schizophrenia etiology involves one or many different molecular risk mechanisms. Although the associated 'T' allele at SNP rs1625579 has a modest overall effect on schizophrenia risk (OR = 1.12), it is of interest as it may implicate a particular molecular risk mechanism. MicroRNAs (miRNAs) are small non-coding RNAs that play a regulatory role in cellular processes, including brain functioning, by regulating the function of potentially hundreds of genes through RNA interference. MIR-137 has been directly implicated in regulation of neuronal maturation [17] and adult neurogenesis [16,18]. In the Psychiatric GWAS Consortium (PGC) study [9], SNPs mapping to the 301 high-confidence predicted gene targets of MIR-137 were more likely to be associated with schizophrenia than would be expected by chance. Gene targets of MIR-137 include the bipolar disorder susceptibility gene CACNA1C, suggesting that MIR-137 mechanism may have a wider impact on psychosis risk. A small GWAS study by Potkin et al. [15] reported modest association between genetic variants in the gene network regulated by MIR-137 and reduced dorsolateral prefrontal cortex (DLPFC) activation during a working memory task. Predicted target genes of MIR-137 include 4 genes which have reached genome-wide significance in schizophrenia studies, namely CSMD1, C10orf26, CACNA1C and TCF4 [12]. There has not been evidence to date of altered MIR-137 expression in either peripheral tissue or brain tissue in individuals with schizophrenia [3].

The wealth of evidence supporting overlap of heritability across schizophrenia, bipolar disorder and schizoaffective disorder [13,19] taken with the identification of the psychiatric diagnosis as "the weak component of modern research" [1], the overlap of symptoms across diagnostic entities, and the differing clinical manifestations of each individual diagnosis throws into relief longstanding debates over the validity of Kraepelin's dichotomy [11]. A convincing argument has been made for the use of more complex models [6] in psychiatric research, to avert the problems associated with a categorical diagnostic approach, which lose information regarding symptomatic experience of illness.

The aim of this study was to investigate whether carriers of the risk allele at MIR-137 represented a specific psychosis subgroup as defined by clinical or neuropsychological features. To test this hypothesis we examined clinical profiles of psychosis patients (n = 821) using a within case design to determine if carriers of the MIR-137 risk allele (rs1625579) had different symptom profiles. Using a dimensional approach facilitated the inclusion of subjects with bipolar disorder and schizoaffective diagnoses, as the dimensional approach favors the capture of subtle differences in clinical manifestations both within and across diagnostic categories.

We also assessed whether carrying this allele was associated with differences in neurocognitive performance in a subset of cases (n = 399) and controls subjects (n = 171).

2. Methods

2.1. Subjects and assessment

Subjects were recruited through community and inpatient mental health facilities throughout the island of Ireland for genetic studies of psychotic disorders. The sample was a convenience sample. Treating teams nominated potential participants, who were then invited to meet researchers. Where individuals were identified in an acute phase of their illness, interview was deferred. Approximately 20% of nominated participants declined to partake. Of the 902 participants recruited by the time of this analysis, 81 were excluded from further analysis (diagnoses of delusional disorder, OCD, intellectual disability, epilepsy, bipolar affective disorder II, psychotic disorder not otherwise specified). All participants provided written informed consent and were interviewed using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID) [8]. Diagnosis of a major psychotic disorder was made by the consensus lifetime best estimate method using DSM-IV criteria with all available information – interview, the Operational Criteria Checklist for Psychotic Illness (OPCRIT) [14], family or staff report, and chart review. All cases were over 18 years of age, of Irish origin (with 4 Irish grandparents) and had been screened to exclude substance-induced psychotic disorder or psychosis due to a general medical condition. The current study included 821 patients with a DSM-IV diagnosis of schizophrenia (N = 573), schizoaffective disorder (N = 123) or bipolar affective disorder I (N = 125). Further demographic details on subjects have been published elsewhere [10].

The sample for neurocognitive testing consisted of 399 cases and 171 controls. Cases consisted of clinically stable patients with a DSM-IV diagnosis of SZ (n = 329) or schizoaffective disorder (n = 70) recruited from 5 sites across Ireland. Other clinical characteristics of the clinical sample are detailed elsewhere [20].

The healthy control sample was recruited on the basis of responses to local media advertisements. Control participants were only included if they were aged between 18 and 65 and satisfied, based on clinical interview, the criteria of having no history of major mental health problems, intellectual disability or acquired brain injury, and no history of substance misuse in the preceding 6 months based on self report. Control participants were also excluded from the study if they reported having a 1st degree relative with a history of psychosis.

The Bipolar Affective Disorder Dimension Scale (BADDS), developed by Craddock et al. [5] as an adjunct to conventional categorical diagnosis, was used as an additional measure of lifetime symptomatology, in order to capture a more complete description of frequency and severity affective and psychotic episodes, which can be lost in hierarchical diagnoses. The BADDS provides a measure of severity over the course of illness for manic, depressive, psychotic, incongruent dimensions. The four dimensions – mania, depression, psychosis and incongruence are each rated as an integer on a 0–100 scale. The range within which the score lies is informed by the severity of the worst episode, and within that range it is determined by the number of episodes. Anchor points are clearly defined. For example, an individual who has experienced a number of hypomanic episodes, but no manic episodes, would score between 40 and 59 in the mania dimension, depending on the number of episodes. An extra point is scored for the number of similar episodes, while half a point would be added for each less severe episode.

The BADDS is a particularly useful instrument as it is able to accommodate sub-clinical features, discriminates between illness severity within disease category and show similarities in course of illness in individuals within different disease categories. The initial validation study included subjects with schizophrenia [5], and a further reliability study has been completed in schizoaffective disorder [21].

The Operational Criteria Checklist for Psychotic Illness (OPCRIT) was developed, by McGuffin and Farmer [14], as a computer suite of programmes to facilitate a polydiagnostic approach. It involves a 90 item checklist. 30 items relate to background information, while 60 items apply to the presence or otherwise of clinical features or symptoms. Scoring is typically between 0 and 2, with 1 typically indicating a symptom having been present for no more than a few days.

Neuropsychological assessment focused on the domains of (1) general cognitive ability (IQ) as measured by an abbreviated version of the Wechsler Adult Intelligence Scale (WAIS-III); (2) verbal episodic and working memory as measured by the Logical

Table 1
RS1625579 SNP genotype groups and demographic variables.

	GG (n = 26) Mean (SD)	TG (n = 231) Mean (SD)	TT (n = 547) Mean (SD)	F/X^2 value	p value
Age at onset	21.17 (7.069)	24.23 (8.297)	24.03 (8.476)	1.388	.250
Duration of illness (years)	14.9148 (15.43135)	19.9946 (12.26666)	19.9617 (12.30284)	1.850	.158
Chlorpromazine equivalent (mg)	412 (412.838)	506.86 (449.29)	468.86 (411.856)	.730	.483
Gender (% male)	84.0%	62.67%	66.16%	4.683	0.096

Memory (LM), Letter Number Sequencing (LNS), and digit span sub-tests from the Wechsler Memory Scales (WMS-III); (3) spatial episodic and working memory as assessed using the CANTAB paired associate learning task and the Spatial Working Memory task; and (4) attentional control as assessed using the CPT-IP. Further details on the neuropsychological assessment are reported elsewhere by Walters et al. [20].

2.2. Genotyping

The SNP rs1625579, was genotyped using a Taqman® SNP Genotyping Assay on a 7900HT Sequence Detection System (Applied Biosystems). The call rate for was >95%. Both case/control samples were in Hardy–Weinberg Equilibrium (HWE; $p > 0.05$). A number of HapMap CEU DNA samples ($n = 90$: www.hapmap.org) were genotyped for quality control purposes. All genotypes were found to be concordant with available online HapMap data.

2.3. Statistical analysis

The risk 'T' allele is common, but because there is insufficient basis to test a specific genetic model (e.g. dominant or recessive) our primary analysis was based on all three genotype groups TT ($n = 547$), TG ($n = 231$) and GG ($n = 26$). Where significant differences were detected these were further explored in a two-group analysis of homozygous 'T' risk allele carriers versus carriers of 1 or 0 risk alleles.

We investigated for association between rs1625579, and demographic variables which could potentially have relationships with lifetime dimension scores, using Chi-squared tests and ANOVAs. ANOVAs were performed examining for interactions between each genotype group and each of the four BADDS dimension scores for manic, depressive, psychotic and incongruent symptoms. For the factor analysis, we selected the 60 signs and symptoms within the Operational Criteria Checklist for Psychotic Illness (OPCRIT) to enter into exploratory factor analysis with VARIMAX rotation, using SPSS 16.0. Variables not directly associated with phenomenology (e.g. source of rating, duration of illness) were excluded. We used the scree plot, displayed in Supplementary Fig. 1 to determine the number of factors that best accounted for the covariance among these items. We selected items with loadings of 0.4 or greater to create factor-derived scales, yielding 44 items. A five factor solution gave an interpretable pattern of factors, namely manic, depressive, positive, disorganized and negative factors. These five factors accounted for 47.58% of the total variance. The Kaiser–Meyer–Olkin Measure of Sampling Adequacy was 0.856, sufficient for a satisfactory factor analysis to proceed. Bartlett's test of sphericity was 14,958.995, $p < 0.0001$. Factor score coefficients were then calculated in SPSS 16.0 using the regression method. Having derived the factor scores, we then performed MANOVAs looking for an association between SNP rs1625579 status and each of the five identified factors.

Association between MIR-137 rs1625579 and the phenotypes of general cognitive function, episodic memory, working memory and attentional control were tested using a general factorial design in the same statistical package. Our analysis was based on a comparison of the three genotype groups (TT versus TG versus GG) and diagnosis (cases versus controls), both of which were entered as fixed effects. In a series of ANCOVAs scores for each neuropsychological subtest were entered as the dependent variables, with age and gender included as covariates where appropriate.

3. Results

The final clinical analysis included 803 cases who passed genotyping QC. Demographic and clinical characteristics by rs1625579 genotype (TT, GT, and GG) appear in Table 1. There was no association between the risk SNP and diagnosis (schizophrenia, schizoaffective disorder or bipolar disorder), current age, gender, or medication dosage in chlorpromazine equivalents (Supplementary Table 1). The risk allele for SNP rs1625579 was associated with lower BADDS incongruence dimension scores ($p = 0.017$) as well as lower OPCRIT-derived positive symptom scores ($p = 0.041$). When analyses of the relationship between the BADDS incongruence groups were restricted to diagnostic groups, the signal

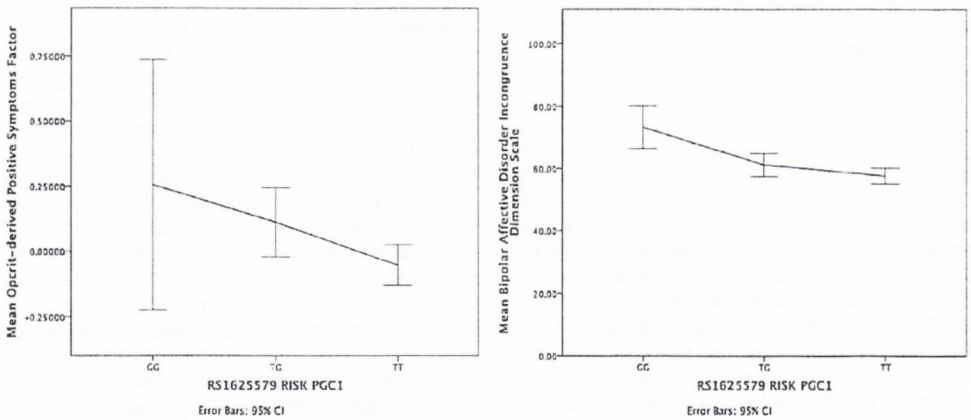

Fig. 1. Relationships between genotype groups and Opcrit-derived positive symptom factor score, Bipolar Affective Disorder Incongruence Dimension Score.

Table 2
RS1625579 (PGC1) stats results table (2 groups).

Cognitive function	Test or subscale	Sample	*n*	Mean (SD) GG/GT	Mean (SD) TT	$F_{\text{Case versus Controls}}$	*p*	$F_{\text{Main effect}}$	*p*	$F_{\text{Interaction effect}}$	*p*
IQ	Abbreviated Full Scale IQ	Patients	275	93.31 (17.82)	90.63 (17.19)	293.1	<.0001	2.384	.123	.410	.522
		Controls	133	124.33 (12.2)	121.82 (15.4)						
	WTAR (adult reading test)	Patients	275	96.45 (11.93)	97.32 (11.32)	.012	<.0001	.012	.461	0	.985
		Controls	133	110.06 (3.73)	109.55 (5.34)						
	Verbal IQ	Patients	275	94.06 (16.02)	93.03 (17.51)	282.1	<.0001	.817	.367	.079	.778
		Controls	133	125.79 (13.8)	123.7 (14.99)						
	Performance IQ	Patients	275	93.75 (20.8)	89.71 (18.59)	173.4	<.0001	3.04	.051	.099	.753
		Controls	133	119.33 (13.5)	116.03 (19.3)						
Working memory	LN sequence	Patients	370	8.162 (3.548)	7.51 (3.24)	.014	.924	1940	.014	.005	.956
		Controls	167	13.38 (2.91)	12.78 (3.34)						
	CANTAB SWM (between error)	Patients	366	−.80 (1.32)	−1.02 (1.3)	96.98	<.0001	.628	.149	1.186	.277
		Controls	159	.237 (.716)	.281 (.823)						
Episodic memory	Logical Memory Immediate	Patients	379	7.01 (3.52)	6.18 (3.31)	346.2	<.0001	1.535	.023	1.72	.19
		Controls	167	12.75 (2.54)	12.72 (2.95)						
	Logical Memory Delayed	Patients	379	7.46 (3.19)	7.21 (3.21)	369.1	<.0001	.035	.244	.354	.552
		Controls	167	13.29 (2.4)	13.34 (2.65)						
	CANTAB PAL (adjusted standard score)	Patients	291	−2.65 (3.84)	−2.77 (3.77)	53.89	<.0001	.001	.297	.092	.762
		Controls	123	.1592 (1.2)	.2654 (1.08)						
	Faces 1	Patients	355	8.69 (2.83	8.76 (2.77)	85.1	<.0001	.221	.639	.031	.861
		Controls	168	11.14 (2.64)	11.54 (2.88)						
	Faces2	Patients	355	9.2 (2.86)	9.34 (2.93)	46.5	<.0001	2.054	.152	.845	.358
		Controls	168	11 (2.46)	11.63 (2.84)						
Vigilant attention	CPT_IP (3 letters)	Patients	74	1.85 (1.01)	2.02 (.973)	–	–	1.457	.229	–	–
		Controls	0								
	IDED (8 shapes adjusted)	Patients	322	10.85 (10.65)	12.93 (10.97)	17.85	<.0001	5.16	.047	.042	.838
		Controls	157	7.05 (7.95)	9.39 (10.15)						
	IDED (6 shapes adjusted)	Patients	322	1.05 (2.85)	.812 (1.49)	2.53	.112	.204	.652	2.738	.099
		Controls	157	.381 (.652)	.814 (2.3)						

was strongest amongst those with a diagnosis of bipolar disorder ($p = 0.024$, partial eta squared = 0.060). These findings were confirmed in a two-group analysis, where having two copies of the risk allele was associated with significantly lower incongruence dimension ($p = 0.019$) and positive symptom factor ($p = 0.015$) scores, compared with having one or no copies of the risk allele in this psychosis sample. Partial eta squared for both scores is 0.007. The relationship between genotype and the scores for these measures (shown in Fig. 1) suggests an allele dosage effect.

Mean scores for each of the four cognitive domains (IQ, working memory, episodic memory and attention) by MIR-137 genotype group for cases and controls are presented in Table 2. As expected, patients performed below controls on all cognitive tests administered ($p < 0.0001$). MIR-137 genotype was not associated with differences in IQ, either as a main effect or as an interaction effect with case–control status. Analysis of the three allele groups (TT, GT, and GG) identified association between groups carrying 'T' risk allele and verbal episodic memory (Logical Memory, Immediate; $F = 1.16$, $p = 0.048$) and extradimensional set shifting (as measured by the IDED-ED scores) (attentional control phenotypes; $F = 3.23$, $p = 0.04$). In each case carrying the risk allele was associated with worse performance; there was no interaction effect with case/control status. Given the small number of homozygous risk carriers, these were repeated in a two-group analysis comparing the homozygous carriers of the 'T' risk allele with all other patients; both findings were confirmed.

4. Discussion

A common genetic variant at MIR-137 has recently emerged as a risk factor for schizophrenia. We investigated the role of the MIR-137 risk SNP (rs1625579) on psychosis symptom factors and dimensional measures of lifetime symptomatology. We identified association between the 'T' risk allele and lower scores for an OPCRIT-derived psychotic symptom factor. Carriers of the risk allele also scored lower on a lifetime measure of psychosis-symptom incongruity. This finding suggests that carriers of the MIR-137 risk allele are more likely to represent a subgroup of psychotic patients with fewer psychotic symptoms, where these symptoms are mood-congruent. There was no converse finding of an increased burden of negative symptoms, and there was no evidence that the variant influenced mood symptomatology directly within our cohort. One possible explanation is that this group represents patients defined by a particular molecular aetiology. Alternatively, the risk variant may have a modifying effect which increases risk of mood congruent psychotic symptoms among psychosis-spectrum patients, where psychotic symptoms are experienced.

While cognitive deficits are less prominent in bipolar disorder than in schizophrenia, where both global and specific cognitive deficits are described, a range of abnormalities have been reported including deficits in episodic and working memory, processing speed and sustained attention [2,4]. The cognitive deficits associated with MIR-137 in this study affected domains reported to be impaired in both disorders. Specifically, risk allele carriers did not show deficits in global function as measured by IQ, but did show subtle deficits in both episodic memory and attentional control. In both cases, the deficits were more prominent in a 2-groups comparison of homozygous risk carriers versus the other genotype groups combined. Previous work by our group suggested that deficits in attentional control disassociate from more general cognitive deficits within the schizophrenia population [7]. Whether the subtlety of these effects reflects this risk variant's particular association with an affective form of psychosis in which cognitive deficits are less pronounced, or simply reflect the modest cognitive effects of this variant remains to be elucidated. Further studies, incorporating functional MRI may be helpful in establishing the true strength of this effect.

Multiple statistical tests were performed in this study and the findings could reflect Type 1 errors. However, although a relatively large clinical sample ($n = 831$), our study was significantly underpowered to detect subtle clinical effects compared to large GWAS samples such as the PGC. We tried to control for the temporal instability of clinical symptomatology by using two different measures of psychopathology in a cohort of psychosis patients with generally well-established illness (mean duration of illness 19 years, SD 12 years). One measure captured the life-time presence of different symptoms (OPCRIT), while the other measured severity and frequency of illness episodes over time. Our psychosis sample was over-represented for schizophrenia cases ($n = 573$) and the life-time measure employed (BADDSs) was developed and validated for use in bipolar disorder subjects. Further studies using serial assessment of clinical symptoms and neurocognitive performance are warranted.

It is important to note that MIR-137 genotype was responsible for <1% of the variance in scores (based on the associated partial eta squared values) on the incongruity dimension and psychosis factor. This indicates that the common risk variant at MIR-137 does not have a clinically meaningful effect on either symptom profile or cognitive performance. In the Psychiatric GWAS Consortium (PGC) study, SNPs mapping to the 301 high-confidence predicted gene targets of MIR-137 were enriched for association signals with schizophrenia, compared with other genes of similar size or genetic marker density in the genome. Excluding the gene itself and the major histocompatibility complex region (MHC), 4 of 9 associated loci in a combined meta-analysis of schizophrenia and bipolar GWAS data had predicted MIR-137 target sites, i.e. TCF4, CACNA1C, CSMD1, and C10orf26 [9]. Further questions raised by this study, are whether having a greater burden of common risk variants from this gene network is associated with increased illness risk, the extent of this risk, and whether this maps a molecular subtype of psychosis characterized by less psychotic or incongruent psychotic symptomatology and subtle cognitive deficits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2012.08.065.

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THE COGNITIVE GENETICS OF NEUROPSYCHIATRIC DISORDERS

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1. Introduction

Classification in psychiatry is heavily dependent on clinical symptoms and illness course. This ignores the critical role that cognitive problems play in neuropsychiatric disorders affecting different domains across the lifespan, from ADHD and autism to schizophrenia and Alzheimer's disease. At this point, it is unclear whether aberrant cognitive mechanisms are specific to disorders, whether multiple disturbances in cognitive processes can contribute to the same disorder, or whether aberrant neural processing can result in many different phenotypic outcomes. Understanding this would allow us to better grasp normal as well as pathological brain function. This could inform diagnostics based on understanding of neurophysiological processes and the consequent development of new therapeutics.

Genetics, and the development of genomic research, offers real opportunities to understand the molecular mechanisms relevant to cognition. This chapter defines and describes the main cognitive phenotypes, which are investigated in psychiatric disorders. We review evidence for their heritability and early progress in the field using cytogenetic, linkage and candidate gene-based research methodologies. With high-throughput genomics it is now possible to explore novel common and rare risk variants for psychiatric disorders and their role in cognitive function at a genome-wide level. We review the results of early genomic studies and discuss the novel insights that they are starting to provide. Finally, we review the analysis of whole-genome DNA sequence data and the challenges that this will bring for cognitive genomics research.

2. Selection of cognitive deficits in neuropsychiatric genetics.

Historically, no aspect of cognition (or any human trait for that matter) has received more attention in terms of its underlying genetics basis than general cognitive ability or intelligence. While this history has not been without controversy (cf. the eugenics movement), the genetic basis of intelligence has been studied widely in both the general population and across psychiatric disorders.

A key question in the selection of specific cognitive phenotypes is what extent performance on a given task is heritable. Differing degrees of relationships within families, associated with more or less sharing of genetic material (e.g. monozygotic twins share 100% of genes, dizygotic twins/siblings 50%, and half-siblings 25%) allow estimation of the proportion of individual differences in performance in a population at a given time that are due to genetic differences (termed heritability (h^2)). The availability of twin and population-based disease register data has confirmed the importance of heritability for many psychiatric disorders. Less information has been available to allow interpretation of the heritability of cognitive deficits within twin samples. Because of this practical sampling constraint, most epidemiological studies have used a family-based design to investigate cognitive deficit in healthy relatives of

patients. The finding that this group has higher rates of a particular cognitive deficit is taken as suggestive evidence of heritability.

The total variance in general intelligence that can be attributed to genetic influences range from 30 to 80%. Broad domains of cognitive ability, such as verbal and perceptual abilities show similar measures of genetic influence (Posthuma et al, 2001), although the genetic influence on sub domains such as memory tends to be smaller, due to measurement error and variance, but being highly correlated with general intelligence, genetic effects overlap. The heritability of general intelligence increases with age to 70–80% in adulthood (Jacobs et al, 2007). Variations in brain structures such as the density and the volume of grey and white matter, amygdala and hippocampus, and overall brain volume are thought to be endophenotypes for intelligence. Therefore, genes involved in intelligence might be more closely associated with variations in brain structure and function than to measures of intelligence.

By comparison, selection of specific cognitive functions for analysis in genetic studies has been heavily influenced by the kinds of deficits observed within specific disorders. This selection has also been strongly influenced by the perceived potential of a cognitive function or process as 'intermediate phenotypes' or 'endophenotypes' for the disorder. The 'endophenotype' concept in psychiatry (described by Gottesman & Gould, 2003) relates to the identification of heritable quantifiable characteristics, which may be useful targets for genetic studies as they represent some intermediate stage between genotype and clinical disorder. The authors suggested that the potential utility of an endophenotype can be judged against a set of criteria:

1. Association with illness in the population
2. Co-segregation of the endophenotype with illness in families
3. Evidence that this is genetically mediated (i.e. heritable)
4. Evidence that the endophenotype is state-independent (i.e. present whether illness is active or not)
5. That the endophenotype can be measured accurately and reliably
6. The endophenotype is also shared by non affected family members of the proband, this may be advantageous for genetic studies

Across disorders, deficits in executive function, memory function and attentional control have each been a particular focus for research. Clinical awareness of these impairments has increased as it has been established that such deficits are predictive of psychiatric morbidity. In the case of schizophrenia for example, cognitive deficits are present from an early stage of the disorder and often predate the emergence of clinical symptoms (Erlenmeyer-Kimling et al, 2000). They are relatively stable over time and closely related to functional outcome (Green et al, 2004). This includes deficits in general cognitive ability (Donohoe et al, 2009b) and specific deficits in working and episodic memory (Donohoe et al, 2009a) and attentional control (Bellgrove & Mattingley, 2008; Donohoe et al., 2009a). Genetic epidemiological research using family and twin studies indicates that some of these deficits may themselves have a substantial genetic component (Goldberg et al., 1990/1995; Cannon et al., 2000). While cognitive deficits are somewhat correlated with clinical symptoms (for example, negative symptoms in schizophrenia) the amount of variance shared by these variables appears to be small, and cognitive function often emerges as a separate factor from clinical symptoms in factor analysis (Donohoe & Robertson, 2003). Taken together these data indicate that cognitive phenotypes in schizophrenia are heritable, trait-like and generally independent of clinical symptomatology.

Mood disorder researchers, investigating bipolar disorder and recurrent depressive disorder have focused less on general cognitive ability and more on memory related phenotypes (Thomas & Elliott, 2009; Frodl, 2009). In attention deficit hyperactivity disorder (ADHD), attention has inevitably been a particularly important focus, both in terms of orienting attention, sustaining attention, and inhibiting processing of task-irrelevant stimuli (Bellgrove & Mattingley, 2008). In autism, impairments in overall cognitive ability are found in 50% of affected individuals. In higher functioning individuals deficits in executive functioning (specifically attention orienting, response inhibition and set shifting) are reported.. The most widely investigated area of cognition in autism relates to impairments in social cognition. Social cognition is the ability to process social information, thought to be fundamentally impaired in autism.

2.1 Social cognition and psychiatric disorder

A significant development in cognitive genetic studies of psychiatric disorders has been the increased focus on the genetics of social cognition that began with research in autism. Social cognition is the sum of those processes that allow individuals of the same species (conspecifics) to interact with one another (Frith & Frith, 2007), specifically it refers to the set of skills that allow us to understand the thoughts and intentions of others and frequently involves the investigation of social information processing, especially its encoding, storage, retrieval, and application to social situations. Essentially it depends upon the exchange of signals, such as speech, facial expression, body posture and eye gaze (Frith & Frith, 2007). Signals such as these can be socially informative in that they tell us what someone may be feeling (Vuilleumier, & Pourtois, 2007), where they are focusing their attention, and what they are intending to do (Frith & Frith, 2006). The ability to process, comprehend and act appropriately to these signals is tantamount to social success and relies on coordination of several cortical regions, e.g. dorsomedial and dorsolateral prefrontal cortices, the paracingulate cortex and the right and left temporoparietal junctions and amygdala (Mitchell 2009). In several psychiatric disorders this ability is impaired leading to social misperceptions, unexpected reactions to and from the person, and social withdrawal (Green et al, 2005), resulting in difficulties with maintaining friendships, employment, and general community functioning (Penn et al, 2008). Alongside these clinical and outcome goals, there is increasing interest in identifying the neural basis underlying social cognitive deficits in psychiatric illness. As such, further research in this domain is viewed as highly valuable (Green et al, 2005).

Currently, social cognition is investigated both in behavioural terms and in terms of neuroanatomy. Some of the tests that fall into the former category include theory of mind (TOM) tests (such as Mind in the eyes, Faux pas and Hinting task), the Hotel Task, Multiple errands task, Iowa gambling task, the Social Cognitive Skills Test (SCST), Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT), the Penn emotional recognition task, Facial Affect recognition, Attribution test and tests of self-control (see Table 1 for task summaries). The latter category however is most often assessed using magnetic resonance imaging (MRI) and involves performing simple social cognitive tests such as facial aspect recognition, and imaging the brain to see what areas are activated during task performance.

	TEST	DEFINITION/USAGE
Emotion Recognition	MSCEIT	Measures emotional intelligence, namely 4 areas: **Perceiving Emotions, Facilitating Thought, Understanding Emotions, Managing Emotions**
	Penn emotional recognition task	A computer-based test that includes 96 color photographs of facial expression of evoked—or felt—emotions: happy, sad, angry, fearful, disgusted, and nonemotional or neutral
	Facial affect recognition	Facial Emotion Identification Test (FEIT) and the Facial Emotion Discrimination Test (FEDT). Both use black and white photographs of facial emotions that are presented on DVD.
Theory of Mind	TOM	A "Theory of Mind" (often abbreviated to TOM) is a specific cognitive ability to understand others as intentional agents, that is, to interpret their minds in terms of theoretical concepts of intentional states such as *beliefs* and *desires*.
	Mind in the eyes	Measure of adult mentalising. The ability to deduce emotion from looking at photographs of people's eyes.
	Faux pas test	The faux pas test ascertained the participants' ability to identify and understand a social faux pas, and to understand the mental states of the characters (the speaker and the recipient) in a conversation with a social faux pas.
	Hinting task	Mental state reasoning, infering . Measures ability of subject to 'read between the lines' in a social context.
Attribution	Attribution test	How one assigns causality in a social context.
Social Perception	Social cognitive skills test (SCST)	Focuses on the assessment of social reasoning skills.
	Iowa gambling task	Decision making abilities. Looks at trust and the ability to analyse intentions.
Self Regulation	Self control test (implicit association test (IAT))	Measure the strength of automatic association between mental representations of objects (concepts) in memory

TABLE 1: Definition and usage of various social cognitive tests

To investigate the utility of these measures of social cognition as psychiatric 'endophenotypes' (based on the criteria outlined above) we undertook a review of the main tests found in the literature. Search included test name, heritability, twin, sibling, state, independent, co-segregation, brain region, and endophenotype. Secondary search terms used in the event of the primary terms yielding no results included family, trait, dependent, gene, psychiatric, schizophrenia and autism. A list of the common measures of social cognition reviewed and evidence of their feasibility as endophenotypes is presented in Table 2. As with

neuropsychological measures most of the evidence of heritability for each of the measures of social cognition reviewed is derived from evidence that family members of psychiatrically affected probands also show deficits at a level intermediate between cases and healthy controls. These deficits have been associated with a number of candidate risk genes for psychiatric disorders, including 5HT2A, Oxytocin, and vasopressin.

3. Testing cognitive deficits in neuropsychiatric genetics.

Cognitive neuroscience approaches to investigating both cognitive performance in the healthy population and cognitive disability associated with psychiatric disorders have included both behavioral and neuroimaging paradigms. Behavioral measures include traditional neuropsychological paper and pen tasks (e.g. the child and adult versions of the Wechsler Intelligence Scales, the Wechsler memory scales) and computer based tasks (e.g. various versions of the Continuous Performance Task (CPT); the Cambridge automated test battery). The main advantage of these neuropsychological tests is that they generally have well-established psychometric properties that can be easily administered to large groups of varying ability, a key requirement for cognitive genetics research. Imaging modalities have included both functional Magnetic Resonance Imaging (fMRI) during tasks of memory, attention and - more recently – affective processing, with the high spatial resolution it offers, and electroencephalogram recordings (EEG), with the high temporal resolution it provides. While, previously, individual groups tended to specialize in one main approach, groups working in this area increasingly aim to integrate findings across modalities. In our own group for example, we have used high density EEG and structural MRI to follow up on specific associations between individual genetics variants and neuropsychological test performance, such as the contribution of abnormal sensory level processing to cognitive performance deficits (Donohoe et al, 2008).

Across disorders, available family and twin studies generally support the concept that the cognitive deficits associated with these disorders are themselves inherited. In the case of schizophrenia, the heritability of a number of specific deficits have been confirmed by twin studies, including general cognitive ability, working memory, and episodic memory (Goldberg et al, 1990/1995; Cannon et al., 2000; Kremen et al, 2006; Touloupoulou et al, 2007/2010). The heritability of deficits in general cognitive ability and working memory in particular appear to overlap strongly with the heritability of illness risk in schizophrenia (Touloupoulou et al, 1997) although not to the point of suggesting an identity between the genetic architecture of schizophrenia and cognition. In autism research, deficits in general cognitive ability, executive functioning, and processing of socially relevant information (e.g. face recognition) have consistently been reported (Adolphs et al, 2001). The most consistent familial traits are language and communication skills, insistence on sameness and non-verbal IQ (Szatmari, White et al. 2007). In ADHD, cognitive studies find widespread abnormalities in children and adults with the disorder, particularly in the executive function domains of response inhibition/delay aversion and sustained attention (Willcutt et al, 2005) and in reaction time variability (Klein et al, 2006), one of the most discriminating between ADHD and controls. Attention, and in particular, sustained attention, deficits are prominent in ADHD, with unaffected siblings performing better than their affected siblings but worse than healthy controls (Slaats-Willemse et al, 2005). Sustained attention has been examined in preschool twins using the Amsterdam Neuropsychological Tasks set with correlations higher for MZ compared to DZ twins suggesting a heritability of 0.46 – 0.72 for this measure (Groot et al, 2004). Yet many proposed endophenotypes in ADHD have not been examined in classical population based twin studies. It is not yet clear if these impairments are related or

perhaps have separate aetiological pathways. Kuntsi, et al (2010) examined this in a large sample of ADHD probands and their siblings using a multivariate familial factor analysis approach. The results suggest that two familial phenotypes, mean reaction time/reaction time variability and omission/commission errors on the go/no-go task reflect 85-98% and 13% of the familial variance of ADHD respectively. The findings for response time variability reflect recent population twin data (Wood et al, 2010).

4. Cytogenetics and cognitive phenotypes

Cytogenetic methods to investigate chromsomes microscopically, initially allowed gross examination of chromosomes, incorporating staining methods which identified cytogenetic bands on chromosomes; these evolved to molecular hybridization methods, known as fluorescent in situ hybridization (FISH). Cytogenetics methods contribute to localising susceptibility genes by identifying chromosomal abnormalities such as deletions or translocations, which segregate with a disorder in families or are found more commonly in cases than in control populations.

A number of these (described further below) have been identified for childhood disorders affecting cognitive ability and mental health, including sex chromomosome aneuploidies such as 47XYY, Klinefelters and Turner's syndrome; fragile X, Williams' syndrome, and autism. In adult onset disorders, two cytogenetic abnormalities have provided consistent evidence that the chromosomal regions involved contribute to disease risk. One is a balanced translocation t(1;11)(q42.1;q14.3) that co-segregates with schizophrenia within a large Scottish pedigree. The other involves one of the commonest known chromosomal abnormalities, small interstitial deletions of chromosome 22q11. The latter cause velo-cardio facial syndrome (VCFS), which increases the risk of psychosis by at least 20-fold, and also presents with cranio-facial dysmorphology and congenital heart disease. In carriers of these cytogenetic abnormalities, both general and specific cognitive effects are associated, including language deficits (Williams syndrome, autism), specific learning deficits (e.g. mathematical ability in Turner's syndrome, Williams syndrome), and memory deficits (Schizophrenia); these are discussed next.

4.1. Cytogenetics of childhood-onset neuropsychiatric disorders

Sex chromosome aneuploidies (SCAs), where there is variation in the number of sex chromosomes, are the most common chromosomal abnormalities occurring in 1 per 400 births. The commonest syndromes include the 47XYY syndrome, Klinefelter Syndrome (47XXY) and Turner's Syndrome (XO). Frequently deficits in speech and language, motor skills and educational achievement are reported in relation to all SCAs although in general affected individuals usually live independently and the severity of cognitive deficits is not clear (Leggett, Jacobs et al. 2010). Males with 47XYY frequently present normally and the phenotype may go undetected. In general in SCAs, the presence of an additional X chromosome is frequently associated with lowered verbal IQ. Klinefelter Syndrome is associated with executive function deficits (Samango-Sprouse 2010). The absence of an X chromosome in girls is also associated with cognitive deficits. Turner Syndrome (XO), an SCA affecting girls is characteristically associated with visual-spatial and executive function deficits and social cognitive impairments (Hong, Scaletta Kent et al. 2009). Mathematical learning difficulties are prevalent in Turner Syndrome which may relate to deficits in executive function or spatial deficits although this is not clear (Mazzocco 2009). Non-verbal learning deficits are present in 80% of girls with Turner Syndrome. The syndrome is also

associated with social cognitive deficits such as facial emotion recognition and gaze perception that are also reflected by reported neuroanatomical abnormalities in the amygdala (Burnett, Reutens et al. 2010).

William Beurens Syndrome (WBS) is a contiguous gene syndrome arising from a deletion of approximately 1.5-1.8Mb affecting 28 genes on chromosome 7q. It is associated with a characteristic physical phenotype and a cheerful, sociable manner. Individuals with WBS frequently have intellectual disability but present with superficially good language ability. They present with poor visuo-spatial ability (Pober 2010). A microduplication syndrome of the same region has also been described with somewhat contrasting phenotypic characteristics; intellectual disability is also reported; speech and language deficits are more common while visuo-spatial functioning is spared (Merla, Brunetti-Pierri et al. 2010). Both the duplication and deletion syndromes are reported to be associated with ADHD and autistic type deficits; social deficits and aggression are more characteristic of the duplication syndrome. A clear relationship between specific genes within the affected region and the cognitive phenotype has not been determined, however several interesting candidate genes exist. Frizzled drosophila homolog of 9 (FZD9) is expressed in the hippocampus and null mouse mutants have defects in memory and learning although WBS related phenotypic features are not universally reported (Ranheim, Kwan et al. 2005). The Syntaxin 1A (STX1A) gene is a brain-expressed protein implicated in presynaptic vesicle docking. Expression levels of this gene are correlated with intelligence in WBS (Gao, Bellugi et al. 2010). LIM kinase 1 (LIMK1) is a serine protein kinase predominantly expressed in the nervous system and implicated in synapse formation (Scott and Olson 2007). LIMK1 knockout mice have abnormalities in the dendritic spine morphology and have impaired fear conditioning and spatial learning (Meng, Zhang et al. 2002). CAP-Gly domain-containing linker protein 2 (CLIP2) is expressed widely in the nervous system, including the hippocampus. Knock-out mice have growth deficits, motor co-ordination deficits and altered synaptic functioning in the hippocampus (Hoogenraad, Koekkoek et al. 2002). Clinically, patients with deletions not including CLIP2 have reduced visuospatial impairments and less deficits in gross and fine motor skills (Ferrero, Howald et al. 2010).

Fragile X syndrome, is the commonest cause of inherited intellectual disability. Not specifically a cytogenetic abnormality, it is caused by a trinucleotide repeat expansion (CGG) in the FMR-1 protein on the X chromosome. The condition is associated with autistic type difficulties in social interaction, e.g. gaze avoidance, social anxiety, poor pro-social behaviour and peer relationships. Decreased prefrontal brain activation is reported and reduced frontal and temporal cortical volumes are reported compared with control subjects or individuals with idiosyncratic autism (Hoeft, Walter et al. 2011). Declines in IQ and adaptive function are also reported in longitudinal studies with greater declines in males (Fisch, Carpenter et al. 2010). Declines in central executive and verbal working memory are also reported in later life in males. Working memory deficits correlate with the length of the CGG expansion (Cornish, Kogan et al. 2009). Attentional difficulties, anxiety, aggression and mood symptoms are also reported (Boyle and Kaufmann 2010).

Prader Willi Syndrome (PWS) is a rare genetic syndrome associated with non-expression of a set of genes on the paternal chromosome 15q11-q13. This may be due to deletion of the region on the paternal chromosome (70%) or uniparental disomy (UPD) (25%) involving the maternal chromosome. The region is imprinted and also associated with Angelman's syndrome caused by lack of expression of the *UBE3A* gene originating on the maternal chromosome. Individuals with PWS have a characteristic physical phenotype and usually intellectual disability (approximately 70% of cases), however they often have strengths in

visual perception, reading and vocabulary. Strengths in verbal IQ are reported in UPD compared with carriers of the deletion (Copet, Jauregi et al. 2010). Deficits in auditory processing are reported as are weaknesses in mathematics, visual and auditory memory and auditory attention. Larger deletions in the region tend to be associated with greater impairments in cognitive ability (Milner, Craig et al. 2005). In Angelman Syndrome, deletions are associated with greater cognitive impairment than UPD and cognitive skills are stronger than motor or language skills with relative strengths in the area of receptive language (Gentile, Tan et al. 2010).

Down syndrome is caused by Trisomy for all or part of chromosome 21. Associated with learning disability and early onset dementia, the cognitive profile of individuals with Down Syndrome can be heterogeneous, but relative strengths in receptive language compared with expressive language are reported.

4.2. Cytogenetics of adult-onset neuropsychiatric disorders

Cytogenetic studies have also implicated both a relatively common deletion of chromosome 22q11.2 and a complex, rare translocation as increasing risk for psychotic disorders. The gene Disrupted-in-Schizophrenia-1 (DISC1) was identified at a balanced translocation between chromosome 1 and 11,which strongly co-segregates with mental illness in a Scottish family. Although the index case had a diagnosis of conduct disorder, within the family 18 of 29 (70%) translocation carriers had a major mental illness (schizophrenia, bipolar disorder or major depressive disorder), whereas none of 38 non-translocation carriers had such a diagnosis (Blackwood et al, 2001). Outside this family, there is equivocal support for involvement of the gene as a risk factor for major mental disorders (Porteous et al, 2006) and there has been substantial investigation of its role in neurodevelopment. At a cognitive level, within the affected Scottish family there was no difference in mean IQ between 12 relatives with the translocation and 8 with a normal karyotype. Despite this, unaffected, as well as affected, translocation carriers had abnormalities on event related potential (ERP) P300-typical of schizophrenia and bipolar disorder-suggesting that specific cognitive processing deficits may be important.

Using methods (described in Section 5), Finnish researchers have identified linkage between a locus containing the DISC1 gene and impaired working memory dysfunction (Gasperoni et al, 2003) and visual working memory (Hennah et al, 2005). Two subsequent studies have reported association between markers at the DISC1 locus and impairments in verbal learning and memory (Cannon et al, 2005); working memory (Callicott et al, 2005); and reduced hippocampal volumes (Cannon et al, 2005; Callicott et al, 2005). In assessing the evidence implicating hippocampal dysfunction in DISC1 it is worth noting that different genetic variants have been investigated, with potentially different phenotypic effects (e.g. conflicting evidence for involvement of a DISC1 SNP and cognitive ageing) but none of these have been confirmed as functionally causal.

The 22q11.2 deletion syndrome (22q11.2DS; also known as velo-cardio-facial syndrome (VCFS)) is caused by the most common large microdeletion in the human genome and has an incidence of 1 in ~4000 live births. The phenotype is highly variable and can affect multiple organs and tissues, but carriers have a 30-fold increased risk of schizophrenia and an increased rate of other psychiatric phenotypes including ADHD and autistic spectrum disorders. Many, but not all, carriers fall into the lower than average IQ (FSIQ 70-75) range or have mild learning disability. Most studies, particularly in children, report higher scores on verbal than non-verbal tasks. Investigation of these non-verbal deficits across age groups

indicate impairments in comparisons of magnitude and time duration, which implicate parietal and frontal circuitry underlying attentional and numerical cognition (reviewed, Karayiorgou et al, 2010). However, the ability to inhibit processing of extraneous information is also critical and impaired performance on inhibition tasks is also reported in affected children. Impairments in other aspects of inhibition including prepulse inhibition and reduced frontal lobe activation during the mismatch negativity paradigm have also been reported. These findings indicate that how information is selected or inhibited in attentional processing is important in the non-verbal deficits evident in this syndrome. Some brain regions are either structurally enlarged or reduced in children with 22q11.2DS compared to healthy controls. There appears to be cortical thinning and reduced cortical gyrus complexity in frontal and parietal cortices. The few reported functional and connectivity studies broadly confirm the findings of the neurocognitive tests with performance on arithmetic and spatial attention tasks correlating with frontal and parietal connectivity and functional measures.

There are a number of ways in which loss of genes at this locus could contribute to the expression of these cognitive phenotypes. First, under-expression of a single gene at this locus could exert a major effect on the phenotype. Second, the effect could be the result of under-expression of a number of proximally located genes. Finally, the microdeletion could unmask the effects of one or more recessive mutations. How these effects can be functionally investigated in an animal (e.g. mouse model) is beyond the scope of this chapter, but is reviewed in Karayiorgou at al (2010). The example of 22q11.2DS identified several key issues in cognitive genetics. Unlike the risk variants reported in the association studies of DISC1, 22q11.2DS is uncommon, so ascertainment of sufficient samples for neurocognitive studies is challenging. Compared to DISC1, we are more informed as to the potential molecular mechanisms involved making this locus more tractable for functional studies in model systems. Unlike the DISC1 example, which implicates one gene, the 22q11.2DS locus potentially involves more than 25 genes, which is more biologically challenging. In the next section we consider how advances in genotyping technology have expanded the range of risk loci available for investigation in cognitive genetic studies of neuropsychiatric disorders.

5. Linkage and candidate gene-based approached to cognitive genetics

The identification of common polymorphic genetic markers shared in individuals within populations was a key step for molecular genetics. This made possible two new approaches to identify genes or genetic loci, which caused or contributed to phenotypes or traits. The first, genetic linkage analysis, capitalized on genetic maps of markers across the genome to investigate large single pedigrees or multiple families affected with a given disorder. Statistical evidence that specific markers co-segregated with illness, could be used to map loci linked to this disorder. Linkage analysis is most effective when there is a strong correlation between the phenotype being measured and the inferred genotype at the markers being tested for co-segregation. Further fine-mapping of linked loci, could then implicate specific risk genes. This approach was highly successful for Mendelian disorders, including many with cognitive phenotypes, but less successful for disorders with a more complex genetic aetiology and weaker correlation between phenotype and inferred genotype.

The second strategy, which could use either case-control or family based designs, directly targeted genetic markers at specific genes which were implicated in a disorder through understanding of the disease process (functional candidate gene studies) or targeted likely candidates within a region implicated by linkage or cytogenetic studies (positional candidate genes studies). Association studies are based on populations instead of pedigrees, and compare frequencies of marker alleles in affected individuals versus unaffected individuals

from the general population. Challenges in identifying such candidate genes include uncertainty regarding the biological aetiology of the disorder, and limited study power to detect common alleles of small effects. This power issue becomes even more relevant for the genome-wide association studies (GWAS) described in the Genomics section.

5.1. Linkage and candidate genes studies for disorders of childhood

5.1.1. Attention deficit hyperactivity disorder (ADHD)

Genetic studies of the ADHD clinical phenotype have followed the traditional pathway of twin and adoption studies to establish heritability, followed by genetic linkage studies, association studies based on candidate genes and more recently genome wide association studies (GWAS) and analysis of rare structural variants (detailed below).

Results from the handful of published linkage studies using the ADHD diagnosis as a phenotype (Fisher et al., 2002; Arcos-Burgos et al., 2004; Asherson et al., 2008; Romanos et al., 2008), show some degree of overlap for regions on chromosomes 5p, 9q, 16q and 17p if nominally significant findings are considered; however, no regions have achieved genome-wide significance using strict criteria. Attempts have been made to include neurocognitive measures in the linkage analysis of ADHD to identify quantitative trait loci linked to these traits. This approach assumes that the neurocognitive impairments in ADHD index a latent trait, or traits, that overlap, at least in part, with the heritable pathophysiology of ADHD. Taking this approach, Rommelse et al., (2008) examined candidate endophenotypes in a genome-wide search for susceptibility loci for ADHD. This study found strong evidence for linkage to 2q21.1 and 13q12.11 for measures of motor timing and digit span measures, respectively, incorporating ADHD symptoms as covariates. Doyle et al, (2008) identified a region on 3q13 showing suggestive evidence for linkage to several neurocognitive traits and inattention symptoms in ADHD. None of these studies have produced convincing evidence for linkage, making the presence of one or a small number of gene variants with a large effect on a given trait measure unlikely.

Early candidate gene studies focused on a range of candidate genes with some a priori evidence for a potential role in ADHD pathophysiology. As with the linkage studies described above, putative ADHD risk variants at candidate genes have been tested for association against clinical and cognitive variables. Kebir et al, (2009) reviewed 29 studies examining 10 genes (DRD4, DAT1, COMT, DBH, MAOA, DRD5, ADRA2A, GRIN2A, BDNF and TPH2) in relation to neuropsychological traits relevant for ADHD. For DAT1, there are conflicting results in relation to omission and commission errors, but more consistent findings that increased reaction time variability (Bellgrove et al, 2005a) and abnormalities in spatial attention (Bellgrove et al., 2005b) are associated with the ADHD associated 10-repeat variant. Against what might have been expected, several studies (Manor et al, 2002; Bellgrove et al, 2005), reported better performance on tests of attention in children with the 7-repeat DRD4 variant previously shown to be associated with ADHD. This is a similar finding to studies in psychoses where the GWAS identified *ZNF804A* risk variant (discussed below) may identify a patient subgroup with relatively spared cognitive performance, suggesting that the DRD4 risk variant indexes a pathophysiological pathway to ADHD not mediated by poor performance on cognitive measures. The effect of the 7-repeat variant was confined to children with ADHD and not seen in controls and in a more recent study (Johnson et al, 2009), spectral analysis of reaction time variability supported the hypothesis that the association of greater variability was with the absence of the 7-repeat allele and was also specific to ADHD. Other ADHD candidate genes examined in relation to

cognitive function include the X-linked steroid sulfatase gene where case reports of deletions have been found in cases with neurodevelopmental disorders associated with abnormal cognitive function and ADHD (Doherty et al., 2003). Stergiakouli et al (2011) showed that ADHD associated risk variants were associated with inattentive symptoms and poor performance on verbal IQ and comprehension subtests in ADHD subjects but not controls.

5.1.2. Autism spectrum disorders

A number of linkage regions, replicated in two or more studies have been identified in autism 2q21–33, 3q25–27, 3p25, 4q32, 6q14–21, 7q22, 7q31–36, 11p12– 13, 17q11–21 (reviewed by (Freitag, Staal et al. 2010). The chromosome 2q region had marginally stronger evidence for linkage in individuals with language delays (Buxbaum et al, 2001). A further study stratifying linkage analyses found non-significant evidence of linkage in individuals with IQ>70 and those with delayed language (Liu, Paterson et al. 2008). Multiple candidate gene studies have been conducted but none have been reliably replicated. Consequently we have focused here on genetic studies in social cognition where a convergence of evidence appears to support the role of neuropeptides oxytocin and vasopressin.

Nonapeptides and social cognition

Oxytocin (OXT) and vasopressin (AVP) are highly conserved neuropeptides with marked diversity in the regulation of their receptors. Modulated significantly by sex steroids, they are likely to have sexually dimorphic effects and are therefore of interest for further investigation in disorders such as autism, which show marked gender bias (M:F~4:1). Considerable evidence from animal literature has implicated nonapeptides oxytocin and vasopressin in social behaviour (Insel 2010) relating particularly to pair bonding, maternal care, social recognition and response to threat (Reviewed by (Donaldson and Young 2008). Administration of oxytocin to humans is associated with reduced anxiety, alteration in parenting behaviour, increases in prosocial behaviour (e.g. trust, generosity, altruism and betrayal aversion), reduction in gaze aversion, improved mentalisation and differential amygdala activity in fMRI in response to face perception and changes in social memory (Skuse and Gallagher 2011). Studies reporting association with OXT and autism are inconsistent and no evidence for association has emerged from GWAS studies. However beneficial effects of exogenous oxytocin on core ASD symptoms have been reported offering the potential possibility of new therapeutics (Green and Hollander 2010). Vasopressin has been implicated in aggression, social recognition and pair bonding. The AVP receptor 1A gene is highly conserved. It contains genetic variation reported to influence species-specific differences in pair bonding in animal studies (Wang and Aragona 2004). Genetic variation in AVPR1A has been investigated in ASD with variable reports of association ((Kim, Young et al. 2002; Wassink, Piven et al. 2004; Yirmiya, Rosenberg et al. 2006). One of the variants in humans has demonstrably reduced expression (Tansey, Hill et al. 2011) possibly demonstrating a functional route for genetic association with the gene in autism. The wide-ranging effects of these neuropeptides on human social behaviour are perhaps not specific to autism and may potentially have utility in a wider range of psychiatric disorders with social cognitive deficits.

5.1.3. Intellectual disability

Notwithstanding the high heritability of intelligence, little progress has been made in identifying loci reliably linked or associated with intelligence in normal population samples. There are exceptions, such as the association, predominantly in older people, between ApoE variants and general cognitive ability, episodic memory and executive function, and the weak

associations reported with COMT and BDNF variants accounting, if true for only a very small proportion of the variance in intelligence. In contrast, several hundred genes are known to be associated with intellectual disability (Chelly et al, 2006).

Genetic forms of ID are divided into syndromic ID, characterized by associated clinical, radiological, metabolic or biological features, and non-syndromic ID in which cognitive impairment represents the only manifestation of the condition. The distinction might be helpful for clinical purposes, but recent phenotype–genotype studies are blurring the distinction. Causes of ID are extremely heterogeneous and include environmental forms (eg. premature birth, perinatal brain ischemia or fetal alcohol syndrome), disorders due to chromosomal abnormalities (including sub-microscopic copy number variation discussed below) and conditions due to monogenic causes or dysregulation of imprinted genes. About 50% of cases with moderate or severe ID have a definable cause with a lower percentage in milder cases. Taken together, the emerging genetic findings in ID are suggesting a neurobiology around synaptogenesis, synaptic activity and plasticity with aberrant length and density of dendritic spines a frequent histological finding. Genetic defects and biochemical abnormalities have been described in several pathways that feed into synaptic function including the RhoGTPase signal transduction pathway; with loss of function at its components PAK3, OPHN1, TM4SF2 and FMRP leading to LD and the Ras/MAPK transcription signaling cascade; with the genes NF1, RKS2, CBP and PAK3 involved in ID. Many of these genes when mutated in animal models affect learning and memory processes that require gene transcription and translation of proteins.

5.2. Linkage and candidate genes studies for disorders of adult onset

5.2.1 Schizophrenia

A meta-analysis of more than 30 schizophrenia linkage studies by Ng and colleagues (2009), suggests the involvement of multiple chromosomal loci in schizophrenia susceptibility. Few investigations focussed on cognitive phenotypes in linkage analysis of such families have been reported. One example, reported by Almasy and colleagues, investigated 43 families and identified significant linkage to the chromosome 5q region for the cognitive phenotypes of abstraction and mental flexibility. A more recent study of 557 sibling pairs of Han Chinese ethnicity identified association with the 12q24.32 locus and undegraded CPT hit rate (Lien et al. 2010). Rather than focussing on individual neurocognitive phenotypes, Hallmayer and colleagues (2005) identified families with co-segregation of more pervasive cognitive deficits and identified linkage in these families to chromosome 6p24, a region that had previously been implicated in schizophrenia risk across multiple studies (Straub et al, 1996). Individual markers at this locus have also been associated with deficits in CPT performance in the Han Chinese population detailed above (Lin et al, 2009). The lack of consistency in measured phenotypes makes replication, and final interpretation of these results difficult. Very few studies of this type have been reported for other psychiatric disorders. For example, in bipolar disorder, where many large-scale linkage analyses have also been reported the focus has been on dividing families according to clinical rather than cognitive covariates.

Candidate gene studies of cognitive phenotypes have received much wider attention. This was prompted by studies of functional variants at two candidate genes, the catechol-O-methyltransferase (*COMT*) and Brain Derived Neurotrophic Factor (*BDNF*) genes. From both animal and human studies it is known that reduced dopamine in prefrontal cortex is associated with impaired performance on cognitive testing. Deficits in working memory can

be reversed with dopamine agonists, but both very low and very high levels of dopamine activity are associated with impaired prefrontal cortex function. The COMT gene appears to be key to dopamine catabolism in the prefrontal cortex (PFC) and is a logical candidate for investigation in disorders such as schizophrenia as well as for studies of cognition. Numerous association studies for neuropsychiatric phenotypes have been performed with equivocal results, with several large meta-analyses failing to find association with schizophrenia (Munafo et al, 2005). In the first cognitive study, Egan and colleagues (2001) reported that the high-activity val allele was associated with poorer performance on the Wisconsin Card Sort Test (WCST) and reduced efficiency of physiological response of the dorso-lateral prefrontal cortex during a working memory task. This finding has received consistent replication and a meta-analysis of 12 studies supports the original WCST finding (Barnett et al, 2007). The WCST is a complex problem-solving task with many cognitive components and several authors have tried to identify simpler tests for specific components of this task involving cognitive stability and flexibility. Because COMT influences the ratio of activation of D1/D2 receptors and D4 receptors are known to have an effect on PFC function, variants in these three genes have also been investigated in cognitive studies of adults with no clear findings emerging.

BDNF is known to have an important role in learning and regulates activity-dependent synaptic plasticity necessary for short and long-term memory storage (Alonso et al 2002). Studies of BDNF have focused on a valine (val) to methionine (met) substitution in the 5' region of the gene, which decreases BDNF activity-dependent secretion. Association between the variant and neuropsychiatric clinical phenotypes has been reported, although replication of these findings has been inconsistent. A meta-analysis of studies across multiple phenotypes reported that the met allele was associated with risk for eating disorders and schizophrenia and a protective effect for substance-related disorders (Gratacos et al, 2007). This may have represented a publication bias. On the basis of BDNF's known role in hippocampal function, it has been suggested that met allele carriers may have impaired performance on memory tasks. Supporting this hypothesis Egan and colleagues (2003) identified poorer episodic memory performance, a disruption in normal hippocampal fMRI findings during a working memory task and reduced hippocampal levels of a marker for neuronal function in schizophrenia patients and healthy controls. The same year, Hariri and colleagues showed that met-carriers had reduced hippocampal engagement during encoding and retrieval of a spatial task and also made more recognition errors on the task. Subsequently it was reported that val/met heterozygotes have lower hippocampal volumes than carriers of the val/val genotype (Pezawas et al, 2004). Within schizophrenia patients, met-carriers are also reported to have poorer medial temporal lobe-related performance and correspondingly smaller temporal and occipital lobar grey matter volume (Ho et al, 2006). Although negative studies have been reported, most of the available data supports a modest association between the met-allele and reduced cognitive performance.

With the emergence of putative candidate genes from the schizophrenia literature these were also systematically investigated for cognitive phenotypes. The list of investigated genes includes Neuregulin-1, Dysbindin and DAO. Unlike the case of COMT and BDNF, no clear functional variants have been identified at these genes and multiple studies have reported different risk variants, alleles or haplotypes. This makes direct comparison of studies, which have often also examined different phenotypes, difficult (reviewed in Gill et al, 2010).

5.2.2. Cognitive ageing and psychiatric disorders

Association between an increased risk for Alzheimers disease and the ε4 allele of the apolipoprotein E (ApoE) gene is one of the most robust findings in complex disorder genetics. Carriers of one copy of this allele are 3-4 times more likely to develop late-onset Alzheimers Disease (LOAD), but carriers of two copies have a more than 10-fold increase in risk (Farrer et al, 1997). Multiple studies have shown that APOE ε4 is associated with cognitive decline in patients with AD. More recently it has been demonstrated that this allele has an effect on cognitive performance in non-patient groups as well. Carriers of the risk allele perform significantly poorer on tests of episodic memory, global cognitive ability, executive functioning and perceptual speed although the effect sizes are small. There was no difference between carriers and non-carriers for tests of attention, primary memory, verbal ability and visuo-spatial skill. For the domains where differences were detected these differences became more significant with increased age (Wisdom et al, 2011). These data lead researchers to explore the effects that other candidate genes may have on cognitive ageing. A study investigating 10 candidate genes (including BDNF, COMT and DISC1) for cognitive function failed to identify association with performance and cognitive ageing in the Lothian birth cohort of over a 1000 Scottish 70-year old individuals (Houlihan et al, 2009).

6. Application of genomics methods

Genome-wide association studies (GWAS), by combining advances in high-throughput genotyping platforms and understanding of common genetic variation in populations, allow most common variation in the genome to be tested in a single, usually case-control experiment. Testing all genes is a powerful hypothesis-free approach and GWAS have proven remarkably successful at identifying common risk variants for complex human disease. However, this comes with a significant multiple testing burden and requires large sample sizes-in the thousands or tens of thousands- to identify what are typically modest gene effects (Corvin et al, 2009).

In psychiatry as with other medical specialties, this requirement has driven collaboration, for example, the formation of the Psychiatric GWAS consortium, which is currently performing meta-analysis of GWAS data for schizophrenia, bipolar disorder, autism, recurrent major depression and ADHD (Psychiatric GWAS Consortium Coordinating Committee, 2009). For many of these disorders novel susceptibility loci have been identified (detailed below). GWAS can also inform on the genetic architecture of psychiatric disorders: identified loci appear in many cases to increase risk across traditional diagnostic boundaries; a substantial proportion of schizophrenia and bipolar disorder risk may involve thousands of overlapping gene variants of small effect (International Schizophrenia Consortium, 2009) whereas smaller numbers of variants of large effect appear involved in autism susceptibility.

GWAS platforms were designed to assay common genetic variation and SNPs with a population frequency of at least 5%, however, rare or even unique genetic variants are much more frequent in the human genome. Until now our ability to test for involvement of this type of variation in human disease 'the rare variant common disease hypothesis' has been very limited. GWAS platforms and custom-designed microarrays using comparative genomic hybridization (CGH) have allowed investigation of one class of rare genetic variation, namely, copy number variation (CNV). This provides some insight into likely challenges for cognitive research in analyzing rare variants: an issue that will become more relevant with the increasing availability of whole genome sequence data.

6.1. Genome-wide association studies (GWAS)

6.1.1. GWAS for adult-onset psychiatric disorders

Schizophrenia

Nine schizophrenia loci have been identified: the zinc finger protein 804A (*ZNF804A*) gene on chromosome 2q32; at the major histocompatibility complex (MHC) region on chromosome 6p21-6p22; upstream of the neurogranin (*NRGN*) gene on chromosome 11q24; at the transcription factor 4 (*TCF4*) gene on chromosome 18q21; downstream from microRNA miRNA137 on chromosome 1p21.3; a 0.5 Mb gene-rich region on chromosome 10q24.32; an intronic SNP in the CUB and sushi multiple domains 1 (*CSMD1*) gene; and common variants in gene deserts on chromosomes 2q32.3 and 8p21.3. Additionally, there is substantial evidence for overlap in particular between schizophrenia and mood disorders, as the schizophrenia risk variant at ZNF804A has also been implicated in bipolar disorder (Williams et al, 2011) and the CACNA1C variant, identified in bipolar disorder has also been implicated in schizophrenia and recurrent major depression (Green et al, 2010). A logical next step is for cognitive studies to test whether specific neural mechanisms underlie this susceptibility and its clinical expression.

The psychosis risk variant at gene *ZNF804A* has received the most attention to date. Esslinger and colleagues investigated the influence of the risk variant (rs1344706) on cortical activity within, and connectivity between, regions during working memory (N-back task) and emotional recognition task performance in a sample of 115 healthy controls. Differences in functional connectivity, but not regional activation, were observed. They reported reduced connectivity in the dorso-lateral prefrontal cortex (DLPFC) between and within hemispheres, but also increased connectivity between the hippocampal formation (HF) and the DLPFC, and between the amygdala and the HF, orbitofrontal cortex and prefrontal cortex. They have subsequently reported evidence for involvement of the variant in aberrant brain activation during social information processing using a theory of mind (TOM) task (Walter et al, 2009) and in state-independent inter-hemispheric processing (Esslinger et al, 2010). Their interpretation that the risk allele has a deleterious effect on cognitive performance has been questioned by several more recent studies. First, Walters et al (2010) found and replicated evidence for better cognitive performance on working memory and episodic memory tasks-which involve the DLPFC and HF-in patient carriers of the risk allele. This effect was not present in controls. In a subsequent study, of a different patient group, the authors found relatively larger hippocampal volumes in risk allele carriers (Donohoe et al, 2011). These data suggest that the *ZNF804A* risk variant may identify a patient subgroup with relatively spared cognitive performance, but possibly more social deficits. Although we note that several smaller equivocal studies have also been reported (Lencz et al, 2010; Ballog et al, 2010). Further studies particularly in the domain of social cognition would be useful.

Of the other identified schizophrenia loci, some are large and implicate many genes (e.g. the MHC region) and some map to gene deserts which are not obvious candidates for involvement in cognitive functioning. Of the identified genes, Neurogranin (*NRGN*) is the most compelling target as it plays an important role in calcium-calmodulin signaling, is abundantly expressed in hippocampus, and NRGN knockout mice have severe deficits in hippocampus-dependent tasks. However, a recent study by Donohoe and colleagues (2011b) failed to identify a strong relationship between the risk allele and neuropsychological performance in either patient or control populations on general cognitive ability, verbal episodic and working memory, spatial episodic or working memory or attentional control.

Bipolar Disorder

In bipolar disorder the best supported loci are the calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C) and the ankyrin 3, node of Ranvier (ANK3) genes (Ferreira et al, 2008). Perceived wisdom is that cognitive deficits are less prominent in bipolar disorder, although a range of abnormalities have been reported including altered identification of emotional stimuli (e.g. facial expression), processing speed, working memory and impairments in sustained attention (Arts et al, 2010). Cognitive phenotypes in bipolar disorder may vary with mood state and have received less attention in family studies (to estimate heritability) than equivalent studies in schizophrenia. Of the common risk variants identified in GWAS studies, the *CACNA1C* gene has received the most attention as non-synonymous mutations of *CACNA1C* cause Timothy syndrome, a multi-organ disorder, which includes cognitive impairments (Splawski et al, 2005). *Cacna1c* heterozygous female mice also demonstrate mood-related phenotypes including reduced risk-taking behaviour and increased anxiety (Dao et al, 2010).

Association studies have been reported in case and control populations between the risk allele at rs1006737 across different neuropsychological testing paradigms and imaging studies. The first reported study (Krug et al, 2010) found reduced semantic verbal fluency with increased activation of the left inferior frontal gyrus and left precuneus in healthy male subjects who carried the risk variant. The authors acknowledged that the data to suggest reduced verbal fluency in euthymic bipolar disorder is limited and their results require independent replication. To date five imaging studies have been published, with somewhat mixed results. An initial report of reduced grey matter volume in healthy UK carriers of this risk variant (Kempton et al, 2009) did not replicate in a much larger German control sample (Franke et al, 2010). The latter reported association between genetic variation at the gene and reduced brainstem volume, but this was with different SNPs at the gene and requires independent replication. Studies using blood-oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) have targeted circuits potentially implicated in bipolar disorder. A study of patients and controls, by Bigos and colleagues (2010) implicated circuits putatively involved in bipolar disorder and schizophrenia. They identified a trend for increased hippocampal activity during emotional processing and also greater prefrontal cortical activity during a working-memory paradigm, a pattern previously associated with putative schizophrenia risk variants. A smaller study of healthy controls targeted increased limbic activity as a bipolar disorder phenotype and identified association with increased amygdala activity in response to reward. Erk et al (2010) in studying brain activation during a declarative memory task identified reduced bilateral hippocampal activation during episodic memory recall and reduced coupling between left and right hippocampal regions in 110 healthy subjects.

Alzheimers disease

Until recently, APOE was the only gene known to increase risk of the common form of Alzheimer's disease with late onset. A number of new susceptibility variants have been identified by GWAS including novel loci for late-onset Alzheimers disease (AD) implicating the genes clusterin (CLU), the phospatidlyinositol-binding clathrin assembly protein gene (PICALM), the complement receptor gene (CR1), the bridging integrator 1 gene (BIN1), the ATP-binding cassette (ABC) transporter gene (ABCA7), MS4A gene, CD2AP, CD33 and EPHA1 (Hollingworth et al 2011). These genes appear to be involved in different processes,

with five being linked to immune function; four to cell membrane endocytosis; and three being involved in lipid processing. As of yet none have been tested for involvement in specific aspects of cognitive functioning or their role in cognitive ageing.

6.1.2. GWAS for childhood-onset psychiatric disorders

Data from GWAS studies to date have not generally supported the linkage regions previously identified for childhood onset disorders. A region on chromosome 5p.14 harbouring cadherin genes CDH9 and CDH10 showed evidence for association in one study (Wang, Zhang et al. 2009). A SNP at 5p.15 was located close to a taste receptor gene (TASR1) and a member of the semaphorin family (SEMA5A), the latter family of genes are implicated in axonal guidance (Weiss, Arking et al. 2009). Genome-wide evidence for association at the MACROD2 gene, a gene of uncertain function, was detected in a further study (Anney, Klei et al. 2010). Stratification in the latter analysis based on IQ and verbal status did not reveal statistically significant genome-wide evidence of association.

Results of the PGC consortium ADHD meta-analysis have yet to be reported and smaller GWAS studies have yet to provide genome-wide significant evidence of association.

6.2. Studies of structural genomic variation in Neuropsychiatric Disorders

As recently as 2004, it was discovered that submicroscopic deletions or duplications involving the gain or loss of entire DNA segments (e.g. from a thousand to several million bases) are common (Sebat et al, 2004) and may encompass more than 10% of the average human genome. This technology also made it possible to test for the involvement of inherited or *de novo* CNVs in disease. It rapidly became evident that CNVs play an important role in susceptibility to neurodevelopmental disorders including autism (Sebat et al, 2007), learning disability (Roubertoux & de Vries, 2011), schizophrenia (Walsh et al, 2008) and ADHD (Williams et al, 2010). For autism, CNVs have been identified in at least 10% of cases, implicating a large number of novel genomic loci and risk genes (reviewed Betancur, 2011). In schizophrenia, the seven most established CNVs collectively account for ~2-4% of susceptibility (reviewed Sebat et al, 2009). An excess of CNVs have also been identified in ADHD including a duplication of chromosome 16p13.11. Data for other neuropsychiatric disorders is more equivocal, with the exception of bipolar disorder where a large study of 1697 cases and 2806 controls found no evidence of either an increased total burden or association with individual CNVs (Grozeva et al, 2010).

6.2.1 Cognitive studies of structural genomic variants and rare mutations implicated in psychiatric disorders

The results thus far challenge many preconceptions about the clinical entities being investigated. The same CNVs are being implicated in different disorders. For example, in autism although 70% of affected individuals have learning disability, almost all of the implicated CNVs have also been associated with learning disability. Many of the CNVs identified in schizophrenia have also been implicated in autism: the duplication reported in ADHD has been reported across all three disorders, being most common in ADHD patients with co-morbid learning disability. This diverse phenotypic expression extends beyond psychiatric phenotypes. The 1q21.1 deletion reported in schizophrenia (International Schizophrenia Consortium, 2008) is now known to be associated with a broad array of

paediatric developmental abnormalities including autism, but also heart defects and cataracts (Mefford et al, 2008). These findings suggest that at least a subset of patients with clinical disorders have underlying rare genomic disorders.

Cognitive deficits are a characteristic feature of many psychiatric disorders but perhaps the most significant relationship is with autistic spectrum disorders, where 50% of children diagnosed with autism fall into the mild to moderate IQ range and a further 20% in the severe category. If autism symptoms or behaviour is considered, it is associated with a large number of rare mutations and chromosomal abnormalities, all implicated in ID suggesting that these two neurodevelopmental conditions have overlapping genetic aetiologies (Betancur 2011). In a genome-wide study of rare CNV's in autism, Pinto et al, (2010) found that genes and CNV's previously associated with ID were more likely to be affected by CNV's in autism cases compared to controls. In a gene set analysis, the authors identified gene-sets involved in cell and neuronal development and function (including projection, motility, and proliferation) previously reported in ASD-associated phenotypes. Additional findings related to gene-sets involved in GTPase/Ras signaling, with component Rho GTPases known to be involved in regulating dendrite and spine plasticity and associated with ID; gene sets linked to microtubule cytoskeleton, glycosylation, CNS development and cell adhesion.

Extrapolating from examples of rare genomic disorders that have already been classified we could suspect that some will share core phenotypic features (e.g. Williams syndrome and Prader-Willi/Angelman syndrome) but others (e.g. chromosomal deletions involving 1q21.1 and 22q11.21) may have such a wide range of phenotypic expression as to encompass several clinical syndromes (Lee & Scherer, 2010). Not all CNVs are causative: some are likely to have more modest effects on risk and probably interact with other genetic or environmental risk factors.

What does this mean for cognitive studies? We know that some of the implicated loci can have a profound effect on cognitive functioning leading to significant general learning disability. Would a general screen of IQ in clinical populations identify these CNV carriers? Or do some CNVs cause more subtle cognitive deficits? Plausibly, specific CNVs may impact on, and be extremely informative about, discrete aspects of cognitive functioning. Performing such studies is problematic because of the numbers involved.

Lessons can be learned from investigation of chr22q11.21, but many of the validated CNVs have a frequency of less than 1 in 500 in case samples, having large-scale collaboration and common assessment methods will be essential. Some consensus on batteries of tests is also essential to allow comparison across CNVs, which may be important in identifying where the phenotypic effects may be a consequence of involvement of the same molecular mechanism or pathway. Obvious targets for investigation are loci where a single, or small number of genes are disrupted as these are currently most tractable for other functional studies. CNVs are often complex and both gain or loss of function at a locus may need to be considered. For example, mutation of the gene encoding methyl-CpG-binding protein-2 (MECP2) causes Rett syndrome a neurodevelopmental disorder almost exclusively found in females, however, duplications or triplications of the gene are associated with developmental delay or learning disability in males.

7. Future directions: new phenotypes and new approaches

A major development in cognitive genetics disorders has, as with psychiatric genetics studies, been the move from single gene studies to genome wide studies of the genetic architecture of cognition. Examples of these studies are already published (e.g. Need et al., 2009; Davis et al., 2010) with several more in progress. These studies are likely to replicate difficulties found in genome wide association studies (GWAS) of psychiatric disorder, in particular the low power to detect small effects in samples. Current estimates (Visscher et al., 2009) suggest the need for more than 10,000 samples to detect small effects in psychiatric disorders; power to detect variants with an odds ratio of 1.1 to 1.2 (the effect size associated with already identified common variants) are likely to require even larger samples. If the effect size of 'cognition' genes is similar this requires us to plan experiments on a scale that is far beyond what was traditionally thought of as large. One example of an attempt to achieve the required scale is the COGENT consortium, which has to date amassed data on ~7000 neuropsychological phenotyped healthy participants for the purposes of a genome wide association study of general cognitive ability or 'g'. In advance of the results of these experiments, the evidence that cognitive phenotypes are unlikely to be much less complex in its genetic architecture than seen in psychiatric disorders should cause us to expect meaningful but small advances in our understanding of cognitive genetics.

At the same time, the increased ability to investigate rare genetic effects is identifying many interesting candidate genes for further cognitive studies. These may be particularly important as they may each be associated with significant risk. For studies of CNV carriers, the logistics of performing studies of sufficient statistical power is challenging, although not insurmountable as we know from the 22q11.2DS studies. One major question will be whether specific CNVs have distinct cognitive or clinical effects. This will be addressed by ongoing large studies in Europe where carriers of these variants are being targeted for neurocognitive and neuroimaging studies (Meyer-Lindenberg, 2010).

This type of analysis will be even more challenging when there are large data resources available with full genome sequencing information. We already know that in the human population, many disease genes can show dozens or even hundreds of independent mutations. Mutations that have more severe functional consequences (i.e. to the production of the gene product) may be lethal or associated with severe phenotypes, such as microcephaly. Research from single gene cognitive disorders suggests that, depending on the functional consequence of the mutation, there may be a range of phenotypic outcomes, which include much more subtle phenotypes (Walsh & Engle 2010). Many of the genes known to be building blocks for important neurodevelopmental processes are likely to harbour mutations with these types of genetic effects. This offers a potential framework for understanding and potentially grouping molecular mechanisms at the level of the gene, or in pathways based on understanding of molecular neurodevelopment. The potential to perform cellular and animal studies as well as the ability to return to families, which carry mutations should offer fascinating insights into the genomic underpinnings of cognitive function. In performing such studies we must not lose sight of the fact that the molecular programmes that govern and modify neurodevelopment are affected by stochastic variation and actively influenced by the world that surrounds us. To maximize what we can learn about cognitive genomics we will need to understand the impact of environment.

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Test	First Author	N	Associated with Illness	Heritability/Present in other family members	State Independent	Cosegregates with illness
Penn emotional recog task	Greenwood, 2007	183 nuclear families ascertained through probands with schizophreni	Yes	Yes		
	Eack, 2010	70 first-degree relatives of schizophrenia probands; 63 healthy controls	Yes	Yes		
	Calkins, 2010	SZ/Szaff =610, Relatives=928, HC=334	Yes	Yes		Yes
	Gur, 2002	14 patients with schizophrenia and 14 matched comparison subjects	Yes			
	Bediou, 2007	drug-naive patients with first-episode schizophrenia (n=40) and their unaffected siblings (n=30) compared with controls (n=26).	Yes	Yes	Yes	
Facial affect recognition	Erol, 2010	Patients with schizophrenia (n=57), their unaffected biological siblings (n=58) and healthy controls (n=58)	Yes	Yes		
TOM task	McKinnon, 2010	14 bipolar, 14 control	Yes			
	Sabbagh, 2008	46 3-year-olds and their parents		Yes		
	Sprong, 2007	29 studies (combined n=1518)			Yes	
	de Achával (2010)	20 Schizophrenia patients, 20 healthy age- and gender-matched individuals, 20 unaffected first-degree relatives of the schizophrenia patients, and 20 healthy individuals matched for age and gender	Yes	Yes		
Hinting Task	Janssen et al, 2003	43 patients with schizophrenia or schizoaffective disorder, 41 first degree non-psychotic relatives and 43 controls from the general population.	Yes	Yes	Yes	
Mind in the eyes	Losh, 2007	Forty-eight parents of individuals with autism (13 of whom were identified as 'aloof'), and 22 control parents	Yes	Yes		Yes
	Bora et al, 2005	Forty-three euthymic bipolar patients and 30 controls			Yes	

Faux Pas	de Achával et al, 2010	20 Schizophrenia patients 20 healthy age- and gender-matched individuals, 20 unaffected first-degree relatives of the schizophrenia patients, and 20 healthy individuals	Yes	Yes		
	Shamay-Tsoory, 2009	19 patients, 20 controls			Yes	
Attribution test (IPSAQ)	Lau, 2008	Childhood(birth-12 yr) (100), School Age (6-12 yrs) (180), Adolescence (13-17yr) (200), Adulthood(18 yr plus) (300), Young Adult(18-29 yr) (320)	Yes	Yes	Yes	
	Janssen, 2006	23 patients with psychosis, 36 first-degree relatives of patients with psychosis, 31 subjects with subclinical psychotic experiences and 46 normal controls.	Yes			
Social cognitive skills test (SCST)	Scourfield,1999	population-based sample of twins aged 5-17		Yes		
	Scourfield, 2004	A population-based sample of twins aged 5-17		Yes	Yes	
	Coleman, 2008	Eight children with ASD and eight control children aged 5-13 years	Yes			
Iowa gambling task	Viswanth, 2009	Twenty-five unaffected siblings of OCD probands with familial OCD, and 25 individually matched healthy controls	Yes	Yes		
	Cavedini, 2010	35 pairs of OCD probands and unaffected first-degree relatives and 31 pairs of HC subjects without a known family history of OCD and their relatives	Yes	Yes		
Implicit association test	Egloff, 2002	Forty-one introductory psychology students (33 women and 8 men)	Yes			
	Sasaki, 2010	Individuals scoring High (n = 26) and Low (n = 18) on Social Anxiety	Yes			
	Glashouwer & de Jong (2010)	Patients (n=2329) and non-clinical controls (n=652)	Yes		Yes	

Table 2 Satisfaction of endophenotypic criteria for social cognitive task

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LETTER TO THE EDITOR

ZNF804A and social cognition in patients with schizophrenia and healthy controls

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The neural mechanism by which zinc-finger protein gene *ZNF804A* increases the risk of psychosis is unclear. Following a recent 'theory of mind' study, we tested the hypothesis that risk is partly mediated by an effect on social cognition. We found that ZNF804A was significantly associated with variation in interpersonal attributions in healthy participants but not in patients.

Following both meta-analysis and extensive re-sequencing, the single nucleotide polymorphism rs1344706, located in intron 2 of the zinc-finger protein gene *ZNF804A*, continues to be the common variant most strongly associated with schizophrenia risk ($P = 1.1 \times 10{-}13$).[1] Functional effects of this variant on both protein expression[2] and altered DNA–protein interaction[3] have been reported, although the neural mechanism by which this contributes to schizophrenia risk is unclear. Although the risk 'A' allele at rs1344706 has been associated with altered functional brain connectivity,[4] so far these alterations do not appear to be strongly associated with deficits in neuropsychological performance; if anything the opposite has been the case.[5] Recently, a functional magnetic resonance imaging study of healthy participants suggested ZNF804A's contribution to schizophrenia risk may alternatively be mediated via effects on aspects of social cognition, particularly theory of mind.[6]

We tested this hypothesis that the increased risk associated with the ZNF804A 'A' allele is partly mediated via deleterious effects on social cognition based on measures of two constructs widely investigated in schizophrenia. First, we indexed 'theory of mind' using two widely used behavioural measures: The 'Eyes of the Mind' task,[7] which indexes mental state decoding and the 'Hinting task',[8] which measures mental state reasoning. Second, we indexed attributional style when interpreting positive and negative events using the 'Interpersonal social attributions questionnaire'.[9] Data on these measures were obtained by re-contacting participants in our earlier neuropsychological study of ZNF804A[6] and from new enrollments since that study's publication, resulting in a sample of 418 patients (ZNF804A genotype groups: CC = 51; AC = 184; AA = 183) with schizophrenia or schizoaffective disorder and 200 (CC = 28; AC = 88; AA = 84) healthy controls; for all participants the criteria for enrollment were as previously described.[5]

No differences between ZNF804A genotype groups were observed in theory of mind scores (Hinting task: $F = 0.11$, $P = 0.89$; Eyes task: $F = 0.92$, $P = 0.40$). However, we did observe an association with attributional style in a direction consistent with our hypothesis. Specifically, healthy carriers of 1 or 2 copies of the 'A' risk allele demonstrated significantly higher personalising bias scores (a measure of the tendency to attribute negative events to other people rather than to situational factors) than non-carriers ($F = 4.79$, $P = 0.01$; Figure 1). In the context of the earlier report by Walter *et al.*[6] it is interesting to note that this effect was only observed in healthy controls. Patients were not observed to show any appreciable changes in social cognition associated with ZNF804A.

These findings are consistent with earlier findings from the Walter *et al.*[6] imaging study in that the ZNF804A risk allele was in both studies associated (and in the same direction) with altered responsiveness in social cognition in healthy participants. The two studies also differed, however, in that we observed this association only for interpersonal attributional style but not for theory of mind. Specifically, the observed difference in personalising bias scores between risk allele carriers versus non-risk carriers suggests their increased tendency to attribute negative events to other people rather than to situational factors. Such differences in personalising bias—the tendency to blame others—have variously been associated with paranoid ideation, anger and learned helplessness.[10] However, observation of these differences in healthy participants but not in patients appears inconsistent with the hypothesis that illness risk is mediated via effects on social cognition. Support for that hypothesis would necessitate a similar effect in patients to that observed in healthy participants.

Following the argument that genetic effects are more penetrant—and hence more identifiable—in functional imaging studies than in behavioural studies, it is possible that ZNF804A's subtle effects on behavioural measures of social cognition in patients were missed, despite including twice as many patients as controls and six times as many participants overall as in Walter *et al.*[6] In particular, subtle effects on social cognition might be particularly difficult to observe behaviourally in patients given a background of general cognitive decline. Such background cognitive 'noise' inevitably increases difficulties with detecting more subtle gene-specific changes in patients, and may explain the apparent

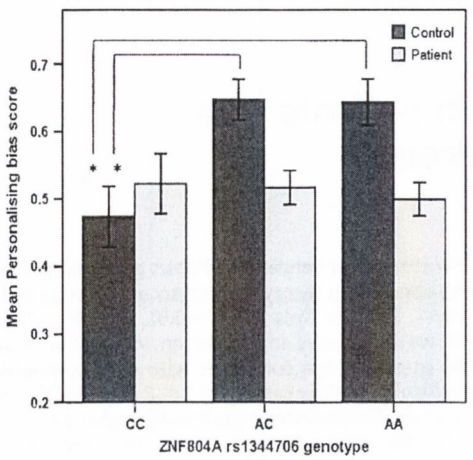

Figure 1 Mean scores (and s.d.) for Interpersonal social attributions questionnaire personalizing bias grouped by genotype. Carriers of 1 and 2 copies of the 'A' risk allele at rs1344706 show significantly increased personalizing bias scores compared with non-risk carriers in healthy controls but not in patients. The asterisks in this figure represents statistical significance.

difference in effects observed in healthy participants. Importantly, however, our earlier neuropsychological study of ZNF804A suggested, in two large independent samples, that the effects of this genotype differed between healthy participants and controls (a finding which persists in the present slightly enlarged sample).[5] If this is the case, these data highlight the need for caution in extrapolating from findings based solely on healthy participant data to neural mechanisms of illness in patients, at least when considering the effects of ZNF804A.

Conflict of interest

The authors declare no conflict of interest.

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Letter to the Editors

A neuropsychological investigation of the genome wide associated schizophrenia risk variant NRGN rs12807809

Dear Editors,

A single nucleotide polymorphism rs12807809 located 3457 bases upstream of the Neurogranin (NRGN) gene was identified in a recent schizophrenia (SZ) genome wide association study (GWAS) as showing genome wide significance in combined samples of 12,945 cases and 34,591 controls (p-value 2.14×10^{-9}; OR1.15; 95CI 1.1–1.2; Stefansson et al, 2009). NRGN plays an important role in the calcium–calmodulin signalling pathway (Hayashi, 2009). Ca^{2+} induced oxidation of NRGN leads to calmodulin's activation of CaMKinase II, which is associated with strengthened NMDA receptor signalling. This cellular role in neuroplasticity, together with the evidence that NRGN is abundantly expressed in areas of the brain that are important for cognitive processing, particularly CA1 pyramidal neurons in the hippocampus (Huang et al., 2007), has led to the suggestion that NRGN may be important in the neurobiology of learning and memory. This hypothesis is supported by evidence of severe deficits on hippocampus-dependent tasks in neurogranin knock-out mice (Pak et al., 2000; Huang et al, 2004). Among currently identified genome wide significant SZ variants therefore, the NRGN risk allele is *a priori* one of the most likely variants to be associated with a deleterious effect on cognition.

We tested this hypothesis in 393 neuropsychologically assessed Irish cases with DSM-IV schizophrenia or schizoaffective disorder and 157 controls, collection of which we have previously described elsewhere (Donohoe et al., 2009; Walters et al., 2010). Neuropsychological assessment focused on the domains of (1) general cognitive ability (IQ) as measured by an abbreviated version of the Wechsler Adult Intelligence Scale (WAIS-III), (2) verbal episodic and working memory as measured by the Logical Memory (LM), Letter Number Sequencing (LNS), and digit span sub-tests from the Wechsler Memory Scales, (3) spatial episodic and working memory as assessed using the CANTAB paired associate learning task and Spatial Working Memory task, and (4) attentional control as assessed using the CPT-IP. To confirm our results, we tested independent samples of German patients (n = 240) and healthy participants (1344) using comparable neuropsychological tests, again as previously described (Donohoe et al, 2009; Walters et al, 2010).

Irish samples were genotyped for rs12807809 using a Taqman SNP Genotyping Assay on a 7900HT Sequence Detection System (Applied Biosystems Inc, Foster City, California). The call rate for the Taqman genotyping was greater than 95%, and both case and control samples were in Hardy–Weinberg equilibrium ($P<.05$). German samples had been genotyped as part of the original study identifying the NRGN SNP rs12807809 tested here (Stefansson et al., 2009). Given the low frequency of homozygous C allele carriers our neuropsychological analyses grouped patients and controls as homozygous T carriers (the risk allele) versus homozygous and heterozygous C risk carriers (i.e. TT v CT/CC). Analysis of covariance was used to test for association between NRGN genotype and neuropsychological performance; performance on individual cognitive tests was entered as the dependent variables, genotype and diagnosis (cases versus controls) were entered as the independent variables, and age and gender were entered as covariates. Differences in educational attainment or medication dosage were not associated with genotype and hence were not included in the analysis.

Patients performed significantly below healthy participants on all measures (all p-values<.0001), showing deficits in performance that were generally in keeping with those previously reported in schizophrenia. No significant differences between TT carriers (patients 284; controls 105) and CT/CC carriers (patients 109; controls 52) were observed in the Irish samples on any neuropsychological measures assessed (all p-values greater>.05). Similarly, while the expected significant differences between cases and controls were observed on all neuropsychological measures, no significant interactions between NRGN genotype and diagnosis was observed for any of the neuropsychological variables assessed (all p-values greater>.05). Re-running the analysis on the basis of genotype (TTvCTvCC) did not change the significance of these results. Undertaking the same analysis in the German samples confirmed these data: no significant results were observed on any measure for either cases or controls (again all p-values> .05). One trend level association with verbal episodic memory (immediate logical memory performance) was observed in the Irish sample ($F3,532 = 2.91$; $p = .089$); however, this did not replicate in the German samples.

We conclude that despite the strong hypothesis that impaired cognition may mediate the risk associated with schizophrenia genes (Kendler and Neale, 2010), and the strong *a priori* evidence that NRGN may influence cognition, no association between the SZ associated NRGN variant rs12807809 and cognition could be detected in these samples. This was despite the fact that many of the samples on which the present analysis was based contributed to the ISC replication of NRGN as a risk variant in the original analysis

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(Stefansson et al., 2009). This is also despite previous evidence of NRGN's involvement in cognitive deficits in animal models (Pak et al., 2000; Huang et al, 2004) and its relationship to CAMKII which is thought to have a major role in short term memory (Wang et al., 2008), a core feature of schizophrenia (Toulopoulou et al, 2007).

Given the modest effect of NRGN on SZ risk observed by Stefansson and colleagues (OR1.15; 95CI 1.1–1.2) was based on an initial discovery sample of 2663 cases and 13,498 controls, the question of whether this study had adequate statistical power to test our hypothesis is important. Our analysis had above 80% power to detect a difference of greater than .33 SD (i.e. a difference of 1.5 scaled score points in Wechsler memory tests or 5 Wechsler IQ points) between genotype groups. Possible smaller effects of NRGN on cognitive function are not ruled out by this study.

In conclusion, the failure to identify a strong relationship between this risk allele and neuropsychological performance highlights the need to consider other, perhaps non-cognitively mediated, mechanisms by which risk is being increased. The absence of significant findings within this study suggests that NRGN's effect on SZ risk is unlikely to have been mediated primarily via an effect on cognition as we initially hypothesised. However, it remains to be determined whether NRGN's effects on cognition will be more readily identifiable in interaction with other risk and non-risk associated variants.

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Increased variation in working memory explained by epistasis versus polygene scores in the ZNF804A pathway

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Association between ZNF804A and variation in neurocognitive functioning has been replicated in several studies. Here we investigated the amount of variation in neuropsychological function explained by both ZNF804A and genes interacting with ZNF804A as identified in a recent siRNA knockdown experiment. Patients with psychosis (N = 424) and healthy controls (N = 89) were assessed in areas of cognitive ability found to be impaired in schizophrenia including IQ, memory, attention and social cognition. Using the PGC dataset to calculate a polygenic score for each individual based on all identified risk variants within this genetic pathway, higher polygene scores were associated with poorer performance amongst patients on IQ, memory and social cognition, explaining 1-3% of variation on these scores (*p*-values ranged from 0.012-0.034). Further, using a narrow psychosis training set and independent test sets of narrow psychosis, other psychosis (bipolar I disorder, major depressive disorder and psychosis NOS), and controls respectively, the addition of two interaction terms containing two SNPs each increased the R^2 for SWM strategy in the independent psychosis test sets from 1.2% using the polygene score only to 4.8% (respective *p*-values = 0.11 and 0.0012). These data support a role for the ZNF804A pathway in IQ, memory and social cognition in cases. Further we show that epistasis can increase variation explained above the contribution of the polygene score.

Keywords: schizophrenia, working memory, epistasis, polygene score, ZNF804A, cognition

Genome wide association studies (GWAS) have been at the forefront of identifying candidate genes for complex genetic disorders such as psychosis. One of the first genetic variants to achieve genome-wide significance for psychosis was rs1344706 located within the zinc finger binding protein 804A (ZNF804A). Several independent replication studies[1,2] have supported an association between schizophrenia and the risk allele of this SNP; further, a recent meta-analysis reported the association exceeded accepted levels of genome-wide significance (*p*-value = $2.5e^{-11}$).[3] ZNF804A, which is expressed in the brain, is predicted to encode a protein with a C2H2 zinc finger domain. This suggests a role in the regulation of gene expression through DNA and/or RNA binding.[4] It has been reported to show association with brain activity and structure[1,2], and in a recent study by Hill and Bray[5], which assessed the effects of its knockdown on the cellular transcriptome, it has been linked to cell adhesion

molecules suggesting a role in neural migration, neurite outgrowth and synapse formation, which are commonly hypothesized to be aberrant in schizophrenia.

Several studies have linked ZNF804A to cognition, based on imaging studies, traditional neuropsychological measures[6-8] and measures of social cognition.[9-10] To date, the results of these studies deviate from what would be expected from a schizophrenia risk gene in that whilst the rs1344706 risk allele appears to convey impairments in cognition in controls based on behavioural and imaging studies[7,11-13], the literature repeatedly points to more preserved cognition in patients.[6,8,14-15] Although some studies suggest that ZNF804A is associated with impaired social cognition in control subjects, it is uncertain if ZNF804A might also confer a disadvantage to the patient population as thus far only one study[10] has included patients in the analysis. Further, one intriguing question remains: if ZNF804A confers risk of psychosis, why does the risk-allele carrying patient population show relatively preserved cognitive function? Perhaps the answer lies in the impact of ZNF804A embedded within its functional pathway. Complex traits are thought to depend on the contribution of a large number of independent and/or interacting genes[16-17], with small individual contributions of each gene. Within the context of case-control analysis, polygene risk scores (a simple or weighted summation of the top sets of SNPs), even including those without nominal uncorrected significance, have been shown to predict case status in related disorders and explained a significant percentage of variability.[18] Limiting this polygenic risk score calculation to only include variants within genes interacting with ZNF804A identified by Hill and Bray[5], we investigated whether more of the variance in patients' neuropsychological function can be explained than is explained by single variants. Further, as variation in complex traits is thought to be not only polygenic, but also epistatic, we sought to examine whether epistasis between SNPs within the ZNF804A pathway could explain variation above that explained by the polygene score.

Specifically, within the Hill and Bray[5] ZNF804A pathway, we used the PGC GWAS schizophrenia case-control results[19] to create a polygene score based on the summation of risk-associated alleles. We used the *p*-values from the PGC case-control analysis to rank SNPs for inclusion in the polygene score. We then determined the amount of variation in patients' cognitive function explained by the polygene scores. To include epistatic effects, we used half of the narrow psychosis (schizophrenia and schizoaffective) set as a training set to test for pairwise epistasis among all SNPs falling under the *p*-value threshold, then assessed whether adding these interactions to the regression model containing the polygene score increased the R^2 among three independent test sets including (1) additional narrow psychosis cases, (2) other psychosis (bipolar disorder, major depressive disorder and psychosis NOS), and (3) healthy controls.

Materials and methods

Participants

Four hundred and twenty-four cases and 89 healthy participants who completed a full neuropsychological assessment battery and for whom full GWAS data were available were included. Cases were clinically stable patients with a DSM-IV diagnosis of schizophrenia (SZ, N = 282), schizoaffective disorder (SZA, N = 58), bipolar disorder (BP, N = 61), major depressive disorder with psychotic features (MDD, N = 11) or psychosis not otherwise specified (PNOS, N = 12) (Table 1) recruited from five sites across Ireland. Inclusion criteria required that participants were clinically stable at neuropsychological assessment, aged 18 to 65 years, had no history of co-morbid psychiatric disorder, no substance abuse in the

preceding six months, no prior head injury with loss of consciousness and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV Axis 1 Diagnoses (SCID).[20] We based our analysis on a narrow psychosis definition of schizophrenia and schizoaffective disorder and a broad definition of psychosis which encompassed all meeting criteria for psychosis. Additional diagnostic details and clinical sample characteristics including symptom severity (SAPS/SANS)[21-22] and medication dosage are detailed elsewhere.[6] Healthy control participants were recruited via online and poster advertising. They were aged 18 to 65 years, with no history of substance abuse in the preceding six months, no prior head injury with loss of consciousness, no history of seizures, and no history of psychosis in themselves or their first-degree relatives. All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All participants were of Irish ancestry (i.e. four grandparents born in Ireland) and all provided written informed consent.

Cognitive assessment

Participants completed a neuropsychological assessment battery designed to target the cognitive deficits typically reported in schizophrenia including general cognitive function, episodic memory, working memory, attentional control and social cognition. General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design and Matrix Reasoning) from the Wechsler Adult Intelligence Scale, 3rd edition[23], yielding a full scale, verbal and performance IQ. Episodic memory was assessed using the logical memory subtest from the Wechsler Memory Scale, 3rd edition (WMS-III).[24] Working memory was assessed using the spatial working memory task (SWM) from the Cambridge Automated Neuropsychological Test Battery (CANTAB)[25] and letter number sequencing (LNS) from WMS-III.[24] Attentional control was assessed using the continuous performance task identical pairs version (CPT-IP)[26], the intradimensional-extradimensional shift task (IDED) (CANTAB)[25] and the sustained attention to response task (SART).[27] Social cognition was assessed using the reading the mind in the eyes task[28] and the IPSAQ[29], which yields two bias scores; externalising bias (EB), which indicates a propensity to attribute positive events to oneself rather than to other people, and a personalising bias (PB), which indicates a propensity to attribute negative events to other people rather than to situational factors.

Genotyping

Genotyping was conducted on DNA extracted from blood in patients and saliva in controls. SNP data was obtained from a recent GWAS using the Affymetrix SNP Array 6.0, described in detail elsewhere.[30]

Statistical analysis

Polygene score calculation

Polygene scores for variants located within the ZNF804A pathway were calculated in four steps.[18] First, all available SNPs within 20Kb of genes in the Hill et al.[5] ZNF804A pathway were identified (Supplementary Table 1). Second, alleles within these SNPs were identified as risk-associated or non-risk-associated using data from the PGC SZ GWAS analysis and

were re-coded so the "target" allele was the risk-associated allele. Third, to account for difference between variants in the magnitude of the association with illness, each risk allele score was weighted as the $\log_{10}$ of the odds ratio described in the PGC dataset. Finally, two polygene scores for each individual were calculated. The first was based on the simple sum of the number of risk-associated alleles they carried averaged across the total number of valid genotypes for that individual. The second, the weighted polygene score, was based on the count polygene score, with the exception that each SNP was weighted by the $\log_{10}$ of the odds ratio from the PGC. To determine subsets of the total number of pathway-based SNPs, we used three *p*-value thresholds from the PGC case-control analysis (*p*-values < $1.0e^{-05}$, < 0.05, < 0.50). These arbitrary *p*-value threshold cut-off points followed those used in previous polygenic analysis[18] (N SNPs: $1.0e^{-05}$, N = 10; 0.05, N = 218; 0.50, N = 1525). As individual ZNF804A SNPs have been associated with cognition, we examined whether it is polygenicity, rather than ZNF804A itself, that accounts for association between the polygene score and cognition by removing ZNF804A SNPs (N = 27) from the pathway and reassessing association between the polygene score and cognition.

Association analysis

Polygene scores

Associations between ZNF804A pathway polygene score and the phenotypes of IQ, episodic memory, working memory, attention and social cognition were tested in multiple regression analyses implemented in SPSS 17[31] and/or the R Statistical Computing Environment.[32] In each case, scores for each neuropsychological phenotype were entered as dependent variables, controlling for age and gender as necessary, followed by ZNF804A polygene score on the second step.

Polygene scores plus epistatic effects

To test whether additional variation could be explained by epistasis beyond that explained by the polygene score, we developed a novel, yet simple, approach within the context of the regression models described above. We restricted our search for epistasis to the models where the polygene score accounted for a small but significant amount of variation in the neuropsychological phenotype in broad or narrow psychosis including SWM strategy, IPSAQ-EB, and performance IQ. First we created equal-sized training and test sets from the narrow psychosis cases. Second, to obtain a stable and consistent estimate of the *p*-value from two-SNP interactions, we took 100 bootstrap samples with replacement from the narrow psychosis training sample and performed linear regression analysis for all-possible pairs of SNPs falling under the threshold at hand (SWM threshold = 0.05, N SNPs = 218; IPSAQ-EB and performance IQ threshold = $1.0e^{-05}$, N SNPs = 10). The linear regression model contained the unweighted polygene score plus an epistatic term which was the product of the number of risk-associated alleles at two SNPs. The unweighted polygene score was used instead of the weighted score so the alleles comprising the polygene score were on the same scale of measurement as those used for the interactions; however, little difference was observed using the weighted polygene score (discussed below). The *p*-value for the interaction term was retained across all pairs of SNPs across all 100 bootstrap replicates. The average *p*-value from the 100 replicates was used to determine which interactions would be evaluated using the three test sets, using an uncorrected *p*-value < 0.05 threshold. Due to linkage disequilibrium, a further condition was the interaction with the smallest *p*-value containing a particular SNP would be tested and all other interactions containing that SNP would not be considered for analysis due to collinearity. A model using

the training data was derived for the polygene score plus interaction term(s). R^2 values on the independent test sets were calculated as the square of the correlation between the fitted values for the test set based on the model calculated on the training data and the observed values from the test set.

Results

Demographic and clinical measures

Demographic and clinical characteristics for patients and healthy participants appear in Table 1. The characteristics of the broad psychosis group were compared to the narrow psychosis group and to the control group using *t*-tests (http://www.quantitativeskills.com/sisa/statistics/t-test.htm). No significant differences were observed between the narrow and broad psychosis groups for age, gender, age at onset, full scale IQ, medication dosage as measured by chlorpromazine equivalents or positive and negative symptoms, with the exception of the 'mania' factor where the broad psychosis group scored significantly higher. The patient group contained significantly more males than the healthy group, was significantly older at the time of assessment and had a significantly lower full scale IQ.

ZNF804A pathway polygene score

ZNF804A weighted polygene scores were associated with measurements of both general and social cognition, including SWM, IPSAQ-EB and performance IQ. Variation in performance on those tasks that was significantly explained by the polygene score was of moderate effect size, ranging between 1.2 and 3% in cases (Table 2; results for all phenotypes are in Supplemental Table 2). Possessing a higher ZNF804A polygene score was predictive of poorer performance on performance IQ in the broad psychosis group at the *p*-value threshold of $1.0e^{-05}$, but did not predict performance IQ in the narrow psychosis group (Table 2). The IPSAQ-EB demonstrated significant association with the ZNF804A polygene score among broad and narrow psychosis at a *p*-value threshold of $1.0e^{-05}$. Patients with psychosis who had greater polygene scores demonstrated a decreased IPSAQ-EB score suggesting that these patients were more likely to attribute positive causality externally. Among both narrow and broad psychosis groups, a higher ZNF804A polygene score led to significantly poorer performance on SWM strategy at a *p*-value threshold of 0.05.

To determine whether the polygene score results were due to SNPs in ZNF804A or its pathway, we excluded all variants in ZNF804A and reassessed association with the polygene score. Without ZNF804A the results were virtually unchanged (Table 2) with the exception of SWM strategy, which showed a slightly reduced R^2 and larger *p*-value in the narrow psychosis set, indicating ZNF804A was not strongly influencing the association with cognition (results for all phenotypes are in Supplemental Table 3).

Epistasis within the ZNF804A pathway

We used the three measures showing association with the polygene score (performance IQ, SWM strategy and IPSAQ-EB) to test for additional variation explained by two-SNP epistasis via a narrow psychosis training-independent test set approach. Due to linkage disequilibrium, 112 average *p*-values across 100 bootstrap samples of the training set were less than 0.05, but of these, only 3 were completely independent of the other sets. Beginning with SWM strategy, adding the most significant interaction, rs17186340:rs140512, to the model containing the polygene count score led to an increase of 1.4% in the narrow psychosis test set (*p*-value = 0.050), 3.7% (*p*-value = 0.035) in the other psychosis test set, and a combined case test set increase of 2.3% (*p*- value = 0.0064) (Table 3). Although adding this interaction increased the variation explained in two independent sets of cases, it reduced the variation explained versus the polygene count score in controls (R^2 = 0.0069, *p*-value = 0.45). Adding a second interaction term to the model (rs2295984:rs34138673) further increased the variation explained in cases: in the narrow psychosis test set the R^2 increased by 1.3% (*p*-value = 0.016) to a total of 4.0%, in the other psychosis test set the R^2 increased further by 1.2% (*p*-value = 0.036) for a total variation explained of 6.2%, and in the combined set of cases the R^2 increase was 1.3% (*p*-value = 0.0012) for a total of 4.8%. In controls, the adding the second interaction did not increase the variation explained (R^2 = 0.001, *p*-value = 0.77). Interestingly, the addition of the epistatic terms to the model containing only the polygene count score in the training data led to a stronger association between the polygene count score and SWM strategy (Table 4). Although the *p*-value for the polygene count score in the training data was 0.12, after adding the first interaction term the polygene count score *p*-value was reduced to 0.093, and after adding the second interaction term the *p*-value was reduced to 0.044. This strengthening of association between the polygene count score and SWM strategy was not due to the interactions being comprised of a small number of SNPs showing the opposite sign of association with the phenotype: of the 218 SNPs at this threshold, 99 showed positive association individually and the remaining showed negative association.

To test whether the effect was truly due to strongly associated SNPs contained in the interactions themselves, interaction SNPs were tested individually for association with SWM strategy. Two SNPs were associated with SWM strategy at an uncorrected *p*- value of < 0.05 and both were in the second interaction term: rs17186340:rs34138673. The improvement in R2 values from the narrow psychosis training set model containing the polygene count score plus rs17186340 on the narrow psychosis, other psychosis and total test cases set were 5.89e-06, 9.1e-04 and 8.0e-05, respectively. Results for the same analysis using rs34138673 were 0.0056, 0.013 and 0.0079, indicating that the interaction term explains more variation than either single SNP.

To see whether the type of polygene score influenced the amount of variation explained, the weighted polygene score was substituted for the count polygene score and R2 values were again calculated for the case test sets. In the narrow psychosis test set, the use of the weighted polygene score in the three models (polygene-only, one and two interactions) increased the R2 value by 9.1e-04 - 0.001, whereas the use of the weighted polygene score in other psychosis reduced the R2 value by 4.7e-04 - 0.0061 suggesting the choice of polygene score was trivial.

For performance IQ and IPSAQ-EB, the p-value threshold was 1.0e-05. Ten SNPs were included, all on chromosome 10 and in tight linkage disequilibrium (r2 ranged from 0.6-1.0), which led to collinearity in the interactions. The resulting training set p-values for all interaction terms for both phenotypes were > 0.05 and were not tested further.

Discussion

We used polygene scores, based on the PGC schizophrenia GWAS dataset, to investigate whether increased numbers of ZNF804A pathway schizophrenia risk-associated alleles correlated with neuropsychological function amongst 424 psychosis patients. Higher polygene scores within the ZNF804A pathway were significantly associated with poorer performance in IQ, working memory, and biased social cognition, and explained 1-3% of the variation in these measures, consistent with estimates previously reported in general intelligence in controls.[33] Removal of the SNPs within ZNF804A reduced the R^2 values only slightly, suggesting the combined contribution of genes within the pathway - not ZNF804A alone - were driving the association. Further, we showed that considering epistasis along with the polygene score resulted in over three times the amount of variation explained in two independent test sets of cases (total R^2 ranged from 4.0-6.2%). Since the SNPs participating in interactions were not in ZNF804A, we provide further evidence that this gene was not the key contributor to our pathway-based results. Thus, our findings are not inconsistent with previous studies showing preserved cognitive function in cases is associated with ZNF804A. Finally, although the polygene score explained a similar amount of variation in controls, improvements due to epistasis were specific to psychosis cases.

Although previous studies have shown that the risk allele of ZNF804A rs1344706 shows differential effects in cases and controls[6-15], we show that, at the pathway level, the effect of the combination of schizophrenia risk alleles leads to poorer performance in patients with psychosis on measures of intelligence, working memory and social cognition. The 4 SNPs participating in epistasis that increased the R^2 values in our test sets were near STAC (rs17186340), MAPK8IP2 (rs140512) and flanking either side of FAM46A (rs2295984 and rs34138673). In mice, Stac is expressed in brain, neurons and postsynaptic densities and within the brain the expression is highest in the hippocampus and cerebellum.[34-35] A Stac knockout mouse model showed reduced social interaction, impaired learning, and deficits in exploration of novel environments.[35] MAPK8IP2 is located within the Chr22q13.3 deletion region associated with autism spectrum disorders and Phelan-McDermid syndrome, which is characterised by developmental delay. Also known as JIP2, it is a scaffold protein that is necessary for N-methyl-D-aspartic acid (NDMA) receptor function and modulates signal transduction.[36] FAM46A is expressed in adult human brain and shows higher expression in human fetal brain.[37] The SNPs participating in the second interaction term, rs2295984 and rs34138673, are located on either side of FAM46A, approximately 19.5K bp apart, possibly indicating a promoter and/or enhancer role. Whether the influence of these novel cognition-related genes acting in epistasis may modulate the effect of ZNF804A on cognition as part of the Hill and Bray[5] pathway is unknown.

How can we reconcile previous research that has shown the risk allele at ZNF804A rs1344706 is associated with less impaired cognition in psychosis patients with the results of the present study, which showed that the polygene score from the ZNF804A pathway was associated with poorer performance IQ, working memory, and social cognition? The *p*-values from the PGC were used to select SNPs for inclusion in the polygene score, and the smallest ZNF804A *p*-value was 0.0015 for rs1344706. Therefore, the set with the most stringent threshold did not include any ZNF804A SNPs, and this set was negatively associated with IQ and social cognition. For working memory, the removal of the ZNF804A SNPs at a *p*-value threshold of 0.05 would have included 12 of the 27 SNPs with p-values ranging between 0.0015-0.047. We have shown that the removal of these SNPs did not lead to significant differences in the magnitude of association between the polygene score and working memory. The use of the weighted polygene score would have ensured a weak contribution of

these SNPs as they were not strongly associated with schizophrenia in the PGC and they comprised only 5.5% of the total number of SNPs at that threshold. Interestingly, the PGC *p*-values for the 4 SNPs participating in epistasis ranged between 0.007 (rs2295984) to 0.043 (rs17186340), showing that although they are marginally associated with schizophrenia they would not have been considered for follow-up at that level of association. As was the case with the previous use of the polygene score[18], we showed that the polygene score and epistatic models based on a narrow psychosis training sample was able to significantly account for variation in working memory in two independent psychosis samples, but not able to predict variation in controls. Although the control sample size was modest (N = 89) the other psychosis sample was of a similar size (N = 84) so a lack of statistical power cannot fully explain the inability to account for variation in controls.

We have introduced a novel method to evaluate the combined effect of the polygene score and epistasis using a training-independent test set approach, which is both simple and computationally tractable at the pathway level. The addition of the epistatic terms also increased the interpretability of the model, as it is difficult to determine which genes were contributing signal to the polygene score. Although it is clear that this method leads to very optimistic results on the training data (Table 2), we show the results are generalisable to two independent test sets of patients – one with narrow psychosis and the other with non-schizophrenia psychosis – similar to previous studies' use of the polygene score.[18] In both instances the variation explained by our epistatic terms is much larger than that explained by the polygene score itself: the polygene score explains between 1.2-1.3% of variation whereas increases in R^2 using our novel approach in the test sets are between 2.7-4.9%. Epistasis is thought to be a key element in complex phenotypes[16-17] and has been shown to influence risk for schizophrenia and inefficient dorsolateral prefrontal cortex processing during a working memory task in healthy controls.[16-17, 38-39]

In conclusion, this study is the first, to our knowledge, to investigate the role of the ZNF804A pathway in the cognitive decline commonly evident amongst psychotic patients. We have identified three new candidate genes for working memory in the ZNF804 pathway: STAC, MAPK8IP2 and FAM46A. Perhaps more critically, we introduced an improvement in the use of polygene scores by adding an epistatic component which explained additional variation in working memory that was specific to cases with psychosis.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary information is available at *Molecular Psychiatry's* website.

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Table 1. Participant demographics, neurocognitive and clinical measures

	Patients		Healthy participants
	Psychosis narrow	Psychosis broad	
	N=340	N=424	N=89
Psychosis subtype SZ SZA BP MDD PNOS	 N = 282 N = 58	 N = 282 N = 58 N = 61 N = 11 N = 12	N/A
Gender (ratio; M:F)	2.6:1	2.2:1	1.4:1
Age (years; mean (SD))	41.3 (12.2)	41.3 (12.4)	36.27 (12.8)
Age at onset (years; mean(SD))	22.8 (7.2)	23.2 (7.5)	N/A
Chlorpromazine equivalent (mg/day; mean(SD))	589.8 (562.4)	555.5 (540.7)	N/A
SAPS/SANS: Manic (mean (SD)) Depression (mean (SD)) Positive (mean (SD)) Disorganised (mean (SD)) Negative (mean (SD))	 -0.18 (0.95) 0.16 (1.07) -0.02 (0.99) -0.22 (0.76) 0.39 (0.9)	 0.04 (1.09) 0.23 (1.06) -0.12 (0.95) -0.31 (0.78) 0.32 (0.87)	N/A
Cognition: full scale IQ (mean (SD))	89.6 (17.8)	90.3 (18.3)	124.6 (13.3)

Table 2. Significant variance explained (R^2) and associated *p*-value for ZNF804A polygene score regression on neuropsychological phenotypes

Phenotype	Narrow Psychosis			Broad Psychosis		
p- value threshold	*1.0e*$^{-05}$	*0.05*	*0.5*	*1.0e*$^{-05}$	*0.05*	*0.5*
Including ZNF804A: Performance IQ (R2, (p-value))	0.009 (0.26)	0.001 (0.51)	0.002 (0.41)	**0.012 (0.028)**	<0.001 (0.78)	0.004 (0.21)
Including ZNF804A: IPSAQ EB (R2, (p-value))	**0.030 (0.012)**	0.0070 (0.21)	0.0030 (0.43)	**0.025 (0.010)**	0.0040 (0.28)	0.0030 (0.39)
Including ZNF804A: SWM Strategy (R2, (p-value))	0.010 (0.09)	**0.017 (0.028)**	0.003 (0.35)	0.0040 (0.23)	**0.013 (0.034)**	0.0020 (0.41)
Including ZNF804A: SWM Strategy (R2, (p-value))[1]	0.010 (0.11)	**0.017 (0.028)**	0.003 (0.36)	0.0040 (0.23)	0.011 (0.052)	0.0010 (0.47)

1. Only the significant *p*-value threshold for SWM strategy (*p*-value < 0.05) included ZNF804A SNPs.

Table 4. Decrease in ZNF804A polygene score regression *p*-value in narrow psychosis training set as number of interactions increases

Model	Polygene Score P-value
Polygene count score	0.12
Polygene Count Score + rs17186340T:rs140512A	0.093
Polygene Count Score + rs17186340T:rs140512A + rs2295984T:rs34138673G	**0.044**

Table 3. Increase in R^2 values using epistasis in conjunction with ZNF804A polygene scores across the narrow psychosis test set and other psychosis set

Model	Train R^2	Train *p*-value	Test R^2	Test *p*-value	Other R^2	Other *p*-value	Total Test Cases R^2	Total Test Cases *p*-value	Control R^2	Control *p*-value
Polygene Count Score	0.017	0.12	0.013	0.17	0.013	0.34	0.012	0.11	0.03	0.11
Polygene Count Score + rs17186340T:rs140512A	0.13	6.70E-005	0.027	0.05	0.05	0.06	**0.035**	**0.0064**	0.0069	0.45
Polygene Count Score + rs17186340T: rs140512A + rs2295984T:rs34138673G	0.16	2.70E-005	**0.04**	**0.016**	**0.062**	**0.036**	**0.048**	**0.0012**	0.001	0.77

The novel psychosis risk variant CNNM2 rs7914558: Effects on social cognition

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Abstract

A single neucleotide polymorphism (rs7914558) within cyclin M2 (CNNM2) was recently identified as showing genome-wide level significance for association with schizophrenia (SZ) risk. CNNM2's role in SZ was not previously hypothesized, and the mechanism by which it contributes to risk is unknown. We investigated the effect of the rs7914558 risk allele on measures of neurocognition, social cognition, and brain structure. Patients with SZ (N=400) and healthy controls (HC; N=160) completed measures of neuropsychological function and and social cognition. In the absence of effects on neuropsychological test performance, a dosage effect of the CNNM2 risk allele was observed for social attributions, such that the risk allele was associated with reduced externalizing bias in both patients and HC (GG>AG>AA; $p<0.05$). Structural imaging data (T1-MRI) were acquired from Irish HC (N=159) and Italian SZ patients (N=82) and HC (N=39). Using voxel based morphometry to compare carriers and non-carriers of the risk allele on neuroanatomical regions putatively supporting social cognition, we found that genotype was associated with GM volume in the right temporal pole and right anterior cingulate (ACC; $p_{corrected}<0.05$)in the Irish sample; neuroanatomical associations between CNNM2 and GM volume in ACC were also observed in the Italian samples. While the biological role of CNNM2/rs7914558 in SZ remains unknown, these data suggest that the increased illness risk for SZ associated with this variant is likely to be mediated, at least in part, via effects on neural systems relevant to social cognition.

Keywords: schizophrenia, CNNM2, social cognition, voxel based morphometry, imaging genetics

Introduction

Schizophrenia (SZ) is a major cause of global disability with a lifetime risk of approximately 1%. Perhaps due to its complex genetics, identifying the genetic variation responsible for disease risk has proven difficult despite SZ's substantial heritability (~80%)[1-4]. Genome wide association studies (GWAS) have identified a small number of genome wide significant ($p<7.2\times10^{-8}$) common risk variants[5-6]. Recently, the psychiatric genetics consortium (PGC) GWAS 'mega-analysis' of over 51,000 cases and controls identified seven risk loci, including five novel findings[7]. Of the novel loci identified, one of these (on 10q 34.33) was unique in harboring two genome wide significant variants, including the rs7914558 single nucleotide polymorphism (SNP) located within an intron of the cyclin M2 gene (CNNM2[1]; OMIM: 607803).

CNNM2 is implicated in the transport of magnesium (Mg^{2+}), which plays a crucial role in many biological processes, including neuronal transmission. Despite the extensive evidence for unique mammalian Mg^{2+} transporters, CNNM2 is one of only a few proteins biochemically identified as fulfilling this role[8]. Sequence variation within CNNM2 increases hypertension risk *and* alters serum Mg^{2+} blood levels, a risk factor for a variety of vascular diseases[9]. Furthermore, CMMN2 mutations are associated with hypomagnesaemia risk[10]. Whether or how this biological role in Mg^{2+} regulation is relevant to CNNM2's association with increased risk for SZ is uncertain.

Approaches to elucidating the biological role of novel SZ genetic susceptibility factors includes delineating their effects on "intermediate" or "endo-" phenotypes - i.e. discrete aspects of behavior or brain strucuture and function that mediate genetic effects on the broader phenotype[11-13]. Neurocognitive intermediate phenotypes widely used in SZ genetics include general cognitive ability, long-term and working memory, and attentional control. In addition to these more 'traditional' neuropsychological phenotypes, social cognition, which includes functions such as emotional recognition and perception, theory of mind (TOM), and attributional style, has been a recent focus of SZ gene studies. This is largely due to the notion that measures of social cognition predict social and occupational function in SZ independently of neurocognitive task performance[14]; thus, studying the contribution of SZ risk variants to potentially heritable deficits in social cognition may index aspects of disability distinct from traditional neurocognitive tasks. For example, while ZNF804A, the first variant to achieve genome wide significance for SZ, has not been reliably associated with general cognitive deficits, it has been associated with poorer performance on indices of social cognition. Healthy individuals carrying the ZNF804A risk allele showed reduced activation during a TOM task, compared to non-risk carriers[15]. Similarly in a large sample of patients and healthy controls, we found that being a ZNF804A risk carrier significantly increased biases in social attributions[16].

This study considered the effects of CNNM2/rs7914558 on indices of neurocognition and social cognition in both patients and healthy individuals, with the aim of testing the hypothesis that the increased risk associated with CNNM2 was mediated via deleterious effects on both these aspects of cognition. Furthermore, we sought to investigate the impact of CNNM2 on brain structure (i.e. grey (GM) and white matter (WM) volume), in order to follow-up significant cognitive associations via delineating the mediating impact of CNNM2/rs7914558 on brain structure in regions underlying associated functions.

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[1] Note: this gene was previously known as ancient conserved domain protein 2 (ACDP2)

Methods

Participants (Table 1)

Irish samples: Neuropsychological testing included 400 clinically stable patients with DSM-IV diagnosis of SZ or schizoaffective disorder and 160 healthy individuals. Participants were recruited from 5 sites across Ireland, and ethics approval was obtained from local ethics committees. Written informed consent was obtained from all subjects. Participants were aged 18 to 65 years and of confirmed Irish lineage (i.e. Irish grandparents on both sides of the family). Healthy individuals had no history of significant neurological or psychiatric conditions and no first degree relatives with a history of psychosis.

Structural magnetic resonance imaging (sMRI) data were acquired from healthy individuals as part of the Trinity College Institute for Neuroscience (TCIN) biobank project [see 17 for details]. Participants were right handed and had no history of significant neurological, psychiatric or other major medical health problems, and had no other contraindication for MRI. In accordance with local ethics committee approval, written, informed consent was obtained for the use of sMRI data and subjects provided a saliva sample (Oragene DNA self-collection kits: DNA Genotek, Ontario, Canada) for genetics analysis. Individuals (N=159) were selected for imaging analysis based upon sMRI data quality and successful CNNM2/rs7914558 genotyping. While the majority were Caucasian Irish or other European (N=129), for 30 participants lineage was not documented. Nonetheless, the relative homogeneity of the local population suggests that these participants were also likely to be Caucasian Irish (92% of the population of Dublin city are of Irish/Caucasian background; www.cso.ie/census). Further, this sub-group did not differ from other imaging participants in gender ratio, age and years of education, or minor allele frequency.

Italian imaging sample: Patients with a confirmed diagnosis of SZ (N=66) were recruited consecutively from two outpatient clinics in central Italy. Patient exclusion criteria included a history of: (1) DSM-IV axis I or II diagnosis except SZ; (2) traumatic head injury with a loss of consciousness; (3) epilepsy, seizures or other relevant neurological or medical illness (e.g. cerebrovascular disease); and/or (4) substance abuse in the six months pre-participation study. Healthy participants (N=37) were recruited from the same geographical area as patients and screened for current or past diagnosis of any DSM-IV axis I or II disorder using the SCID-I and II[18-19]. In addition to the exclusion criteria outlined for patients, those individuals with a history of schizophrenia or any other psychiatric disorder diagnosis among first-degree relatives were also excluded. All participants were of Caucasian Italian ancestry.

>>Table 1<<

Procedure

Cognitive assessment: Participants completed a neuropsychological battery that included measures of IQ (i.e. WAIS-III[20]), working and long term memory (i.e. letter number sequencing and logical memory subtests from the WMS-III[21] and the CANTAB spatial working memory task[22]), and attentional control (i.e. the CANTAB intradimensional/extradimensional set-shifting task[22]).

Social cognitive assessment: Social cognitive function was assessed using the: (1) 'Hinting' Task (HT)[23]; (3) Reading the Mind in the Eyes (RME)[24]; and (4) Internal, Personal, and Situational Attributions Questionnaire (IPSAQ)[25]. The IPSAQ is concerned with attributional style and uses subscale scores that delineate the extent of internal and external attributions for hypothetical events to calculate two indices of cognitive bias, i.e. externalizing bias (EB) and personalizing bias (PB). While EB scores index the propensity of an individual to attribute positive *or* negative events internally (i.e. the extent of self-serving bias), PB is indicative of the tendency to credit external attributions to personal (i.e. actions or omissions of another) *or* situational factors (i.e. circumstance or chance). In contrast, HT and RME target TOM processes. These data were collected as part of an ongoing multisite project, consequently

all assessments were not available in the entire cohort (IPSAQ: N=387 256 patients & 131 controls); HT: N=408 (276 patients & 132 controls); RME: N=201 (151 patients & 50 controls)).

Neuropsychological data were analyzed in SPSS (Release 16; SPSS Inc., Chicago, IL, USA). For each measure, univariate ANOVA considered both group (patient vs. control) and rs7914558 genotype as fixed effects, and included age, gender and IQ as co-variates.

sMRI: Irish structural images were acquired on a Philips Intera Achieva 3T MRI system, and involved the acquisition of a 180 slice T1-weighted image using a TFE gradient echo pulse sequence (TR=8.4ms, TE=3.8ms, flip angle=8°, slice thickness=0.9mm, voxel size=0.9mm^3, 180slices, duration=6min). Italian sMRI involved a 1.5T whole-body Siemens Vision Magnetom scanner and the acquisition of T1-weighted images using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR=11.4ms, TE=4.4ms, flip angle=151°, slice thickness=1mm, no interslice gap, voxel size=1mm^3).

Voxel based morphometry (VBM)[26]: sMRI analysis was performed within SPM5 (http://www.fil.ion.ucl.ac.uk/spm) running under Matlab (v7.8; The MathWorks, Cambridge, UK) and utilized the VBM toolbox (v5.1; http://dbm.neuro.uni-hen.dc/vbm). Volumes passing initial data quality control (i.e. visual inspection for scanner artifacts and gross anatomical abnormalities) were segmented into GM, WM, and CSF, without tissue priors and using a Hidden Markov Random Field weighting of 0.15. Segmented images were normalized using DARTEL[27], in which GM and WM templates were created using standard parameters. Jacobian scaled ('modulated') warped tissue classes were subsequently created for both GM and WM for each subject and the resultant images smoothed with an 8mm^3 Gaussian kernel.

sMRI data were analyzed using full factorial ANOVA models in SPM5. Separate analyses were carried out for the Irish and Italian imaging samples. Modeling included genotype as a fixed factor with either three (i.e. AA vs. AG vs. GG) or two (i.e. risk homozygotes vs. non-risk carriers) levels plus age and gender as covariates. To account for normal variation in brain volume, total volume was also included as a covariate. Analysis of the Italian dataset also included group (patient vs. control) as a fixed-factor. In addition to preliminary whole brain analyses of GM and WM, based upon the outcomes of our cognitive analyses (see Results) *post hoc* region-of-interest (ROI) analyses considering GM variation in regions that support social cognitive functions (i.e. anterior cingulate cortex (ACC)/BA32; superior temporal sulcus/BA22; and temporal pole/BA38)[28-30] were also carried out.

Since the non-uniform smoothness of VBM data can influence the interpretation of these types of analysis[26, 31], prior to the determination of significance a non-stationarity cluster extent correction utilizing the random field theory version of cluster inference under non-stationarity[32] was implemented using the NS toolbox for SPM5 (http://fmri/wfubmc.edu/cms/NS-General).

Genotyping: Genetics analysis was carried out using DNA extracted from blood or saliva samples. The rs7914558 SNP was genotyped using a Taqman® SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was >95% and the samples were in Hardy-Weinberg equilibrium ($p>0.05$). Along with these samples a small number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for rs7914558 for quality control purposes and were found to be concordant with available HapMap data for this SNP.

Results

Demographics: There was no genotype-related variability in gender ratio, age, or years of education in any of the samples (Table 1). Moreover, in patient samples, rs7914558 was not associated with age of onset, illness duration, nor medication (i.e. chlorpromazine equivalents) and there was no difference in genotype frequency between patients and controls. There was, however, a significant difference in age and years of education between groups, such that patients were older (Italian VBM: $F_{(1,97)}=10.79$, $p=0.001$; Irish Neuropsych.: $F_{(1,537)}=4.36$,

$p<0.05$) and had less years of education (Italian VBM: $F_{(1,81)}=21.59$, $p<0.001$; Irish Neuropsych.: $F_{(1,537)}=105.61$, $p<0.001$).

Irish neuropsychology

General cognition (Table S1): While patients performed worse than controls on all measures of general cognitive function ($p\leq0.001$), there was no genotype-related variability on indices of IQ, memory or attentional control.

Social cognition: Regarding TOM processes, HT performance was unaffected by group, yet patients performed significantly worse on the RME ($F_{(2,121)}=8.11$, $p<0.05$). Performance on TOM measures was unaffected by genotype.

IPSAQ cognitive bias scores were differentially impacted by group and genotype. For example, PB was significantly lower in patients than controls ($F_{(2,375)}=6.12$; $p<0.05$) but was unaffected by genotype. Conversely, EB varied according to genotype ($F_{(2,375)}=5.46$, $p=0.004$) but not group (Figure 1). This effect was due to the linear impact of genotype on EB ($F_{(1,389)}=10.14$, $p<0.001$), such that 'GG' individuals scored highest on this subscale, followed by 'AG' and then 'AA' individuals ($p<0.05$).

Irish sMRI/VBM

GM (Figure 2): There were no significant effects of genotype that survived correction for multiple comparisons in either the three (AA vs. AG vs. GG) or two (AA vs. AG/GG) genotype group whole brain analyses. Conversely, *post hoc* anatomical ROI analyses were indicative of a main effect of CNNM2/rs7914558 status. In the three group analysis, there was a main effect of genotype in the right temporal pole/BA38 (MNI: 40 16 -33; $F_{(2,154)}=7.88$; number of voxels/cluster extent (K_E)=217; $p_{CORRECTED}<0.05$) and ACC/BA32 (MNI: 10 39 19; $F_{(2,154)}=7.26$; $K_E=50$; $p_{CORRECTED}<0.05$). In both clusters *post hoc* t-tests indicated that this main effect was due to relatively greater GM volume in homozygous 'A' individuals compared to heterozygotes ($t_{(154)}=3.95$ & $t_{(154)}=3.77$; $p_{CORRECTED}<0.05$). Similarly, the two group analysis was also indicative of a significant main effect of genotype in the right temporal pole (MNI: 39 15 -33; $F_{(1,155)}=14.92$; $K_E=948$; $p_{CORRECTED}<0.05$) and right ACC (MNI: 13 41 18; $F_{(1,155)}=11.47$; $K_E=118$; $p_{CORRECTED}<0.05$). Again this was due to relatively greater volume in 'A' homozygous individuals (AA vs. AG/GG: $t_{(155)}=3.39$ & $t_{(155)}=3.86$; $p_{CORRECTED}<0.05$).

WM: Following correction for multiple comparisons at the whole brain level there were no WM regions where volume varied as a function of genotype, irrespective of whether rs7914558 was considered as a fixed factor with three or two levels.

Italian sMRI/VBM

GM: Both three and two genotype group analyses were indicative of a main effect of group ($p_{CORRECTED}<0.05$) on GM volume in a wide range of regions (Table S2). However, there were no whole brain effects of genotype (main effect or interaction effects) on GM volume that survived correction. Similarly, *post hoc* ROI analyses modeling genotype as a fixed factor with three levels failed to reveal a significant main effect of genotype or any interaction between group and genotype on GM volume. However, when 'A' homozygotes were compared to the combined group of hetero- and homozygous carriers of the rs7491558 non-risk 'G' allele there was a significant interaction between group and genotype in the left anterior cingulate (MNI: -7 7 52; $t_{(97)}=3.80$; $K_E=135$; $p_{CORRECTED}<0.05$). Within this cluster, patients who were homozygous for the risk allele showed reduced GM volume ($t_{(97)}=3.49$; $K_E=101$; $p_{CORRECTED}<0.05$), while control homozygous risk carriers had greater GM volume ($t_{(97)}=3.79$; $K_E=69$; $p_{CORRECTED}<0.05$), compared to carriers of the 'G' allele.

WM: As with the GM analyses, there was a main effect of group on WM volume in a number of clusters ($p_{CORRECTED}<0.05$; Table S2), but no impact of CNNM2/rs7149558 genotype on WM volume in either three or two genotype group whole brain analyses.

Discussion

This study sought to ascertain the neurocognitive effects of the putative SZ risk variant CNNM2/rs7914558. In Irish patients and healthy participants the risk 'A' allele was associated with variability in social cognitive function, in the absence genotype-related deficits in general cognitive function that are typically altered in SZ. Specifically, carriers of the risk allele exhibited a *reduced* 'self-serving' bias – i.e. the adaptive tendency to attribute more positive than negative events to oneself. The same CNNM2 variant was also associated with variation in GM volume in putative 'social cognition' regions (i.e. anterior cingulate and temporal pole), compared to non-risk individuals (AG and GG). Collectively, these data suggest that one mechanism by which this gene increases SZ risk is via an impact on the neural underpinnings for the processing of social stimuli.

CNNM2/rs7914558 and social cognition

Differences in attribution style are commonly reported in psychiatric conditions. In paranoia, for example, attributional style is characterized by the tendency to attribute negative events to *external*, *global* and *stable* factors [33]. While healthy individuals are more likely to attribute positive events to internal factors, and attribute negative events to external factors (i.e. in a manner consistent with self-serving bias), attributional style in depression is defined by a tendency to consistently attribute negative events internally. Our observations suggest that carriers of the risk 'A' allele lacked a 'healthy' self serving bias and made fewer positive and more negative internal attributions for negative events, i.e. in a manner similar to attributional profiles in depression [34]. Jolley and colleagues found that depression and grandiosity in SZ symptomology were significantly associated with attributional style, such that high externalizing bias scores were associated with grandiose symptoms, while depression was related to reduced self-serving bias and an externalizing attributional style for positive events[35]. Since persecutory delusions are thought to be associated with high EB scores, this observation may suggest that the CNNM2/rs7914558 risk might delineate a subgroup of patients where positive symptoms are less pronounced.

CNNM2/rs7914558 and brain structure

Anatomical ROI analysis of sMRI data were indicative of CNNM2-related variability in GM volume in putative social cognition regions (i.e. right ACC and temporal pole) in both imaging samples. Although these analysis were subsequent to the observed association between rs7914558 risk and IPSAQ/EB scores, it is unclear how these structural variations may be associated with behavioral differences between the genotype groups. A recent functional imaging investigation, which may speak to this issue, found that activity in dorsal ACC (dACC) was associated with self-serving bias in causal attribution[36]. Presuming a causal relationship between brain structure and function in the ACC, our data may indicate that variability associated with the risk 'A' allele in ACC alters activation in a manner that contributes to changes in self-serving bias. In addition, dACC volume is reduced in psychiatric disorders that involve emotional dysregulation (including depression)[37-39] and a recent study in healthy female participants found that dACC volume was related to cognitive reappraisal in emotion regulation[40]. Therefore, CNNM2/rs7914558 may confer risk for SZ and changes in attributional style via an impact on emotional processing mediated by alterations in GM volume in regions that regulate these functions, such as the ACC. Although this notion is intriguing, it is curious that distinct genotype effects on GM were noted in healthy individuals vs. patients. That similar effects were seen in Irish and Italian controls (e.g. AA >AG/GG in ACC), despite the latter sample being relatively underpowered, suggests that in unaffected individuals CNNM2-risk is reliably associated with increased volume. By comparison, patients who were homozygous carriers of the risk allele had relatively reduced GM volume in this same region. This findings was not hypothesized in our study, and may be the result of multiple factors,

including CNNM2 interaction with other SZ genetic risk factors and/or environmental influences that are specific to the disease phenotype. These interpretations are clearly speculative, and could not be tested given the relatively small sample size available for sMRI; however this diagnostic distinction in the influence of CNNM2 genotype on ACC volume is intriguing and warrants further investigation.

Bioinformatic analysis of CNNM2/rs7914558

The SNP under consideration here, rs7914558, is located on chromosome 10q24 in a large region of high and complex linkage disequilibrium (LD). Analysis of HapMap Phase II + III data (release 28 NCBI build 36) indicates that rs7914558, positioned at 104,765,898(hg18) is in high LD (r2>0.8) with 42 other SNPs in this region (including 22 SNPs at r2=1). These SNPs span a 322kb region from rs11191438 at position 104,627,854 to rs4307650 at position 104,949,842. This region contains four RefSeq genes: C10orf32, AS3MT, CNNM2 and NT5C2. Analysis of SNP x gene expression databases including brain (see Supplementary information) does not identify a direct functional link between rs7914558 (or proxy) and altered expression of any of the four genes in this region, thus all four remain candidate schizophrenia loci.

Study Limitations

Interpretation of these data are limited by two key factors. Firstly, little is known regarding the functional pathways of the CNNM2/rs7914558 SNP considered here, either in general or with regards to SZ specifically. Certainly, our understanding of how or why this particular variant contributes to social cognitive processes involved in attributional style and variability in brain volume, and how this speaks to SZ risk, will be significantly enhanced by the elucidation of the functional biology of this particular variant. Secondly, we are limited by the absence of SZ patients in the Irish imaging sample, and as such are unable to more fully determine patterns of similarity and/or difference between the Irish and Italian samples. Finally, the relatively small number of patients available for sMRI means that we are unable to accurately model other factors that might contribute to patterns of altered brain volume due to variability in the CNNM2/rs7914558 genotype.

Conclusions

In conclusion, the present study provides evidence that a novel genome-wide associated risk variant for SZ, CNNM2 (rs7914558), may contribute to illness risk via a role in social cognitive processing and brain volume in regions that support these functions. Moreover, these effects seem to be independent of a more global effect of illness on cognitive functioning. Although further exploration of this variant and replication of these results is necessary, these data provide support for the utility of studies of brain structure and behavior in the investigation of GWAS-identified variants for elucidating the contribution of risk variants to illness etiology.

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	Total Sample		CNNM2/rs7914558						Comparison
			GG		AG		AA		
Neurocognition	***N=560***		***N=89***		***N=258***		***N=213***		
	Patients N=400	Controls N=160	Patients N=66	Controls N=23	Patients N=174	Controls N=84	Patients N=160	Controls N=53	
Gender (F:M)	111: 288	76: 84	22: 44	12: 11	49: 125	43: 41	41: 119	21:32	ns
Age (years; mean(s.d.))	41.45 (12.25)	37.14 (12.70)	39.96 (10.45)	39.61 (14.14)	42.52 (11.86)	37.13 (12.33)	42.32 (11.79)	36.12 (13.02)	Controls < Patients $F_{(1,537)}$=4.86, p <0.05
Years of Education (mean (s.d.))	13.09 (2.55)	15.98 (2.42)	13.86 (2.47)	16.13 (2.63)	12.95 (2.45)	16.17 (2.60)	13.11 (2.71)	15.61 (2.00)	Controls > Patients $F_{(1,537)}$=105.61, p<0.001
Age at onset (years; mean (s.d.))	22.79 (7.32)	n/a	21.57 (5.52)	n/a	23.28 (8.22)	n/a	21.87 (6.03	n/a	ns
Chlorpromazine Equivalents (mg/day; mean (s.d.))	582.84 (543.76)	n/a	549.22 (478.55)	n/a	570.17 (511.79)	n/a	597.11 (618.99)	n/a	ns
sMRI: Irish sample	***N = 159***		***N = 21***		***N = 67***		***N = 71***		
Gender (F:M)	87: 72		14: 7		37: 30		36: 35		ns
Age (years; mean(s.d.))	27.86 (12.42)		31.33 (16.19)		26.75 (10.91)		27.87 (12.51)		ns
Years of Education (mean (s.d.))	17.00 (2.30)		17.06 (3.06)		17.02 (2.11)		16.97 (2.26)		ns
sMRI: Italian Sample	***N = 103***		***N = 23***		***N = 45***		***N = 35***		
Group (frequency)	Patients (N = 66)	Controls (N = 37)	Patients (N = 17)	Controls (N = 6)	Patients (N = 23)	Controls (N = 22)	Patients (N = 26)	Controls (N = 9)	ns
Gender (F:M)	25: 41	18: 19	5: 12	3: 3	6: 17	9: 13	14: 12	6: 3	ns
Age (years; mean (s.d.))	40.67 (11.67)	32.28 (12.58)	38.41 (10.83)	31.00 (17.54)	41.65 (12.01)	34.09 (12.89)	41.27 (12.15)	29.11 (7.89)	Controls < Patients $F_{(1,97)}$ = 10.79, p = 0.001
Years of Education (mean (s.d.))	10.98 (4.02)	15.38 (3.20)	11.00 (3.93)	15.60 (4.93)	9.65 (3.14)	15.28 (3.10)	12.58 (4.59)	15.50 (2.26)	Controls > Patients $F_{(1,81)}$ = 21.59, p < 0.001
Age at onset (years; mean (s.d.))	24.11 (8.34)	n/a	24.00 (6.24)	n/a	23.05 (7.55)	n/a	25.42 (10.69)	n/a	ns
Duration (years; mean (s.d.))	18.18 (10.41)	n/a	15.12 (8.85)	n/a	19.55 (10.21)	n/a	19.16 (11.76)	n/a	ns
Chlorpromazine Equivalents (mg/day; mean (s.d.))	507.64 (606.42)	n/a	517.50 (297.92)	n/a	611.95 (912.49)	n/a	384.05 (309.31)	n/a	ns

Table 1: Participant demographics

Figures

Figure 1: Main effect of CNNM2/rs7914558 genotype on IPSAQ EB score. Note: Error bars show +/- 1 standard error. * $p < 0.05$

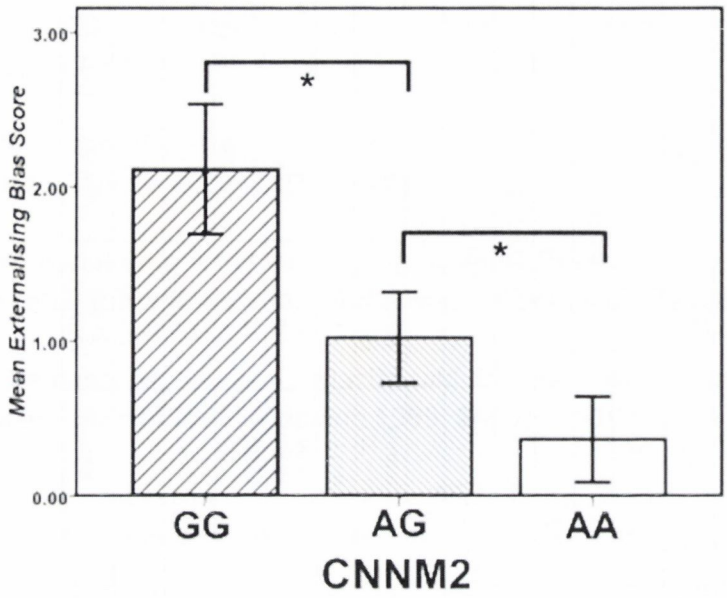

Figure 2: The impact of CNNM2/rs7914558 genotype on GM volume in *post hoc* anatomical ROI analysis in regions supporting social cognitive function (ACC, superior temporal gyrus and temporal pole) in: **A.** Irish healthy individuals (AA vs. AG vs. GG); **B.** Irish healthy individuals (AA vs. AG/GG); and **C.** Italian patients and healthy individuals (AA vs. AG/GG) – (i) 3-plane view of GM volume differences (ii) SPM contrast. Note: Clusters showing an impact of genotype are shown rendered on the ch2better brain template from MRIcron (www.nitrc.org/projects/mricron).