Cognition, psychopathology and the role of genetic variation in Catechol-O-Methyltransferase in children at increased risk of schizophrenia

Maria Niarchou

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Statements and Declarations

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

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Contributions

With regards to the projects based on the ALSPAC birth cohort (i.e., Chapters 6 & 7), I have performed all the analysis used in this thesis, I have derived all the cognitive variables and the haplotypes, interpreted the results, conducted the literature reviews, read and summarized all background information used in this work.

Concerning the project on 22q11.2DS (i.e., Chapter 8), I have administered the psychiatric interviews of 11 children and the cognitive testing of 10 children that form part of the project’s sample. I have entered, double-checked and cleaned all the data for errors. I have derived all the psychiatric diagnoses which were also double-checked by one or two of my colleagues and the team’s psychiatrist, Dr Jane Scourfield. I have done all the analyses, the literature review and the writing-up. I also did the DNA extractions and DNA quantification of approximately 60 samples that form part of this thesis.

All of my work was followed by the guidance of my supervisors, Dr Marianne van den Bree, Professor Michael Owen and Dr Stanley Zammit. Professor Glyn Lewis and Dr James Walters commented on the publication that arose from Chapter 6 and their suggestions were incorporated in this thesis. Professor Anita Thapar and Professor Stephanie van Goozen commented on the publication that arose from Chapter 8 and their suggestions were also included in this thesis.
Publications based on this thesis


Niarchou M, Zammit S, Owen MJ, van den Bree MB. Exploring the indirect effects of Catechol-O-Methyltransferase (COMT) on psychotic experiences through cognitive function and anxiety disorders in children. (in submission)
Publications to which I have contributed


*joint first authors

Summary

In this thesis I explored cognition, psychopathology and the role of Catechol-O-Methyltransferase (COMT) in children at increased risk of schizophrenia with the aim of making a contribution to our understanding of the processes that take place early in the development of psychosis. Two samples were studied. The first sample came from the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) where I examined the relationships between a priori selected cognitive domains and psychotic experiences (PEs). The results indicated that impaired processing speed and attention were related to greater risk of PEs in children, with processing speed being a key cognitive feature. Moreover, the relationships between cognition and later occurrence of PEs were similar to those that have previously been reported between cognition and schizophrenia. I also examined whether genetic variation in COMT was associated with PEs indirectly through cognition and anxiety disorders. The findings showed that COMT was indirectly associated with PEs through processing speed, IQ and attention. The second sample comprised children with 22q11.2 Deletion Syndrome (22q11.2DS). I examined the nature and prevalence of psychopathology and cognitive dysfunction in the sample and their siblings and to what extent the children’s intellectual impairment indirectly influences the risk of psychopathology associated with the deletion. There were high rates of psychopathology and cognitive impairments in children with 22q11.2DS. However, I found no evidence for an indirect association between the deletion and the risk of psychopathology through cognition. Finally, there was no evidence that COMT is related to the susceptibility of children with 22q11.2DS to cognitive and psychiatric problems.

These findings have potentially important implications for our understanding of the development of psychosis during childhood and they also show that using different research designs to investigate specific aims in samples at increased risk enables the researcher to widen their scope of interpretation.
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SECTION I GENERAL INTRODUCTION

Chapter 1 Overview of schizophrenia

Schizophrenia is a severe mental disorder with an incidence of 15-20 cases per 100000 per year, a lifetime prevalence of 0.3-0.7% and a lifetime risk of approximately 0.7-0.9%. Onset is usually in early adulthood with peak onset of 20-24 years in men and 25-29 years in women. The disorder has a male preponderance (male to female ratio; 1.4:1) (van Os and Kapur 2009).

Schizophrenia is characterized by positive, negative and cognitive symptoms. Positive symptoms include delusions (strongly held irrational ideas or beliefs that persist despite the absence of supporting evidence), hallucinations (perceptions of experiences without an external stimulus) and thought disorder. Negative symptoms refer to a reduction in social and occupational functioning and include social withdrawal, anhedonia, loss of motivation and blunted affect. It is noteworthy that many of these symptoms also appear in other psychotic disorders. Another core symptom of schizophrenia is impaired cognitive functioning which was recognised as such since its first diagnostic conception.

According to a survey by the World Health Organization, schizophrenia is among the top ten disorders that contribute to the global burden of disease (WHO 2008) and a leading cause of suffering for patients and their family members (Arango and Carpenter 2011). The financial burden of this devastating disorder is enormous and wide-ranging due to the frequent requirements for hospitalisation and long-term psychiatric care, the lost productivity, the costs of treatment and the increased mortality rates that are associated with the disorder (Knapp, Mangalore et al. 2004). Current therapeutic interventions provide only limited
treatment of the positive symptoms and often do not improve patients’ functioning. Addressing the cognitive impairments of this disorder could potentially improve the morbidity of this condition and enhance the quality of life of the patients.

1.1 A brief historical perspective

The historical roots of schizophrenia are poorly defined (Adityanjee, Aderibigbe et al. 1999). There is evidence that schizophrenia has existed for 2000 or more years (Bark 1985, Bark 1988), but there are also those who believe that Kraepelin was the first to describe dementia praecox because the illness increased in frequency around these times (Cooper and Sartorius 1977, Hare 1988).

It seems that the first concise description of schizophrenia was given by Pinel, in 1801, in France. He used the term démence (loss of mind) to describe the decline in cognitive functioning of chronically ill, hospitalised patients (Pinel 1962). This term had already been used in the French literature since 1381 to characterise mental decline (Berrios 1987). Fifty years later, Morel (also in France) was the first to coin the term démence précoce to describe young patients with premature dementia (Wender 1963). During the same period, Kahlbaum and Hecker in Germany, developed the concepts catatonia and hebephrenia (meaning youthful insanity, derived from the Greek goddess of youth Hebe), respectively (Palha and Esteves 1997).

The integration, however, of these variable clinical syndromes into a single nosological entity was realised by Kraepelin who conceptualized what subsequently became known as schizophrenia as dementia praecox (Kraepelin 1919). The term dementia was given to describe the chronic and debilitating course of the disorder and the term praecox to emphasize the early onset of the disease (Andreasen 2011). Kraepelin recognised the
diversity of the clinical syndromes associated with *dementia praecox*, and stressed the underlying *fundamental* disorders associated with it. These were the *general decay of mental efficiency* (i.e., cognitive deficits) and *loss of mastery over volitional action* (i.e., executive dysfunction) (Jablensky 2010).

In the early twentieth century Bleuler introduced the term *schizophrenia* (splitting of mind) and widened the concept to include disorders that do not necessarily lead to severe deterioration (Bleuler 1950). He also stated that schizophrenia is a group of diseases and distinguished between basic and supplementary symptoms. The basic symptoms are at the core of the disease and are also known as the four As; looseness of associations, blunted affect, ambivalence and autistic isolation (Insel 2010). The supplementary symptoms develop due to the patient’s inability to adapt to the basic symptoms (Bleuler 1950).

In the 1950s, Schneider further developed these concepts by specifying a group of psychotic symptoms he called *first-rank symptoms* (e.g., audible thoughts, thought withdrawal). It is noteworthy that his approach was descriptive rather than aetiological. Schneider’s symptoms were included in the early and recent diagnostic systems such as the Diagnostic Statistical Manual of Mental Disorders version IV (DSM-IV) and the International Classification of Diseases version 10 (ICD-10) (Adityanjee, Aderibigbe et al. 1999). Another, less influential, but more detailed classification of psychoses was introduced by Leonhard in his *endogenous* psychoses theory which grouped psychotic disorders based on a presumed localised cerebral dysfunction (Jablensky 2010).

Later, in the 1980s, Crow further characterised schizophrenia based on the presence of positive (hallucinations, delusions and formal thought disorder) and negative (social withdrawal, avolition, affective flattening and poverty of speech) symptoms (Crow 1980), a distinction still forming the basis for current diagnostic systems (Adityanjee, Aderibigbe et al.)
Another related concept, which has not been formalised in diagnostic criteria, was introduced by Carpenter and collaborators, i.e., the \textit{deficit schizophrenia} (a concept referring to enduring negative symptoms) (Carpenter, Heinrichs et al. 1988).

It should be noted that diagnostic systems such as Research Diagnostic Criteria (RDC), DSM-III and DSM-IV are still relying on Kraepelinian principles (Jablensky, Hugler et al. 1993, Hegarty, Baldessarini et al. 1994). Kraepelin himself, however, did express doubts about the dichotomisation of dementia praecox and manic depressive insanity (equivalent to bipolar disorder) and the controversial validity of this distinction is still being questioned today (Craddock and Owen 2010, Owen 2011).

Another way to conceptualise schizophrenia is the dimensional approach. The observation of attenuated psychotic symptoms in biological relatives of patients with schizophrenia (Kendler, McGuire et al. 1993), gave rise to theories supporting a continuum of psychosis/schizophrenia (for a review see: (Linscott and van Os 2013)). Sub-clinical levels are often referred to as psychosis proneness, psychotic-like experiences, schizotypy or at-risk mental states (Chapman, Chapman et al. 1994, Verdoux, van Os et al. 1998, Stefanis, Hanssen et al. 2002, Siever and Davis 2004) and are assumed to share etiological factors with schizophrenia (van Os and Linscott 2012). The etiological and biological validity of these psychotic symptoms however, is not yet clear (Lawrie, Hall et al. 2010) (also see Chapter 6.1) and the question of whether schizophrenia is better described categorically or dimensionally remains to be resolved (Jablensky 2010).

In view of the concerns regarding both the categorical and dimensional approaches, the most recent psychiatric classification revision (DSM-V) systems seem to keep the categorisation intact, but also emphasise the importance of dimensional approaches (American Psychiatric Association 2011). Cognition did emerge as a dimension in this revised diagnostic system.
A recent development in support of the dimensional approach is the NIMH's Research Domain Criteria (RDoC) project, an experimental approach whose focus is to incorporate multiple dimensions instead of diagnoses that are based on behaviour, genetics, cognition and neurobiology providing a more effective framework for research and in the long term in clinical practice.

1.2 Aetiology of schizophrenia

Despite a large amount of research, the complexity of human behaviour complicates our understanding of the aetiology and the underlying pathological mechanisms that operate in schizophrenia. It seems that schizophrenia is a complex and multi-factorial disorder that is the consequence of the action of both genetic and environmental factors.

There is considerable evidence that schizophrenia has a strong genetic component (see Chapter 3), with heritability estimates ranging from 60 to 90% (McGuffin, Farmer et al. 1984, Cardno, Marshall et al. 1999, Kendler 2002).

The neurodevelopmental hypothesis of schizophrenia has been proposed by many researchers for more than two decades and provides a framework for understanding the pathogenic mechanisms of schizophrenia by focussing on antecedents involved in early brain development (Owen, O'Donovan et al. 2011). It also has implications for public health as it supports early prevention and intervention at multiple ages (Weinberger and Levitt 2011).

According to this model, pathogenesis is due to a combination of genetic and environmental abnormalities in brain development (Murray 1987, Weinberger 1987). Indeed, there is considerable evidence that adult-onset schizophrenia is associated with abnormalities in brain development as well as delays and impairments in cognitive, emotional, motor and social
development (Fish, Marcus et al. 1992, Jones, Rodgers et al. 1994, Cannon, Bearden et al. 2000, Rosso, Bearden et al. 2000, Lawrie and Pantelis 2011, Meyer-Lindenberg and Bullmore 2011). Premorbid psychopathology, including attention deficit hyperactivity disorder (ADHD), conduct disorder, depression and anxiety as well as the presence of psychotic experiences in childhood and adolescence have frequently been observed in individuals who later develop schizophrenia (Schaeffer and Ross 2002, Kim-Cohen, Caspi et al. 2003). With regards to environmental effects, these can be at different stages of development, from complications during foetal development (Cannon, Jones et al. 2002) through to cannabis use in adolescence (Zammit 2004). Other environmental factors that have been related to increased risk of schizophrenia are urbanicity (Krabbendam and van Os 2005) and migration (Veling, Susser et al. 2008).

Nevertheless, it is not yet known whether these premorbid impairments cause schizophrenia or whether they are non-specific markers of an underlying pathological process that may cause both the premorbid impairments and psychosis. If they are on the causal pathway, then modifying the cognitive or social factors could reduce the risk of developing the disorder (Hollis and Rapoport 2011).

1.3 Clinical course and treatment

With regards to the clinical course of schizophrenia there is great unexplained inter-individual variability and it can range from short, acute episodes of illness to chronic disabling conditions.

Patients with schizophrenia typically experience a premorbid state.

Bleuler (as cited in (Reichenberg 2005)) wrote in 1911:
‘...there are early character anomalies which can be demonstrated by careful case histories in more than half the individuals who later become schizophrenic: the tendency to seclusion, withdrawal, together with moderate or severe degrees of irritability.’

There is evidence that the time between developing the first psychotic symptom and receiving treatment is associated with long-term functional outcome (Marshall, Lewis et al. 2005, Perkins, Gu et al. 2005). Early intervention services have been adopted in the UK and other countries that aim to identify, engage and treat those at high risk of developing psychosis. These interventions seem to improve outcome and are financially beneficial, though their long-term effectiveness remains questionable (Gafoor, Nitsch et al. 2010, McCrone, Craig et al. 2010).

Antipsychotic drugs, especially those that block dopamine D2 receptors, form the mainstay of pharmacological treatment (Kapur and Mamo 2003). Despite the advances and the development of newer atypical antipsychotics, approximately one third of patients remain symptomatic and the majority are unable to attain functioning goals such as taking up employment (van Os and Kapur 2009). Moreover, cognitive impairments cannot yet be adequately treated (Reichenberg, Harvey et al. 2009). Alternative approaches include the development of non-pharmacological interventions that seek to reduce the effects of the cognitive impairments. Cognitive remediation is an example of such an approach. There is evidence of a small to moderate effect which could ameliorate the cognitive impairments seen in schizophrenia (Wykes, Huddy et al. 2011). Developing treatments that can address the cognitive symptoms is important, especially given their relation to long-term patient outcome.
Chapter 2  Cognition in schizophrenia

As demonstrated in Chapter 1, the role of cognition in schizophrenia has been recognized from the very early formulation of the disorder concept. Moreover, impaired cognition has often been regarded as a core feature of psychosis and a critical feature of schizophrenia (Seidman 1983, Green 1996). Indeed, the average cognitive impairment that has been related to schizophrenia is 1 standard deviation (SD) below that of healthy comparison subjects (Dickinson, Ramsey et al. 2007). Much data derives from IQ testing and shows that persons who later developed schizophrenia are characterized by an IQ decline during developmental years and stabilization at a lower level than that predicted from parental socio-demographic variables (Seidman, Giuliano et al. 2006, Woodberry, Giuliano et al. 2008). Yet, given that schizophrenia is a highly heterogeneous disorder, it also follows that not all patients with schizophrenia have cognitive deficits. Approximately 15% to 30% of patients with schizophrenia have neuropsychological profiles in the normal range (Kremen, Seidman et al. 2000, Allen, Goldstein et al. 2003, Leung, Bowie et al. 2008).

Furthermore, deficits in cognitive function undermine treatment response and long-term patient outcome as well as the ability to function in the community and acquire social skills (Green, Kern et al. 2000). The resurgence of interest over the last decade in cognition has led some to ask for the inclusion of such symptoms in the schizophrenia diagnosis (Keefe and Fenton 2007, Keefe 2008). However, the lack of consistent evidence for a specific cognitive impairment in schizophrenia might prevent this from happening (Bora, Yücel et al. 2010).

2.1 Core cognitive deficits in schizophrenia

Over the last decades, many prominent investigators have focused on identifying fundamental cognitive mechanisms that are associated with schizophrenia that can clarify the underlying
neuropathology of the disorder as well as contribute to developing treatments and effective rehabilitation programmes (Andreasen 1997, Palmer, Dawes et al. 2009).

Several domains have been suggested as ‘core’ deficits, the most prominent of which are described below. It should also be mentioned that the following cognitive domains have also received support for using them as endophenotypes in schizophrenia; i.e., a phenotype that is on the pathway between gene(s) and disease and may provide a more direct association with the gene(s) than a psychiatric phenotype further removed from it (Cornblatt and Malhotra 2001, Gottesman and Gould 2003, Walters and Owen 2007).

2.1.1 Processing speed

Processing speed is defined as the time that is required to complete a task or the amount of work that can be completed in a specific amount of time and is considered to be one of the most important processes of the human mind; it is necessary for efficient neural and cognitive cortical functioning and in everyday life functions (DeLuca 2008). It was first studied by Galton, Wundt and Cattell in the late 1800s and was initially defined as reaction time. Processing speed was thought to be at the heart of individual differences in intelligence. This notion, however, waned in the early 1900s as the speed with which mental operations function was not considered as important as obtaining an understanding of the fundamental operations of the mind itself. Therefore, the study of processing speed was abandoned for the next 40 to 50 years only to be rediscovered again in the last 30 years (O'Brien and Tulsky 2008). Despite the growing interest in studying this concept, a clinically and cognitively accepted model of processing speed does not yet exist (DeLuca 2008).

Evidence in support of processing speed as an endophenotype for schizophrenia includes that impairments in processing speed 1) appear more often in individuals with a schizophrenia
diagnosis than in the general population (Niendam, Bearden et al. 2003, Dickinson, Ramsey et al. 2007, Leeson, Barnes et al. 2010); 2) are heritable (Byrne, Clafferty et al. 2003, Niendam, Bearden et al. 2003, Hawkins, Addington et al. 2004, Glahn, Almasy et al. 2007, Wang, Chan et al. 2007); 3) share genetic risk factors with schizophrenia as documented by twin studies (McClearn, Johansson et al. 1997, Swan and Carmelli 2002) and; 4) do not depend on the stage, duration or severity of the illness, nor on medication response (Verdoux, Magnin et al. 1995, Hill, Schuepbach et al. 2004, Dickinson, Ramsey et al. 2007, Glahn, Almasy et al. 2007, Reichenberg, Caspi et al. 2010). Finally, processing speed impairments also help differentiate patients from relatives and those who are premorbid but will later develop the illness (Niendam, Bearden et al. 2003).

Even though processing speed has been documented as the single largest cognitive impairment in schizophrenia patients, there is a recognised dearth of studies into the construct (Goldberg, David et al. 2011). There are several reasons for this, which mainly have to do with the loose definition of the concept. For example, processing speed has long been considered a unitary concept, however, there is now evidence it may in fact be multidimensional (DeLuca 2008). Based on the statistical tool of factor analysis Chiaravalloti and colleagues identified two underlying components of processing speed, simple and complex processing speed that seem to capture separate cognitive processes (Chiaravalloti, Christodoulou et al. 2003). However, this research group is still working towards the best definition for complex processing speed (DeLuca 2008). Another complication is that processing speed is strongly related to other cognitive processes such as working memory and attention. As a result, these terms have often been used interchangeably.
2.1.2 Attention

Attention refers to the selective processing of environmental stimuli while ignoring others (Eysenck and Kean 2005). It is one of the most examined cognitive constructs and reportedly amongst the most severely affected domains in schizophrenia patients (Braff 1993, Cornblatt and Keilp 1994, Saykin, Shtasel et al. 1994, Censits, Ragland et al. 1997, Nuechterlein, Pashler et al. 2006). Attentional deficits are present during psychosis (Nuechterlein, Dawson et al. 1992) as well as during remission in schizophrenia (Wohlberg and Kornetsky 1973, Asarnow and MacCrimmon 1978). Furthermore, impairments in attention occur in first-degree relatives of schizophrenia patients, indicating they are likely to be part of the genetic susceptibility to schizophrenia (Cannon, Zorrilla et al. 1994, Faraone, S et al. 1995, Asarnow, Nuechterlein et al. 2002, Nuechterlein, Pashler et al. 2006). Despite the importance and severity of attentional deficits in schizophrenia patients, the processes that underlie the impairments in attention in schizophrenia remain unknown (Nuechterlein, Pashler et al. 2006). As with processing speed, this is mainly due to the fact that the concept of attention has not been clearly defined, nor, as a result, optimally measured (Goldberg, David et al. 2011).

2.1.3 Working and episodic memory

Memory refers to the encoding, storage and retrieval of information. Working and episodic memory have received most research attention in the schizophrenia literature and particularly so during the last two decades.

Working memory is typically operationalized as the processes that maintain and manipulate information for a limited amount of time (Baddeley 1992). Working memory impairment has been suggested as one of the core features and biological markers of schizophrenia.
Working memory deficits have been found in relatives of patients with schizophrenia (Park, Holzman et al. 1995, MacDonald Iii, Pogue-Geile et al. 2003), in individuals with schizotypal personality disorder (Park, Holzman et al. 1995, Tallent and Gooding 1999) as well as in adolescents at high-risk of schizophrenia (Smith, Park et al. 2006).

Episodic memory refers to the memory of events (Tulving 2002). Several meta-analytic studies have shown that defects in episodic memory are amongst the cognitive impairments with the largest effect sizes in individuals with schizophrenia (Aleman, Hijman et al. 1999, Dickinson, Ramsey et al. 2007). Moreover, this cognitive concept has direct practical applicability because of evidence that measures of episodic memory are robustly correlated with the functional outcome (Green 1996).

Working and episodic memory share a number of similarities (i.e., they are not modality specific, the severity of impairment is not affected by increasing the delay between the initial learning and later examination and finally they both seem to implicate deficits in the formation of representations). For this reason, Goldberg and colleagues (2011) have speculated that these systems are both affected by a more general mechanism that compromises the formation of representations (Goldberg, David et al. 2011).

2.1.4 Executive functioning

According to traditional cognitive or neuropsychological perspectives, executive function is an umbrella term (Geurts, Corbett et al. 2009) that includes a set of separate cognitive domains that involve vigilance or sustained attention (Pennington and Ozonoff 1996, Smith and Jonides 1999), inhibition (Jonides and Nee 2005, Luna, Padmanabhan et al. 2010), set shifting (Ravizza and Carter 2008), planning (Smith and Jonides 1999) and working memory.
(Goldman-Rakic 1996). It has been argued that, because of these various separate functions the concept is too general to be used in scientific theory (Miyake, Friedman et al. 2000).

Early brain lesion studies in animal (Jacobsen 1936) and neuroimaging studies of brain lesions in humans (Milner 1982, Shallice 1982, Leimkuhler and Mesulam 1985, Owen, Downes et al. 1990) have indicated the involvement of the prefrontal cortex in successful performance on tasks tapping into executive function. Research attention into executive function in patients with schizophrenia stemmed initially from the phenomenological similarities that were observed between patients with schizophrenia and those with frontal lobe syndromes (Wobrock, Ecker et al. 2009). This was subsequently supported by behavioural (Chan, Chen et al. 2006), psychophysiological (Weisbrod, Kiefer et al. 2000) and functional imaging findings (Holmes, MacDonald et al. 2005). Moreover, evidence from meta-analyses has indicated large effect sizes demonstrating considerable differences in performance on measures of executive functioning between schizophrenia patients and normal controls (Dickinson, Ramsey et al. 2007).

### 2.2 Generalized versus specific cognitive deficits in schizophrenia

There is considerable controversy concerning whether the cognitive impairments in schizophrenia represent a generalized deficit or comparatively independent or different deficits in distinct cognitive processes (Dickinson, Iannone et al. 2004, Green, Horan et al. 2013).

During the 1980s and in light of evidence that schizophrenia affects brain structure and function, clinical neuropsychological assessments were introduced to the field in order to detect localized brain lesions. As a result, most of the research focused on examining
executive and episodic memory performance as these were thought to correspond to prefrontal and temporo-hippocampal brain regions that had been identified as impaired in schizophrenia in the neuroimaging literature (Goldberg, David et al. 2011). However, in light of accumulated evidence and following the seminal meta-analysis of Heinrichs and Zakzanis (1998), these localizationist views were challenged (Heinrichs and Zakzanis 1998). This meta-analysis demonstrated that patients with schizophrenia showed substantial impairments on all cognitive assessments. A later meta-analysis by Dickinson and colleagues (2007) provided further support for a generalized cognitive deficit in schizophrenia, by indicating that processing speed as well as overall IQ, two measures that cannot be pinned to a specific brain region, had the largest effect sizes (Dickinson, Ramsey et al. 2007).

Further evidence came from studies showing that the schizophrenia patients’ performance across assessments that are thought to measure relatively independent cognitive processes are moderately to highly correlated (Dickinson, Ramsey et al. 2007). Also, several factor analysis studies have shown the existence of a robust first factor on which almost all the cognitive measures load (Gladsjo, McAdams et al. 2004, Keefe, Bilder et al. 2006).

However, these findings do not necessarily rule out the existence of different and/or independent substantial deficits (Goldberg, David et al. 2011). There are investigators who emphasize the fact that there is a considerable portion of variance that remains unexplained by such a general cognitive factor as well as the fact that there are assessments and domains that are relatively independent (Stankov and Roberts 1997, Green, Horan et al. 2013). Another argument comes from the cognitive experimental paradigms that indicate that certain cognitive mechanisms, such as attentional control in the context of visual working memory encoding, remain relatively intact in schizophrenia (Gold, Fuller et al. 2006). Whereas neuropsychological tests require the effective contribution and co-operation of several cognitive processes and have been criticized for failing to measure separate and distinct
cognitive functions, these paradigms have been designed to isolate specific cognitive functions (MacDonald Iii and Carter 2002, Jonides and Nee 2005). As a possible explanation of these contradictory findings, Goldberg and colleagues (2011) have suggested that cognitive deficits in schizophrenia could lie mainly in the reduced capacity to coordinate different widespread networks in the brain and that it is this deficiency that generalized neuropsychological tests, but not specific experimental paradigms, capture (Goldberg, David et al. 2011).

2.3 Premorbid cognitive deficits in schizophrenia and course of impairment

It has been repeatedly and robustly shown that cognitive deficits are manifest in individuals during their first episode of schizophrenia (Mesholam-Gately, Giuliano et al. 2009); in prodromal patients with psychosis (Lencz, Smith et al. 2006); during the early developmental stages of psychosis (Isohanni, Jones et al. 2001, Cannon, Caspi et al. 2002, Ang and Tan 2004, MacCabe, Lambe et al. 2008); and also in individuals well before any evidence of any psychotic symptomatology (Reichenberg, Weiser et al. 2006).

Research in young people specifically indicates that development of schizophrenia in adulthood is preceded by impaired cognitive performance in relation to normal peers (for a review see: (MacCabe 2008)). Cognitive deficits include low IQ, and impairments in verbal memory, attention, and receptive language (Jones, Rodgers et al. 1994, Erlenmeyer-Kimling, Rock et al. 2000, Cannon, Caspi et al. 2002, Reichenberg A 2002, Zammit, Allebeck et al. 2004). These premorbid cognitive deficits provide further support for the neurodevelopmental hypothesis of schizophrenia (Murray 1987, Weinberger 1987, Cornblatt, Lencz et al. 2003, Owen, O'Donovan et al. 2011). These impairments in conjunction with
genetic, epigenetic and environmental factors are considered to lie on the risk pathway to psychosis (Polanczyk, Moffitt et al. 2010).

Much remains unclear, however, including for example, when these cognitive impairments present themselves during childhood; whether they remain stable or change over time; if specific cognitive functions follow different developmental patterns; or whether they are due to deterioration of brain function, environmental risk factors or both (Seidman, Giuliano et al. 2006).

With regards to the developmental course of cognitive impairments, a number of studies have shown that general intellectual ability (IQ) declines between premorbid to postmorbid examination (Sheitman, Murray et al. 2000, Seidman, Buka et al. 2006). Others, however, have found no evidence of decline (Russell, Munro et al. 1997, Cannon, Bearden et al. 2000).

Studies have also found evidence that decline during school years in academic achievement scores (Bilder, Reiter et al. 2006), relative deterioration between childhood and midlife in receptive vocabulary (Kremen, Vinogradov et al. 2010) and decline in language between the ages of 8 and 11 was associated with schizophrenia in adulthood (Fuller, Nopoulos et al. 2002). Also, developmental lag in working memory ages 7, 9, 11, and 13 was found to be associated with schizophreniform disorder in adulthood (Reichenberg, Caspi et al. 2010). The course of cognitive impairment during the early and later stages of schizophrenia, even in patients receiving psychotropic medication, seems to be stable (Goldberg, Hyde et al. 1993, Bilder, Goldman et al. 2000, Buchanan, Ball et al. 2005). In later life, however, there is a minority of patients who show pronounced cognitive decline that is more excessive in relation to healthy controls (Bowie, Reichenberg et al. 2006).
2.4 The relation between cognition and schizophrenia

The examination of the association between cognition and schizophrenia is important in order to gain a better understanding of the nature of the disease as well as for the development of therapeutic strategies (Bark, Revheim et al. 2003).

Two possible models have been proposed (figure 1).

Figure 1 Proposed models of the relation between cognition and schizophrenia\(^1\).

According to the first model (figure 1, Model A), the cognitive and psychotic characteristics of the illness are correlated through neurodevelopmental disruption. Several studies support this model (Cuesta and Peralta 1995, Suslow, Junghanns et al. 1998, O'Leary, Flaum et al. 2000, Nieuwenstein, Aleman et al. 2001). Moreover, the differential impact of medication on

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\(^1\) According to Model A, the cognitive and psychiatric characteristics of the illness are correlated through neurodevelopmental disruption. According to Model B, the cognitive impairments lie on the pathway between neurodevelopmental disruption and psychopathology and are the underlying cause of the psychiatric characteristics of the illness.
schizophrenia and cognition (Meltzer and McGurk 1999) suggests distinctive underlying pathological mechanisms. The fact that the course of cognition is stable, even after the first episode of psychosis, whereas the course of psychosis is not (an der Heiden and Hafner 2011) further supports this model.

This model does not exclude possible interactions between the cognitive and psychiatric symptoms of the illness. Psychosis, for example, can affect cognition, since it often appears at a period when the person’s learning style is not yet stable, and often leads to academic under-achievement as well as decreased motivation to learn and thus less efficient cognitive functioning. Impaired cognition, on the other hand, can function as an additional stressor by making social and independent functioning more difficult and therefore aggravating the psychotic symptoms (Green 1998). The fact that individuals with youth-onset schizophrenia have worse cognitive deficits in relation to those with late-onset is also in favour of this model (Rajji, Ismail et al. 2009). Although, it is also possible that early-onset schizophrenia represents a more severe and possibly more genetic form of schizophrenia (Nicolson and Rapoport 1999).

Other researchers support the view that the underlying cognitive abnormalities are on the causal pathway of schizophrenia (figure 1, Model B) (Fletcher and Frith 2009). This is supported by studies that have found associations between cognition and psychiatric symptomatology (Buchanan, Strauss et al. 1994, Mahurin, Velligan et al. 1998, Verdoux, Liraud et al. 1999, Milev, Ho et al. 2005, Carlsson, Nyman et al. 2006).

To summarize, even though the relation between cognition and schizophrenia has been thoroughly studied, results are inconsistent (Pijnenborg, van Beilen et al. 2003, Goldberg, David et al. 2011). A reason for these inconsistencies could be the fact that studies differ with regards to the clinical state of the patients under study (Nieuwenstein, Aleman et al. 2001).
Some studies have included acutely schizophrenic patients (Addington, Addington et al. 1991), whereas others have included only chronic patients (Liddle and Morris 1991). Associations might differ for patients in different phases of the illness (Baxter and Liddle 1998). Methodological differences across the studies may also contribute to the inconsistencies. Differences in symptom scales would result in differences in the correlations between cognition and psychotic symptomatology and this includes differences that can arise from studies using individual cognitive measures compared to those derived from clusters of symptoms based on factor analysis (Nieuwenstein, Aleman et al. 2001).

Chapter 3  Genetic studies of schizophrenia

The best established etiological factors in schizophrenia are genetic. Initial efforts from genetic linkage and association studies met with limited success. Whilst, recent advances in genetic technology have provided useful insights, the understanding of the mode of transmission of schizophrenia is hampered by the great complexity of the disorder. Nevertheless, there is optimism that the collaborative efforts of schizophrenia research groups around the world, the actualization of highly powered genome wide association studies and the study of rare risk alleles could potentially elucidate the pathophysiological mechanisms that underlie the disease.

This chapter will give a brief overview of the field of the genetic study of schizophrenia. In conclusion, I will discuss the findings and the future directions.
3.1 Genetic epidemiology of schizophrenia

The question genetic epidemiology aims to answer is whether relatives of patients with schizophrenia are at greater risk of developing schizophrenia in relation to the population baseline and if this is the case, whether this risk is due to genetic or environmental factors or both. A large amount of data from family, twin and adoption studies has consistently shown that there is a large genetic component to liability to schizophrenia.

Genetic epidemiological studies of schizophrenia have shown that (a) the genes contributing to schizophrenia risk act in aggregate; (b) schizophrenia is a multifactorial disease with both genetic and environmental factors contributing to risk; (c) the pattern of inheritance is complex and therefore not Mendelian; also, (d) schizophrenia is a complex trait that is influenced by a large number of risk factors, many of which seem more likely to be within the range of normal human variation and each produce only small increases in risk (Riley and Kendler 2011).

3.1.1 Family, twin and adoption studies of schizophrenia

There is compelling evidence from family studies that the risk of schizophrenia is higher for relatives of patients with schizophrenia than the general population. Gottesman and Shields (1991), combined results of approximately 40 family and twin European studies published between 1921 and 1987 (Gottesman 1991). The lifetime morbid risk in the siblings of patients with schizophrenia was found to be almost 10 times higher than in the general population. Smaller but consistent increases in risk were seen in second- and third-degree relatives. Similar results were obtained from more recent and methodologically stronger studies (Lichtenstein, Yip et al. 2009, Gottesman, Laursen et al. 2010).
Twin studies help to estimate the relative contribution of genetic and environmental factors to risk. This is mainly done by comparing concordance/ correlation or shared variance for schizophrenia between monozygotic (MZ) twin pairs and dizygotic (DZ) pairs. Since DZ twins share on average 50% of their genetic material in relation to MZ twins who are assumed to be entirely identical, greater MZ concordance can be ascribed to genetic influence. The necessary assumption for this inference is that the environments of MZ and DZ twins are comparable, i.e., that MZ twins are not treated more similarly than DZ twins because of their greater likeness (equal/stable environment assumption) (Donovan and Susser 2011).

Twin studies of schizophrenia, though they have tended to employ different methodologies and diagnostic methods, have consistently indicated a genetic effect with a higher concordance in MZ (35-58%) than DZ (9-27%) twins. Recent reviews and meta-analyses estimate the heritability of schizophrenia at around 80%, supporting the reasoning behind looking for genetic and common environmental etiological factors (Cardno and Gottesman 2000, Sullivan, Kendler et al. 2003).

The question adoption studies seek to address is whether the increased risk of biological relatives of patients with schizophrenia is equal if these relatives are raised in different environments. Consistent with the twin studies, adoption studies have found that the rates of schizophrenia are not different for people raised in the adoptive families compared to those raised in their biological families (Tienari 1991, Prescott and Gottesman 1993, Kety, Wender et al. 1994). It should be noted, however, that because the risk of a MZ twin of a patient with schizophrenia is less than 100%, what is inherited is not the certainty of the condition but rather a predisposition or liability to develop the disorder (Owen, O'Donovan et al. 2002).
3.2 Molecular genetics of schizophrenia

3.2.1 Linkage studies

Genetic linkage analyses are mainly used with Mendelian or genetic illnesses that are caused by mutations in a single gene that is located at a single place on a chromosome. Given the rarity of these disorders, these rare risk alleles segregate within families, from parents with a family history to their affected child or might arise as an even rarer de novo mutation. The risk allele can therefore be identified following the segregation of the allele from the affected parents and grandparents to the affected offspring. This phenomenon of segregation of the risk allele within multiple families is called linkage (Riley and Kendler 2011).

During the late 1980s and the early 1990s, linkage studies were the main tool for conducting systematic molecular genetic analysis of schizophrenia (Owen, O'Donovan et al. 2002). However, results were not very promising. This may be because linkage analyses are most powerful in Mendelian disorders, where a relatively small number of families can often provide strong evidence for linkage to a small chromosomal region (Owen 2005). Schizophrenia differs from Mendelian disorders in many ways; a) risk does generally not seem to be explained by the action of a few rare highly penetrant alleles; b) there seems to be locus and allelic heterogeneity; c) environmental factors seem to play a role in the observed patterns of risk; d) diagnostic boundaries are unclear and; e) many risk variants seem to be common rather than rare (Riley and Kendler 2011). It should also be noted that linkage studies only identify regions and not actual genes (Owen 2005).

Despite these difficulties, linkage studies have identified some candidate regions. Results from two meta-analyses of genome scan linkage data (Badner and Gershon 2002, Lewis, Levinson et al. 2003) found tentative evidence for schizophrenia susceptibility regions. These
studies used different statistical approaches and found evidence for a number of non-overlapping regions (13q, 3p, 11q, 6p, 1q, 20q, 2q and 14p (for a review see: (Riley and Kendler 2011)). For two regions, however, both meta-analyses provided support: 8p and 22q. The 8p region harbours the candidate gene neuregulin 1 (NRG1) (see below), while the 22q region has received considerable research attention because deletions in 22q11.2 cause a syndrome with high risk for development of schizophrenia (see Chapter 8).

3.2.2 Association studies

Association studies examine whether affected individuals are more likely to possess risk variants than unaffected individuals. These studies have greater power for identifying genes of small effect, but the researcher needs to have an a priori hypothesis about the involvement of the specific gene in the disease (Risch and Merikangas 1996). This hypothesis can be biased, especially given the number of genes in the human in the human genome (around 40,000) and the limited understanding of the underlying biological mechanisms of schizophrenia.

There are two additional issues that should be borne in mind before examining the evidence for specific genes. First, given the polygenic nature of schizophrenia, each sample examined is likely to vary in the extent to which specific environmental risk factors may be present. For example, it could be that certain high-risk variants become expressed when environmental risk factors are present. Moreover, there is a variety of methodological reasons why association might be unrelated to disease etiology. One is population stratification, which refers to situations where the cases and controls come from different population groups or subgroups, and the association is actually the result of background genetic population differences between the two groups rather than the disease (Riley and Kendler 2011). Second,
many studies conducted in the past have been underpowered for the identification or confirmation of alleles of small effect (Owen, O'Donovan et al. 2002).

3.2.2.1 Positional candidate studies

Positional candidate studies aim to identify schizophrenia susceptibility genes by mapping positional candidate genes based on their chromosomal location as identified by linkage analysis.

There is support for several positional candidate genes in schizophrenia. These include regulator of G-protein signaling 4 (RGS4) (Chowdari, Mirnics et al. 2002), fibroblast growth factor receptor 2 (FGFR2) (O'Donovan, Norton et al. 2009), Protein Kinase C, alpha (PRKCA) (Carroll, Williams et al. 2010) and disrupted in schizophrenia 1 (DISC1) (Schumacher, Laje et al. 2009). The genes that have received most support to date are:

1. neuregulin 1 (NRG1);
2. dystrobrevin binding protein 1 or dysbindin (DTNBP1);
3. D-amino acid oxidase activator (DAOA).

NRG1 was first identified after linkage findings pointed to the chromosome 8p region in a very large sample of Icelandic families (Stefansson, Sigurdsson et al. 2002). Further support for this locus was subsequently provided by case-control studies on samples from Scotland (Stefansson, Sarginson et al. 2003) and Ireland (Corvin, Morris et al. 2004). NRG1 is known to interact with the receptor tyrosine-protein kinase erbB-4 (ERBB4) gene, which has also been related to risk of schizophrenia (Norton, Moskvina et al. 2006). Replications for this association have been obtained by several independent studies in multiple populations,
although negative findings have been reported (for a review see: (Tosato, Dazzan et al. 2005)). Therefore the link of NRG1 with schizophrenia remains questionable.

Furthermore, the way NRG1 might lead to schizophrenia remains unknown (Kirov, O'Donovan et al. 2005). NRG1 is thought to encode approximately 15 proteins with a variable range of developmental functions in the brain, including neuronal migration, synaptogenesis and neurotransmission. Any of these could confer risk to schizophrenia (Corfas, Roy et al. 2004).

Evidence that DTNBP1 (chromosome 6p) is associated with risk of schizophrenia was first provided by a study in an Irish sample (Straub, Jiang et al. 2002). Subsequent studies have confirmed this association in samples from diverse ethnic backgrounds (e.g., (Kirov, Ivanov et al. 2004) and (Tochigi, Zhang et al. 2006)), but as was the case with NRG1, not all studies have replicated these associations (e.g., (Sanders, Duan et al. 2008) and (Holliday, Handoko et al. 2006)).

The function of the DTNBP1 protein in the brain remains largely unknown (Riley and Kendler 2011). There is evidence that reduced DTNBP1 expression in cultured neurons results in a reduction in glutamate release (Numakawa, Yagasaki et al. 2004) and one hypothesis is that variation in DTNBP1 might lead to risk of schizophrenia by altering presynaptic glutamate function (Kirov, O'Donovan et al. 2005). There are also studies that have found that the expression of DTNTBI in the hippocampus and cerebellum of patients with schizophrenia is reduced at both the RNA (Weickert, Straub et al. 2004) and protein levels (Talbot, Eidem et al. 2004).

DAOA (chromosome 13q) was first implicated to be involved with schizophrenia in 2002 (Chumakov, Blumenfeld et al. 2002). Since then, several studies have replicated this association (e.g., (Addington, Gornick et al. 2004) and (Korostishevsky, Kaganovich et al. 2004)).
but also there is evidence of non-replication (Hall, Gogos et al. 2004) and (Sanders, Duan et al. 2008). Overall, three meta-analyses have shown weak positive (Li and He 2007) to strong positive (Detera-Wadleigh and McMahon 2006, Shi, Badner et al. 2008) evidence of an association.

The function of *DAOA* protein remains mostly unknown. It has been reported to interact with D-amino acid oxidase, which may indirectly affect glutamate signaling by reducing the concentration of D-serine in the brain, which in turn can lead to decline in NMDA receptor potentiation (Hashimotom, Fukushima et al. 2003).

### 3.2.2.2 Functional candidate studies

Another way of looking for schizophrenia susceptibility genes is by examining genes that might be involved in the pathogenesis of the disease (so-called candidate genes). Despite the large amount of functional candidate studies, there are no ‘fully convincing robustly replicated findings’ (p.275, (O'Donovan and Owen 2011)). Limited power, the utilization of variants that have small genetic effects that do not cover the common variation in genes and the lack of understanding on the processes that are involved in the disease constitute some of the reasons for the inconsistent findings (O'Donovan and Owen 2011). Amongst the most studied candidate genes are those involved in dopaminergic and serotonergic neurotransmission.

**Dopamine genes**

The *COMT* gene has long been considered a positional candidate gene for schizophrenia based on linkage studies implicating region 22q (Badner and Gershon 2002, Lewis, Levinson
et al. 2003), as well as because of its position in the deleted region of 22q11.2 Deletion Syndrome which is associated with high rates of schizophrenia (see Chapter 8). COMT is also an attractive functional candidate gene for schizophrenia because of its important role on catabolizing dopamine in the prefrontal cortex (Weinberger, Egan et al. 2001). The involvement of prefrontal dopaminergic dysfunction in schizophrenia is well documented (Howes Od and et al. 2012). It follows that genes related to dopamine activity in this brain region might also be involved in the disorder (O'Donovan and Owen 2011).

COMT is one of the enzymes that degrades catecholamines such as dopamine, epinephrine and norepinephrine and accounts for >60% of the dopamine degradation in the prefrontal cortex (PFC) (Karoum, Chrapusta et al. 1994). It contains a functional polymorphism (Val108/158Met, rs4680) that affects the enzyme’s temperature sensitivity (Lachman, Papolos et al. 1996). Homozygosity for the low-activity Met allele is related to an almost 4 times reduction of COMT enzyme activity in relation to the Val allele, presumably resulting in lower dopamine catabolism and thus higher levels of dopamine particularly in the prefrontal cortex (Lotta, Vidgren et al. 1995).

COMT has been widely studied with results showing both positive and negative findings. For example, some studies report the Val allele being associated with schizophrenia (Ohmori, Shinkai et al. 1998, Kotler, Barak et al. 1999) while others report the Met allele (Kim, Kim et al. 2008, Gupta, Bhatnagar et al. 2009, Hoenicka, Garrido et al. 2010). A recent meta-analysis did not support the role of COMT Val/Met polymorphism in schizophrenia risk, although there might be significant association with another two functional COMT polymorphisms (rs737865 and rs4818) (Allen, Bagade et al. 2008). Rs737865 has been reported to be in linkage disequilibrium with rs2097607, a SNP that is located within the P2 promoter region and has also been implicated in schizophrenia (Palmatier, Pakstis et al. 2004). Rs4818 has been reported to account for more functional variation in COMT activity in relation to the
Val108/158Met (Nackley, Shabalina et al. 2006) (for a possible explanation of these positive and negative findings on COMT see Chapter 7).

Other approaches to identifying mechanisms by which variation in COMT might predispose to the disorder have been studies of gene-gene and gene-environment interactions. Many studies have been reported on possible interactions between COMT and other genes, including RGS4, DAOA, glutamate receptor, metabotropic 3 (GRM3) and Disrupted in schizophrenia 1 (DISC1) (Nicodemus, Kolachana et al. 2007) as well as the dopamine transporter (DAT1) (Talkowski, Kirov et al. 2008).

COMT is also one of the first genes that were examined in studies of gene-environment interaction. Although one study suggested that carriers of the Val allele may be more likely to develop psychosis if they use cannabis, compared to those that are homozygous for the Met allele (Caspi, Moffitt et al. 2005), there have been at least seven studies that did not replicate these findings (for a review see: (Zammit, Owen et al. 2010)).

In circumstances where extreme patterns of interactions occur, gene-environment and gene-gene interactions can provide insights in the pathogenesis of the disease. However, such extreme interactions are rare. There are substantial problems of gene-environment interactions to date, including issues with multiple testing (O'Donovan and Owen 2011) and methodological problems associated with their interpretations (Zammit, Owen et al. 2010, Zammit, Wiles et al. 2010).

Finally, a number of other dopaminergic genes have also been examined based on the premise that they represent dopamine receptor genes and are thus involved in dopamine neurotransmission (i.e., DRD3 (Jonsson, Flyckt et al. 2003), DRD1, DRD4 and DRD5 (Allen, Bagade et al. 2008), and furthermore the tyrosine hydroxylase (TH) gene, but there is no consistent evidence for replication.
Serotonergic genes

Current pharmacological treatments for schizophrenia also focus on the serotonergic system (Waddington, O'Tuathaigh et al. 2011). Two genes, the solute carrier family 6, member 4 (SLC6A4) and the 5-hydroxytryptamine receptor 2A, (HTR2A) have received most research attention (O'Donovan and Owen 2011), but thus far no consistent evidence for association has been found for either (Ikeda, Iwata et al. 2006) (Allen, Bagade et al. 2008).

3.2.2.3 Genome Wide Association Studies

The recent advent of Genome Wide Association Studies (GWAS) has made important contributions to the field of schizophrenia research (Riley and Kendler 2011). This method has several advantages over the previous ones mentioned above:

1. By allowing for genome-wide coverage, the possible biases of selecting individual candidate genes based on usually unclear disease processes are avoided;
2. The non-parametric techniques of association analysis can be used rather than parametric linkage analysis methods that assume an explicit mode of inheritance for the disease;
3. Issues arising from the large number of tests performed are avoided by applying rigorous correction for multiple testing (p<10^-7);
4. Multiple genes can be simultaneously detected.

The first GWAS studies in schizophrenia (Shifman, Johannesson et al. 2008, Kirov, Zaharieva et al. 2009) were based on DNA pooling, whereby DNA samples (both cases and controls) are mixed together and allele frequencies are estimated. Results from case and
controls are then compared. The main advantage of this method is its reduced cost in relation to GWAS based on individual sample genotyping. One important finding was that the gene *reelin* was associated with schizophrenia in females (Shifman, Johannesson et al. 2008). *Reelin* could be a candidate gene for schizophrenia given its role in processes such as neuronal migration, formation of the cerebral cortex and neuroplasticity and its reduced expression in schizophrenia (Shifman, Johannesson et al. 2008). More research is needed in order for the evidence to become more conclusive.

Pooled DNA comparisons, however, have some limitations, including the additional variation that might be due to the pool construction (i.e., variation in DNA amounts among individuals contributing to the pool) or due to the fact that both minor and major alleles might not be amplified to the same extent and this could result in biased cases versus controls comparisons (Hoogendoorn, Norton et al. 2000). Therefore, GWAS studies that are based on individual sample genotyping are preferable.

The first significant GWAS result based on individual sample genotyping showed that the strongest supported locus was at 2q32.1, close to *zinc finger protein 804A* (ZNF804A) (O'Donovan, Craddock et al. 2008). Furthermore, results from a large meta-analysis showed that this marker was also associated with bipolar disorder, suggesting its possible involvement in a broader psychosis phenotype (Williams, Norton et al. 2011). Not much is known about the biological function of ZNF804A, though it might be a putative transcription factor (Esslinger, Walter et al. 2009).

Furthermore, a large meta-analysis of datasets from three consortia, the Molecular Genetics of Schizophrenia (Shi, Levinson et al. 2009), the International Schizophrenia Consortium (Purcell, Wray et al. 2009) and SGene (Stefansson, Ophoff et al. 2009), giving a total of 12945 cases and 34591 controls, identified the following loci as genome-wide significant:
two that are included in a broad region of chromosome 6p which encompasses the Human Leucocyte Antigen (HLA) region, one that is close to neurogranin (NRGN) and the other is an intron of transcription factor 4 (TCF 4) (Stefansson, Ophoff et al. 2009).

The region of chromosome 6 that was implicated in the findings includes a large number of genes whose alleles are highly correlated and therefore it is not yet known which exact gene or genes are implicated. With regards to NRGN, it is highly expressed in the hippocampus (Huang, Huang et al. 2007). It encodes a protein that is implicated in the regulation of calcium that in turn regulates NMDA receptor activation and therefore synaptic plasticity and long-term potentiation (Zhong, Cherry et al. 2009). Finally, TCF4 is involved in neuronal development (Blake, Forrest et al. 2010) and it has been shown that highly disruptive mutations in this gene can cause Pitt-Hopkins syndrome, a condition involving among other characteristics learning disability, epilepsy and poor motor development.

Another GWAS study by the Schizophrenia Psychiatric GWAS Consortium (Consortium 2011) including overall 51,695 individuals, identified five new loci that were found to be associated with schizophrenia (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 1-q24.32-q24.33) and two loci that have been implicated previously (6p21.32-p22.1 and 18q21.2). The strongest signal was at rs1625579 at 1p21.3 which includes the gene MicroRNA MIR137. MIR137 has been involved in neuronal maturation (Smrt, Szulwach et al. 2010) and therefore could have a role in the brain abnormalities in schizophrenia (Consortium 2011). Amongst the previously implicated loci was TCF4. However, though there was a GWAS signal at 11q24.2, this was distant from NRGN that was previously reported.

Another important finding that arose from these GWAS studies is that there is a considerable polygenic component to the etiology of schizophrenia. Using a different method for analyzing the GWAS data, the International Schizophrenia Consortium observed that a
summary score that included the top sets of thousands of alleles could (weakly but significantly) discriminate cases from controls (Purcell, Wray et al. 2009). It was also found that the same sets of alleles could also discriminate people with bipolar disorder from controls. According to the authors, these results show that a) there is a large number of loci of small effect (OR<1.1) that contribute to risk of schizophrenia; b) that these together account for at least 30% of the genetic and non-genetic variance in liability to the disorder and; c) that there seems to be a common genetic etiology between schizophrenia and bipolar disorder, providing further support for the continuum hypothesis of schizophrenia. Similar findings were found with a different approach where the variation in liability to schizophrenia was estimated from the average genome-wide similarity between all pairs of individuals using all SNPs (Lee, DeCandia et al. 2012). Genetic variation was calculated when pairs of cases and pairs of controls are more similar that cases-controls pairs across the genome.

3.2.3 Structural genomic variation

There are many forms of structural genomic variation, including deletions, duplications, inversions and chromosomal translocations. Deletions and duplications of chromosomal segments (copy number variants or CNVs), in particular, have received considerable research focus and clinical attention. CNVs are by definition at least 1000 bases in size and affect the number of copies of a specific stretch of DNA sequence. CNVs also seem to account for a major proportion of genomic variation between individuals in the general population (Pinto, Marshall et al. 2007).

The hypothesis of common disease/rare variant (in conjunction with the common disease/common variant hypothesis that predicated GWAS studies) of genetic risks for complex traits has been recently proposed in schizophrenia (McClellan, Susser et al. 2007)
mainly based on the decrease in fertility observed in cases. The CNV most strongly implicated in schizophrenia is a deletion in the chromosomal region 22q11.2 that causes 22q11.2 Deletion Syndrome (see Chapter 8).

One clear line of evidence for the involvement of CNVs in schizophrenia is that genome wide, cases have been reported to have a greater load of low-frequency CNVs than controls. It seems, that the CNV burden in schizophrenia might be relatively small (O'Donovan and Owen 2011).

Results from large GWAS studies have implicated the following loci for risk of schizophrenia: deletions mapping to 1q21.2, 2p16.3, 3q29, 15q13.3, 22q11.2 (Levinson, Duan et al. 2011), 17q12 (Moreno-De-Luca, Mulle et al. 2010) and duplications mapping to 1q21.2, 16p11.2 (Levinson, Duan et al. 2011) and 7q36.3 (Levinson, Duan et al. 2011, Vacic, McCarthy et al. 2011). It should be noted that though these CNVs are rare in both cases and controls, the associated relative risk is considerable, with estimates ranging from 5 to 20 (Sullivan, Daly et al. 2012). The fact these CNVs are found in controls emphasizes they are not sufficient to cause the disorder and they are also not necessarily specific to schizophrenia (O'Donovan and Owen 2011, Kirov, Rees et al. 2013). Indeed, almost all are nonspecific since they often confer risk for other disorders like epilepsy, somatic dysmorphism and autism spectrum disorder (for a review see: Sullivan, Daly et al. 2012)). Moreover, these CNVs contribute to the pathogenesis of schizophrenia since they have also been found to be enriched for N-methyl-D-aspartate receptor (NMDAR), a synaptic protein complex that affects synaptic plasticity and cognition (Kirov, Pocklington et al. 2012).
3.3 Conclusions and future directions

Although there has been considerable advancement in the genetic study of schizophrenia, our understanding of the molecular mechanisms that underlie the disease remain elusive. There is strong evidence of a large genetic component in schizophrenia, that seems to also increase liability to other psychiatric disorders, but due to the complexity of the disorder the majority of heritability in schizophrenia is currently still unaccounted for.

At this point, however, I would like to comment on the issue of unreplicability in genetic association studies, which is part of broader concerns about replication in biomedicine (Ioannidis 2005), psychology (Laws 2013) and neuroscience (Button, Ioannidis et al. 2013). Several authors and empirical reviews have shown that only a minority of associations has been consistently replicated and this is mainly to do with the increased likelihood of type I errors arising from multiple statistical comparisons and numerous analytical strategies and the increased likelihood of type II errors given the small effects observed from single markers in multifactorial diseases related to small (underpowered) studies (Walters 2012). For example, (Sullivan 2007) showed using simulations that false positive findings are very highly likely to occur in candidate gene studies. The solutions suggested from several authors (e.g., (Ioannidis 2005)) involve specified a priori and exploratory research hypotheses, full and accurate information on the experiments performed and finally appropriate correction for multiple testing. Moreover, it is necessary that replication is highly precise in order to minimize the propagation of a false positive finding (I am further commenting on the caveats of genetic association studies in Chapter 7).

Nevertheless, several promising positional candidate genes have been found and it is unlikely these will all turn out to be false (Owen 2012). The overall conclusions from the genetic studies of schizophrenia are as follows: (1) what is inherited seems to be a genetic
predisposition rather than certainty of developing the disease; (2) genetic susceptibility includes a considerable range of risk alleles; (3) risk alleles can range from common to rare, and effect sizes from small to large, with each contributing only a small proportion to variation in the population; (4) some of risk genes might interact with others and/or the environment; (5) in addition to schizophrenia, some, possibly many may also be associated with increased liability to other psychiatric disorders.

Indeed, findings on the study of CNVs have shown a genetic overlap between schizophrenia and a range of other neurodevelopmental disorders, such as autism, ADHD, and intellectual disability (Purcell, Wray et al. 2009, Williams, Zaharieva et al. 2010, Smoller, Craddock et al. 2013). These findings support a continuum of neurodevelopmental causality (Owen 2012) and question the view that these disorders are unrelated diagnostic entities (Owen, O’Donovan et al. 2011). In view of my findings in Chapter 8, I will be further discussing this issue in the General Discussion.

In conclusion, the developments in genetic technology and phenotyping are likely to shed light on the specific genes that predispose to schizophrenia. Moreover, increasingly more highly powered genome-wide association studies, the advent of next generation sequencing and the necessary accompanying advances in genetic statistics, might unambiguously begin to point at susceptibility genes for schizophrenia.

Chapter 4  Genetic studies of cognition

Cognitive abilities vary greatly among individuals. These differences in cognition (also referred to as intelligence, mental ability and IQ) are strongly genetic (~80%) (Deary, Johnson et al. 2009) and highly predictive of important life outcomes including income (Strenze 2007), social mobility, quality of life, as well as health outcomes and health-related
behaviours (Batty, Deary et al. 2007). The study into the genetic origins of cognitive abilities can provide important insights into how the brain functions and how disruptions in these systems could lead to mental health outcomes such as schizophrenia.

The purpose of this chapter is to provide a summary of the findings on the genetic and non-genetic components of cognition.

4.1 Basic heritability of ‘g’

Studies have shown that approximately 40 to 50% of the variance in a wide spectrum of cognitive abilities is accounted by a general cognitive factor (g). This factor was discovered by Spearman in 1904 and since has become one of the most replicated findings in psychology (Deary, Spinath et al. 2006). There are also small amounts of variance that are attributable to separable cognitive domains, and there is also an important amount of variance that is unique to highly specific cognitive abilities (Deary, Johnson et al. 2009, Donohoe, Deary et al. 2013).

Galton, Darwin’s half-cousin, concluded, around 150 years ago, that cognitive abilities are substantially genetic in origin and transmitted genetically from one generation to the next and these notions remain supported to date (Plomin, DeFries et al. 2008).

To be more exact, studies that have been based on twins (Bouchard, Lykken et al. 1990), adoptive and biological siblings (Scarr and Weinberg 1977), and parents and their adoptive and biological offspring (Plomin, Fulker et al. 1997) have all shown a substantial genetic component in the ‘g’ factor that ranges from 30% to 80% of its total variance. Similar findings have derived from studies examining broad cognitive abilities (Posthuma, de Geus et al. 2001, Johnson, Bouchard Jr et al. 2007), with the exception of memory that seems to be less genetically influenced (Finkel, Pedersen et al. 1995, Johnson, Bouchard Jr et al. 2007).
Moreover, it is well established that the heritability of $g$ increases with age; from around 30% in childhood to 80% in adulthood (McCartney, Harris et al. 1990, Spinath, Ronald et al. 2003, Edmonds, Isaacs et al. 2008). The mechanism underlying this change is not yet understood. Two possible interpretations have been suggested (Deary, Johnson et al. 2009). According to the first, given that several brain structures and functions are strongly genetically influenced and undergo changes over development (Shaw, Greenstein et al. 2006), heritability of $g$ might increase with age because of $g$ being related to similar genetic influences to those that underlie some brain structures and functions (e.g., gray and white matter volumes).

Another hypothesis involves neuronal repair; there must be some processes that protect the brain from the accumulation of environmental insults that occur with increasing age and harm the neurons. These individual differences in brain repair over time might influence the cognitive phenotypes to the extent that there is genetic variation of these processes that in turn influence cognition. The association of the gene for apolipoprotein E with general cognitive ability at age 79 but not age 11 years, provides some support for this argument (Wilson, Schneider et al. 2002).

### 4.2 Molecular genetics of cognition

Although there is a substantial genetic component involved in cognition, there is lack of replicated molecular genetic findings and no genetic locus has unambiguously been associated with it.

With regards to rare mutations, there have been at least 300 genes in which rare highly penetrant mutations have been associated with mental retardation. Interestingly, out of these 300 genes, 20% are located on the X chromosome. Moreover, approximately 10% of patients
with mental retardation have either deletions or duplications of likely pathogenic significance (Shaw-Smith, Redon et al. 2004).

Moreover, in common with other complex traits, like schizophrenia, many associated genetic variants are expected to be common (>1%) and individually contribute well below 1% of the variance, which necessitates the examination of large samples (Plomin, Kennedy et al. 2006).

In the following section, I will provide an overview of the current findings and methods used to identify specific genetic variants for normal range intelligence.

4.2.1 Candidate gene, linkage and association studies

Candidate gene studies have been conducted in small samples and the findings have not been replicated. They suffer from many of the shortcomings associated with similar studies of schizophrenia reviewed above.

One candidate gene that has received some support is the Apolipoprotein E (APOE), which is also implicated in Alzheimer’s disease. To be more exact, the E4 allele of APOE has been found to have a small protective effect on cognition, but only in old age (Small, Rosnick et al. 2004). The mechanisms, however, that underlie this effect are not yet understood. Other candidate genes that seem to be related to intelligence, with small effect sizes, consistent with a polygenic view of heritability of intelligence, are the cholinergic muscarinin 2 receptor (Comings, Wu et al. 2003), cathepsin D (Payton, Holland et al. 2003), the brain-derived neurotrophic factor (BDNF) (Tsai, Hong et al. 2004) and the formin binding protein 1-like (FNBP1L) (Benyamin, Pourcain et al. 2013). Nevertheless, a recent study that used a large combined sample of almost 10000 individuals did not replicate any of these findings (Chabris, Hebert et al. 2012).
Another candidate gene is COMT (see Chapters 3, 7 and 8). Cognitive performance, in line with the dopaminergic effects on behavior has been proposed to follow an inverted U shape, with both deficient and excessive amounts of dopamine activity predicting poor performance (Tunbridge, Harrison et al. 2006). Indeed, studies have shown that healthy individuals who are homozygous for the Met allele have better working memory, make less perseverative errors in the Wisconsin Card Sorting Test (WCST) and have higher IQ, followed by individuals who are heterozygous for this allele with the lowest scores on these measures for individuals who are homozygous for the Val allele (Egan, Goldberg et al. 2001, Malhotra, Kestler et al. 2002, Goldberg, Egan et al. 2003, Diamond, Briand et al. 2004). Not all studies, however, have replicated these associations (e.g., (Stefanis, Van Os et al. 2004)). Moreover, a large meta-analysis on the influence of COMT on cognition suggested that it may have a small effect on IQ, but this finding appeared to be unreliable given the evidence for significant publication bias (Barnett, Jones et al. 2007).

Several linkage studies of intelligence have been conducted that have suggested regions of linkage harbouring potential candidate genes but there is not much evidence of replication. These studies have shown linkage in the following regions: 1q41, 1q43, 2q21-23, 3q13, 6p25-p22, 7q31-36, 8p12, 11p15, 11q22-q23, 11q25, 14q11,14q24, 14q13-q21, 14q32, 14q23, 17q12, and 22q12 (for a review see: (Deary, Johnson et al. 2009)).

Candidate gene association studies of intelligence are inconclusive due to lack of replicated results and I am therefore not elaborating on these studies.

In general, results across candidate, linkage and association studies have been inconsistent and none of these findings has accounted for much of the large genetic component of intelligence. Similarly to the genetic association studies of schizophrenia, the genetic studies
of cognition face methodological problems including high rates of false positive findings and unreplicated results.

4.2.2 Genome Wide Association Studies

The first positive finding from a GWAS on cognition reported that the sodium channel, voltage-gated, type I, alpha subunit (SCN1A) gene was associated with short-term memory (Papassotiropoulos, Henke et al. 2011).

The most recent GWAS, however, did not replicate this finding. There was initial evidence for the formin-binding protein 1-like (FNBP1L) gene, but this was also not replicated (Davies, Tenesa et al. 2011). Another interesting finding that arose from this latter study was that a substantial proportion (approximately 40 to 50%) of variation in human intelligence was associated with common SNPs that are in linkage disequilibrium (LD) with unknown causal variants. These results suggest that a large part of the heritability of intelligence may be due to common variants.

In conclusion, it seems that a possible reason why GWAS analyses of cognition have failed to detect replicable signals might be because the effects of the common SNPs are too small to pass the stringent genome-wide significance levels. Therefore, in line with schizophrenia research, this suggests that very large samples are needed to detect such very small individual effects.

4.3 Conclusions and future directions

In conclusion, it seems that in cognition as in schizophrenia both common and rare allele variants play a role. A possible explanation for this is that a mutation-selection balance, or the
accumulation of many mildly harmful mutations that natural selection has not yet wiped from the population have led to this genetic variance in intelligence (Penke, Denissen et al. 2007). This also explains why highly penetrant alleles are rare in the population.

Hence, examining relationships between specific gene variants and cognition and whether or how these are on the pathway to schizophrenia can provide important insights into our understanding of the mechanisms that operate in schizophrenia (see Chapters 7 and 8).

Chapter 5 High-risk research in schizophrenia

5.1 Rationale

As stated in Chapter 1, support for the neurodevelopmental hypothesis of schizophrenia according to which the pathogenesis of the disorder is due to abnormalities in brain development (Murray 1987, Weinberger 1987) comes from several streams of evidence, including epidemiological, neuroimaging and developmental studies (van Os and Kapur 2009). For example, minor physical anomalies (MPAs) (slight defects of the eyes, head, ears, mouth, hands and feet) that are thought to be attributed to an early brain insult during the first or second trimester of foetal life, are more frequent in individuals with schizophrenia than in healthy controls (Murphy and Owen 1996, Compton and Walker 2009). Furthermore, neurological soft signs (NSS) (neurological abnormalities that are not localized to a specific brain region) also occur in excess in individuals with schizophrenia in relation to healthy controls (Arango and Carpenter 2011). Additionally, perinatal factors, such as obstetric complications, are associated with higher risk of development of schizophrenia in adulthood (Cannon, Jones et al. 2002, Khashan, Abel et al. 2008). Infections and malnutrition during
pregnancy might also increase the risk for later developing schizophrenia (Brown and Susser 2002).

If schizophrenia is due to abnormal neurodevelopment, then we can expect that this abnormality in development will somehow be evident during childhood. Indeed, follow-back, cohort and conscript studies have shown that cognitive (Kremen, Buka et al. 1998, Cannon, Bearden et al. 2000), emotional (Walker, Grimes et al. 1993), motor (Jones, Rodgers et al. 1994, Rosso, Bearden et al. 2000) and social (Davidson, Reichenberg et al. 1999) developmental delays in childhood or adolescence are associated with adult-onset schizophrenia. Nevertheless, there are also discrepancies. For example, there are studies that did not find differences in school marks between children who later developed schizophrenia and those who didn’t (Isohanni, Jarvelin et al. 1998, Cannon, Jones et al. 1999). To the contrary, one of these found that males who later developed schizophrenia had higher school marks in relation to those that did not develop a psychiatric disorder (Isohanni, Jarvelin et al. 1999).

The chief weakness of these follow-back and conscript studies is that due to schizophrenia being a rare outcome, these rely on historical cohorts that have limited data of interest. For example, the sources of information are indirect and not intended to be used in research studies. Therefore, information is non-systematic, incomplete and non-specific.

To address these limitations, a study method has been devised to enrich the sample for individuals who have greater risk of later development of schizophrenia. This method is called the High-Risk (HR) method.
5.2 The High-Risk method

The High-Risk (HR) method aims to examine schizophrenia by studying individuals who are considered to have a higher statistical risk of developing the disorder in relation to individuals in the general population (Obuchowski and Michael 1997). There are numerous ways to define risk. For example, factors such as maternal influenza or other pre- and post-natal infections, brain injuries early in childhood or characteristics that occur later in development such as psychotic experiences can be used to define individuals at increased risk (see Chapters 6 and 7). Another possibility is to study populations at high genetic risk. Some of the work in this thesis uses this approach by studying individuals at risk through possession of a pathogenic CNV, i.e., 22q11.2 Deletion (see Chapter 8).

The advantages of these studies compared to follow-back or conscript studies are the following: 1) the outcome is not as rare as schizophrenia; 2) information is prospectively collected and can therefore be detailed and systematic; 3) they are useful in studying cognitive functions because the functioning of persons who already have schizophrenia may simply reflect epiphenomena related to the disorder (Niemi, Suvisaari et al. 2003).

To date the most frequent approach in HR studies has been to study individuals with high familial risk. HR studies have typically studied the offspring of (an) affected parent(s), because their risk of development of schizophrenia is approximately 10% compared to 1% in the general population and increasing to almost 50% if both parents have schizophrenia.

The first HR studies started in the 1920s and were small, consisting of children of psychiatrically ill mothers. The first study to add a longitudinal follow-up to the study design was the New York Infant Study, which began in 1952 (Fish 1992). The overall goal of these studies was to detect early indicators of schizophrenia, by examining the genetic and environmental risk factors and their interaction (Niemi, Suvisaari et al. 2003).
These studies have shown that HR children have more developmental abnormalities than controls. For example, offspring of parents with schizophrenia have greater risk of deviations in neurologic maturation (Blennow and McNeil 1991, Fish, Marcus et al. 1992) and abnormalities in neuromotor development (Rieder and Nichols 1979, McNeil, Harty et al. 1993) as well as lower IQ compared to controls (e.g., (Byrne, Hodges et al. 1999, Goldstein, Seidman et al. 2000)). There are, however, some studies that did not find IQ differences (Sohlberg and Yaniv 1985) or even the opposite effect (Worland, Weeks et al. 1982). Other reported cognitive deficits include attention deficits (Erlenmeyer-Kimling and Cornblatt 1992), concentration difficulties (Sohlberg and Yaniv 1985), and impaired executive function and memory (Byrne, Hodges et al. 1999, Erlenmeyer-Kimling 2000). It is noteworthy that not all (i.e., 58%) of the HR offspring showing impaired attention in childhood develop schizophrenia in adulthood (Erlenmeyer-Kimling 2000). Other risk indicators include instability of the rearing environment (Cannon, Bearden et al. 2000) and problems in social adjustment, with HR offspring having more difficulties with peer relationships, and being more likely to show social withdrawal, disruption and aggression while growing up (Dworkin, Cornblatt et al. 1993, Johnstone, Ebmeier et al. 2005).

There are, however, some limitations associated with these findings. These developmental abnormalities are not specific to offspring at high risk of schizophrenia. Indeed, findings of HR offspring of other disorders show similar impairments. Also, the extent to which these findings reflect risk for schizophrenia rather than being reared in a less stable environment is unknown. Finally, the findings are plagued by low positive predictive value, as only 10% of these HR children develop schizophrenia and small sample sizes. Therefore, unless very large effects are present, statistical power will be fairly low.

One way to overcome these issues is to examine other HR risk populations. For example, by examining individuals with 22q11.2 Deletion Syndrome, who are at considerable higher risk
of schizophrenia than HR offspring (25% instead of 10%). Moreover, given that this syndrome mostly occurs de novo rather than being familial, behaviour cannot be ascribed to being raised in a higher-risk environment to the same degree as is the case for the offspring of parents with schizophrenia. Another option is to examine children with psychotic experiences in the general population. Children reporting psychotic experiences are at increased risk of developing schizophrenia, and although this risk is relatively small, examining processes related to this risk can potentially provide significant insights into the developmental mechanisms taking place in schizophrenia. Moreover, children with psychotic experiences can be studied in ongoing cohort studies that have used detailed and systematic assessments. Another additional advantage of examining general population samples is that they also include individuals that might never appear to clinical services (Zammit, Kounali et al. 2013).

### 5.3 Summary of Introduction

Despite the large amount of research that has taken place, the complexity of human behaviour has made advances in our understanding of the aetiology and the underlying pathological mechanisms that operate in schizophrenia a slow process. It is clear that schizophrenia is a complex and multi-factorial disorder originating from the action of both genetic and environmental factors, with impaired cognition playing a fundamental role.

One way that could assist in our gaining a better understanding of the nature of the disease is examining the association between cognition and schizophrenia. If cognitive impairments are a major underlying cause of the psychiatric characteristics of the illness, this could inform intervention strategies, by focussing more on cognitive rehabilitation programmes, which may be put in place even before the development of severe psychiatric symptoms and which
could potentially be delivered through educational services (rather than psychiatric) and therefore be less stigmatising.

There is strong evidence of a large genetic component in schizophrenia, but despite the advancement in genetic research, our understanding of the underlying molecular mechanisms remains elusive. It is clear that what is inherited is a genetic predisposition rather than certainty of developing the disease. Similarly, cognition seems to have a substantial genetic component with the underlying molecular mechanisms being equally elusive.

Detecting specific variants that predispose to schizophrenia will enable translational research and the development of personalized drugs based on knowledge of the pathophysiology of the disease. Knowledge of how environmental and genetic risk factors co-participate in disease will enhance our ability to identify high-risk individuals and implement appropriate prevention strategies.

Several streams of evidence have shown that schizophrenia is a neurodevelopmental disorder. There are, however, some limitations with follow-back and conscript studies that have mainly to do with sampling problems and non-systematic information. The High-Risk method is much more advantageous in that respect, because information is systematic and is collected prospectively.

The overall aim of this thesis is to examine whether and how cognition is related to psychopathology in children that are at increased risk of schizophrenia, as well as the possible roles in these relationships of specific genetic variants that have received evidence of predisposing to schizophrenia. In order to explore these questions, I am utilizing two different samples.
The first sample comes from a large population-based study where children that report psychotic experiences are compared to children that do not. Psychotic experiences in children are associated with increased risk of development of psychosis. Therefore, I will be examining whether there are specific cognitive deficits that may be associated with increased risk of psychopathology (Chapter 6). Moreover, I will explore whether COMT has an effect on cognition and psychotic experiences in children in this sample (Chapter 7).

The second sample comprises children with 22q11.2 Deletion Syndrome and their siblings. 22q11.2DS represents one of the strongest risk factors for developing schizophrenia. Therefore, given that cognitive and psychiatric deficits are predictors of schizophrenia in the general population, examining these relationships in such a high-risk sample can improve our understanding of the neurodevelopmental processes that occur in schizophrenia in the general population as well as inform us on the way genetic risk influences the brain and behaviour. I will discuss in Chapter 8 to what extent we can assume that people with this deletion who develop schizophrenia can be considered to be representative of patients with schizophrenia without the 22q11.2 deletion. I will also examine the role of COMT, which lies within the deleted region (i.e., people with this syndrome are hemizygous for COMT) (Chapter 8).
SECTION II EXPERIMENTAL CHAPTERS

Chapter 6  Cognition and non-clinical psychotic experiences in children: longitudinal analysis in a large birth cohort

6.1  Chapter Overview

Patients with psychosis have been reported to have deficits in a range of cognitive domains, with processing speed representing a core deficit. However, it remains unclear to what extent these deficits are affected by the presence of psychosis and by use of psychotropic medication. It is also unknown whether the pattern of cognitive deficits present in patients with psychosis also exists in children with non-clinical non-medicated psychotic experiences within the general population. In this chapter, I describe the longitudinal analyses I conducted to examine the relationships between a priori selected key cognitive domains and psychotic experiences in the large population-based Avon Longitudinal Study of Parents and Children (ALSPAC).

The results provided evidence that within this population, similar patterns between cognition and later occurrence of psychotic experiences existed as have been previously reported between cognition and psychosis in patients with schizophrenia. It was also found that, taking all cognitive domains into account, defective processing speed showed the largest independent association with later psychotic experiences. These findings have potentially important implications for our understanding of the development of psychosis during childhood as well as the specific cognitive deficits that may be associated with this development.
6.2 Introduction

6.2.1 Psychotic experiences (PEs) and the dimensional model of psychosis

Presence of psychotic experiences (PEs) is an integral part of a diagnosis for any psychotic disorder. Recent studies have shown that PEs are not only experienced by patients with psychiatric disorders but are also reported by approximately 5%-10% of the general population (Horwood, Salvi et al. 2008, van Os, Linscott et al. 2009, Polanczyk, Moffitt et al. 2010, Kelleher and Cannon 2011, Linscott and van Os 2013). Estimates, however, can differ substantially as a result of different assessment methodologies, definitions of PEs, and variation in the number of items assessed (Laurens, Hodgins et al. 2007).

Findings from large-scale community studies provide support for the dimensional model of psychosis according to which PEs are on a phenotypic continuum with disorders such as schizophrenia lying at its extreme end (van Os, Hanssen et al. 2000). This implies that similar aetiological mechanisms may underlie the range of phenotypes on this continuum, including cognitive and neural processes (Dutta, Greene et al. 2007).

Support for this model comes from several streams of evidence that have been extensively reviewed elsewhere (e.g., (Myin-Germeys, Krabbendam et al. 2003, van Os, Linscott et al. 2009, Kelleher and Cannon 2011, Kaymaz, Drukker et al. 2012, Linscott and van Os 2013)). A summary of the evidence is provided below.

PEs are related to increased risk of developing psychosis later in life
It has been reported that PEs in childhood increase the risk for psychotic disorder later in life. An influential study of the Dunedin Multidisciplinary Health and Development Study, followed up children who had reported PEs at age 11 and assessed them at age 26. It was shown that children who reported PEs at age 11 were at 5 to 16-fold increased risk of schizophreniform disorder (Poulton, Caspi et al. 2000). Similar results were obtained when the same subjects were assessed at age 38 (Fisher, Caspi et al. 2013). An Australian study also showed that children who self-reported hallucinations at age 14 were at increased risk for developing psychotic disorder at age 21 (Welham, Scott et al. 2009). Similarly, a recent study in the ALSPAC birth cohort demonstrated that the risk of psychotic disorder at age 18 was increased to those that reported PEs at age 12 (Zammit, Kounali et al. 2013).

Furthermore, the Netherlands Mental Health Survey and Incidence Study showed that there is a dose response relationship between the levels of psychotic disorder in a population and the levels of PEs (van Os, Hanssen et al. 2001). To be more exact, PEs and psychotic disorder were assessed in 7076 individuals that were assigned to five groups on the basis of their place of residence (i.e., 5 urbanicity levels). Results indicated that higher levels of urbanicity were associated with higher risk of psychotic disorder and, accordingly, higher risk of PEs (van Os, 2001).

**PEs are familial and heritable**

As stated in the Chapter 3, there is compelling evidence that the risk of schizophrenia is higher for relatives of patients with schizophrenia than the general population. Similar findings have been reported with regards to PEs. For example, a twin study showed that PEs are heritable, with greater concordance in monozygotic than dizygotic twins (Lataster, Tineke
et al. 2009). Moreover, a study on 257 subjects from the general population showed significant familiar clustering of PEs (Hanssen, Krabbendam et al. 2006).

**PEs show similar co-morbid psychopathology with psychosis**

Patients with psychotic disorders frequently meet criteria for other psychiatric disorders (for a review see: (Buckley, Miller et al. 2009)). Substance use, depression and anxiety disorders are the most commonly diagnosed. Similar patterns of comorbidity have been reported for people with PEs (van Os, Hanssen et al. 2000, Polanczyk, Moffitt et al. 2010). For instance, a study on 2232 children, showed that children who reported PEs had more symptoms of depression and anxiety and also exhibited an increase in antisocial behaviour in relation to children who did not report PEs (Polanczyk, Moffitt et al. 2010). Moreover, the robust associations that have been reported between cannabis use and psychosis (Zammit, Allebeck et al. 2002, Moore, Zammit et al. 2007), have been replicated in adolescents and young adults with PEs (Miettunen, Tormanen et al. 2008, Harley, Kelleher et al. 2010). Similarly, associations between tobacco dependence and PEs have also been found (Wiles, Zammit et al. 2006).

**PEs share similar risk factors with psychosis**

A number of risk factors have been associated with psychosis (also see Chapter 6.3.7). These include demographic risk factors, adverse prenatal and perinatal circumstances as well as traumatic childhood experiences.

With regards to the demographic risk factors, urbanicity, ethnic minority status and low socio-economic background have been associated with higher risk of psychosis (for a review
see: (McGrath, Saha et al. 2008)). Similar associations have been reported with PEs (Polanczyk, Moffitt et al. 2010, Zammit, Kounali et al. 2013).

Furthermore, a study in 6356, twelve year-old children from the ALSPAC birth cohort showed associations between PEs and maternal infection during pregnancy, maternal diabetes, need for resuscitation and 5-min Apgar score (Zammit, Odd et al. 2009). Another study in the same cohort also showed associations with advanced paternal age (Zammit, Horwood et al. 2008) that has also been associated with schizophrenia (Zammit, Allebeck et al. 2003).

Finally, childhood abuse, bullying and victimization that have been associated with psychosis have also been associated with higher risk of PEs (Lataster, van Os et al. 2006, Kelleher, Harley et al. 2008).

**PEs share similar schizophrenia related cognitive deficits**

Cognitive impairments constitute a core deficit in schizophrenia (also see Chapter 2) and similar associations between IQ, social cognition and executive function with PEs have also been observed (for a review see the following section). However, research is lacking with regards to whether other cognitive domains that appear to be impaired in patients with schizophrenia are also associated with higher risk of PEs.

**Strengths and weaknesses of this model**
There has been considerable discussion with regards to the dimensional approach (van Os, Linscott et al. 2009, David 2010, Lawrie, Hall et al. 2010, Sommer 2010, Murray and Jones 2012).

The strengths and weaknesses of this model have been eloquently described (David 2010). Among the strengths of this model is its consistence with the medical model, since psychotic symptoms are seen as more severe forms of commonly experienced phenomena. Another strength is that this model expands research to healthy subjects that are more accessible than patients and it can also help reduce stigma by not assigning people in categories of ‘normal’ and ‘pathological’ (David 2010). The dimensional model could potentially be a fruitful and productive alternative to the diagnostic categorizations whose validity has been widely criticized (van Os, Linscott et al. 2009).

One limitation, however, is the potential methodological issues arising from the way PEs are defined. Even though the dimensional model assumes a continuum, researchers still need to decide on a threshold above which experiences can be characterized as psychotic (David 2010). Another limitation is that the complexity of the psychopathological phenomena, reduces our ability to set dimensions (David 2010), a limitation that also applies to the categorical approach.

The specificity of PEs to psychosis

It is noteworthy that the majority of PEs are benign and transient (Blanchard, Jacobson et al. 2010) and their emergence does not necessarily signify that they are strong precursors of psychosis (Murray and Jones 2012). Studies have showed that the positive predictive value for the increasing frequency of PEs predicting psychotic disorders is low (Poulton, Caspi et
al. 2000, Welham, Scott et al. 2009). However, this is expected given that schizophrenia is a rare outcome.

PEs are also related to a range of other disorders like mood and substance-use disorders, suggesting they may be common psychological indicators of risk for a wide range of mental disorders (Scott, Martin et al. 2009, Varghese, Scott et al. 2011, Fisher, Caspi et al. 2013).

However, this does not imply that PEs cannot be informative. Especially when taking into account the above evidence supporting the dimensional model of psychosis, studying the development of PEs and the risk factors associated with their development is of clinical and theoretical importance. Clinically, it is useful to obtain a clear picture of the risks associated with the emergence of PEs as well as whether there are particular features that warrant treatment (Polanczyk, Moffitt et al. 2010). Theoretically, understanding more about the onset of these experiences and their development throughout the life course can help our understanding of schizophrenia and psychotic disorders.

6.2.2 Cognition and schizophrenia revisited

As stated in Chapter 2, impaired cognition has frequently been regarded as a core feature of schizophrenia and a risk factor for psychosis (Seidman 1983, Green 1996). Deficits have been repeatedly and robustly detected in several cognitive domains including attention, working memory and executive function (Reichenberg, Harvey et al. 2009). Processing speed has been identified as the single largest impairment, though it has been suggested that this observation may, at least in part, reflect the effects of psychotropic medication (Knowles, David et al. 2010). Research in young people also shows that development of schizophrenia
in adulthood is preceded by impaired cognitive performance in relation to normal peers (for a review see: (MacCabe 2008)) (see Chapter 2 for more details).

6.2.3 Cognition and Psychotic Experiences

The relationship between PEs and stage of cognitive development or change over time is not clearly established. Large-scale population-based studies have focused on the relationships between IQ and PEs while the associations between other cognitive domains and PEs have remained unexplored.

With regards to PEs and IQ, results from the Dunedin birth cohort have shown that poor performance on standardized intelligence tests at ages 3, 5, 7, 9 and 11 is associated with the emergence of PEs at age 11 (Cannon, Caspi et al. 2002). Similar findings were found in the Environmental Risk Longitudinal Twin Study (Polanczyk, Moffitt et al. 2010) which reported that low IQ at age 5 was associated with PEs at age 12. Results from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort indicated that below average IQ and to a lesser degree, high IQ is associated with increased rates of PEs at age 12 (Horwood, Salvi et al. 2008). However, it is noteworthy, that previous studies of IQ and PEs did not test non-linear relationships.

Concerning PEs and other areas of cognitive functioning, findings from a longitudinal birth cohort twin study indicated that impaired social cognition was related to increased risk of PEs but that there was only weak evidence for association between executive function and PEs. According to the authors, this could be because the children in this study were too young (age
5) to exhibit executive function deficits in relation to psychosis (Polanczyk, Moffitt et al. 2010). Whether or how other cognitive domains impaired in psychosis are related to PEs in children has not yet been examined in a large population-based study.

I am aware of only two studies that have examined cognitive change in relation to development of psychotic symptoms, both based on relatively small high-risk samples. Kremen and colleagues (1998) studied a sample of 18 individuals with probable or definite psychotic symptoms and found that decline in IQ between ages 4 and 7 predicted psychotic symptoms by age 23 (Kremen, Buka et al. 1998). Preliminary findings from the Edinburgh High Risk study (Cosway, Byrne et al. 2000) involving 78 high risk participants and 22 age and sex matched healthy controls also indicated that decline of IQ and memory between an 18th months to 2 years time period preceded the development of psychotic symptoms in young adults.

Apart from the small sample sizes, another limitation of these two studies is the statistical methodology they used to examine change over time. One of the issues that can arise when one deals with multivariate data and complex structures is collinearity. Collinearity is the phenomenon in which two or more of the predictors in a multiple regression are highly correlated leading to regression coefficients that are difficult to interpret (Kirkwood and Sterne 2003). This issue is very common in longitudinal designs given the dependencies within the data arising from the longitudinal nature of the design (i.e., performance in the same tests in different time points of the same people are very likely to be highly correlated) as well as the multi-factorial nature of risk that is associated with complex diseases such as schizophrenia. Approaches such as regressions and ANOVAs that have been used in the previous studies do not take into account potential collinearity issues.
6.2.4 Description of aims

I set out to address this gap in the literature, by exploring in a birth cohort the longitudinal relationships between PEs at age 12 and key cognitive domains at ages 8, 10 and 11, selected \textit{a priori} based on their association with psychosis and schizophrenia (Nuechterlein, Barch et al. 2004).

This chapter study has two primary hypotheses:

1. Impaired performance in these cognitive domains during childhood, would be related to PEs in children aged 12;
2. Decline in these domains over time would be related to PEs in children aged 12.

6.3 Method

In this section I will first provide a general background on epidemiological methodology, causal inference, measures of associations and cohort studies. I will then proceed with describing the Avon Longitudinal Study of Parents and Children (ALSPAC) and the more specific methodology of the current study.
6.3.1 Epidemiological methodology

According to Maxcy, an epidemiologist of the past century, ‘Epidemiology is that field of medical science which is concerned with the relationship of various factors and conditions which determine the frequencies and distributions of an infectious process, a disease, or a physiologic state in a human community’ (1951, cited by (Lilienfeld 1978)). Another more recent definition was given by John Last, ‘Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems’ (p.42, (Last 1988)). Central to these definitions is the hypothesis that disease is not randomly distributed in populations. Hence, identifying the causes of this non-random distribution can provide important insights into the risk factors for disease and the underlying biological mechanisms (Detels 1999).

6.3.1.1 Causal inference

Epidemiology has been characterized as the ‘art of the possible’. Given that solid scientific knowledge can only be derived from experimental studies (Rothman and Greenland 2005), the basis of this argument is that epidemiologists cannot control events or the environment as can laboratory scientists, therefore rendering ‘proof’ unobtainable. However, the most influential idea that arose as a result of continuous and vigorous philosophical debates can be summarized in the following quote by Hume (p.3, (Hume 1740)):

‘The mind can always conceive any effort to follow from any cause, and indeed any event to follow upon another: whatever we conceive is possible, at least in a metaphysical sense: but
wherever a demonstration takes place, the contrary is impossible, and implies a contradiction’.

Therefore, cause-effect relations are only tentative and not certain. Laboratory experiments, do involve a greater degree of observer control compared to what is possible in epidemiology, but this only strengthens the inference and not the level of observation (Rothman and Greenland 2005). Nevertheless, the tentative nature of scientific work, experimental or non-experimental does not prevent striking scientific discoveries from happening.

Given this argument and with regards to the enquiry into causal phenomena Rothman and Greenland (2005) have defined as causal the necessary event, characteristic or condition that occurs before the manifestation of a disease. This event, by itself, however, is not sufficient to affect the disease. Rather, this event is only an element of a ‘complete causal mechanism’. The authors subsequently defined ‘sufficient cause’ (i.e., the complete causal mechanism) as those minimal conditions and events that take place and actually cause the disease. The word ‘minimal’ is used to imply that all of these conditions are necessary for the occurrence of the disease. The onset of disease is the realization of the ‘sufficient cause’. These causal events are not sufficient or necessary to cause the disease but their removal can lead to the prevention of a substantial portion of the disease risk (Rothman and Greenland 2005).

Furthermore, before making any inference about the etiological nature of an association, the following explanations should be taken into account (Rothman and Greenland 2005):

(1) Chance: where the association occurs because of random variation;

(2) Bias: where the association occurs because of bias, be it selection or information bias;
(3) Confounding: where the observed association is the consequence of another factor which is associated with both exposure and outcome;

(4) Reverse causation: where the exposure occurs as a consequence of the outcome;

The degree to which any of these explanations are present instead of a true, causal association, also depends on the study design and the quality and validity of the measurements.

6.3.1.2 Measures of association

Various measures are used to examine the strength of an association between an exposure and an outcome. Usually, the rates in an ‘exposed’ population are compared with those in an ‘unexposed’ population. The exposure might be to risk factors suspected to cause or protect against a disease.

*Attributable risk* (AR) is the difference between the disease rate in exposed persons and the disease rate in unexposed persons.

\[ \text{AR} = I_e - I_u, \]

where

\[ \text{AR} = \text{Attributable risk} \]

\[ I_e = \text{Incidence in exposed} \]

\[ I_u = \text{Incidence in unexposed} \]
Ie is calculated by dividing the number of exposed people who get the disease by the total number of the exposed people. Similarly, Iu is calculated by dividing the number of people who are not exposed and get the disease by the total number of unexposed people. This measure of association tends to be used by policy makers in planning public health interventions (Coggon, Barker et al. 2003).

The measure most often used by epidemiologists, however, is the Relative Risk (RR). Relative risk is the ratio of the probability of the disease occurring in the exposed group versus the unexposed group over a specific period of time:

\[
RR = \frac{\text{risk in exposed group (p1)}}{\text{risk in unexposed group (p0)}}
\]

It is related to the AR by the formula:

\[
AR = Ru \times (RR - 1)
\]

Ru = rate of disease in unexposed persons

RR can be calculated for a wide range of study designs such as cohort and cross-sectional studies and is therefore used more widely by epidemiologists than the AR. It is, however, informative with regards to public health-related decision making.

Relevant to RR and equally used in epidemiology is the odds ratio (OR) that I will be using in my analyses. The odds of a disease are defined as the probability of the disease happening divided by the disease not happening:
The odds are calculated by:

\[
Odds = \frac{\text{prob}(D \text{ happens})}{\text{prob} (D \text{ does not happen})} = \frac{\text{prob}(D)}{1 - \text{prob}(D)}
\]

The odds are calculated by:

\[
Odds = \frac{p}{1 - p} = \frac{D/n}{(1 - D/n)} = \frac{D/n}{h/n} = \frac{D}{h}
\]

D= number of people who experience the disease

h=number of people who do not experience the disease

The OR is also known as the cross-product ratio and is estimated by:

\[
OR = \frac{\text{odds in exposed group}}{\text{odds in unexposed group}} = \frac{D_1/h_1}{D_0/h_0} = \frac{D_1 \times h_0}{D_0 \times h_1}
\]

ORs are widely used in the statistical analyses of binary outcomes and therefore can be calculated from case-control, as well as cohort and cross-sectional studies (Kirkwood and Sterne 2003).
6.3.1.3 Applications of epidemiology: Cohort studies

Cohort or longitudinal studies follow groups or cohorts of individuals without the disease and identify risk factors that are associated with the subsequent occurrence of the disease. The major advantage of cohort studies in comparison with case-control or cross-sectional studies is that they can establish a temporal relationship between risk factors and diseases, and therefore provide a direct measure of risk. On the other hand, case-control and cross-sectional studies only compare exposure frequency between individuals who have the disease and those who do not. Another advantage of cohort studies is that they measure multiple outcomes of a given risk factor and thus can be used to elucidate the spectrum of disease that can be the consequence of a specific risk factor (Detels 1999).

Nevertheless, even though cohort studies establish risk of disease, they are still subjected to measurement errors and selection biases. Therefore, several possible explanations need to be considered before concluding the most likely interpretation may be causation.

6.3.2 The Avon Longitudinal Study of Parents and Children (ALSPAC)

The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort, known to its participants as ‘Children of the 90s’ (http://www.bristol.ac.uk/alspac/) started in 1991-1992, when all pregnant women from a geographically defined region (Avon) in the southwest of England were recruited. The initial ALSPAC cohort consisted of 14,062 live births and 13,988 infants still alive at 12 months (Boyd, Golding et al. 2012, Fraser, Macdonald-Wallis et al. 2012). Participants are now in early adulthood. There have been 68 assessment points between birth and 18 years of age. These include 34 child-completed questionnaires, 9
clinical assessments and 25 questionnaires about the child completed by the mother or other main caregiver (Boyd, Golding et al. 2012).

### 6.3.2.1 Representativeness of the ALSPAC

The representativeness of the sample was assessed by the ALSPAC study team by comparing the socio-demographic characteristics of the mothers who participated in ALSPAC with 1991 British census data from mothers in the whole of Great Britain as well as in the Avon area specifically (see table 1 as taken from the ALSPAC official website [http://www.bristol.ac.uk/alspac/](http://www.bristol.ac.uk/alspac/) (ALSPAC 2012)).

Compared to mothers in the whole of Britain, mothers of infants living in Avon were slightly more likely to occupy their own accommodation and own their own car and less likely to have more than one person per room and be non-White. When comparing ALSPAC mothers to mothers from Avon and Britain, the former are more likely to live in owner-occupied accommodation, to own their own a car and be married and less likely to be non-white. On the other hand, although the ALSPAC participants generally have slightly better socio-economic position in relation to the women in Avon and Britain, they seem to live in slightly overcrowded conditions (more than one person per room). It should be taken into account that ~80% of the cohort completed the 8-month postnatal questionnaire. Therefore, these comparisons will to some degree be influenced by socio-demographic differences resulting from both incomplete enrolment and lack of response to the questionnaire (Fraser, Macdonald-Wallis et al. 2012).
Table 1 Comparison of socio-economic characteristics of mothers living in Great Britain, in the Avon area and those who participated in ALSPAC.

<table>
<thead>
<tr>
<th>Socio-economic characteristic</th>
<th>Whole of Great Britain</th>
<th>Avon area¹</th>
<th>ALSPAC participants²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owner occupier</td>
<td>63.4%</td>
<td>68.7%</td>
<td>79.1%</td>
</tr>
<tr>
<td>1+person/room</td>
<td>30.8%</td>
<td>26.0%</td>
<td>33.5%</td>
</tr>
<tr>
<td>Car in household</td>
<td>75.6%</td>
<td>83.7%</td>
<td>90.8%</td>
</tr>
<tr>
<td>Married couple</td>
<td>71.8%</td>
<td>71.7%</td>
<td>79.4%</td>
</tr>
<tr>
<td>Non-white mother</td>
<td>7.6%</td>
<td>4.1%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

Notes: ¹Mothers with infants <1 year of age, ²Assessed by questionnaire administered at 8-months postnatal.

The representativeness of the ALSPAC study was also assessed in a research article by members of the ALSPAC team (Boyd, Golding et al. 2012). They presented comparisons of demographic and standard school assessment data, taken at 16 years of age between a national sample (i.e., National Pupil Database ‘Key Stage 4’ records, pupil census and assessment data for all pupils in English schools), the ALSPAC ‘enrolled sample’ (i.e., those children on whom data has been obtained at each and every assessment point) and individuals with incomplete enrolment and participation histories. These comparisons showed that the children in the ALSPAC ‘enrolled sample’ have higher educational attainment in relation to the national average. Also, it seems that the difference in educational attainment increases with increasing completeness of participation in the ALSPAC. Children who have not recently participated or are lost to follow-up due to attrition have lower educational attainment than the national average. Also, children in the ALSPAC ‘enrolled sample’ are more likely to be white and less likely to be eligible for free school meals. Nevertheless, these differences could reflect regional differences, for example in ethnicity or socio-economic status. As shown in table 1, mothers from the Avon area are more likely to be white in relation to mothers from the all of British sample, while furthermore economically
the southwest of England ranks fourth highest amongst the 12 UK regions (Marais and Schuster 2007).

In conclusion, there is an over-representation of more affluent groups and an under-representation of non-white minority ethnic groups in the ALSPAC study in comparison with the national as well as local Avon population. These factors might influence findings based on prevalence but when taken into account by using proper statistical methods, they should not adversely influence the longitudinal results (Boyd, Golding et al. 2012).

### 6.3.2.2 Strengths and weaknesses of the ALSPAC

ALSPAC is considered one of the world’s largest and most comprehensive population-based studies (Wise 2001). The aim of the ALSPAC birth cohort is to examine the gene-environment relationships influencing child health and development as well as parental characteristics (Golding, Pembrey et al. 2001). Therefore, one additional advantage of the study is the availability and variety of information from even before birth and throughout development using a variety of sources. These sources include i) questionnaires completed by the mothers, their partners and from age 5 onwards, the children; ii) medical, educational and other records; iii) measurements of the environment, including noise and magnetic radiation; iv) in depth-interviews of particular sub-groups (focus clinics) and; v) biological samples (Golding, Pembrey et al. 2001). The breadth and frequency of data collection, the duration of the follow-up, the ongoing support and commitment from the study families are also great strengths of the ALSPAC (Boyd, Golding et al. 2012, Fraser, Macdonald-Wallis et al. 2012).
With regards to the weaknesses, the main limitation of the ALSPAC birth cohort, similar to other cohort studies, is the loss to follow-up (Boyd, Golding et al. 2012). Nevertheless, attrition can potentially be addressed with relatively new statistical techniques, including multiple imputation techniques (Royston 2004). Other weaknesses include issues with the generalisability of the study given the biases in the representativeness of the sample (Boyd, Golding et al. 2012, Fraser, Macdonald-Wallis et al. 2012). Finally, despite the large sample size, the ALSPAC cohort still does not provide adequate power to study rarer exposures and outcomes (Boyd, Golding et al. 2012).

### 6.3.3 Current study sample size

6,784 individuals out of potentially 13,988 completed the Psychosis-Like Symptoms interview (PLIKSi) at age 12. The number of individuals with available information from both the PLIKSi and various cognitive tests varies, as the latter were completed by different numbers of individuals.

### 6.3.4 Ethical approval

Ethical approval was obtained from the ALSPAC’s Law and Ethics Committee and the Local Research Ethics Committees. Parents who enrolled their children into ALSPAC provided written informed consent at the time of the enrolment and they or their child are free to withdraw at any time.
6.3.5 Cognitive assessments

6.3.5.1 Introducing the MATRICS framework and its development

The Measurement and Treatment Research in Cognition in Schizophrenia (MATRICS) which was developed by the U.S. National Institute of Mental Health (NIMH) reflects the increasing recognition that cognitive deficits are part of the core pathology and likely determinants of the functional outcome in schizophrenia (van Os and Kapur 2009).

The MATRICS initiative was based on the premise that there was lack of agreement on the best way to measure these cognitive impairments as well as the recognition of the need to develop treatments that can alleviate these cognitive deficits (Gold 2004). Therefore, the NIMH aimed to establish a consensus battery with which these deficits would be best assessed and against which cognition-enhancing agents could be evaluated, paving the way for approval of new medications by the U.S. Food and Drug Administration (FDA) (Marder and Fenton 2004).

Hence, the MATRICS group adopted a structured consensus-building process based on the RAND panel method (Fitch, Bernstein et al. 2001). Initially, there was a survey of 68 experts which established the desirable features of the battery. Also, the MATRICS Neurocognition Committee reviewed the results from all the available factor-analytic studies of cognition in schizophrenia in order to derive separable cognitive domains. A conference was then held, during which a panel including more than 130 scientists from academia, government and the pharmaceutical industry agreed to focus on seven cognitive domains:
(1) Speed of processing;
(2) Attention/vigilance;
(3) Working memory;
(4) Verbal learning;
(5) Visual learning;
(6) Reasoning and problem solving;
(7) Social cognition.

In addition, the panel agreed on the following five criteria necessary for test selection:

(1) High test-retest reliability;
(2) Utility as a repeated measure;
(3) Demonstrated relationship to functional status;
(4) Potential changeability in response to pharmacological agents;
(5) Practicality for clinical trials and tolerability for patients.

After this conference, the MATRICS Neuropsychology Committee gathered 90 nominated tests for each of the above domains which met these criteria. They then selected a group of experts from the academia and industry who formed a RAND panel. This group was sent all the potential information that could be used for rating how each of these tests met the criteria. The results of the ratings by the RAND panel were reviewed by the MATRICS Neuropsychology Committee, who formed a beta version of the battery that included 2-5 tests for each cognitive domain (table 2). The beta battery was then formally evaluated in a sample of 176 outpatients with schizophrenia. The results from this assessment were then used to rank the tests within the domains and after a final meeting, the MATRICS battery was decided (Nuechterlein, Green et al. 2008). This battery was subsequently co-normed and
standardized following administration in a representative sample of 300 community controls (Kern, Nuechterlein et al. 2008).

This battery is considered to be the gold standard cognitive assessment tool in schizophrenia research because it was derived using systematic methods that relied on consensus expert-led guidance. Finally, it is noteworthy that although the MATRICS battery was originally developed to assess the effects of cognition-enhancing agents in clinical trials, the lead investigators state that it is their hope that the battery will also be used in broader settings including observational and genetic research (Nuechterlein, Green et al. 2008).
Table 2 Descriptions of tests that were selected for the MATRICS Beta Battery.

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Test</th>
<th>Description of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of processing</td>
<td><strong>Category fluency</strong>&lt;br&gt;<strong>Brief Assessment of Cognition in Schizophrenia (BACS): Symbol-coding</strong>&lt;br&gt;Wechsler Adult Intelligence Scale 3rd edition (WAIS-III): Digit Symbol-Coding&lt;br&gt;<strong>Trail Making Test: Part A</strong></td>
<td>Subject has to name as many animals as they can in 1 minute&lt;br&gt;Digit symbol pairs are followed by a list of digits. The subject needs to write under each digit the corresponding symbol as quickly as possible.&lt;br&gt;Similar test to the one above.</td>
</tr>
<tr>
<td>Attention/Vigilance</td>
<td><strong>Continuous Performance Test-Identical Pairs (CPT-IP)</strong>&lt;br&gt;3-7 Continuous Performance Test (3-7 CPT)</td>
<td>A measure of sustained attention in which the subject has to press a button each time they see matching numbers on the computer screen.&lt;br&gt;Numbers appear on the computer screen and the subject needs to press the button after the digit 7 of each 3-7 sequence.</td>
</tr>
<tr>
<td>Working Memory (verbal)</td>
<td><strong>U. of Maryland Letter-Number Span</strong>&lt;br&gt;Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III): Letter-Number Sequencing&lt;br&gt;Brief Assessment of Cognition in Schizophrenia (BACS): Digit Sequencing</td>
<td>The subject mentally reorders strings of numbers and letters and repeats them to administrator.&lt;br&gt;A test requiring the reordering of an initially unordered set of letters and numbers</td>
</tr>
</tbody>
</table>
| Working Memory (nonverbal) | **Wechsler Memory Scale-3rd edition (WMS-III): Spatial Span**<br>**Spatial Delayed Response Task**<br>Using a board on which 10 cubes are irregularly spaced, respondent taps cubes in same (or reverse) sequence as test administrator.<br>Subjects focus on a central fixation cross on a computer screen. While fixated, a dot-shaped cue flashes in one of 32 possible locations at the screen. Then a delay period occurs during which a series of shapes appear on the screen. Subjects must press a button whenever a diamond shape
<table>
<thead>
<tr>
<th>Verbal Learning and Memory</th>
<th>Hopkins Verbal Learning Test-Revised (HVLT-R)</th>
<th>During this test, a list of 12 words from three taxonomic categories is presented and the subject is asked to recall as many as possible after each of three learning trials. The subject is asked to learn and remember medication instructions and a name, address and phone number.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropsychological Assessment Battery (NAB)-Daily Living Memory</td>
<td>Test involving reproducing six geometric figures from memory.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual Learning and Memory</th>
<th>Brief Visuospatial Memory Test-Revised (BVMT-R)</th>
<th>Test involving three learning trials of nine target stimuli.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropsychological Assessment Battery (NAB)-Shape Learning</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Reasoning and Problem Solving</th>
<th>Neuropsychological Assessment Battery (NAB)-Mazes</th>
<th>Test involving seven timed paper-and-pencil mazes of increasing difficulty that measure foresight and planning. Subjects are asked to repeat lists of digits of increasing lengths in reverse order. Subjects are shown two pictures simultaneously. Each picture shows 3 balls of different colours arranged on 3 pegs, with the balls in a unique arrangement in each picture. Patients are instructed to determine the fewest possible moves of the balls in one picture to make the arrangement of balls identical to that of the other, opposing picture.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wechsler Adult Intelligence Scale 3rd edition (WAIS-III) Block Design</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BACS - Tower of London</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Social Cognition</th>
<th>Mayer Salovey-Caruso Emotional Intelligence (MSCEIT) – Managing Emotions (D&amp;H)</th>
<th>Test involving multiple choice questions in which the subjects’ task is to judge the actions that are most effective in obtaining the specified emotional outcome for an individual in a story.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer Salovey-Caruso Emotional Intelligence (MSCEIT) – Perceiving Emotions (A&amp;E)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:** Tests in bold and Italics are those who were selected for the final MATRICS battery.
The MATRICS framework and the current study

The cognitive tests that were used in ALSPAC are described in figure 2. All cognitive measures were administered by trained psychologists. Cognitive tests were selected for analysis in this study \textit{a priori} according to the Measurement and Treatment Research in Cognition in Schizophrenia (MATRICS) initiative (Nuechterlein, Barch et al. 2004) on the basis that they were representative of the key cognitive domains identified as impaired in schizophrenia. The MATRICS framework served only as a loose concept as not all the selected cognitive measures corresponded perfectly to the MATRICS domains.
Figure 2 Graphic representation\(^2\), providing information on the cognitive measures used in ALSPAC

\(^2\) created with the Mindgenius Mind Mapping Software programme
There were tests available corresponding to four of the seven MATRICS cognitive domains.

The domains and tests examined were as follows:

**Processing Speed (ages 8 and 11)**

Processing speed at age 8 was assessed using measures from two different tasks:

(i) the Sky Search task, which was taken from the Tests of Everyday Attention for Children (TEACh) (Robertson 1996)


Processing speed at age 11 was assessed as in (i) above. However, the WISC-III was not conducted at this age and so test (ii) was not available at age 11.

**Attention/vigilance (ages 8 and 11)**

The ‘Opposite Worlds’ task from TEACh (Robertson 1996) was used to assess this domain at both ages. It should be noted that this test is related but somewhat different from attention/vigilance as measured in the MATRICS.

**Working memory (ages 8 and 10)**
Working memory at age 8 was assessed by Backwards Digit Span test and Arithmetic task from the WISC-III.

At age 10, the Counting Span Task (Case 1982) was used to assess working memory. Therefore, there is incomplete correspondence, as the tests used at age 8 were not repeated at age 10.

Reasoning and problem solving (age 8)

This cognitive domain was assessed by the following WISC-III tasks:

1) Picture Completion;

2) Picture Arrangement;

3) Block Design;

4) Object Assembly.

A description of these cognitive tests is provided in table 3.
Table 3 Description of cognitive tests

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Cognitive test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>Coding</td>
<td>Task involving copying shapes that match to different numbers as quickly as possible within a designated time limit.</td>
</tr>
<tr>
<td></td>
<td>Sky Search</td>
<td>Children were presented with pairs of identical and non-identical spaceships and asked to circle identical pairs as quickly as possible. A selective attention score derived from this task which reflects the time taken (in secs) for the task, divided by the number of spaceships pairs correctly circled, adjusted for motor speed. Motor speed was the time taken for the task when the task was repeated with the non-identical spaceships removed.</td>
</tr>
<tr>
<td>Attention/vigilance</td>
<td>Opposite Worlds</td>
<td>This is a ‘Stroop-Like’ task; children were shown a total of 24 numbers in succession. They were asked to read the numbers out loud as quickly as possible and they were asked to call out ‘two’ when they reach number 1 and ‘one’ when they reach number 2. As a score for this task was the time taken to make the response.</td>
</tr>
<tr>
<td>Working memory</td>
<td>Backwards Digit Span</td>
<td>Children were asked to repeat lists of digits of increasing lengths in reverse order.</td>
</tr>
<tr>
<td></td>
<td>Arithmetic</td>
<td>Task comprising of timed orally administered arithmetic questions which are presented in story format.</td>
</tr>
<tr>
<td></td>
<td>Counting Span Task</td>
<td>A number of red and blue dots were shown on a white screen and children were asked to count the red dots out loud. After each set of screens children recalled the number of dots presented on each screen within each set. At the start, children were shown two practice sets of two screens, followed by three sets of two, three, four and five screens. The main outcome score for</td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>Picture Completion</td>
<td>this task was a span score reflecting the number of correctly recalled sets, weighted by the number of screens within each set.</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Picture Arrangement</td>
<td>For this task, children pointed out what is missing from each of a series of incomplete pictures, arranged in order of difficulty.</td>
</tr>
<tr>
<td></td>
<td>Block Design</td>
<td>Task involving the ordering of pictures into meaningful stories within a specific time limit and with increasing difficulty. A high score reflected both rapid response and accuracy.</td>
</tr>
<tr>
<td></td>
<td>Object Assembly</td>
<td>Children were shown specific patterns of blocks and asked to copy them using real blocks. Patterns were presented in order of difficulty. A high score reflected both rapid response and accuracy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Puzzles were put together within a constrained time limit. Puzzles were presented in order of increasing difficulty. A high score reflected both rapid response and accuracy.</td>
</tr>
</tbody>
</table>

The MATRICS procedure was used to incorporate these measures into cognitive domains, allowing adherence to a wider theoretical framework and limiting the number of statistical comparisons. As in the development of the MATRICS battery I included multiple tests within domains, where suitable tests were available and this inclusion was supported by the observed high correlations of the tests within domains (table 4).
Table 4 Pearson’s correlations of the cognitive tests within each cognitive domain

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Cognitive tests</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>Coding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sky Search</td>
<td>0.38</td>
</tr>
<tr>
<td>Working memory</td>
<td>Arithmetic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Backwards digit span</td>
<td>0.29</td>
</tr>
<tr>
<td>Reasoning and problem</td>
<td>Picture arrangement</td>
<td></td>
</tr>
<tr>
<td>solving</td>
<td>Picture completion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Block design</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Object Assembly</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Object Assembly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Block design</td>
<td>0.43</td>
</tr>
</tbody>
</table>

6.3.6 Assessment of psychotic experiences

The semi-structured Psychosis-like Symptoms Interview (PLIKSi) was conducted at age 12 (Horwood, Salvi et al. 2008). The interviewers were psychology graduates who were trained by experienced child and general psychiatrists. They were also trained by experts of the widely used semi-structured interview Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing, Babor et al. 1990). There were regular training sessions as well as monthly workshops to discuss complex cases. The interviewers had to reach 95% rating agreement in two gold-standard interview videotapes prepared by the psychiatrists. All interviews were audio-recorded and each interview was also rated by a second independent interviewer. Test-retest reliability was established in 3% of the cohort who were invited back for a second interview two weeks after the initial interview. The average kappa value for inter-rater reliability was 0.72. The PLIKSi is based on the psychosis section of the Diagnostic
Interview Schedule-Children (DISC-IV) (Shaffer, Fisher et al. 2000), the Schedule for Affective Disorders and Schizophrenia for School-Age children (K-SADS) (Puig-Antich and Chambers 1978) and the SCAN version 2.0 (SCAN – Version 2.0) (Sheitman, Murray et al. 2000), with slight modifications after piloting. It comprises of 12 core questions regarding the three main domains of positive symptoms: hallucinations (auditory and visual), delusions (being spied on; persecutory; thoughts being read; reference; control; grandiose ability, not otherwise specified) and experiences of thought interference (thought broadcasting; thought insertion; thought withdrawal). If the child answered ‘yes’ or ‘maybe’ to any of these questions, the interviewer used additional probe questions to rate whether the concept was definitely present, suspected as present or not present. Questions were also included to clarify that symptoms were not attributable to hypnagogic and hypnopompic states, fever or substance misuse. The time frame for assessment of symptoms was presence during the last six months. For the analyses presented below, I excluded those symptoms that were attributable to sleep, fever and substance misuse.

The primary outcome measure was suspected or definite PEs (1) versus no PEs (0). This approach was chosen rather than grouping suspected PEs together with no PEs to be consistent with previous studies on PEs in the ALSPAC birth cohort (e.g., (Zammit, Owen et al. 2011)) as well as because the latter approach has been criticized as being over conservative (Polanczyk, Moffitt et al. 2010).
6.3.7 Confounders

In the following section I provide a description of the potential confounding variables that were taken into account.

6.3.7.1 Demographic variables

Gender: Gender differences have been reported in individuals with schizophrenia (McGrath, Saha et al. 2008). For example, males with schizophrenia have earlier age of onset than females (e.g., (Gorwood, Leboyer et al. 1995)). The results with regards to cognition are mixed, with studies reporting greater cognitive abnormalities in men than women with schizophrenia (e.g., (Goldstein, Seidman et al. 1994)), other studies reporting evidence to the contrary (e.g., (Lewine, Walker et al. 1996)) and others reporting no evidence (e.g., (Albus, Hubmann et al. 1997)). Gender differences could, therefore, account for differences in the associations between cognition and PEs. For this reason, I added gender as a potential confounder in my analysis.

Parental education, social class and crowding index: Low social class or variables that are linked to low social class have been related to increased risk for schizophrenia in a number of studies (e.g., (Castle, Scott et al. 1993, Harrison, Gunnell et al. 2001, Goldberg, Fruchter et al. 2011)). Some authors have argued that low social class, or other factors related to low social class are causally related to schizophrenia (Wicks, Hjern et al. 2010). An alternative explanation, however, is that of social drift where individuals with schizophrenia drift down the social ladder as a result of disease onset (Byrne, Agerbo et al. 2004). It could also be that
both of these explanations operate. Similarly, associations have been observed between low socio economic status and cognitive performance (e.g., (Kaplan, Turrell et al. 2001, Hackman, Farah et al. 2010).

**Ethnic group:**

Immigrant status has been associated with risk of schizophrenia (for a review see: (McGrath, Saha et al. 2008)) as well as impaired cognitive performance (Shadlen, Larson et al. 2001).

### 6.3.7.2 Parental psychiatric problems

As stated in Chapter 1, family members of subjects with schizophrenia have a 10-fold increased risk of also developing the disease and this can also impact upon their cognitive performance, therefore, parental psychiatric problems can potentially act as confounders.

### 6.3.7.3 Development

Developmental delay has also been associated with both risk of schizophrenia (Isohanni, Murray et al. 2004) and impaired cognitive performance (First and Palfrey 1994).
6.3.7.4 Behavioural/Emotional difficulties

I included this confounder to account for the possibility that the child’s performance at the cognitive tasks was influenced by hyperactivity or other emotional problems. Behavioural and emotional difficulties are also related to risk of PEs (Cannon, Caspi et al. 2002). As stated in Chapter 1, premorbid psychopathology has been associated with increased risk of schizophrenia.

6.3.7.5 Summary of selected confounding variables and their coding in my analyses

Apart from gender (which came from birth certificates), these confounding variables were obtained from the following sources of information: 1) children face-to-face interviews; 2) main caregiver reports on offspring; 3) maternal self-reports and; 4) mother’s partner reports. Below is a summary of the confounding variables that I selected for my analysis and figure 3 shows their graphic representation.

- Demographic (gender, parental education, parental social class, crowding index, ethnic group);

**Gender:** Gender information was obtained from birth certificates and was a dichotomous variable coded as “Boy=0” and “Girl=1”.

**Parental education:** All variables were created by the ALSPAC Study Team. I categorised the maternal and paternal education variables into three levels based on the UK educational
system. The low level included O’ levels and secondary education, the middle level included A’ levels and vocational training and high level included University degree. This variable was derived by maternal and partner’s self-reports at 32 weeks of gestation.

**Parental occupation:** Parental occupation was categorised by the ALSPAC Study Team into six levels according to the 1991 Office of Population Censuses & Surveys (OPCS) classifications, with the 1st class being the highest. Occupational status was derived by maternal and partner’s self-reports at 32 weeks of gestation.

**Crowding index:** The crowding index variable was derived by the ALSPAC study team and estimated by dividing the number of people in the household by the number of rooms. This variable was derived by maternal self-reports at 8 weeks of gestation.

**Ethnic group:** This variable was derived by the ALSPAC Study Team from two questions in which the children’s mother indicated her own group and the one of the biological father of the child (if provided). This variable was derived from maternal self-reports at 32 weeks of gestation and was coded as a dichotomous variable: “Caucasian=0” and “Non-Caucasian=1”.

- Parental psychiatric problems;

These variables were derived from maternal and paternal self-reports at 12 weeks of gestation. I created two summary scores for maternal and paternal psychiatric problems respectively, including psychiatric problems such as bulimia, anorexia nervosa, alcoholism, drug addiction, depression, schizophrenia and other psychiatric problems.

- Child developmental delay;
This variable was derived from the Denver Developmental Screening Test (Frankenburg and Dodds 1967), a questionnaire which was completed by the mother when the child was 6 months old.

- Behavioural/emotional difficulties.

The total problems scale of the Strengths and Difficulties Questionnaire (Goodman 1997) was completed by the main carer when the child was 81 months.
Figure 3 Graphical representation³, providing information on the age, source and type of the confounding variables

³ created with the Mindgenius Mind Mapping Software programme
6.3.8 Data analyses

Data analyses were conducted using in STATA/IC (version 11) for Windows.

All cognitive measures were standardized to have a mean of zero and a standard deviation (SD) of one. Individuals scoring more than 3 SDs from the mean were excluded from further analysis, because their scores were unlikely to accurately reflect cognitive performance. Moreover, I was concerned that an erroneous extreme score (outlier) is more likely to affect the associations in comparison with scores in the middle of distribution and if a true effect exists then it would be identifiable without the extreme scores. I conducted sensitivity analyses whereby I included individuals with scores above or below 3 SDs from the mean by collapsing measures into quintiles, and the results were very similar to those excluding individuals with extreme scores. Where domains were comprised of more than one test (e.g., working memory at age 8), a summary statistic was calculated by averaging and standardizing the z-scores from the tests that comprised each domain. All variables were normally distributed. This procedure parallels the methodology used in the MATRICS, generating a summary statistic that represents the score of each individual in this domain.

Logistic regression analyses were conducted to estimate odds ratios (ORs) and 95% confidence intervals for associations between cognitive domains at ages 8, 10 and 11 and PEs at age 12. To facilitate interpretation, the cognitive domains were recoded so that higher score indicated worse performance. Seven independent regressions were conducted to determine whether the independent variables (i.e., scores on each of the seven neurocognitive domains), predicted group status (0=individuals without psychotic experiences, 1=individuals with psychotic experiences). Table 5 shows the correlations between the cognitive domains.
Table 5 Correlations between cognitive domains.

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Processing speed age 8</th>
<th>Attention age 8</th>
<th>Working memory age 8</th>
<th>Reasoning and problem solving age 8</th>
<th>Working memory age 10</th>
<th>Processing speed age 11</th>
<th>Attention age 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed age 8</td>
<td>1</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention age 8</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory age 8</td>
<td>0.30</td>
<td>0.33</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reasoning and problem solving age 8</td>
<td>0.29</td>
<td>0.29</td>
<td>0.37</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory age 10</td>
<td>0.23</td>
<td>0.29</td>
<td>0.37</td>
<td>0.22</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed age 11</td>
<td>0.43</td>
<td>0.31</td>
<td>0.16</td>
<td>0.20</td>
<td>0.13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Attention age 11</td>
<td>0.45</td>
<td>0.58</td>
<td>0.30</td>
<td>0.23</td>
<td>0.29</td>
<td>0.36</td>
<td>1</td>
</tr>
</tbody>
</table>
These associations were subsequently adjusted for possible confounders. To examine whether associations for any of the cognitive domains were independent of each other, I subsequently conducted three regressions where I also adjusted for all other cognitive domains (cognitive domains at ages 10 and 11 were adjusted for earlier (i.e., age 8) measures of the different cognitive domains only).

I examined non-linear relationships between the cognitive domains and PEs by examining likelihood ratio tests for the addition of quadratic as well as linear terms to the models.

I examined change in cognitive performance over time by applying Principal Components Analysis (PCA) to measures of both time points. This approach has been suggested by several authors (e.g., (Hotelling 1957, Kendall 1957, Mosteller and Tukey 1977, Mardia, Kent et al. 1979, Gunst and Mason 1980)) but has not received appropriate attention by the psychological and medical research.

PCA transforms the data linearly and orthogonally to a new coordinate system where the greatest variance by any projection of the data is on the first coordinate (called the first principal component), the second greatest variance on the second coordinate and so on up to a maximum number which is the total number of variables included in the PCA (Kirkwood and Sterne 2003). PCA has been used to reduce the number of dimensions in exploratory data analysis and also for predictive models in more complex modern computational algorithms like these appearing in computational statistics such as neural networks (e.g., (Selamat and Omatu 2004)) and pattern recognition (e.g., (Horn and Gottlieb 2001)). PCA is closely related to factor analysis but PCA is the simplest dimensionality reduction technique (Dunteman 1989). I used PCA as a means of transforming the original variables (which are
correlated by definition as these were measuring the same phenomenon at the same scale repeatedly) in order to avoid problems associated with collinearity that ANOVAs and regressions that were used in the previous studies do not account for (Kremen, Buka et al. 1998, Cosway, Byrne et al. 2000).

The PCA identified two factors (s1 and s2) for each cognitive domain, where s1 represented the average performance across both time points and s2 represented change. Logistic regression analysis was used to test for associations between s2 and PEs after adjusting for s1.

6.3.9 Dealing with missing data – Multiple Imputation by Chained Equation Model

Missing data are a common problem in epidemiological and clinical research. For example, there are subgroups of people that are more likely to drop out, such as young people with behavioural problems (Wolke, Waylen et al. 2009). Where loss to follow-up is related to possible aetiological factors then the results of the study can be biased.

The specific reasons why data is missing determines the risk of bias. Possible reasons are:

a) Missing completely at random (MCAR);

b) Missing at random (MAR);

c) Missing not at random (MNAR).
When it is likely that there are systematic differences between the missing and the observed values (i.e., the data are not MCAR), analyses that are based on complete cases (i.e., those individuals that have no missing data at all) can be biased (Sterne, White et al. 2009).

Because ALSPAC is a large population-based cohort and the current study in particular focuses on psychosocial problems, missing data could represent a potential bias that could be addressed using multiple imputation analysis (Sterne, White et al. 2009).

Imputation in this study was conducted using a Multiple Imputation by Chained Equation (MICE) approach (Royston 2004, Royston 2005, Carlin, Galati et al. 2008) that was performed using the ‘ICE’ module that was developed by Patrick Royston (Royston 2005) in STATA/IC (version 11) for Windows (StataCorp 2009).

### 6.3.9.1 Characteristics of Multiple Imputation by Chained Equation model

MICE is a flexible and practical statistical technique for handling missing data (Royston 2004). It is performed using the ICE command which imputes missing values by using ‘switching regression’, a multiple multivariate imputation technique that is based on the methodology that was initially developed by Donald Rubin (Rubin 1976) and described by van Buuren (van Buuren, Boshuizen et al. 1999).

MICE uses a two-stage approach. The first stage consists of the creation of multiple copies of the dataset in which the missing values are sampled from their predictive distribution based on the observed values (Sterne, White et al. 2009). Hence, multiple imputation uses a Bayesian inference theory according to which the likelihood that a specific hypothesis is true
(i.e., the value of imputed data) is ascertained by the observed evidence (i.e., the values of the observed data). This is also called the ‘posterior probability’ of the hypothesis (Anscombe 1961). Every variable that includes missing data is imputed using a particular imputation equation based on a regression model in which the variable to be imputed serves as the dependent variable and the other variables as independent variables (Royston 2005).

It is not possible to know the true values of the missing data, but in order to better account for the uncertainty in predicting the missing values, the imputation should be based on a multiple iterative procedure. Single imputation procedures often treat the imputed values as true values and cause type I errors, while also considerably reducing the standard errors and narrowing the confidence intervals (Sterne, White et al. 2009).

It is important for the MICE model to also include the outcome variables, in addition to the predictor variables and the confounders. The outcome variables carry information about the potential reasons why the predictor variables have missing values (Sterne, White et al. 2009). It has also been found that imputation models that omit the outcome variables produce biased coefficients (Moons, Donders et al. 2006).

With multiple imputation techniques, the unknown missing data is replaced using M>1 possible sets of values that are independent and are drawn from the predictive distribution of the missing values conditional on the observed values (White, Royston et al. 2011).

In the second stage, when the multiple imputations have been developed, each imputed dataset can subsequently be analysed separately using standard statistical methods. The results of these analyses are variable due to the missing values being replaced by different values due to the different imputations. These results can be interpreted only when they are
combined into an overall estimate. This happens by using Rubin’s rules and the Bayesian framework that take into account the variability in the results of the different datasets and produce standard errors, confidence intervals and significance levels (Sterne, White et al. 2009, White, Royston et al. 2011).

In STATA the imputed datasets are stored in a vertically stacked format, in which each entry is repeated for the M number of imputations. The analysis of the datasets can then be performed using the prefix ‘micombine’ which fits a variety of regression models, combining the estimates using Rubin’s rules (Royston 2005).

### 6.3.9.2 Addressing missing data in this study

Children who did not attend the PLIKSi interview at age 12 were more likely to perform worse in all the cognitive domains: processing speed at age 8 (mean [SD], -0.11 [1.0] versus 0.03 [1.0]; p<0.001); attention at age 8 (-0.01 [0.7] versus 0.05 [0.6]; p=0.004); working memory at age 8 (-0.12 [1.0] versus 0.03 [1.0]; p<0.001); reasoning and problem solving at age 8 (-0.18 [1.0] versus 0.05 [1.0]; p<0.001); working memory at age 10 (-0.11 [1.0] versus 0.02 [1.0]; p<0.001); processing speed at age 11 (-0.01 [0.8] versus 0.07 [0.8]; p=0.004) and attention at age 11 (-0.09 [1.0] versus 0.01 [1.0]; p=0.006).

As a sensitivity analysis I used imputation analysis with chained equations (Royston 2004, Royston 2005) to determine whether attrition of the cohort had biased the observed associations. Missing data was imputed for both the outcomes and confounders. Fifty two measures were included in the imputation model including variables associated with both
cognition and PEs, such as the individual score measures of Strengths and Difficulties Questionnaire (SDQ) (Goodman 1997) measures at ages 4, 8 and 12; the Short Moods and Feelings Questionnaire (SMFQ) (Angold, Costello et al. 1995) at ages 9 and 12; as well as variables relating to parental socio-demographic characteristics including parental educational qualifications, financial difficulties and house crowding indices. These variables were used because they provide information on the missing data and therefore make the assumption of MAR more plausible. Fifty datasets were imputed.

6.4 Results

6.4.1 Cognitive domains and psychotic experiences at age 12

Among the children that were interviewed, 787 (11.6%, 95% CI=10.9 to 12.4%) had suspected or definite PEs at age 12. A descriptive summary of the children’s cognitive performance is presented in table 6.
Table 6 Descriptive statistics of standardized cognitive performance scores in children with and without psychotic experiences

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Age</th>
<th>Psychotic experiences n</th>
<th>Mean (SD)</th>
<th>No psychotic experiences n</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>8</td>
<td>650 -0.15 (1.0)</td>
<td>5220 0.05 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention</td>
<td>8</td>
<td>625 -0.02 (0.7)</td>
<td>5019 0.06 (0.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory</td>
<td>8</td>
<td>647 -0.09 (1.0)</td>
<td>5191 0.05 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>8</td>
<td>648 -0.01 (1.1)</td>
<td>5197 0.06 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory</td>
<td>10</td>
<td>639 -0.06 (1.0)</td>
<td>5161 0.03 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>11</td>
<td>688 0.07 (0.8)</td>
<td>5433 0.07 (0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention</td>
<td>11</td>
<td>655 -0.11 (1.0)</td>
<td>5279 0.03 (1.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7 provides descriptive statistics of gender, maternal education qualifications and SDQ behavioural rating in children with and without PEs. This table shows that children with PEs are less likely to be males, more likely to have higher SDQ scores in relation to children without PEs. Also, children with PEs are more likely to have a mother whose educational qualification is low or middle in relation to children without PEs who are more likely to have a mother whose educational qualification at degree-level.
Table 7 Descriptive statistics of gender, maternal educational qualifications and SDQ behavioural ratings in children with and without psychotic experiences.

<table>
<thead>
<tr>
<th></th>
<th>Psychotic Experiences (n=787)</th>
<th>No psychotic experiences (n=5997)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% males)</td>
<td>45.49</td>
<td>49.51</td>
</tr>
<tr>
<td>SDQ</td>
<td>8.51 (5.35)</td>
<td>7.17 (4.60)</td>
</tr>
<tr>
<td>Maternal educational qualifications (%)^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>22.35</td>
<td>21.17</td>
</tr>
<tr>
<td>Middle</td>
<td>64.8</td>
<td>61.99</td>
</tr>
<tr>
<td>High</td>
<td>12.85</td>
<td>16.84</td>
</tr>
</tbody>
</table>

**Abbreviations:** SDQ, Strengths and Difficulties Questionnaire, total difficulties score

**Notes:** ^a Based on the UK examination system, refers to the highest maternal educational attainment

Poorer performance in the domains of processing speed (age 8), attention (ages 8 and 11), and working memory (ages 8 and 10) was associated with increased risk of PEs (table 8). There was weak evidence of association with the domain reasoning and problem solving (age 8).

There was weak evidence for a non-linear relationship between performance in the reasoning and problem solving domain (OR: 1.06, 95%CI (1.10 to 1.12), p=0.032) and risk of developing PEs.
Table 8 Psychotic experiences at age 12 in relation to cognitive domains before and after adjustment for confounders.

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Age</th>
<th>n</th>
<th>Non-adjusted OR (95%CI)</th>
<th>Adjusted OR(^a) (95%)</th>
<th>Adjusted OR(^b) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>8</td>
<td>4909</td>
<td>1.27 (1.16 -1.39)</td>
<td>1.30 (1.18 -1.43)</td>
<td>1.24 (1.12 -1.36)</td>
</tr>
<tr>
<td>Attention</td>
<td>8</td>
<td>4714</td>
<td>1.24 (1.09 -1.43)</td>
<td>1.23 (1.07 -1.41)</td>
<td>1.16 (1.00 -1.33)</td>
</tr>
<tr>
<td>Working memory</td>
<td>8</td>
<td>4888</td>
<td>1.16 (1.06 -1.27)</td>
<td>1.13 (1.03 -1.24)</td>
<td>1.07 (0.97 -1.17)</td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>8</td>
<td>4893</td>
<td>1.09 (0.99 -1.19)</td>
<td>1.05 (0.96 -1.16)</td>
<td>1.01 (0.92 -1.11)</td>
</tr>
<tr>
<td>Working memory</td>
<td>10</td>
<td>4742</td>
<td>1.14 (1.04 -1.24)</td>
<td>1.12 (1.03 -1.23)</td>
<td>1.09 (1.00 -1.20)</td>
</tr>
<tr>
<td>Processing speed</td>
<td>11</td>
<td>4976</td>
<td>1.00 (0.89 -1.12)</td>
<td>1.03 (0.91 -1.16)</td>
<td>1.00 (0.89 -1.13)</td>
</tr>
<tr>
<td>Attention</td>
<td>11</td>
<td>4843</td>
<td>1.17 (1.08 -1.28)</td>
<td>1.18 (1.08 -1.29)</td>
<td>1.14 (1.04 -1.25)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; OR, odds ratio

**Notes:** \(^a\) adjusted for gender, maternal education, \(^b\) adjusted for gender, maternal education, and SDQ total difficulties
Table 9 Psychotic experiences at age 12 in relation to cognitive domains before and after adjustment for maternal social class and maternal psychiatric problems.

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Age</th>
<th>n</th>
<th>Non-adjusted OR (95%CI)</th>
<th>Adjusted OR&lt;sup&gt;a&lt;/sup&gt; (95%)</th>
<th>Adjusted OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>8</td>
<td>4754</td>
<td>1.24 (1.13 -1.36)</td>
<td>1.23 (1.12 -1.34)</td>
<td>1.23 (1.13 -1.35)</td>
</tr>
<tr>
<td>Attention</td>
<td>8</td>
<td>4562</td>
<td>1.22 (1.06 -1.41)</td>
<td>1.21 (1.05 -1.40)</td>
<td>1.21 (1.05 -1.40)</td>
</tr>
<tr>
<td>Working memory</td>
<td>8</td>
<td>4729</td>
<td>1.16 (1.06 -1.28)</td>
<td>1.15 (1.05 -1.26)</td>
<td>1.16 (1.05 -1.27)</td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>8</td>
<td>4735</td>
<td>1.07 (0.98 -1.18)</td>
<td>1.06 (0.97 -1.17)</td>
<td>1.07 (0.98 -1.18)</td>
</tr>
<tr>
<td>Working memory</td>
<td>10</td>
<td>4627</td>
<td>1.17 (1.06 -1.28)</td>
<td>1.16 (1.06 -1.27)</td>
<td>1.16 (1.06 -1.28)</td>
</tr>
<tr>
<td>Processing speed</td>
<td>11</td>
<td>4854</td>
<td>1.01 (0.90 -1.14)</td>
<td>1.01 (0.90 -1.14)</td>
<td>1.01 (0.90 -1.14)</td>
</tr>
<tr>
<td>Attention</td>
<td>11</td>
<td>4714</td>
<td>1.19 (1.09 -1.31)</td>
<td>1.19 (1.09 -1.30)</td>
<td>1.19 (1.09 -1.36)</td>
</tr>
</tbody>
</table>

<sup>a</sup> adjusted for maternal social class, <sup>b</sup> adjusted for maternal psychiatric problems

**Abbreviations:** CI, confidence interval; OR, odds ratio

**Notes:** This table shows the odds ratios for psychotic experiences at age 12 in relation to cognitive domains before and after adjustment for maternal social class and maternal psychiatric problems.
Of the potential confounders investigated, parental social class, crowding index, ethnic group, parental psychiatric problems and developmental delay showed no effect on the associations and therefore they were not included in the analyses. An example is provided at table 9 which shows the odds ratios before and after adjustment for maternal social class and maternal psychiatric problems.

Most associations changed only slightly after adjusting for gender and maternal education (table 8). Adjusting for behavioural and emotional difficulties attenuated most associations somewhat, but associations between PEs and processing speed at age 8 and attention at age 11 remained (table 8). There was only weak evidence for associations between PEs and attention at age 8 and working memory at age 10 after these adjustments.

After exploring the separate effects of the SDQ subscales, it seemed that the hyperactivity and peer problems subscales had the greatest effects on attenuating the associations between cognition and psychotic experiences (see table 10 and the example of processing speed).
Table 10 Psychotic experiences at age 12 in relation to processing speed at age 8 before and after adjusting for the total and separate SDQ subscales (n=4978).

<table>
<thead>
<tr>
<th></th>
<th>Odds ratios (95% CIs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.27 (1.17 -1.39)</td>
</tr>
<tr>
<td>Adjusted for total difficulties</td>
<td>1.21 (1.10 -1.32)</td>
</tr>
<tr>
<td>Adjusted for hyperactivity subscale</td>
<td>1.22 (1.11 -1.34)</td>
</tr>
<tr>
<td>Adjusted for prosocial subscale</td>
<td>1.27 (1.16 -1.38)</td>
</tr>
<tr>
<td>Adjusted for emotional symptoms subscale</td>
<td>1.26 (1.16 -1.38)</td>
</tr>
<tr>
<td>Adjusted for conduct problems subscale</td>
<td>1.26 (1.15 -1.38)</td>
</tr>
<tr>
<td>Adjusted for peer problems subscale</td>
<td>1.23 (1.13 -1.34)</td>
</tr>
</tbody>
</table>

I subsequently examined the relationships with individual tests comprising each cognitive domain in order to further identify whether there is an individual test instead of their sum that has the largest effect. The results of the individual tests are described in table 11. Both tests comprising the processing speed domain at age 8 were associated with PEs. Similarly, both tests comprising the working memory domain had similar effects, though the association of the arithmetic with PEs seemed stronger as the evidence for association remained after adjusting for gender and maternal education whereas there was no evidence for association with the backwards digit span. With regards to the reasoning and problem solving domain, the picture completion and picture arrangement tests showed the weakest associations with PEs in relation to the block design and object assembly tests.
Table 11 Psychotic experiences at age 12 in relation to individual tests before and after adjustment for confounders.

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Test</th>
<th>Age</th>
<th>n</th>
<th>Non-adjusted OR (95%CI)</th>
<th>Adjusted OR\textsuperscript{a} (95%)</th>
<th>Adjusted OR\textsuperscript{b} (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>Coding</td>
<td>8</td>
<td>4861</td>
<td>1.31 (1.18 -1.44)</td>
<td>1.33 (1.20 -1.47)</td>
<td>1.27 (1.14 -1.40)</td>
</tr>
<tr>
<td></td>
<td>Sky Search</td>
<td>8</td>
<td>4651</td>
<td>1.20 (1.07 -1.35)</td>
<td>1.21 (1.07 -1.37)</td>
<td>1.16 (1.03 -1.31)</td>
</tr>
<tr>
<td>Working memory</td>
<td>Backwards digit span</td>
<td>8</td>
<td>4767</td>
<td>1.09 (0.99 -1.19)</td>
<td>1.08 (0.97 -1.18)</td>
<td>1.03 (0.94 -1.12)</td>
</tr>
<tr>
<td></td>
<td>Arithmetic</td>
<td>8</td>
<td>4836</td>
<td>1.18 (1.08 -1.28)</td>
<td>1.15 (1.04 -1.25)</td>
<td>1.10 (0.99 -1.21)</td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>Picture completion</td>
<td>8</td>
<td>4842</td>
<td>1.04 (0.95 -1.14)</td>
<td>1.02 (0.93 -1.12)</td>
<td>1.00 (0.91 -1.09)</td>
</tr>
<tr>
<td></td>
<td>Picture arrangement</td>
<td>8</td>
<td>4817</td>
<td>0.98 (0.89 -1.06)</td>
<td>0.96 (0.89 -1.05)</td>
<td>0.95 (0.87 -1.04)</td>
</tr>
<tr>
<td></td>
<td>Block design</td>
<td>8</td>
<td>4825</td>
<td>1.10 (1.01 -1.21)</td>
<td>1.06 (0.97 -1.18)</td>
<td>1.03 (0.94 -1.14)</td>
</tr>
<tr>
<td></td>
<td>Object assembly</td>
<td>8</td>
<td>4601</td>
<td>1.10 (1.01 -1.21)</td>
<td>1.08 (0.98 -1.19)</td>
<td>1.05 (0.96 -1.16)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio

Notes: \textsuperscript{a} adjusted for gender, maternal education, \textsuperscript{b} adjusted for gender, maternal education, and total difficulties
I also examined to what extent performance on these cognitive domains showed effects on PEs independently of each other by adjusting for the other cognitive domains. These results indicated that, taking the other cognitive domains into account only processing speed remained associated with higher risk of psychotic experiences at age 12 (table 12).

Table 12 Psychotic experiences at age 12 in relation to cognitive domains adjusted for the other cognitive domains.

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Age</th>
<th>n</th>
<th>Non-adjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>8</td>
<td>5601</td>
<td>1.22 (1.12 -1.33)</td>
<td>1.20 (1.09 -1.33)</td>
</tr>
<tr>
<td>Attention</td>
<td>8</td>
<td>5601</td>
<td>1.19 (1.05 -1.34)</td>
<td>1.01 (0.86 -1.17)</td>
</tr>
<tr>
<td>Working memory</td>
<td>8</td>
<td>5601</td>
<td>1.12 (1.03 -1.23)</td>
<td>1.08 (0.98 -1.19)</td>
</tr>
<tr>
<td>Reasoning and problem solving</td>
<td>8</td>
<td>5601</td>
<td>1.05 (0.96 -1.14)</td>
<td>0.97 (0.88 -1.06)</td>
</tr>
<tr>
<td>Working memory</td>
<td>10</td>
<td>5024</td>
<td>1.10 (1.01 -1.20)</td>
<td>1.04 (0.95 -1.15)</td>
</tr>
<tr>
<td>Processing speed</td>
<td>11</td>
<td>5017</td>
<td>1.14 (1.05 -1.25)</td>
<td>0.95 (0.84 -1.08)</td>
</tr>
<tr>
<td>Attention</td>
<td>11</td>
<td>5272</td>
<td>1.16 (1.07 -1.26)</td>
<td>1.05 (0.95 -1.16)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; OR, odds ratio

**Notes:** 1Regressions were conducted where associations of PEs with the cognitive domains were non-adjusted and subsequently adjusted for cognitive domains at the same age. In the case of working memory at age 10, processing speed at age 11 and attention at age 11, I adjusted for cognitive domains at ages 8.
Further logistic regressions were conducted as sensitivity analyses where I adjusted all cognitive domains at age 8 separately for processing speed at age 8. This gave similar results to the analysis that adjusted for all the cognitive domains at the same time (i.e., attention [p=0.76]; working memory [p=0.15]; reasoning and problem solving [p=0.80]). Generally, these findings showed that processing speed had the strongest effect of all the cognitive domains examined.

Finally, it is noteworthy, that of the children who scored below the 25th percentile in the domains of processing speed (age 8) and attention (age 11), only 14% and 12%, respectively reported PEs at age 12.

6.4.2 Change over time in cognitive performance and psychotic experiences at age 12

All domains were positively correlated with one another across ages at the level of p<0.001, supporting the subsequent examination of change over time (table 13).

Table 13 Correlations between the same cognitive domains at different ages.

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td></td>
</tr>
<tr>
<td>(age 8)</td>
<td></td>
</tr>
<tr>
<td>(age 11)</td>
<td>0.43</td>
</tr>
<tr>
<td>Attention</td>
<td></td>
</tr>
<tr>
<td>(age 8)</td>
<td></td>
</tr>
<tr>
<td>(age 11)</td>
<td>0.58</td>
</tr>
<tr>
<td>Working memory</td>
<td></td>
</tr>
<tr>
<td>(age 8)</td>
<td></td>
</tr>
<tr>
<td>(age 10)</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Summary scores of change are presented in table 14.

**Table 14 Summary statistics of change in standardized cognitive performance over time.**

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Age</th>
<th>n</th>
<th>mean (SD)</th>
<th>n</th>
<th>mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>8</td>
<td>590</td>
<td>-0.13 (1.0)</td>
<td>4869</td>
<td>0.07 (1.0)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td>0.09 (0.8)</td>
<td>0.09 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Attention</td>
<td>8</td>
<td>549</td>
<td>-0.00 (0.7)</td>
<td>4572</td>
<td>0.07 (0.6)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td>-0.09 (1.0)</td>
<td>0.06 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Working memory</td>
<td>8</td>
<td>561</td>
<td>-0.07 (0.9)</td>
<td>4630</td>
<td>0.05 (1.0)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>-0.06 (1.0)</td>
<td>0.05 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

Logistic regression analyses based on factors identified by the PCA (see Methods section) showed that improvement of processing speed over time was associated with increased odds of PEs (OR=1.29, 95% CI=1.15 to 1.45, p<0.001). As a sensitivity analysis I also calculated change by using only the Sky Search task for processing speed at both time points and results were similar. There was no evidence of association between development of PEs and change in attention (OR=1.01, 95% CI=0.91 to 1.13, p=0.33) or working memory (OR=0.99, 95% CI=0.88 to 1.10, p=0.78) and odds of PEs.
6.4.3 Missing data

Results from the multiply imputed datasets were very similar to the main dataset when both outcomes and confounders or only confounders were imputed, and did not change any of the substantive findings. If anything, sample attrition appears to have led to an underestimation of the associations between the cognitive domains and psychotic experiences. Table 15 shows the example of working memory.

**Table 15 Example of results of imputation analysis**

<table>
<thead>
<tr>
<th>Working memory at age 10</th>
<th>Non-adjusted OR (95%CI)</th>
<th>Adjusted OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimputed data</td>
<td>1.16 (1.06 -1.27)</td>
<td>1.07 (0.97 -1.17)</td>
</tr>
<tr>
<td>Imputed confounders</td>
<td>1.16 (1.07 -1.26)</td>
<td>1.09 (1.00 -1.17)</td>
</tr>
<tr>
<td>Imputed confounders and outcome</td>
<td>1.16 (1.07 -1.27)</td>
<td>1.09 (1.00 -1.17)</td>
</tr>
</tbody>
</table>

6.5 Discussion

Cognitive domains and psychotic experiences

The findings of the current study indicate that lower performance in the domains of processing speed, attention and working memory is associated with higher risk of later development of PEs in a population-based sample of children. The associations were not explained by the background characteristics adjusted for, but for some domains were attenuated substantially when adjusting for behavioural and emotional difficulties.
Associations between PEs and processing speed at age 8 and attention at age 11 remained after these adjustments.

By adjusting for behavioural and emotional difficulties over and above background factors, I may have been overly conservative if these lie on the causal pathway between cognitive ability and PEs. However, it is also possible that such behavioural problems contribute to impaired performance on cognitive tests (e.g., poor concentration), in which case adjustment presents a clearer picture of the relationships I sought to evaluate in this study.

**Impaired processing speed and psychotic experiences**

When adjusting for all other cognitive domains, processing speed was most strongly related to later PEs. This is in line with existing literature that has described processing speed as a fundamental cognitive construct that is close to the core of psychosis (Dickinson, Ramsey et al. 2007). This finding linking processing speed performance with PEs cannot be ascribed to the effects of medication as has been recently suggested (Knowles, David et al. 2010), given that none of the children in this study had ever taken psychotropic medication.

Nevertheless, taking into account that a proportion of the genetic variance for schizophrenia is shared with that of cognition (Toulopoulou, Goldberg et al. 2010, Fowler, Zammit et al. 2012), the association between processing speed and PEs might result from pleiotropic genetic effects and other confounders. Furthermore, reverse causation, whereby PEs present before the age of 8 result in impaired cognitive performance, cannot be excluded as measures of PEs at this young age are not available. It is, however, very difficult to distinguish magical thinking from PEs in younger children. Indeed, the ages of 11 and 12 have been suggested as ideal for the assessment of PEs as children at this age are still unaware of the consequences
(i.e., stigmatization, social rejection) of revealing such experiences and are therefore less inhibited about sharing them (Polanczyk, Moffitt et al. 2010).

If processing speed deficits are causally related to PEs then one possible mechanism for this might be an increase in false prediction errors, i.e., failure to efficiently inform one’s existing beliefs about what the world probably looks like in face of new evidence (Fletcher and Frith 2009). Disturbed brain connectivity has been suggested as a probable mechanism that could lead to false prediction errors (Fletcher and Frith 2009) and has also been associated with both hallucinations (Kubicki, McCarley et al. 2007) and reduced processing speed (DeLuca 2008). Studies in healthy children and adolescent-onset psychotic patients have shown that cognitive slowing might restrict performance in other cognitive processes such as executive functions (Fry and Hale 1996). Therefore, abnormal brain connectivity could affect processing speed which in turn could restrict the optimal performance of other cognitive operations resulting in false prediction errors and in turn positive symptoms.

Attention

Attention also appeared related to PEs independent of confounding, although these effects did not persist when processing speed was adjusted for. Impaired attention has been systematically reported in children at high risk of schizophrenia (Niemi, Suvisaari et al. 2003). Moreover, according to the Israeli High Risk study findings, impaired attention at age 11 predicted development of schizophrenia (Marcus, Hans et al. 1987). Similar results were reported by the New York High Risk study (Erlenmeyer-Kimling and Cornblatt 1992). It should be noted, however, that processing speed was not adjusted for in these studies.

Change in cognition over time
To my knowledge, this is the first study to examine change in performance of cognitive domains over time, in relation to PEs in a population-based sample of children. The finding that improvement in processing speed was associated with later development of psychotic experiences is contrary to my hypothesis and previous findings (Reichenberg, Caspi et al. 2010). One possible explanation is that the results reflect a ‘catch-up’ effect. Processing speed improves rapidly in early childhood, but improves at a much slower rate closer to adolescence (Kail 2008). If this normal development is delayed in children with PEs they might show greater deficits relative to their peers at age 8, but show little difference by age 11, resulting in a relatively greater improvement over time. Unfortunately as there is data at only two time points I was not able to test whether the change in processing speed in children with psychotic experiences is due to such a ‘catch-up’ effect.

Whether or how premorbid decline in attention over time manifests in individuals with schizophrenia has not yet been examined. In contrast to previous findings (Reichenberg, Caspi et al. 2010), I did not find evidence of association between change over time in working memory and PEs. I cannot exclude that this discrepancy may be due to different measures of working memory over time in the present study. Other reasons for not finding evidence of decline can include repeated testing and differential practice effects between the children with and without PEs.

**Theoretical implications**

These findings support a dimensional model of PEs with psychotic disorder at the extreme end. In clinical populations, the cognitive domains that were examined have been found to be associated with schizophrenia and in accordance with the dimensional model I would expect the same associations to exist between cognitive function and psychotic manifestations, if to a
lesser degree. Indeed, the effect sizes of the cognitive domains reported in this study, though smaller, follow the same pattern as those reported in a number of meta-analyses in schizophrenia patients and healthy controls (Heinrichs and Zakzanis 1998, Laws 1999, Fioravanti, Carlone et al. 2005, Henry and Crawford 2005, Dickinson, Ramsey et al. 2007, Knowles, David et al. 2010). For example, at age 8, processing speed in this study had the largest effect size (Hedge’s g=-0.195, 95% CI=-0.111 to -0.279), though this is still a small effect, followed by working memory (g=-0.115, -0.031 to -0.199) and attention (g=-0.114, 95%CI=-0.030 to -0.198).

On the other hand, not all cognitive domains were associated with PEs. The effect size of the reasoning and problem solving domain was not significant (g=-0.041, 0.043 to -0.125), whereas the effect sizes reported in the meta-analyses of schizophrenia range from -0.53 (Laws 1999) to -1.06 (Henry and Crawford 2005) respectively. Even though type II errors cannot be excluded, another explanation could be that the tasks used in the meta-analyses (e.g., the Wisconsin Card Sorting Test (Heaton 1981)) were different than the ones used in this study. Furthermore, although the reasoning and problem solving domain has been identified as a separate impaired cognitive domain in schizophrenia, there is evidence that the impairments of patients with positive symptoms are not of a general reasoning nature (Kemp, Chua et al. 1997) but more specific and such specificity could explain the observed lack of associations.

Furthermore, the fact that the associations between processing speed at age 11 with PEs were weak, could be due to practice effects or because the children had developed cognitively and therefore performed better at the test.

**Strengths and limitations**
This study focuses on PEs and not psychotic disorder. Nevertheless, such experiences are an integral part of a diagnosis for any psychotic disorder; are associated with substantial adverse outcomes in social achievement and functioning in their own right (Rössler, Riecher-Rössler et al. 2007); and PEs in childhood are strongly associated with psychotic disorders in adult life (Poulton, Caspi et al. 2000). The finding that processing speed is the strongest predictor of PEs, irrespective of psychotropic medication, indicates that the mechanisms mediating the association between impaired cognition and schizophrenia also mediate the association between impaired cognition and PEs. Thus, this study provides further insight into understanding of the pathogenesis of psychotic phenomena and the cognitive deficits that may place children at higher risk of developing schizophrenia. The predictive value of the findings is rather limited (i.e., only 14% of the children who scored below the 25th percentile for the processing speed domain developed psychotic experiences), whilst the predictive value of PEs for uncommon disorders such as schizophrenia is also likely to be low (Dominguez, Wichers et al. 2011). Nevertheless, these findings assist in better characterizing and defining the cognitive mechanisms associated with increased psychopathological risk as well as informing potential prevention strategies. For example, several studies have shown that processing speed training can improve everyday functioning for older adults (e.g., Edwards, Wadley et al. 2005). Therefore, cognitive remediation for processing speed could also be helpful for children.

One of the limitations of this study is that I was not able to test all the MATRICS domains, because not all had been assessed in the study sample. Therefore, results may be confounded by another underlying cognitive domain that was not examined. Moreover, a common criticism of neuropsychological tests is that they do not measure cognitive processes in isolation (Palmer, Dawes et al. 2009) and these tests may have less than perfectly captured
the specific cognitive domains. For example, the arithmetic test (which I classified as part of the working memory domain) might overlap to some extent with the reasoning and problem solving domain. Differential loss to follow-up could introduce attrition bias, though the imputation analyses indicated that missingness is unlikely to have biased the observed relationships.

**Conclusions**

I examined the longitudinal relationships between cognitive domains that have been previously associated with schizophrenia (Nuechterlein, Barch et al. 2004) and psychotic experiences in children in a large birth cohort. The findings suggest that processing speed and attention are related to greater risk of psychotic experiences in children, with processing speed representing the key cognitive feature. Nevertheless, this study does not indicate whether it is impaired processing speed that causes later psychotic experiences or whether a third factor causes both. One way that this could be elucidated is by examining whether specific genetic variants that have been associated with schizophrenia are also associated with psychotic experiences and also whether impaired cognitive performance mediates these associations. I will examine this in Chapter 7.
Chapter 7 Exploring the indirect effects of Catechol-O-Methyltransferase (COMT) on psychotic experiences through cognitive function and anxiety disorders in children.

7.1 Chapter Overview

In Chapter 6, I showed that defective processing speed and attention were more strongly associated with PEs, than a range of other cognitive functions that were selected \textit{a priori} based on their associations with schizophrenia. If this association is causal then one possible mechanism would be an increase in false prediction errors. Disturbed brain connectivity could potentially lead to false prediction errors through its effect on processing speed and attention. To expand on this hypothesis, and given that dopamine (DA) has been shown to alter brain connectivity (e.g., (Honey, Suckling et al. 2003)), I examined in this Chapter whether genetic variation in \textit{COMT}, an enzyme that degrades DA and has been considered a plausible candidate for schizophrenia and cognitive deficits, is associated with PEs indirectly, through its effect on processing speed, attention. Taking into account the literature indicating that \textit{COMT} is also associated with anxiety disorders, with the Met allele being associated with both better cognition and higher risk of anxiety disorders, and anxiety disorders also being associated with PEs, I also examined whether there is an indirect effect of \textit{COMT} to PEs through anxiety disorders.

This is the first study to examine indirect effects of \textit{COMT} on PEs. The findings showed that \textit{COMT} was indirectly associated with PEs through processing speed and attention but there was no evidence for a total or indirect effect of \textit{COMT} on PEs through anxiety disorders.
Evidence of an indirect association suggests a complex developmental pathway underlies the emergence of PEs in children with possible implications for prevention and intervention strategies. These findings also provide additional support for processing speed and attention as endophenotypes in psychotic disorders.

### 7.2 Introduction

Children reporting psychotic experiences (PEs) are at increased risk of developing schizophrenia, and although this risk is relatively small, understanding the mechanisms by which PEs arise might provide important insights into the developmental mechanisms taking place in the early stages of schizophrenia. Indeed, the findings of Chapter 6 showed that processing speed and attention were strongly related to PEs in children within the general population. It was also shown that the pattern of associations between cognition and PEs was similar to the one between cognition and schizophrenia. As stated in Chapter 6, if these associations are causal, one possible explanation would be disturbed brain connectivity leading to impaired processing speed and attention which would lead to an increase in false prediction errors and consequently positive symptoms (Fletcher and Frith 2009).

One neurotransmitter that has long been associated with psychosis and schizophrenia is Dopamine (DA) (for a review see: (Howes and Kapur 2009)). DA has been shown to alter brain connectivity within the corticostriatal thalamic loops in humans (Williams, Tijssen et al. 2002, Honey, Suckling et al. 2003) by modulating the strength of the connections within these loops (Bamford, Zhang et al. 2004). DA imbalance has long been associated with schizophrenia (Howes and Kapur 2009) and there is also consistent evidence indicating the importance of prefrontal dopamine function for modulating cognitive processes such as attention, inhibition, working memory and planning (Goldman-Rakic, Lidow et al. 1990,
Daniel, Weinberger et al. 1991, McCarthy, Blamire et al. 1994, Sokolowski and Salamone 1994, Granon, Passetti et al. 2000, Kirrane, Mitropoulou et al. 2000). To be more specific, there is evidence from a wide range of studies (e.g., neuropsychological studies in humans and animals as well as neuroimaging work) to suggest that the relationship between DA levels and cognitive performance follows an inverted ‘U’ shape with too low or too high levels of DA affecting optimal prefrontal function (e.g., (Williams and Goldman-Rakic 1995, Kimberg, D'Esposito et al. 1997, Frank, Callicott et al. 1999, Mattay, Callicott et al. 2000, Mehta, Owen et al. 2000, Mattay, Goldberg et al. 2003, Meyer-Lindenberg, Kohn et al. 2005, Tunbridge, Harrison et al. 2006, Vijayraghavan, Wang et al. 2007)).

Even though there are other potential candidate genes acting on DA subsystems, like Monoamine Oxidase (MOA) and the Dopamine Transporter (DAT) that regulate DA catabolism in the prefrontal cortex and the striatum respectively (Savitz, Solms et al. 2006), one of the most well characterized and studied genes is COMT.

### 7.2.1 COMT revisited

COMT is one of the enzymes that degrades catecholamines such as dopamine, epinephrine and norepinephrine and is mainly expressed in the prefrontal cortex (Williams, Owen et al. 2007) (also see Chapter 4.2). Although, in the striatum, the synaptic action of DA is terminated mainly by DAT reuptake into pre-synaptic terminals (Giros, Jaber et al. 1996, Gainetdinov, Jones et al. 1998), DAT is expressed in low density in prefrontal cortex. Thus, the COMT enzyme accounts for >60% of the DA degradation in the prefrontal cortex, but <15% of dopamine degradation in the striatum (Karoum, Chrapusta et al. 1994).
COMT contains a functional polymorphism (Val108/158Met, rs4680) that impacts on the enzyme’s activity (Lachman, Papolos et al. 1996). The low-activity Met allele is less active in reducing COMT enzyme activity compared to the high-activity Val allele. Hence, carriers of the Met allele catabolize DA at a slower rate than carriers of the Val allele and have higher levels of DA in the prefrontal cortex (Lotta, Vidgren et al. 1995, Egan, Goldberg et al. 2001).

As stated in Chapter 3.2, although COMT has been widely studied as a positional candidate gene for schizophrenia, results have failed to show consistent evidence of association. If there is an effect, which is likely to be small (Munafo, Bowes et al. 2005), the reasons that studies have not succeeded to detect it could be several, including low statistical power and publication bias (i.e., the tendency to publish positive findings) (Barnett, Jones et al. 2007).

An alternative explanation might be that studies have overlooked the effects of other COMT functional single nucleotide polymorphisms (SNPs) or haplotypes that might explain the variability in COMT enzymatic activity and protein levels (Scheggia, Sannino et al. 2012). One of the most comprehensive accounts of COMT function was reported by Nackley and colleagues (Nackley, Shabalina et al. 2006). They described a haplotype consisting of rs4680, rs6269 (in the P1 promoter), rs4633 (in exon 3) and rs4818 (in exon 4). COMT expression differed mostly between the haplotypes that were different in the two synonymous SNPs (rs4633 and rs4818) resulting in differences in the stability of mRNA secondary structure. It is noteworthy that the Val allele of rs4680 was included in both high and low activity haplotypes.

A different haplotype, comprised of rs4680, and two SNPs, one in intron 1 (rs737865) and the other in the 3’ untranslated region (rs165599) was associated with schizophrenia in a sample of Ashkenazi population (Shifman, Bronstein et al. 2002). Moreover, mRNA expression studies showed that this haplotype differentially affected the expression of COMT.
rs4680 in human brain tissue (Bray, Buckland et al. 2003). Also, they showed that the strongest haplotype association included a SNP at intron 1. Even though rs737865 is located at the 5’ end of intron 1 it is more likely that another SNP, the rs2097603, which is in the P2 promoter, located upstream rs737865 at intron 1 is more relevant, since the P2 promoter drives transcription of the predominant form of COMT in the brain (MB-COMT), while rs737865 is not functional. The relevance of rs2097603 to schizophrenia was further supported by a large population genetic study (Palmatier, Pakstis et al. 2004). This SNP has also been found to affect COMT activity in lymphocytes and post-mortem brain tissue (Chen, Lipska et al. 2004). Studies have shown stronger effects of a haplotype including rs4680, rs165599 and rs2097603 than those observed for single SNPs on working memory and prefrontal activation in healthy controls (Meyer-Lindenberg, Nichols et al. 2006) and on working memory in patients with schizophrenia (Diaz-Asper, Goldberg et al. 2008).

Similarly with regards to PEs, although studies have repeatedly shown that increased striatal dopamine levels are strongly related to the positive symptoms of the disorder (Laruelle and Abi-Dargham 1999, Abi-Dargham, Rodenhiser et al. 2000, Howes Od and et al. 2009, Woodward, Cowan et al. 2011, Sorg, Manoliu et al. 2013), a study in ALSPAC using children’s self-reports of PEs (at age 16) did not show evidence for an association with COMT (Zammit, Owen et al. 2011). It is noteworthy, that there was no evidence for associations between a number of other COMT SNPs (rs4680, rs737865, rs2097603, rs6269, rs481, rs16559), the Nackley haplotypes and PEs. A more recent study in the same cohort however, that used semi-structured interview of PEs (at ages 12 and 18) found evidence for associations between rs2097603, but this association did not survive correction for multiple testing (Zammit, Hamshere et al. 2013).

One way that COMT could be related to schizophrenia and PEs is through its role in catabolizing dopamine in the prefrontal cortex (Williams, Owen et al. 2007). There is a large
body of evidence that the cognitive dysfunction and the negative symptoms seen in schizophrenia are mostly related to prefrontal DA dysfunction (Abi-Dargham, Mawlawi et al. 2002, Goldman-Rakic, Castner et al. 2004). Even though the nature of this dysfunction remains unclear, post-mortem studies have shown decreased tyrosine hydroxylase immunolabeling in the entorhinal cortex and the prefrontal cortex suggesting that schizophrenia might be associated with decreased innervations in these regions (Akil, Pierri et al. 1999, Akil, Edgar et al. 2000).

Nonetheless, the exact effect of COMT on sub-cortical DA neurotransmission is not clear since it is not known whether these decreased innervations in the PFC are the cause or the result of the DA imbalance in schizophrenia. It has been hypothesized that decreased activity in the PFC can lead to increased levels of DA in the striatum by inhibiting sub-cortical DA neurotransmission but there is also evidence to the contrary, that increased DA levels in the striatum can lead to decreased PFC activity (for a review see: (Kuepper, Skinbjerg et al. 2012)).

Several studies have examined the effects of rs4680 on cognitive performance and they have showed that homozygous carriers of the low activity Met allele had better attention (Bellgrove, Domschke et al. 2005, Galderisi, Maj et al. 2005), verbal learning (Minzenberg, Xu et al. 2006), episodic and semantic memory (De Frias, Annerbrink et al. 2004), working memory (Bertolino, Blasi et al. 2006, Han, Kee et al. 2006, Minzenberg, Xu et al. 2006, Caldú, Vendrell et al. 2007, Diaz-Asper, Goldberg et al. 2008) and executive function (Egan, Goldberg et al. 2001, Malhotra, Kestler et al. 2002, Rosa, Peralta et al. 2004, Galderisi, Maj et al. 2005, Caldú, Vendrell et al. 2007) in relation to homozygous carriers of the Val allele, while the cognitive performance of the heterozygotes was at intermediate levels. Not all studies, however, have replicated these associations (Joober, Gauthier et al. 2002, Tsai, Yu et al. 2003, Rosa, Peralta et al. 2004, Stefanis, Van Os et al. 2004, Barnett, Jones et al. 2007,
Wang, Li et al. 2013, Wardle, de Wit et al. 2013). The most recent meta-analysis showed a small effect of rs4680 on IQ, but there was no evidence for associations with attention, verbal fluency, working memory and executive function (Barnett, Scoriels et al. 2008). Reduced statistical power, publication bias and examination of single SNPs rather than haplotypes could explain these weak and inconsistent findings.

It should be noted, however, that cognition is a complex psychological trait that is likely to be affected by many genes. Therefore, the magnitude of the individual genetic effects on cognition is likely to be small (Savitz, Solms et al. 2006).

The relation between COMT and processing speed is relatively under-examined in both adults and children. This is mainly because studies have focused on examining cognitive measures mostly supported by the PFC (for a review see: (Dickinson and Elvevåg 2009)). Also, the first studies that were conducted employing tasks measuring IQ and attention did not show evidence for associations with COMT (Egan, Goldberg et al. 2001, Goldberg, Egan et al. 2003). However, Bilder and colleagues did examine possible associations between rs4680 and cognitive domains measuring processing speed and attention, executive functions, verbal learning and memory, motor skills and general cognitive ability in 58 patients with chronic schizophrenia (Bilder, Volavka et al. 2002). They found that the low activity allele was associated with better performance in the domains of processing speed and attention. Nevertheless, they did not examine whether other SNPs or haplotypes within COMT were associated with processing speed and attention. In children, there is one study that examined processing speed using the Sky Search task (for more information on this task see Chapter 6, table 3), which found a gender specific effect of rs4680, i.e., boys with the low activity allele performed better, whereas this was not found for girls. This study, however did not examine the Coding subtest of the WISC-III (which is the equivalent of the Wechsler Digit Symbol
subtest – patients with schizophrenia show the greatest impairments in this test), nor did the authors examine possible effects of other COMT SNPs and haplotypes (Barnett, Heron et al. 2007). With regards to attention, one study has reported that the low activity allele of rs2097603 and the low activity Nackley haplotype were associated with better attention in children, but rs4680 was not (Barnett, Heron et al. 2009). Finally, rs4680 has been associated with verbal IQ but no evidence for associations was reported for rs2097603, rs6269, rs4818, rs165599 and the Nackley haplotypes and verbal or performance IQ (Barnett, Heron et al. 2007, Barnett, Heron et al. 2009).

In summary, existing evidence seems to support a relation between high levels of COMT and better performance in the domain of processing speed but there is less evidence for a relation with attention and IQ. Given the evidence associating cognitive deficits with PEs, it could be hypothesized that COMT would be associated indirectly to PEs through processing speed, attention and IQ with the high activity alleles/haplotypes being associated with higher risk of PEs through worse cognitive performance.

In addition to association with cognitive performance, the low activity allele of rs4680 has also been studied in relation to anxiety (Woo, Yoon et al. 2002, Pooley, Fineberg et al. 2007), where high levels of anxiety have been related to the high activity (Val) allele. However, in this case too, there are studies that have not replicated these results (Ohara, Nagai et al. 1998). There has also been a study reporting the opposite effect, i.e., associations between anxiety and low activity allele (McGrath, Kawachi et al. 2004).

This balance between advantages in cognitive function (Met allele) and emotional resiliency (Val allele) has been proposed as the basis of the warrior – worrier model. According to this model, balancing advantage results in a stable frequency of both alleles in the population (Goldman, Oroszi et al. 2005). To be more specific, even low levels of stress can increase
dopamine levels in the prefrontal cortex (Arnsten 2009). Therefore, carriers of the high activity Val allele clear out the released dopamine quickly from the prefrontal cortex, before the harmful consequences of high levels of dopamine due to stress are seen. On the other hand, carriers of the Met allele have high levels of dopamine even when they are not stressed. Thus, they are less protected against the excessive levels of dopamine in the PFC than the carriers of the Val allele (Diamond 2007).

To my knowledge, studies to date have not examined genetic variation in COMT and anxiety disorders in children in the general population (figure 4, path a2). With regards to the anxiety disorders and PEs (figure 4, path b2), associations have been reported in children in two large birth cohorts (Polanczyk, Moffitt et al. 2010, Fisher, Schreier et al. 2012). It should also be mentioned that anxiety disorders are also associated with schizophrenia (Ayalew, Le-Niculescu et al. 2012). A recent study found significant genetic overlap between schizophrenia and anxiety disorders and suggested the introduction of the nosological domain of schizoanxiety disorders (Ayalew, Le-Niculescu et al. 2012).

Therefore, it could be that genetic variation in COMT has an indirect effect on PEs through anxiety disorders.

In addition, for these hypotheses to hold, anxiety disorders would be, if at all, associated with better processing speed, attention and IQ. According to the literature, anxiety disorders are not related to neuropsychological deficits in children (Mayes and Calhoun 2007). To the contrary, a study including 980 children with behavioural problems found that children with anxiety disorders had high scores in the Processing Speed Index of the WISC-III (Calhoun and Mayes 2005). There is also evidence indicating that when anxiety disorders are comorbid with ADHD, children have better attention and executive function in relation to children who only have ADHD (Vloet, Konrad et al. 2010).
To sum up, *COMT* could be associated with PEs indirectly, through its effects on cognition and anxiety disorders that are also associated with the PEs. Figure 4 shows a representation of these hypotheses.

**Figure 4 Representation of the possible indirect effects of *COMT* on psychotic experiences**
Research on mediation analyses has shown (figure 4) that the total effect of X on Y (c) can be quantified as the sum of the direct effect of X on Y (c’) plus the sum of the products of the indirect effects of M and W (a1b1 + a2b2). When indirect effects operate in opposite directions, the main effect could be zero as these indirect effects could cancel each other out (Hayes 2009, Xinshu Zhao, Jr et al. 2010). Similarly, the total effect of COMT Val158Met on PEs (c) can be quantified as the sum of the direct effect of COMT Val158Met (c’) and the products of the indirect effect of Val158Met on PEs through cognition (a1b1) and the indirect effect of Val158Met on PEs through anxiety (a2b2). The formula for the calculation of the total effect is the following:

\[ c = c' + a1b1 + a2b2. \]

Since both alleles can be associated with PEs in two different indirect ways, it is possible that the total effect of COMT on PEs would be close to zero. This could explain previous findings where no evidence for an association between COMT and PEs was found (Zammit, Owen et al. 2011, Zammit, Hamshere et al. 2013).

Studies thus far have not examined possible indirect effects of COMT on PEs in children. Addressing this gap in the literature is important as it could improve our understanding on the possible pathways to PEs with potential implications for treatment and prevention in children at high risk of psychotic disorders.

### 7.2.2 Description of aims

The overall aim of this project is to explore whether COMT affects PEs through processing speed, attention, IQ and anxiety disorders. IQ was included in my analysis, as a more general
construct of cognition which has also been associated with PEs (Horwood, Salvi et al. 2008). Therefore, this project has the following aims:

1. To examine whether variation within COMT, as indexed by a number of Single Nucleotide Polymorphisms (SNPs) and haplotypes of interest, is associated with PEs, processing speed, attention, IQ and anxiety disorders in children from the general population. I hypothesized that the low activity alleles/haplotypes would be associated with better cognitive performance but also higher risk of anxiety disorders;

2. To explore whether the association between COMT and PEs is indirect (i.e., mediated) by cognition and anxiety disorders.

7.3 Method

The methodology of this chapter is identical to the one in Chapter 6. Please see Chapter 6.3 on epidemiological methodology and the Avon Longitudinal Study of Parents and Children (ALSPAC).

7.3.1 Current study sample size

As stated in Chapter 6.3, the initial Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort (http://www.bristol.ac.uk/alspac/) consisted of 14,062 live births and 13,988 infants still alive at 12 months (Boyd, Golding et al. 2012, Fraser, Macdonald-Wallis et al. 2012). 6,784 individuals completed the Psychosis-Like Symptoms interview (PLIKSi) at age 12. The number of individuals with available information from the PLIKSi, the
cognitive tests and the genotype data varies, as these were completed by different numbers of individuals.

### 7.3.2 Ethical approval

Ethical approval was obtained from the ALSPAC’s Law and Ethics Committee and the Local Research Ethics Committees. Parents who enrolled their children into ALSPAC provided written informed consent at the time of the enrolment and they or their child are free to withdraw at any time.

### 7.3.3 Outcome: Psychotic experiences

The semi-structured Psychosis-like Symptoms Interview (PLIKSi) was conducted at age 12 (Horwood, Salvi et al. 2008). Information on the PLIKSi interview is described in Chapter 6.4.5.

The primary outcome measure was suspected or definite PEs (1) versus no PEs (0) to be consistent with the study in Chapter 6.

### 7.3.4 Exposure: COMT

The following SNPs were examined individually: rs4680 (Val158Met), rs165599, rs2097603, rs4818, rs6269 and rs737865. These were selected because they have been shown in previous studies to be associated with schizophrenia (Allen, Bagade et al. 2008). Among these only rs4680 and rs2097603 affect COMT activity, while rs4818 and rs6269 form part of the Nackley et al. haplotype (Nackley, Shabalina et al. 2006). Although there is no evidence that
it affects COMT function (Chen, Lipska et al. 2004), rs737865 has been shown to be associated with schizophrenia risk in a large meta-analysis of genetic-association studies (Allen, Bagade et al. 2008).

I also examined the following haplotypes:

a. the three – SNP marker haplotype (rs6269-rs4818-rs4680) that defines the Nackley et al. haplotype (Nackley, Shabalina et al. 2006) which has been shown to alter mRNA’s secondary structure. Rs4633 which was originally included in this haplotype was omitted because it was in perfect linkage disequilibrium (LD) with rs4680, with D’ and r² of 1.

b. the three-SNP marker haplotype (rs2097603-rs4680-rs1655999) that was examined in (Meyer-Lindenberg, Nichols et al. 2006). I first examined the two-SNP marker haplotype (rs2097603-rs4680) and then added rs165599 following the procedure reported by the authors. I will refer to this haplotype as the Meyer-Lindenberg haplotype as these were the first to formally test this. This haplotype was shown to be associated with prefrontal working memory response in healthy controls (Meyer-Lindenberg, Nichols et al. 2006).

Genotyping was performed by KBioscience (http://www.kbioscience.co.uk); SNPs were genotyped using the KASP SNP genotyping system. KASP is a competitive allele-specific polymerase chain reaction incorporating a fluorescent resonance energy transfer quencher cassette (for more information see: http://www.kbioscience.co.uk/reagents/KASP.html).

Genotyping was successful for over 97% of the sample across the six SNPs, with error rates of approximately 0.5% (Zammit, Owen et al. 2011).

7.3.5 Mediators: Cognition and anxiety disorders
7.3.5.1 Cognition

I used the cognitive variables that were found to be most strongly associated with PEs in Chapter 6. These were:

Processing Speed (age 8)

Processing speed at age 8 was assessed using measures from two different tasks:

(i) the Sky Search task, which was taken from the Tests of Everyday Attention for Children (TEACh) (Robertson 1996).


Attention/vigilance (11)

The ‘Opposite Worlds’ task from TEACh (Robertson 1996) was used to assess this domain.

I also included an estimate of IQ at age 8 as measured by the WISC-III (10 subtests).

For more information on the cognitive tests see table 3.

7.3.5.2 Anxiety disorders

The Development and Well Being Assessment (DAWBA), a semi-structured interview which provides DSM-IV diagnoses (Goodman, Ford et al. 2000), was completed by the parents when the children were 10 years old. The main interview is fully structured and includes sections that assess a range of psychiatric disorders. The questions resemble closely the diagnostic criteria set forth in the DSM-IV and ICD-10 manuals. There are 20 to 25 questions which are only administered when children report having problems in initial screening questions. Due to low prevalence of anxiety disorders in children, specific subtypes of
anxiety disorders were examined using a composite measure of the presence of one or more of the following disorders: Generalized Anxiety Disorder, Obsessive Compulsive Disorder, Separation Anxiety, Social Phobia and Specific Phobia. I also used the DAWBA bands instead of the binary diagnostic predictions as these are suggested as more appropriate when a dose-response relationship between an outcome and a risk factor is under examination (Goodman, Heiervang et al. 2011). These bands were generated using a computer algorithm and are included in an ordered categorical measure that consists of up to 6 levels that are the following:

Level 0; <0.1% of children in this band have any anxiety disorder

Level 1; ~0.5% of children in this band have any anxiety disorder

Level 2; ~3% of children in this band have any anxiety disorder

Level 3; ~15% of children in this band have any anxiety disorder

Level 4; ~50% of children in this band have any anxiety disorder

Level 5; >70% of children in this band have any anxiety disorder

7.3.6 Data analyses

Overall, the SNPs did not deviate from Hardy-Weinberg equilibrium which was calculated with the $\chi^2$ test using the software PLINK (version 1.07) for Windows (Purcell, Neale et al. 2007, Purcell 2013) (table 16). Rs737865 did deviate from Hardy-Weinberg equilibrium in children without PEs (p=0.02), but not in those with PEs or in the overall sample.
Table 16 Hardy-Weinberg Equilibrium results

<table>
<thead>
<tr>
<th>SNP</th>
<th>A1</th>
<th>A2</th>
<th>Group</th>
<th>Genotype counts</th>
<th>Observed (Het)</th>
<th>Expected (Het)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>G</td>
<td>A</td>
<td>All</td>
<td>1103/2283/1268</td>
<td>0.4905</td>
<td>0.4994</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PEs</td>
<td>136/302/178</td>
<td>0.4903</td>
<td>0.4977</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No PEs</td>
<td>967/1981/1090</td>
<td>0.4906</td>
<td>0.4995</td>
<td>0.26</td>
</tr>
<tr>
<td>rs737865</td>
<td>C</td>
<td>T</td>
<td>All</td>
<td>409/1864/2390</td>
<td>0.3997</td>
<td>0.4098</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PEs</td>
<td>35/258/322</td>
<td>0.4195</td>
<td>0.3911</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No PEs</td>
<td>374/1606/2068</td>
<td>0.3967</td>
<td>0.4124</td>
<td>0.02</td>
</tr>
<tr>
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<td>C</td>
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<td>817/2241/1730</td>
<td>0.468</td>
<td>0.4818</td>
<td>0.05</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>PEs</td>
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<td>0.4749</td>
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</tr>
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<td></td>
<td></td>
<td>No PEs</td>
<td>717/1953/1489</td>
<td>0.4696</td>
<td>0.4828</td>
<td>0.08</td>
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<tr>
<td>rs6269</td>
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<td>0.4816</td>
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<td></td>
<td></td>
<td></td>
<td>PEs</td>
<td>98/285/242</td>
<td>0.456</td>
<td>0.4735</td>
<td>0.35</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>No PEs</td>
<td>703/1919/1461</td>
<td>0.47</td>
<td>0.4828</td>
<td>0.09</td>
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<td>A</td>
<td>All</td>
<td>441/2000/2338</td>
<td>0.4185</td>
<td>0.4212</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>0.15</td>
</tr>
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<td></td>
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<td>0.4219</td>
<td>0.97</td>
</tr>
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<td>A</td>
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<td>1</td>
</tr>
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<td></td>
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<td>0.4968</td>
<td>0.34</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No PEs</td>
<td>752/2000/1361</td>
<td>0.4863</td>
<td>0.489</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Abbreviations:** SNP=Single-Number Variant, A1= Minor Allele, A2=Major Allele, Het=Heterozygosity

I only included data from white participants of European origin as the frequencies of this allele have been shown to be different in different ethnicities. As shown in table 17, rs4680
was in strong LD with rs4818 ($r^2=0.71$, $D'=0.99$) and rs6269 ($r^2=0.71$, $D'=0.99$). Similarly, rs4818 was in strong LD with rs6269 ($r^2=0.97$, $D'=0.99$). Therefore, when examining the effects of individual SNPs, I excluded rs4818 and rs6269 from the analysis.

Table 17 Linkage Disequilibrium results

<table>
<thead>
<tr>
<th>SNP A</th>
<th>SNP B</th>
<th>$r^2$</th>
<th>$D'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>rs165599</td>
<td>0.18</td>
<td>0.63</td>
</tr>
<tr>
<td>rs2097603</td>
<td></td>
<td>0.175</td>
<td>0.497</td>
</tr>
<tr>
<td>rs4818</td>
<td></td>
<td>0.71</td>
<td>0.986</td>
</tr>
<tr>
<td>rs6269</td>
<td></td>
<td>0.71</td>
<td>0.986</td>
</tr>
<tr>
<td>rs737865</td>
<td></td>
<td>0.238</td>
<td>0.741</td>
</tr>
<tr>
<td>rs165599</td>
<td>rs2097603</td>
<td>0.045</td>
<td>0.369</td>
</tr>
<tr>
<td></td>
<td>rs4818</td>
<td>0.043</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>rs6269</td>
<td>0.046</td>
<td>0.269</td>
</tr>
<tr>
<td></td>
<td>rs737865</td>
<td>0.058</td>
<td>0.249</td>
</tr>
<tr>
<td>rs4818</td>
<td>rs6269</td>
<td>0.974</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>rs737865</td>
<td>0.331</td>
<td>0.746</td>
</tr>
<tr>
<td>rs6269</td>
<td>rs737865</td>
<td>0.335</td>
<td>0.752</td>
</tr>
</tbody>
</table>

I used UNPHASED version 3.1.6 for Windows (Dudbridge 2008) to derive the haplotypes. I coded the individual SNPs and haplotypes based on the number of copies of the high-activity allele/haplotype as 0, 1 and 2, thus using the same approach with previous studies (Zammit, Owen et al. 2011). 64% of the sample had a probability greater than 0.97 of having the high activity Nackley haplotype (i.e., G-G-G) while 65% had a probability greater than 0.86 of having the high activity two-marker Meyer-Lindenberg haplotype (i.e., A-G) and 42% of the
sample had a probability greater than 0.67 of having the high activity three-marker Meyer-Lindenberg haplotype (i.e., A-G-G).

As in the previous analysis (Chapter 6), all cognitive measures were standardized to have a mean of zero and a standard deviation of one. Individuals scoring more than 3 standard deviations (SDs) from the mean were excluded from further analysis. Where domains were comprised of more than one test (i.e., processing speed), a summary statistic was calculated by averaging and standardizing the z-scores from the tests that comprised each domain.

Regression analyses were conducted in STATA/IC (version 11) for Windows to estimate regression coefficients and 95% confidence intervals for associations between COMT (i.e., 4 individual SNPs (rs165599, rs2097603, rs4680, rs737865), the Nackley haplotype, the 2-marker Meyer-Lindenberg haplotype and the 3-marker Meyer-Lindenberg haplotype) and PEs (aim 1), COMT and cognition (aim 1), COMT and anxiety disorders (aim 1). I also examined whether anxiety disorders were associated with PEs using logistic regression (aim 1). Associations between cognition and PEs have been examined previously (Chapter 6 and for IQ (Horwood, Salvi et al. 2008)).

Mediation analysis (aim 2) was conducted using the binary_mediation command, where there was evidence of association between COMT and cognition and/or anxiety disorders (path ‘a’ in the mediation model). This command uses the product of coefficients approach, which is considered to have good power and a low type I error in relation to other approaches such as the difference of coefficients approach or the causal steps approach (MacKinnon, Lockwood et al. 2002). Standard errors and biased-corrected confidence intervals were also calculated using bootstrapping with 5000 replications. As this command uses a binary outcome for the exposure, individuals who had no copy of the high-activity allele/haplotype were coded with zero (0) and those with at least one copy of the high-activity allele/haplotype with one (1).
7.3.7 Missing data

Children that did not complete the PLIKSi interview at age 12 did not differ by $COMT$ genotypes (p values: 0.141 to 0.883) with the exception of SNP rs165599 in which children that completed the interview were more likely to have the high activity allele (p=0.02). Imputation of genetic data is not possible when participants have not been genotyped and therefore in this study attrition bias could not be addressed.

7.4 Results

Table 18 shows the distribution of PEs in children in relation to $COMT$. 
Table 18 Number of children with PEs in relation to *COMT*

<table>
<thead>
<tr>
<th><em>COMT</em></th>
<th>No PEs</th>
<th>PEs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>rs4680</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (low activity)</td>
<td>1090</td>
<td>178(14)</td>
</tr>
<tr>
<td>AG (intermediate activity)</td>
<td>1981</td>
<td>302(13)</td>
</tr>
<tr>
<td>GG (high activity)</td>
<td>967</td>
<td>136(12)</td>
</tr>
<tr>
<td><strong>rs2097603</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (low activity)</td>
<td>752</td>
<td>127(15)</td>
</tr>
<tr>
<td>AG (intermediate activity)</td>
<td>2000</td>
<td>325(14)</td>
</tr>
<tr>
<td>GG (high activity)</td>
<td>1361</td>
<td>177(12)</td>
</tr>
<tr>
<td><strong>rs165599</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (low activity)</td>
<td>2017</td>
<td>321(14)</td>
</tr>
<tr>
<td>AG (intermediate activity)</td>
<td>1751</td>
<td>249(13)</td>
</tr>
<tr>
<td>GG (high activity)</td>
<td>378</td>
<td>63(14)</td>
</tr>
<tr>
<td><strong>rs737865</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (low activity)</td>
<td>2068</td>
<td>322(9)</td>
</tr>
<tr>
<td>CT (intermediate activity)</td>
<td>1606</td>
<td>258(14)</td>
</tr>
<tr>
<td>CC (high activity)</td>
<td>374</td>
<td>35(14)</td>
</tr>
<tr>
<td><strong>Nackley haplotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No high activity haplotype</td>
<td>1349</td>
<td>219(14)</td>
</tr>
<tr>
<td>At least 1 high activity haplotype</td>
<td>1758</td>
<td>261(13)</td>
</tr>
<tr>
<td>2 high activity haplotypes</td>
<td>638</td>
<td>85(12)</td>
</tr>
<tr>
<td><strong>2 marker M-L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No high activity haplotype</td>
<td>1304</td>
<td>217(14)</td>
</tr>
<tr>
<td>At least 1 high activity haplotype</td>
<td>1911</td>
<td>285(13)</td>
</tr>
<tr>
<td>2 high activity haplotypes</td>
<td>564</td>
<td>74(12)</td>
</tr>
<tr>
<td><strong>3 marker M-L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No high activity haplotype</td>
<td>2181</td>
<td>351(14)</td>
</tr>
<tr>
<td>At least 1 high activity haplotype</td>
<td>1408</td>
<td>197(12)</td>
</tr>
<tr>
<td>2 high activity haplotypes</td>
<td>169</td>
<td>26(13)</td>
</tr>
</tbody>
</table>

*Abbreviations:* M-L: Meyer Lindenberg

### 7.4.1 COMT and psychotic experiences

Apart from the low activity of rs2097603 that was found to be associated with higher risk of PEs, there was no evidence for associations between *COMT* and PEs (table 19).
### Table 19 COMT and PEs

<table>
<thead>
<tr>
<th>PEs (suspected/definite vs. none)</th>
<th>Non-adjusted OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual SNPs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs165599</td>
<td>4779</td>
<td>0.97(0.85-1.11)</td>
</tr>
<tr>
<td>rs2097603</td>
<td>4742</td>
<td><strong>0.87(0.77-0.98)</strong></td>
</tr>
<tr>
<td>rs4680</td>
<td>4654</td>
<td>0.93(0.82-1.05)</td>
</tr>
<tr>
<td>rs737865</td>
<td>4663</td>
<td>0.89(0.78-1.02)</td>
</tr>
<tr>
<td><strong>Haplotypes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nackley</td>
<td>4310</td>
<td>0.91(0.80-1.03)</td>
</tr>
<tr>
<td>2-marker M-L</td>
<td>4355</td>
<td>0.89(0.78-1.02)</td>
</tr>
<tr>
<td>3-marker M-L</td>
<td>4332</td>
<td>0.91(0.78-1.06)</td>
</tr>
</tbody>
</table>

**Abbreviations:** M-L=Meyer-Lindenberg  
**Notes:** The individual SNPs and haplotypes are coded based on the number of copies of the high-activity allele/haplotype as 0, 1 and 2.

#### 7.4.2 COMT and cognition

Table 20 shows the associations between COMT and cognition. Rs2097603, rs4680 and the 2-marker Meyer-Lindenberg haplotype were associated with processing speed such that the high activity alleles/haplotypes were associated with worse performance in processing speed. The high activity allele of rs2097603 and the high activity 3-marker Meyer-Lindenberg haplotype were associated with worse performance in attention. Finally, the high activity allele of rs2097603 and the high activity Nackley and 2-marker Meyer-Lindenberg haplotypes were associated with lower IQ. Next, the associations between COMT and the separate tasks that comprise the processing speed domain (table 21) were examined to identify whether any associations are specific to one task. Results were substantively similar, consistent with the high correlations between these two tests (rho=0.37, p<0.001).
<table>
<thead>
<tr>
<th>Individual SNPs</th>
<th>n</th>
<th>Processing Speed</th>
<th>p</th>
<th>R²</th>
<th>n</th>
<th>Attention</th>
<th>p</th>
<th>R²</th>
<th>n</th>
<th>IQ</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs165599</td>
<td>5377</td>
<td>-0.02(-0.06 to 0.02)</td>
<td>0.33</td>
<td>0.0002</td>
<td>4793</td>
<td>-0.03(-0.07 to 0.02)</td>
<td>0.22</td>
<td>0.0003</td>
<td>5298</td>
<td>-0.01(-0.05 to 0.04)</td>
<td>0.82</td>
<td>0.0000</td>
</tr>
<tr>
<td>rs2097603</td>
<td>5337</td>
<td>-0.06(-0.10 to -0.02)</td>
<td>0.002</td>
<td>0.0017</td>
<td>4758</td>
<td>-0.04(-0.08 to -0.003)</td>
<td>0.04</td>
<td>0.0009</td>
<td>5257</td>
<td>-0.04(-0.08 to -0.00)</td>
<td>0.03</td>
<td>0.0009</td>
</tr>
<tr>
<td>rs4680</td>
<td>5229</td>
<td>-0.04(-0.08 to -0.01)</td>
<td>0.02</td>
<td>0.0010</td>
<td>4654</td>
<td>-0.01(-0.05 to 0.03)</td>
<td>0.73</td>
<td>0.0000</td>
<td>5155</td>
<td>-0.03(-0.07 to 0.00)</td>
<td>0.07</td>
<td>0.0006</td>
</tr>
<tr>
<td>rs737865</td>
<td>5238</td>
<td>-0.02(-0.07 to 0.02)</td>
<td>0.25</td>
<td>0.0003</td>
<td>4669</td>
<td>-0.03(-0.07 to 0.02)</td>
<td>0.23</td>
<td>0.0003</td>
<td>5162</td>
<td>-0.02(-0.06 to 0.02)</td>
<td>0.35</td>
<td>0.0002</td>
</tr>
<tr>
<td>Haplotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nackley</td>
<td>3908</td>
<td>-0.03(-0.07 to 0.02)</td>
<td>0.20</td>
<td>0.0004</td>
<td>3845</td>
<td>-0.03(-0.08 to 0.01)</td>
<td>0.14</td>
<td>0.0006</td>
<td>3852</td>
<td>-0.05(-0.10 to -0.01)</td>
<td>0.02</td>
<td>0.0014</td>
</tr>
<tr>
<td>2-marker M-L</td>
<td>3947</td>
<td>-0.06(-0.10 to -0.014)</td>
<td>0.01</td>
<td>0.0016</td>
<td>3883</td>
<td>-0.03(-0.08 to 0.02)</td>
<td>0.21</td>
<td>0.0004</td>
<td>3891</td>
<td>-0.05(-0.09 to 0.00)</td>
<td>0.05</td>
<td>0.0010</td>
</tr>
<tr>
<td>3-marker M-L</td>
<td>3924</td>
<td>-0.05(-0.11 to 0.001)</td>
<td>0.06</td>
<td>0.0009</td>
<td>3864</td>
<td>-0.05(-0.11 to 0.000)</td>
<td>0.05</td>
<td>0.0010</td>
<td>3869</td>
<td>-0.04(-0.09 to 0.015)</td>
<td>0.16</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*Abbreviations:* M-L=Meyer-Lindenberg

*Notes:* The individual SNPs and haplotypes are coded based on the number of copies of the high-activity allele/haplotype as 0, 1 and 2.
Table 21 *COMT* and processing speed

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Coding</th>
<th>P</th>
<th>Sky Search</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual SNPs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs165599</td>
<td>5041</td>
<td>-0.003 (-0.04 to 0.04)</td>
<td>0.88</td>
<td>-0.03 (-0.06 to 0.01)</td>
<td>0.10</td>
</tr>
<tr>
<td>rs2097603</td>
<td>5006</td>
<td><strong>-0.05 (-0.09 to -0.01)</strong></td>
<td><strong>0.008</strong></td>
<td><strong>-0.03 (-0.06 to 0.00)</strong></td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>rs4680</td>
<td>4912</td>
<td>-0.03 (-0.07 to 0.00)</td>
<td>0.08</td>
<td><strong>-0.04 (-0.06 to -0.01)</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>rs737865</td>
<td>4918</td>
<td>-0.02 (-0.07 to 0.02)</td>
<td>0.24</td>
<td>-0.01 (-0.04 to 0.02)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Haplotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nackley</td>
<td>3664</td>
<td>-0.02 (-0.07 to 0.02)</td>
<td>0.31</td>
<td>-0.02 (-0.05 to 0.02)</td>
<td>0.27</td>
</tr>
<tr>
<td>2-marker M-L</td>
<td>3707</td>
<td>-0.04 (-0.08 to 0.01)</td>
<td>0.11</td>
<td><strong>-0.05 (-0.08 to -0.01)</strong></td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>3-marker M-L</td>
<td>3686</td>
<td>-0.04 (-0.09 to 0.01)</td>
<td>0.13</td>
<td><strong>-0.04 (-0.08 to 0.00)</strong></td>
<td><strong>0.05</strong></td>
</tr>
</tbody>
</table>

*Abbreviations:* M-L=Meyer-Lindenberg
7.4.3 COMT and anxiety disorders

The high activity allele of rs2097603 was associated with reduced odds of anxiety disorders (p=0.03). There was no further evidence of association between any other COMT SNPs or haplotypes and anxiety disorders (table 22).
Table 22 COMT and anxiety disorders

<table>
<thead>
<tr>
<th>Individual SNPs</th>
<th>n</th>
<th>Non-adjusted regression coefficients (95% CI)</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs165599</td>
<td>5290</td>
<td>0.01 (-0.02 to 0.04)</td>
<td>0.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>rs2097603</td>
<td>5258</td>
<td>-0.03 (-0.06 to -0.01)</td>
<td>0.03</td>
<td>0.0009</td>
</tr>
<tr>
<td>rs4680</td>
<td>5167</td>
<td>-0.02 (-0.05 to 0.00)</td>
<td>0.07</td>
<td>0.0006</td>
</tr>
<tr>
<td>rs737865</td>
<td>5195</td>
<td>-0.01 (-0.04 to 0.02)</td>
<td>0.63</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Haplotypes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>n</th>
<th>Non-adjusted regression coefficients (95% CI)</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nackley</td>
<td>3730</td>
<td>-0.02 (-0.05 to 0.02)</td>
<td>0.35</td>
<td>0.0002</td>
</tr>
<tr>
<td>2-marker M-L</td>
<td>3776</td>
<td>-0.02 (-0.06 to 0.01)</td>
<td>0.15</td>
<td>0.0006</td>
</tr>
<tr>
<td>3-marker M-L</td>
<td>3754</td>
<td>-0.00 (-0.01 to 0.01)</td>
<td>0.65</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: M-L=Meyer-Lindenberg

Notes: The individual SNPs and haplotypes are coded based on the number of copies of the high-activity allele/haplotype as 0, 1 and 2

Anxiety disorders were associated with PEs (OR: 1.31, 95% CIs: 1.17 to 1.47, n=4940).

7.4.4 Indirect association of COMT and PEs via cognition and anxiety disorders

The mediation analysis (table 23) indicated evidence for indirect effects of rs2097603, rs4680 and the 2-marker Meyer-Lindenberg haplotype on PEs through processing speed. There was also evidence for an indirect effect of the 3-marker Meyer-Lindenberg haplotype on PEs through attention, with the high-activity alleles/haplotypes showing association with increased risk of PEs through worse processing speed and attention. Similarly, there was also evidence for indirect effects of rs2097603 on PEs through attention and IQ. No other evidence was found for association between COMT and PEs, either directly or indirectly through cognitive function or anxiety disorders. As the estimates for the indirect and total
effects were in opposite directions, it was not possible to calculate the proportion of the total effect of COMT that was mediated by the indirect effects in a way that is easily interpretable (personal communication with Drs Dustin Hingley and Raymond Hicks who devised the mediation command).
# Table 23 Mediation results

<table>
<thead>
<tr>
<th></th>
<th>Indirect effect</th>
<th>Direct effect</th>
<th>Total effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual SNPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2097603</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>0.006(0.002 to 0.011)</td>
<td>-0.036(-0.083 to 0.011)</td>
<td>-0.030(-0.078 to 0.016)</td>
</tr>
<tr>
<td>Attention</td>
<td>0.003(0.001 to 0.008)</td>
<td>-0.036(-0.081 to 0.013)</td>
<td>-0.033(-0.078 to 0.017)</td>
</tr>
<tr>
<td>IQ</td>
<td>0.003(0.003 to 0.007)</td>
<td>-0.035(-0.081 to 0.014)</td>
<td>-0.032(-0.079 to 0.017)</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td>-0.002(-0.006 to 0.001)</td>
<td>-0.027(-0.074 to 0.021)</td>
<td>-0.028(-0.076 to 0.020)</td>
</tr>
<tr>
<td>rs4680</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>0.004(0.003 to 0.009)</td>
<td>-0.021(-0.068 to 0.028)</td>
<td>-0.017(-0.064 to 0.032)</td>
</tr>
<tr>
<td><strong>Haplotypes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nackley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>0.002(-0.002 to 0.006)</td>
<td>-0.034(-0.083 to 0.016)</td>
<td>-0.032(-0.081 to 0.018)</td>
</tr>
<tr>
<td>IQ</td>
<td>0.001(-0.001 to 0.005)</td>
<td>-0.032(-0.081 to 0.021)</td>
<td>-0.030(-0.080 to 0.023)</td>
</tr>
<tr>
<td>2-marker M-L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processing speed</td>
<td>0.005(0.001 to 0.010)</td>
<td>-0.043(-0.092 to 0.010)</td>
<td>-0.038(-0.089 to 0.015)</td>
</tr>
<tr>
<td>IQ</td>
<td>0.001(-0.002 to 0.005)</td>
<td>-0.036(-0.086 to 0.016)</td>
<td>-0.034(-0.085 to 0.018)</td>
</tr>
<tr>
<td>3-marker M-L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention</td>
<td>0.003(0.000 to 0.006)</td>
<td>-0.04(-0.092 to 0.011)</td>
<td>-0.038(-0.089 to 0.013)</td>
</tr>
</tbody>
</table>

**Abbreviations:** M-L=Meyer-Lindenberg

**Notes:** Individuals were coded with (0) if they had no copy of the high-activity allele/haplotype and with (1) if they had at least one copy of the high-activity allele/haplotype(1)
7.5 Discussion

**COMT and PEs**

The high activity allele of rs2097603 was associated with reduced odds of PEs. This is not in agreement with other studies in the same cohort that reported no evidence for associations (Zammit, Owen et al. 2011, Zammit, Hamshere et al. 2013). It should be noted that the study that used a semi-structured interview of PEs (at ages 12 and 18) found evidence for associations between rs2097603 but these did not survive correction for multiple testing (Zammit, Hamshere et al. 2013). One explanation for the inconsistent findings could be the different sample composition between the studies; different numbers of people completed the semi-structured interviews at ages 12 and 18 as well as the questionnaire at age 16. With regards to the other study (Zammit, Owen et al. 2011), not finding associations could also be because of the use of questionnaires that tend to overestimate the prevalence of PEs (Zammit, Owen et al. 2011). This, in turn, might have confounded the associations between COMT and PEs.

**COMT and cognition**

Rs2097603, rs4680 and the 2-marker Meyer-Lindenberg haplotype were associated with processing speed, with the markers for high COMT enzyme activity associated with worse performance in this cognitive domain. This study replicates the previous finding in schizophrenia patients (Bilder, Volavka et al. 2002) but in addition provides evidence that processing speed is associated with other COMT SNPs and haplotypes in children from the general population.
It is noteworthy, however, that these effects were not independent, given that rs2097604 and rs4680 are correlated and that the 2-marker Meyer-Lindenberg haplotype consists of these two SNPs. Indeed, when I added these two SNPs and the Meyer-Lindenberg haplotype to the same model, only rs2097603 remained as an independent predictor of processing speed (OR: 0.11, 95%CI: -0.18 to -0.04). The evidence for association with rs4680 became weaker (OR: -0.08, 95%CI: -0.17 to 0.00) while there was no further evidence for association with the 2-marker Meyer-Lindenberg haplotype (OR: 0.08, 95%CI: -0.03 to 0.19).

These findings are not in line with one of the theories on DA regulation, the tonic-phasic hypothesis. According to this theory, DA neurotransmission is regulated within subcortical regions via two different processes. Phasic DA release is driven by bursts of neuron firings in response to immediate, behaviourally relevant stimuli. On the other hand, tonic DA release is driven by slow and low-level neuron firing and is regulated by corticostriatal glutamatergic inputs. Tonic DA levels regulate the responsivity of the DA system to behavioural stimuli. Thus, tonic and phasic DA release are related in a way that low levels of tonic DA facilitate phasic DA release while the opposite happens with high levels of tonic DA. This relationship is preserved via feedback loops that maintain the homeostatic balance of the DA system (Jarcho, Mayer et al. 2012). COMT is suggested to modulate phasic and tonic DA release in the PFC with downstream effects on DA function in subcortical regions (Bilder, Volavka et al. 2004).

For example, in the case of rs4680, it is suggested that the low activity Met allele, by catabolising DA in the PFC at a lower rate, leads to larger glutamatergic input to striatal regions and therefore increases tonic DA levels while decreasing the amplitude of the phasic DA release. On the contrary, the high activity Val allele, which enables faster catabolisation of DA in the PFC, leads to increased amplitudes of the phasic DA release. In this way, it is
proposed, that the carriers of the Met allele have better stability in the DA system resulting in them having better performance at cognitive tasks requiring stability while they are less flexible in responding to stimuli. The opposite pattern applies to carriers of the Val allele (Bilder, Volavka et al. 2004).

Even though the tasks that measure processing speed in this study require a considerable degree of sustained attention, they mostly require speeded response and therefore higher flexibility and faster update of information. Based on this theory, we would expect that the high activity alleles/haplotypes would be associated with better performance in processing speed. These findings, however, provide evidence to the contrary (i.e., that the low activity alleles/haplotypes were associated with better performance in processing speed).

These findings also show that research involving COMT should not exclusively focus on examining cognitive processes that predominantly tap into the DA function in the PFC (Bilder, Volavka et al. 2002). Processing speed involves the optimal recruitment of multiple brain regions. Thus, its associations with genetic variation in COMT are consistent with a widespread effect of this gene in other regions of the brain that also modulate cognitive performance (Bilder, Volavka et al. 2002, Akil, Kolachana et al. 2003).

Overall, in relation to the effects of COMT polymorphisms on IQ and attention, as predicted, the high activity alleles and haplotypes tended to be associated with impaired performance. There was no evidence for associations between rs165599 and rs737865 and cognitive function, in line with previous studies (Diaz-Asper, Goldberg et al. 2008, Gaysina, Xu et al. 2013).
These results are not entirely consistent with previous studies based on the same sample (Barnett, Heron et al. 2007, Barnett, Heron et al. 2009, Barnett, Xu et al. 2010), in that associations between rs2097603 and IQ, rs2097603 and the Sky Search measure of processing speed, as well as the Nackley haplotype and IQ were not previously observed. This could be due to the different filters used for including individuals in the analysis. In particular, these previous studies included children with scores >3SDs from the mean, who were omitted from the analyses in the current study. Indeed, if the methodology of the previous studies is followed, and children with scores >3SDs from the mean are included, the association between rs2097603 and IQ is attenuated and consistent with the previous report (regression coefficient: -0.04, 95%CI: -0.08 to 0.00, p=0.06). Similarly, with regards to the Nackley haplotype and IQ, the previous studies assigned the individuals to six possible diplotypes, whereas in the current study the haplotypes were coded based on the possible number of copies of the high-activity haplotype to 0, 1 and 2.

The choice to exclude extreme scores in the current study was an a priori decision. I was concerned that an erroneous extreme score (outlier) is more likely to affect the associations in comparison with scores in the middle of distribution. I considered that if a true robust effect exists then it would be identifiable even in a sample without the +-3SDs from the mean score.

However, this inconsistency between the studies could also suggest that the associations between COMT and measures of cognition are not particularly robust given that conclusions differ when analyzing data within the same sample using slightly different procedures, a finding of relevance to the methodology of molecular genetic studies in general.

**COMT and anxiety disorders**
This is the first study to examine whether genetic variation in *COMT* is associated with anxiety disorders in children. The findings provide support for the warrior-worrier hypothesis, as there was an association of rs2097603 with anxiety disorders in the expected direction (i.e., the high activity allele was associated with lower risk of anxiety disorders). One possible mechanism is *COMT* modulating dopamine-related hippocampal activity (Matsumoto, Weickert et al. 2003, Smolka, Schumann et al. 2005, Wittmann, Schott et al. 2005, Drabant, Hariri et al. 2006). For example, a study on 101 subjects with *COMT* information underwent fMRI scanning. The results showed that carriers homozygous for the Met allele showed greater hippocampal formation and greater functional connectivity between the amygdala and the orbitofrontal (OFC)/ventrolateral prefrontal cortex (vPFC), the hippocampus and the OFC/vPFC and the vPFC and parahippocampal gyrus relative to carriers homozygous of the Val allele. This neural circuitry, especially the hippocampus, in turn has been associated with high levels of anxiety (Ploghaus, Narain et al. 2001, Rusch, Abercrombie et al. 2001, Bannerman, Rawlins et al. 2004).

**Lack of total effect - existence of indirect effects**

This is the first study to examine possible indirect effects of *COMT* on PEs. Despite lack of evidence for a total effect (i.e., combined direct and indirect effects), there was evidence for an indirect effect of rs2097603 on PEs through processing speed, attention and IQ, an indirect effect between rs4680 on PEs through processing speed, and indirect effects of the Meyer-Lindenberg haplotypes on PEs through processing speed and attention. Detection of indirect effects in the absence of a total effect is possible and it has been argued that where there are theoretical reasons to predict an indirect pathway, this should be explored regardless of whether a total or direct effect is present (Hayes 2009, Xinshu Zhao, Jr et al. 2010, Rucker, Preacher et al. 2011).
Low power could explain the lack of evidence of total effect. This is in part supported by the initial finding of a relationship between rs2097603 and PEs. There was a methodological difference between these two analyses that might have accounted for the lack of total effect; in the mediation analysis a binary outcome for rs209603 was used instead of an outcome comprised of three categories (i.e., no copy/ at least one copy of the high activity allele/haplotype versus no copy /at least one copy/two copies of the high activity allele/haplotype). Using a binary instead of a three-category outcome, reduces sensitivity and in turn, power.

Indeed, the existence of indirect effects in this study is in line with what would be expected had this study been conducted in patients with schizophrenia. Even though type I errors cannot be excluded, the existence of these indirect effects is in agreement with the initial hypothesis that genetic variation in COMT, indexing higher COMT activity and therefore lower dopamine levels in the PFC, can lead to PEs through impairments in processing speed, possibly as a result of an increase in false prediction errors. Thus, these findings suggest a potential mechanism that could contribute towards the emergence of PEs in children.

Despite suggestions that PEs are more closely linked with anxiety disorders than with psychotic disorders (Wigman, van Nierop et al. 2012) and recent evidence indicating common genetic susceptibility between anxiety disorders and schizophrenia (Ayalew, Le-Niculescu et al. 2012), there was no evidence that anxiety disorders were on the causal pathway between COMT and PEs.

Finally, competitive mediation could remain as a possible explanation for lack of total effect. Even though there was lack of evidence of an effect through anxiety disorders, there might be other intermediate pathways operating in opposing ways that were not included in this
analysis. For example, this could be emotion recognition, which is impaired in schizophrenia and is also associated with COMT (Mier, Kirsch et al. 2010). Given that the pathway between gene and phenotype is complex, competitive mediation is a possibility that needs to be further explored by future studies.

Implications

These findings are consistent with the neurodevelopmental hypothesis of schizophrenia, whereby genetic variation influences cognitive function, which in turn leads to increased risk of PEs in childhood and potentially greater vulnerability for schizophrenia in adulthood.

The associations of COMT with processing speed are consistent with COMT having widespread effects on cognition that are not exclusive to the PFC (Bilder, Volavka et al. 2002). Even though the amount of variation that COMT explains in cognitive function in this study is less than 1%, this finding is important; studies of typically developing children show that processing speed mediates the developmental course of other cognitive abilities, such as working memory and response inhibition (Fry and Hale 1996, McAuley and White 2011), whilst cognitive slowing might restrict performance in other cognitive abilities such as executive functions (Fry and Hale 1996, Christ, White et al. 2001, McAuley and White 2011). The impact of processing speed on other cognitive abilities has been eloquently described by Salthouse (1996). According to his theoretical model, processing speed can influence other cognitive abilities in two discrete ways: (a) the limited time mechanism according to which the slower the processing speed is the less cognitive operations can be performed in a given amount of time and; (b) the simultaneity mechanism according to which when a more complex cognitive operation is executed, information from earlier cognitive operations might disappear and therefore further complex processing might be halted.
Considering the model above, these findings are also consistent with previous studies associating rs4680 with working memory sub-processes that involve rapid information updating (Goldberg, Egan et al. 2003, Bruder, Keilp et al. 2005, Aguilera, Barrantes-Vidal et al. 2008).

The existence of an indirect effect, albeit small, provides potentially important clues with respect to the mechanism by which genetic variation can affect behaviour, and this has the potential to inform intervention strategies.

**Strengths and limitations**

This is the first study to examine the possible indirect effects of COMT on PEs in a large population-based sample of children. This study also includes well-established psychometric measures. Preliminary evidence of indirect effects of COMT on PEs indicates that testing for intermediate phenotypes might be an important strategy for identifying mechanisms underlying disease susceptibility.

The difficulty with theories of competitive mediation as an explanation for lack of evidence of total effect of SNP on phenotype is that there are likely to be many candidates as possible mediators. Therefore it is important that the direction of these effects is *a priori* hypothesized, based on well-established literature and theoretically plausible, as in the current study. Moreover, studies should ideally be based on variants associated with demonstrated functional effect on genes of known function. Examining these paths also requires longitudinal data with detailed phenotypic information and genetic data on large samples, as was the case with the current study. Finally, clear, robust replication of any putative competitive mediation reported is essential.
Even though overall the COMT genotype was not associated with data missingness, one limitation is possible attrition bias as a consequence of differential loss to follow-up. Given the potentially complex relationship between genotype, cognition, anxiety and PEs, it is difficult to predict how loss to follow-up may have impacted upon the findings from this study. This further highlights the requirement for robust replication of these findings.

Conclusions

I examined probable indirect effects of COMT on PEs through cognitive function and anxiety disorders. COMT was indirectly associated with PEs through processing speed and attention but there was no evidence for a total effect of COMT on PEs, nor for an indirect effect through anxiety disorders. These findings support the role of processing speed and attention as endophenotypes in psychotic disorders such as schizophrenia and provide additional evidence for the widespread effects of COMT on the brain. The existence of a potential causal pathway between COMT and PEs in children can be the basis for further research on its neurobiological correlates.
Chapter 8 Psychopathology, cognition and the role of genetic variation in Catechol-O-Methyltransferase (COMT) in children with 22q11.2 Deletion Syndrome (22q11.2DS).

8.1 Chapter Overview

In my previous chapters I examined whether and how cognitive function is associated with psychotic experiences (PEs) and also whether genetic variation in Catechol-O-Methyltransferase (COMT) is indirectly associated with PEs through cognitive function and anxiety disorders in children from the general population.

The overall aim of this thesis is to examine whether and how cognitive function is associated with psychopathology in children that are at increased risk of schizophrenia as well as potential associations with genetic variation in COMT. Another way to approach these questions is by examining psychopathology and cognition in a high-risk sample, for example in children with 22q11.2 Deletion Syndrome (22q11.2DS). As I already stated in the General Introduction, 22q11.2DS is one of the strongest known risk factors for developing schizophrenia. Therefore, examining cognitive function and psychopathology in such a high risk sample can improve our understanding of the neurodevelopmental processes that occur in schizophrenia in the general population.

In this Chapter, I examined the nature and prevalence of psychiatric and cognitive problems in children with 22q11.2DS compared to their siblings and also explored to what extent risk
of psychopathology that is associated with the deletion may be influenced indirectly (mediated) by the children’s intellectual impairment. Finally, I also examined whether genetic variation in COMT is associated with the psychiatric and cognitive problems experienced by children with 22q11.2DS.

It was found that children with 22q11.2DS are at risk of deficits in a wide range of psychiatric disorders as well as high rates of cognitive impairments. Moreover, it was found that psychopathology was not mediated by the children’s intellectual impairment, indicating that psychopathology in 22q11.2DS is not a non-specific consequence of intellectual impairment. Finally, there was no evidence that genetic variation in COMT played a role in the susceptibility of children with 22q11.2DS to the cognitive and psychiatric problems.

8.2 Introduction

As I already mentioned in the General Introduction, the high risk of intellectual disability in schizophrenia and conversely, of schizophrenia in individuals with intellectual disability is well established (Owen 2012). Shared genetic etiology has been proposed as a potential explanation of this frequent comorbidity (Morgan, Leonard et al. 2008, Toulopoulou, Goldberg et al. 2010, Fowler, Zammit et al. 2012). A number of recent reports have indicated that large, rare copy number variations (CNVs) increase the risk for intellectual disability and schizophrenia, as well as for a range of other neurodevelopmental disorders (O'Donovan, Kirov et al. 2008). It remains unclear why these CNVs affect multiple phenotypes. Two possible explanations are that: 1. there is a causal relationship between intellectual disability and psychopathology; or 2. that the genes affected by these CNVs exert pleiotropic effects.
resulting in a range of manifestations crossing different diagnostic boundaries (Walters and Owen 2007).

22q11.2DS can offer important insights into these issues. The syndrome is caused by a deletion CNV in region 22q11.2 and is one of the most common genetic causes of intellectual disability and psychopathology (Bish, Chiodo et al. 2007) as well as one of the strongest known risk factors for developing schizophrenia in adulthood (Murphy, Jones et al. 1999, Bassett, Chow et al. 2005).

To be more exact, approximately 25% of the individuals with 22q11.2DS develop schizophrenia in adulthood (Murphy, Jones et al. 1999, Bassett, Chow et al. 2005), which renders 22q11.2DS the third strongest known risk factor for developing schizophrenia, only surpassed by having an identical twin with schizophrenia or having two parents with the disorder (Tsuang and Vandermey 1980). Furthermore, 22q11.2 micro-deletions account for 1 to 2% of schizophrenia cases (Karayiorgou, Morris et al. 1995, Consortium 2008, Stefansson, Rujescu et al. 2008). Prevalence of PEs in individuals with 22q11.2DS range from 17% (Debbane, Glaser et al. 2006) to 60% (Debbane, Glaser et al. 2008). The peak onset of PEs in individuals with 22q11.2DS seems to be around adolescence (Vogels, Verhoeven et al. 2002) and this could explain lower prevalence in studies focussing on children, for example a study in children aged 6 to 19 (Debbane, Glaser et al. 2006) reported prevalence of 17%.

Although there is a paucity of studies into this issue, to date it appears that there is no evidence for an idiopathic form of schizophrenia in individuals with 22q11.2DS (Murphy, Jones et al. 1999, Bassett, Chow et al. 2003). Parental age, parental history of psychosis, parental origin of the deletion or the size of the deletion an individual carries (typically 1.5 or 3 Mega bases (Mb)) do not seem to distinguish individuals with 22q11.2DS with or without
schizophrenia (for a review see: Bassett and Chow 2008)). Low power, however, cannot be excluded, as these studies have been based on small sample sizes (n= 4 to 33).

It is also currently unclear whether there are any neuroanatomical abnormalities that distinguish those individuals with 22q11.2DS that will develop schizophrenia from those who will not (Drew, Crabtree et al. 2011). Inconsistencies in studies to date are mainly attributable to the use of different measurement protocols, while studies have also tended to be based on relatively small samples with wide age ranges (Karayiorgou, Simon et al. 2010). Nevertheless, findings do indicate similarities between patients with 22q11.2DS and patients with schizophrenia but without 22q11.2DS. For example, neuro-imaging studies have reported enlarged ventricles (Chow, Mikulis et al. 1999, Eliez, Schmitt et al. 2000, Kates, Burnette et al. 2001, Chow, Zipursky et al. 2002), reduced cortical thickness (Bearden, van Erp et al. 2007, Bearden, van Erp et al. 2009) and polymicrogyria (Sztriha, Guerrini et al. 2004, Robin, Taylor et al. 2006) in studies of children and adults with 22q11.2DS similarly to what has been reported in patients with schizophrenia (for a review see: Shenton, Whitford et al. 2010)). It remains unknown, however, whether this profile is associated with risk of schizophrenia or with impaired brain development (Ellegood, Markx et al. 2013).

Taking these considerations into account, studying this high risk sample of children offers the potential to investigate the relationships between cognitive impairment and schizophrenia before the onset of severe symptoms and their sequelae (e.g., the introduction of antipsychotic medication) which may confound interpretation of any observed relationships (Lewandowski, Shashi et al. 2007).

Furthermore, as I mentioned previously (Chapter 3.2) it has been hypothesized that impaired cognitive processes tapping prefrontal cortex function are associated with risk of
schizophrenia and genetic variation in COMT (Tunbridge, Harrison et al. 2006). COMT resides within the 22q11.2 deleted region and according to this theory the behavioural and cognitive impairments seen in 22q11.2DS could be related to the dopamine dysregulation resulting from possessing only one copy of COMT (Graf, Unis et al. 2001, Murphy 2002). Obtaining an understanding of the way COMT affects cognition and behaviour in 22q11.2DS could ultimately inform the treatment of the disorder (Fallgatter and Lesch 2007).

Before I proceed with the aims of the study, the purpose of the following section is to introduce the reader to 22q11.2DS by providing an overview of the findings on 22q11.2DS with a particular focus on the behavioural and cognitive profile of individuals with this deletion as well as the findings related to COMT in this population.

### 8.2.1 Definition

22q11.2 Deletion Syndrome (22q11.2DS), also known as Velo-cardio-facial syndrome (VCFS) is the most frequent currently known chromosomal microdeletion syndrome (Scambler 2000, Eliez, Antonarakis et al. 2001, Murphy 2004, Gothelf 2007). It was first described in 1955 (Sedlackova 1955) (see Shprintzen 2008)) and the microdeletion causing the syndrome was first reported in 1992 (Scambler, Kelly et al. 1992). Before the identification of the deletion and because of the syndrome’s extensive phenotype, 22q11.2DS has also been known as DiGeorge sequence or DiGeorge syndrome (Delachapelle, Herva et al. 1981), conotruncal anomaly face syndrome (CAFS) (Matsuoka, Takao et al. 1994), CATCH 22 (Wilson, Burn et al. 1993), Cayler cardiofacial syndrome (Giannotti, Digilio et al. 1994, Bawle, Conard et al. 1998) and Shprintzen syndrome (Shprintzen, Goldberg et al. 1978).
8.2.2 Prevalence

A number of studies have reported a range of prevalence from 1:2000 to 1:9700 (Tezenas Du Montcel, Mendizabai et al. 1996, Goodship, Cross et al. 1998, Botto, May et al. 2003, Oskarsdottir, Vujic et al. 2004, Shprintzen 2008). The discrepancies are mainly because of the phenotypic variability of the disorder and the fact that many of its clinical features become apparent only later in life or even remain undetected (i.e., silent heart anomalies) (Bales, Zaleski et al. 2010). Place of birth is also a factor contributing to the differences in the prevalence rates (Shprintzen 2008). For example, infants are more likely to die in places where the intensive or surgical care units are difficult to access or run less efficiently. 22q11.2DS can also cause other developmental disorders such as holoprosencephaly sequence or Potter syndrome (Wraith, Super et al. 1985, Devriendt, Moerman et al. 1997), which result in malformations that critically reduce the chances for the infants to survive. Hence, the prevalence of 22q11.2DS is likely to be higher. The currently most accepted prevalence rate is 1 in 4000 live births (Karayiorgou, Simon et al. 2010).

8.2.3 Deletion size

The disorder is characterized by microdeletions of chromosome 22q11. These microdeletions are usually detected with fluorescence in situ hybridization (FISH) analysis. This is a genetic mapping technique in which a deoxyribonucleic acid (DNA) probe is labelled by attaching a colour of fluorescent dye and then hybridized with target DNA (Langer-Safer, Levine et al. 1982). Nevertheless, more recent techniques, like comparative genome hybridization (CGH) and genome-wide arrays of single nucleotide polymorphisms (SNPs) are more precise in
identifying chromosomal aberrations and therefore FISH is increasingly replaced by these methods in clinical diagnosis (Bejjani, Saleki et al. 2005).

Approximately 87% of the deletions are 3 Mb in size (including approximately 48 known genes) and about 10% are 1.5 Mb in size (including approximately 30 known genes) (Edelmann, Pandita et al. 1999, Shaikh, Kurahashi et al. 2000). There are reports of rare deletions that are either embedded in the 3Mb typically deleted region (TDR) or distant from the TDR (Carlson, Sirotkin et al. 1997) and are detectable only via microarray (Williams 2011), rather than FISH. Due to the regular recurrence of de novo deletions in chromosome 22q11.2, it has been proposed that parts of this chromosomal region tend to rearrange.

### 8.2.4 Origin of deletion

Only approximately 10% of the deletions are inherited whereas the majority (approximately 85-90%) occur de novo (Ryan, Goodship et al. 1997). In de novo cases, the prevalence of maternal or paternal origin of the deletion is similar (Ryan, Goodship et al. 1997, McDonald-McGinn, Kirschner et al. 1999, Delio, Guo et al. 2013). With respect to familial cases, most reports indicate that maternal inheritance is more common than paternal inheritance (Ryan, Goodship et al. 1997, Swillen, Devriendt et al. 1997, Green, Gothelf et al. 2009). This has been attributed to social factors. For example, it may be possible that the women with 22q11.2DS tend to procreate more often than men with 22q11.2DS (Swillen, Vogels et al. 2000).
If the parent carries the 22q11.2 deletion, there is 50% recurrence risk (Swillen, Vogels et al. 2000). In addition, there is a low, yet indefinite risk of germline mosaicism which increases risk in some de novo cases (Hatchwell, Long et al. 1998, Sandrin-Garcia, Macedo et al. 2002, Bassett, McDonald-McGinn et al. 2011).

8.2.5 Physical phenotype

22q11.2DS has been associated with over 180 physical manifestations, the majority of which relate to disruptions of neural crest cell development (Murphy 2004, Gothelf, Hoeft et al. 2007). Among the commonly reported features are congenital anomalies of the palate; congenital heart defects; velopharyngeal insufficiency; hypocalcemia; T-cell deficiency; thymic hypoplasia; thrombocytopenia; infections (pneumonia and otitis media) and short stature (Ryan, Goodship et al. 1997). Other symptoms less frequently reported include hernias, obesity, hearing impairments, and hypothyroidism (Wilson, Burn et al. 1993, Lindsay, Morris et al. 1995, Bassett, Chow et al. 2005).

In adults, there is no evidence of gender differences in physical symptoms (Bassett, Chow et al. 2005). In children and adolescents, one study of 33 individuals reported that males had more physical symptoms than girls (Oskarsdottir, Belfrage et al. 2005).

The most noticeable characteristic of the 22q11.2DS is the facial dysmorphology. This entails a prominent nose with squared nasal root and narrow alar base, impairment of the malar area, narrow palpebral fissures and ear abnormalities (Motzkin, Marion et al. 1993) (figure 5).
This characteristic facial dysmorphology, however, is not as typical or abnormal as in Down or Williams Syndrome. As a result, many cases with 22q11.2DS remain undiagnosed as infants and young children until severe hyper nasality (75% of the cases) brings the patient to the notice of speech pathologists and cleft palate teams (Shprintzen 2008).

8.2.6 Language

Studies have shown developmental delays in language acquisition (Solot, Gerdes et al. 2001). For example, Briegel and colleagues examined 22 infants and found that 71% could not speak more than 3 words before the age of 19 months (Briegel, Schneider et al. 2007) and

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4 Adapted from “Practical Guidelines for Managing Patients with 22q11.2 Deletion Syndrome” by Bassett A. S. et al. 2011, The Journal of Pediatrics, 2, p.332. Adapted with Permission
Solot and colleagues examined 53 pre-school children (aged 4 to 70 months) and found that 90% could either not speak at all or use only single words (Solot, Gerdes et al. 2001). Other language deficits have also been reported (Solot, Knightly et al. 2000, Woodin, Wang et al. 2001, Persson, Niklasson et al. 2006, Lima, Folling et al. 2010). Expressive language seems to be less impaired than receptive language (Baker and Skuse 2005, Roizen, Antshel et al. 2007) though this is not always the case (Lajiness-O'Neill, Beaulieu et al. 2005). Maternal origin of the deletion has been related to lower receptive language ability (Glaser, Mumme et al. 2002). According to the authors, a mechanism that could explain this observed association could be genetic imprinting in which the parental origin of the chromosome modulates gene expression (Reik and Walter 2001). Nevertheless, the difference in parent of origin effect disappeared when the child’s IQ was adjusted for. It could be that the IQ differences reflect structural brain abnormalities between the groups. However, the small sample size of the study (i.e., 12 children with maternal origin of the deletion and 8 with paternal origin) needs to be taken into account when interpreting these results.

Language skills have been found to be negatively correlated with age (Persson, Niklasson et al. 2006). As age and communication demands increase, children with 22q11.2DS find it harder to cope (Golding-Kushner, Weller et al. 1985). With regards to gender differences, a study on a sample of 50 boys aged 6 to 15 (mean age: 11) and 40 girls aged 6 to 15 (mean age: 10.8) found that girls performed better than boys in an oral and composite language test (Antshel, AbdulSabur et al. 2005) which seems to be in line with findings in the general population (Hyde, J et al. 1988, Fenson, Dale et al. 1994).
8.2.7 Motor skills

Studies have reported that children with 22q11.2DS have delayed motor development (Gerdes, Solot et al. 1999, Oskarsdottir, Belfrage et al. 2005, Briegel, Schneider et al. 2007) and poor psychomotor skills (Swillen, Vandeputte et al. 1999). For example, 48 out of 77 (63%) children with 22q11.2DS aged 4 to 17 years old could not walk by the age of 19 months (Briegel, Schneider et al. 2007). Another study found that out of 33 children with 22q11.2DS, 49% had never crawled in a normal way, 78% had poor balance, 52% poor coordination and 52% impaired motor function, with another 15% borderline motor function (and only 15% with normal motor function) (Oskarsdottir, Belfrage et al. 2005). Gender differences in motor skills have not been reported.

8.2.8 General psychopathology

Children with 22q11.2 DS have been reported to be at high risk of developing a wide range of behavioural and psychiatric problems. There is considerable discrepancy, however, in the reported rates of the psychiatric problems that have been associated with the 22q11.2 deletion. For example, rates for Attention Deficit Hyperactivity Disorder (ADHD) range from 12%, (95%CIs 2.5% to 31%) (Sobin, Kiley-Brabec et al. 2005) to 68% (95%CIs 57% to 77%) (Antshel, Kates et al. 2005); for autism from 0% (95%CIs 0% to 10%) (Sobin, Kiley-Brabec et al. 2005) to 20% (95%CIs 9% to 35%) (Antshel, Aneja et al. 2006); for Oppositional Defiant Disorder (ODD) from 1% (95%CIs 3% to 15%) (Antshel, Fremont et al. 2006) to 43% (95%CIs 25% to 63%) (Feinstein, Eliez et al. 2002); and for anxiety disorders from 3% (95%CIs 1% to 8.5%) (Niklasson, Rasmussen et al. 2009) to 55% (95%CIs 32% to 77%) (Stoddard, Niendam et al. 2010).
Evidence is inconclusive with regards to whether the deletion is more likely to be present in patients with these disorders than in patients with schizophrenia. There are studies supporting this, for example for ADHD (Williams, Zaharieva et al. 2010) and Autism Spectrum Disorder (ASD) (Marshall, Noor et al. 2008, Guilmatre, Dubourg et al. 2009), whereas others have found no differences, for example, for ADHD (Elia, Gai et al. 2010) and ASD (Ogilvie, Moore et al. 2000, Glessner, Wang et al. 2009).

Some psychiatric and behavioural problems seem to increase with age in individuals with 22q11.2DS, e.g., Generalized Anxiety Disorder (GAD), ODD and Major Depressive Disorder (MDD), whereas disruptive disorders such as ADHD seem to decrease (Aneja, Fremont et al. 2007, Briegel, Schneider et al. 2008, Green, Gothelf et al. 2009, Antshel, Shprintzen et al. 2010). However, there are also studies that have found no associations between psychopathology and age, including ADHD (Zagursky, Weller et al. 2006), psychotic symptoms (Stoddard, Niendam et al. 2010), psychopathological summary scores or schizotypy ratings (Baker and Skuse 2005). A sample of 40 adults that was ascertained through an adult cardiac clinic found an elevated rate of GAD whereas no significant differences were found for MDD, ADHD and substance use disorders when compared to general population estimates (Fung, McEvilly et al. 2010) suggesting at least some disorders may be elevated in childhood but not in adulthood. Two studies have reported an increase of anxiety symptoms with age (age range of the studies was 6 to 11 and 4 to 17 respectively) (Swillen, Devriendt et al. 2001, Jolin, Weller et al. 2009). There is evidence from a rare longitudinal study of children/adolescents with 22q11.2DS followed into adolescence/adulthood (n=24) that affective disturbance and anxieties at age 12 as assessed by parental report are predictive of psychotic disorder at age 18 (Gothelf, Feinstein et al. 2007).
The majority of studies have not found gender differences in psychopathology (Murphy, Jones et al. 1999, Swillen, Devriendt et al. 1999, Antshel, AbdulSabur et al. 2005, Bassett, Chow et al. 2005, Vorstman, Morcus et al. 2006, Jolin, Weller et al. 2009). However, (Gothelf, Presburger et al. 2004) have found that ADHD may be more common in males than females with 22q11.2DS (as has also been reported for ADHD in the general population) and it has also been reported that there are more males than females with 22q11.2DS and mental retardation and/or ADHD and/or ASD (Niklasson, Rasmussen et al. 2009). In contrast, one study reported a preponderance of ADHD in females with 22q11.2DS (Zagursky, Weller et al. 2006). Another study found more total and internalizing problems in males compared to females with 22q11.2DS (Briegel, Schneider et al. 2008).

No relationship has been reported between risk of psychopathology, deletion size (Weksberg, Stachon et al. 2007) and whether the deletion is familial or de novo (Gothelf, Aviram-Goldring et al. 2007).

However, it should be noted in this instance too, that studies in 22q11.2DS are likely to be underpowered to detect differences. Type I errors are also likely (Christley 2010).

8.2.9 Psychiatric comorbidity

According to Burke and colleagues (1990), comorbidity is defined as ‘the presence of more than one specific disorder in a person in a defined period of time’ (Burke, Wittchen et al. 1990) (as cited in (Desai 2006), p. 75). It is important to examine comorbidity, because if comorbidity is ignored, the effects of one disorder might actually be attributable to the comorbid disorder (Caron and Rutter 1991).
Nevertheless, there are several issues that need to be considered when examining comorbidity. For example, rates and characteristics of comorbidity could be due to an artefact of referral bias; this can occur when a clinician is known to be interested in a specific pattern of comorbidity and therefore more likely to receive referrals of cases with these specific conditions (Caron and Rutter 1991).

Furthermore, the apparent overlap between psychiatric disorders could be due to diagnostic misconceptualization of the disease entities (Bukstein, Brent et al. 1989). For instance, the fact that the same item of behaviour is included in the diagnostic criteria for several diagnostic categories (e.g., agitation is one of the criteria for anxiety disorders, MDD and ODD) might lead to artefactual rates of comorbidity (Caron and Rutter 1991). Another issue might be the artificial subdivision of the syndromes in subcategories. For example, anxiety disorders are divided in syndromes characterized by how general the anxiety is (e.g., GAD), whether it is specific (e.g., Specific Phobia), or according to the presence or absence of a particular trait (e.g., Agoraphobia with or without Panic Disorder) (Caron and Rutter 1991).

The above issues as well as methodological issues deriving from studies adopting different designs and diverse diagnostic assessments lead to significant variation in the magnitude of reported comorbidity in epidemiological and clinical studies (Wittchen 1996). Nevertheless, even though the above issues are difficult to overcome, understanding comorbidity can lead to significant insights in the mechanisms underlying psychiatric disorders (Simonoff, Pickles et al. 2008).

Individuals with 22q11.2DS often meet criteria for more than one co-occurring psychiatric diagnosis (Jansen, Duijff et al. 2007). Studies have especially shown that children with the deletion who have ASD tend to have more psychiatric diagnoses. For example, it has been
reported that 94% of 17 children with ASD had a co-occurring psychiatric diagnosis compared to 60% of 24 children without ASD (Antshel, Aneja et al. 2006). ADHD in particular has been found to co-occur with ASD (Niklasson, Rasmussen et al. 2009). Also, ASD symptomatology has been suggested to be indicative of psychosis-related deficits in children with 22q11.2DS (Vorstman, Morcus et al. 2006).

8.2.10 The cognitive profile

In the following section, I will describe the cognitive findings from previous studies of 22q11.2DS, where I have grouped together domains according to the schizophrenia literature (see Chapter 2.1).

8.2.10.1 IQ

IQ in individuals with 22q11.2DS varies from moderate mental retardation to normal intelligence. The majority of patients have borderline intelligence (IQ between 70-75) while severe learning disability is rare (Moss, Batshaw et al. 1999, Murphy, Jones et al. 1999). A number of studies have indicated that variability in IQ cannot be explained by physical anomalies such as cardiac deficiencies or palatal abnormalities (Swillen, Devriendt et al. 1997, Gerdes, Solot et al. 1999, Bearden, Woodin et al. 2001, Gerdes, Solot et al. 2001, Solot, Gerdes et al. 2001, Oskarsdottir, Belfrage et al. 2005, De Smedt, Devriendt et al. 2007, Niklasson and Gillberg 2010).
With respect to children and adolescents in particular, there seems to be a similar IQ pattern. Scores mainly fall within the borderline intelligence range (70-75) (50-60%), it is less common for children with 22q11.2DS to have mild intellectual disability (55-75) (35-40%), while low average intelligence (IQ<85) is also found, but relatively rare (15-20%) (McDonald-McGinn, Kirschner et al. 1999, Bearden, Woodin et al. 2001, Solot, Gerdes et al. 2001, De Smedt, Devriendt et al. 2007, Shashi, Veerapandiyan et al. 2012, Hooper, Curtiss et al. 2013).

Verbal abilities are usually more preserved than nonverbal abilities (McDonald-McGinn, Kirschner et al. 1999, Solot, Gerdes et al. 2001, Lajiness-O'Neill, Beaulieu et al. 2005, Niklasson, Rasmussen et al. 2005, De Smedt, Devriendt et al. 2007), and this has led some to hypothesize that 22q11.2DS represents a prototype of Non-Verbal Learning Disability (NVLD) (Swillen, Vandeputte et al. 1999, Bearden, Woodin et al. 2001, Solot, Gerdes et al. 2001, Woodin, Wang et al. 2001). On the other hand, there are studies reporting no differences in impairments between verbal and nonverbal abilities (Niklasson, Rasmussen et al. 2002, Gothelf, Presburger et al. 2004, Lewandowski, Shashi et al. 2007, Debbane, Van der Linden et al. 2008) and other findings yet which have indicated that some children have better nonverbal than verbal ability (Niklasson, Rasmussen et al. 2002, De Smedt, Devriendt et al. 2007). Moreover, in general children with NVLD show large differences between verbal IQ (VIQ) and performance IQ (PIQ), whereas mean differences between VIQ and PIQ in 22q11.2DS tend to be between 4 to 5 points (McDonald-McGinn, Kirschner et al. 1999, Feinstein, Eliez et al. 2002, Lajiness-O'Neill, Beaulieu et al. 2005, Niklasson, Rasmussen et al. 2005, Bearden, van Erp et al. 2006, De Smedt, Devriendt et al. 2007, Andersson, Glaser et al. 2008, Campbell, Azuma et al. 2010, Roizen, Higgins et al. 2010). Mixed receptive and expressive language impairments are also common which is not characteristic of NVLD.
Finally, a (admittedly small) study of 19 adults with 22q11.2DS (mean age (SD): 35.2 (11.4)) (no age range reported) and 19 age, gender and IQ matched control participants found no differences between VIQ and PIQ (Henry, van Amelsvoort et al. 2002) suggesting that in adults there is no evidence for NVLD.

Several studies have indicated that IQ in individuals with 22q11.2DS declines with increasing age (Gothelf, Penniman et al. 2007, Evers, De Die-Smulders et al. 2009, Niklasson and Gillberg 2010). For example, a study on 172 individuals with 22q11.2DS (mean age 15.9 (9.1)) aged 5 to 54 years found that IQ was inversely associated with age with the most striking difference found for VIQ (Green, Gothelf et al. 2009). However, this could also be a cohort effect, with younger participants with 22q11.2DS receiving better education.

With respects to parent of origin effects, there is no evidence of association with IQ (Bassett, Marshall et al. 2008). However, because around 80% of the deletions occur de novo, studies into this issue have been underpowered.

IQ has been shown to be influenced by whether the deletion is de novo or familial. 22q11.2DS individuals with de novo deletions have higher IQ in comparison to individuals with familial deletions (Swillen, Devriendt et al. 1997, Gerdes, Solot et al. 1999, Murphy 2004, De Smedt, Devriendt et al. 2007, Gothelf, Aviram-Goldring et al. 2007). The IQ of affected parents is lower than of unaffected parents and therefore, this could in turn influence the children’s IQ (De Smedt, Devriendt et al. 2007). This influence could be because of shared genetic effects or because of the home environment (Bradley, Whiteside et al. 1993). For example, studies have shown that parents with high IQ can provide a more stimulating and intellectually richer environment for their children compared to parents of lower IQ (Drotar and Sturm 1989, Watson, Kirby et al. 1996, Baker-Henningham, Powell et al. 2003).
Gender differences have also been reported, with girls having higher full scale IQ (FSIQ), PIQ and VIQ (Antshel, AbdulSabur et al. 2005, Oskarsdottir, Belfrage et al. 2005, Vorstman, Morcus et al. 2006, Niklasson and Gillberg 2010). Negative associations between age and FSIQ have also been reported for girls (Antshel, AbdulSabur et al. 2005). Finally, other studies have not found evidence for gender differences in IQ (e.g., (De Smedt, Devriendt et al. 2007).

8.2.10.2 Processing speed

In line with the schizophrenia literature, children with 22q11.2DS have been found to exhibit processing speed impairments. For example, 50 children with 22q11.2DS aged 6 to 16 (mean age: 11 (3)), were compared with 31 siblings of the same age range and found to score lower (p=0.005) than their siblings on the processing speed factor index score of the WISC-III (Campbell, Azuma et al. 2010). Similar results were obtained in a study of 20 children and young adults aged 3 to 19 (mean age: 8.6) where over half (n=11) were slow in their performance on visual reaction time tasks and had difficulty with control of impulses in choice reaction time tasks (Oskarsdottir, Belfrage et al. 2005). Also, another study of 32 individuals with 22q11.2DS aged 5 to 33 years (mean age: 12.3) reported that more than half of children with 22q11.2DS were 'slow performers’ characterized by a lack of ‘mental energy’ which could be interpreted as being slow in processing speed (Niklasson, Rasmussen et al. 2001). Processing speed performance was negatively related to age in a cross-sectional study of children with 22q11.2DS and not related to heart malformation (Niklasson and Gillberg 2010). With regards to gender differences, a study including 50 boys aged 6 to 15 (mean age: 11) and 40 girls aged 6 to 15 (mean age: 10.8) with 22q11.2DS found that girls performed better than boys in the coding and symbol search tests of the WISC-III (Antshel,
AbdulSabur et al. 2005). This was also found in a Swedish study (Niklasson and Gillberg 2010).

8.2.10.3 Attention

Attention deficits have frequently been reported in children with 22q11.2DS (Swillen, Vandeputte et al. 1999, Woodin, Wang et al. 2001, Sobin, Kiley-Brabeck et al. 2005, Antshel, Fremont et al. 2006, Bish, Chiodo et al. 2007, Niklasson and Gillberg 2010). For instance, Sobin and colleagues (2005) examined attention in 40 children with 22q11.2DS aged 5 to 12 (mean age: 7.7) using NEPSY (a developmental neuropsychological battery that assesses language, memory, visuospatial function, sensorimotor function, attention, and executive function) and found that their performance was lower than average (comparison with population-based norms). In this study, children with 22q11.2DS had more errors of omission (i.e., they failed to identify a target) on the visual attention task, while on the auditory attention task they had more difficulty maintaining a response set (i.e., to inhibit a prepotent response) (Sobin, Kiley-Brabeck et al. 2005). Furthermore, children with 22q11.2DS have been reported to exhibit deficits in focussed and sustained attention as measured by the Continuous Performance Task (CPT) and the trail making test A and B (Lewandowski, Shashi et al. 2007, Niklasson and Gillberg 2010). Pre-attentive processing deficits have also been described (Sobin, Kiley-Brabeck et al. 2005). In contrast, a study of 50 school-aged children aged 6 to 17 (mean age: 10.3 (3.2)) with 22q11.2DS (Woodin, Wang et al. 2001) found they had average performance on the Trails A task (which assesses focussed attention). Another, small, study including 14 children with 22q11.2DS aged 7 to 17 (mean age: 12.6) and 8 siblings aged 6 to 13 (mean age: 11.6) did not find group differences
in attention as measured by the Digits Forwards and Letter Forwards task (Lajiness-O'Neill, Beaulieu et al. 2006).

Finally, gender differences have been reported in only one study (Kates, Antshel et al. 2006) which found that girls outperformed boys on the Continuous Performance Task, which assesses sustained attention.

In adults, one small study (n=19) found no evidence of poor performance on tests of sustained and selective attention (Henry, van Amelsvoort et al. 2002), which has led to the suggestion that attention deficits might not persist in adulthood (Baker and Vorstman 2012). The overall inconsistencies in the findings could be because of the different tasks used to measure attention or due to the different sample sizes (where smaller studies can have inadequate power to detect differences between the groups) and age-ranges.

8.2.10.4 Working and episodic memory

Working and episodic memory are also affected in children with 22q11.2DS. For example, a study on 33 children aged 3 to 19 (mean age (SD): 8(8)) demonstrated that 19 out of 26 children showed impaired performance in the arithmetic test of the WISC-III and 12 out of 15 on the digit span forwards of the WISC-III (Oskarsdottir, Belfrage et al. 2005), which are tests of working memory. Deficits in episodic memory have also been reported. A study by Woodin and colleagues (2001) on 50 children aged 6 to 17 (mean age: 10(3)) showed impaired performance in the story memory of the Wide Range Assessment of Memory and Learning (Sheslow and Adams 1990) test (Woodin, Wang et al. 2001).
Regarding gender differences, there is evidence that girls are less impaired than boys with regards to performance on memory-based tasks and also that memory performance declines with age only in girls (Antshel, AbdulSabur et al. 2005). However, another study did not find age or gender differences in memory performance (Campbell, Azuma et al. 2010).

In adults, there is no evidence of poor performance on a test of memory (Henry, van Amelsvoort et al. 2002). Nevertheless, as stated above, this study was based on a relatively small sample and therefore interpretations should be considered in this light.

8.2.10.5 Executive functions

In addition, deficits in inhibitory processes and self-monitoring have been reported, which have not been found to be related to age (Debbane, Van der Linden et al. 2009, Campbell, Azuma et al. 2010).

8.2.10.6 Relations between IQ and other cognitive measures

Obtaining a clear understanding of the relations between IQ and other cognitive measures is important because learning disability is relatively common in 22q11.2DS (approx. 35 to 40% of children) and therefore might potentially influence performance on the other cognitive measures.

Nevertheless, only two studies have examined the relations of IQ and other cognitive measures. VIQ has been reported to be moderately associated ($r=-0.499$, $p<0.05$) with executive function as measured by perseverative errors on the Modified Card Sorting Test (MCST) in a study of 20 adolescents aged 17 to 27 (mean age: 22.1(3.2)) with 22q11.2DS (Rockers, Ousley et al. 2009). Another study in a different age group, however, did not find any relationship between IQ and verbal or visuospatial task performance (Bearden, Woodin et al. 2001) even though the sample size was similar (n=29, mean age: 10.3, aged 5 to 17). Studies have not shown any relations between diagnosis of language disorder and impaired speech (Solot, Gerdes et al. 2001) or self-monitoring impairments (Debbane, Van der Linden et al. 2009).

Finally, it is not yet known whether the pattern of cognitive associations in children with 22q11.2DS is similar to that of children with uncompromised cognitive functions. Examining
this pattern could help identify the extent to which cognitive functions in 22q11.2DS are impaired.

8.2.11 Psychopathology and cognition

It remains unclear to what extent psychopathology in children with 22q11.2DS is a direct consequence of their deletion (figure 6, Model A), or whether this relationship is better explained by children’s intellectual impairment (figure 6, Model B). That is, does the deletion increase risk of intellectual impairment, which subsequently predisposes to risk of psychopathology? If this were the case it might be argued that psychopathology in 22q11.2DS is a non-specific consequence of generalized intellectual impairment. Or, alternatively does the deletion predispose independently to both cognitive impairment and psychopathology? This would imply that cognitive impairments index genetic risk factors for psychiatric disorders, rather than mediate the risk of psychopathology. Moreover, evidence of a strong indirect association through cognitive function could inform intervention strategies, by placing more focus on cognitive remediation programs.
A number of studies have looked at the associations between IQ and psychopathology in 22q11.2DS but the results have been inconsistent, with some studies reporting no relationship (e.g., between IQ and ADHD (Gothelf, Presburger et al. 2004, Antshel, Fremont et al. 2006, Vorstman, Morcus et al. 2006, De Smedt, Devriendt et al. 2007, Jolin, Weller et al. 2009), phobias (Antshel, Fremont et al. 2006); MDD (Baker and Skuse 2005, Antshel, Fremont et al. 2006); anxiety, Obsessive Compulsive Disorder (OCD), Conduct Disorder (CD) (Baker and Skuse 2005), ASD (Niklasson, Rasmussen et al. 2002, De Smedt, Devriendt et al. 2007), or psychotic symptomatology (Baker and Skuse 2005)), whereas other studies have found evidence of such relationships (e.g., between IQ and ASD (Niklasson, Rasmussen et al. 2002, De Smedt, Devriendt et al. 2007); psychotic disorder (Gothelf, Feinstein et al. 2007);

5 According to Model A., psychopathology is a direct consequence of the deletion, whereas according to Model B, psychopathology can be better explained by the intellectual impairment caused by the 22q11.2 deletion.
psychotic symptoms (Dufour, Schaer et al. 2008); and Formal Thought Disorder (Green, Gothelf et al. 2009)).

None of these studies have included other more specific measures of neuro-cognitive impairment and none have used more formal testing of an indirect association between deletion status and psychopathology via IQ, for which a control sample is necessary. Figure 7 shows the mediation model that I am proposing to examine.

Figure 7 The mediation model which examines the role of group status (children with 22q11.2Ds versus siblings) on psychiatric diagnosis through IQ.

8.2.12 COMT and 22q11.2DS

It has been suggested that the behavioural and cognitive impairments seen in 22q11.2DS could be related to dopamine dysregulation resulting from possession of only one copy of
COMT (Graf, Unis et al. 2001, Murphy 2002). Individuals with 22q11.2DS possessing a hemizygous copy of the Met allele would be at higher risk of behavioural and cognitive problems because they are exposed to larger amounts of dopamine in the prefrontal cortex (Gothelf, Eliez et al. 2005) (for a review on COMT see Chapter 3.2 and Chapter 7). Results of COMT genotyping in individuals with 22q11.2DS, however, have been inconsistent. Some studies have reported that the Met allele was associated with worse performance in executive function and working memory as well as ADHD, OCD and Bipolar Disorder (BD) (Lachman, Morrow et al. 1996, Michaelovsky, Gothelf et al. 2008, van Amelsvoort, Zinkstok et al. 2008), whereas other findings have indicated no associations with neuropsychological performance (Glaser, Debbane et al. 2006) nor psychopathology (Murphy, Jones et al. 1999, Baker, Baldeweg et al. 2005, Bassett, Caluseriu et al. 2007). Other studies in samples of more narrow age range (only children), have sometimes found the Met allele to be associated with better cognitive performance (Bearden, Jawad et al. 2004, Kates, Antshel et al. 2006, Shashi, Howard et al. 2010), higher rates of anxiety disorders (Shashi, Howard et al. 2010) and other behavioural problems (Bearden, Jawad et al. 2005), while another study reported an opposite effect, i.e., that the Met allele was associated with worse attention (Takarae, Schmidt et al. 2009). As with psychiatric findings, inconsistencies are likely attributable to small sample sizes and wide age ranges.

**8.2.13 Summary**

22q11.2 Deletion Syndrome (22q11.2DS) is one of the most common genetic causes of intellectual disability and psychopathology (Bish, Chiodo et al. 2007) and one of the strongest known risk factors for developing schizophrenia in adulthood (Murphy, Jones et al.
1999, Bassett, Chow et al. 2005). Studies have shown that children with 22q11.2DS have high rates of psychopathology, especially ADHD, anxiety disorders and ODD, and a range of cognitive problems (e.g., (Green, Gothelf et al. 2009, Shashi, Veerapandiyan et al. 2012, Hooper, Curtiss et al. 2013)).

There are, however, considerable discrepancies in the reported rates of psychiatric disorders stemming from the difficulties associated with recruiting large sample sizes at similar developmental stages in such a rare syndrome. Moreover, a specific neuro-cognitive profile has not been identified (Karayiorgou, Simon et al. 2010). It is also not clear whether learning disability (as assessed by IQ test) in children with 22q11.2DS influences performance on other cognitive measures and whether the pattern of associations between cognitive tests is similar to this of a sample with uncompromised cognitive functions. It also not known whether the relationship between the deletion and risk of psychopathology is better explained by the children’s intellectual impairment.

As mentioned above, given the discrepancies in methodology and the wide age ranges of previous studies, better understanding of the role of COMT in cognitive function and psychiatric diagnosis in 22q11.2DS is also another important issue.

To address these issues I utilized one of the largest samples of children with 22q11.2DS and siblings to date and one of the few studies to have included both detailed psychiatric and cognitive assessments.
8.3 Description of aims

The primary goals of this study were: 1) to explore differences in the prevalence and nature of psychiatric and cognitive problems in children with 22q11.2DS compared to their siblings; as a secondary analysis, I also explored the relations between IQ and other cognitive measures as well as whether the pattern of associations between the cognitive measures is similar for children with 22q11.2DS and their siblings; 2) to examine whether any association between deletion status and psychopathology is mediated through intellectual impairment; 3) to examine the role of COMT in these relations.

Based on preliminary findings on other CNVs (O'Donovan, Kirov et al. 2008) I hypothesized that IQ does not mediate the relationship between the deletion and psychiatric problems (Model A, figure 6) (O'Donovan, Kirov et al. 2008). As an exploratory analysis, I also examined whether any other neuro-cognitive deficits may mediate this relationship.

With regards to COMT, I hypothesized that the Met allele would be associated with worse cognitive functions and greater levels of psychiatric problems. This hypothesis was based on the premise that individuals possessing a hemizygous copy of the Met allele would be exposed to larger amounts of dopamine in the prefrontal cortex in relation to individuals hemizygous for the Val allele. Consequently, this would pose them on higher risk of cognitive and psychiatric problems.
8.4 Method

8.4.1 The ECHO study

The Experiences of CHildren with cOpy number variants (ECHO) study started in Cardiff University in 2009. The Principal Investigators of the study are my supervisors Dr Marianne van den Bree and Professor Michael Owen. The initial focus of the study was to recruit children with 22q11.2 Deletion Syndrome.

8.4.2 Current sample

As part of the ECHO study, 80 children (50% females) with 22q11.2DS aged 6.6-14.1 years (mean age (SD): 10.2 (2.1)) and 39 sibling controls (56% females) aged 6.3-14.3 years (mean age (SD): 10.9 (2.0)) were recruited from 14 genetics clinics across the UK (figure 8), the British 22q11 Deletion Syndrome charities MaxAppeal and the 22 crew, and our ECHO study website (http://medicine.cardiff.ac.uk/psychological-medicine-neuroscience/areas-research/copy-number-variant-research/research-projects/).
However, numbers of children differ for different measures as 5 children with 22q11.2DS were not able to complete all the cognitive assessments. Furthermore, due to geographical considerations, neuro-cognitive testing was not conducted in 6 children (in these cases psychiatric interviews were conducted using video-call). Table 24 provides a socio-demographic description of the sample.
Table 24 Socio-demographic information of the sample

<table>
<thead>
<tr>
<th>Family ethnic background</th>
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<tr>
<td>European</td>
<td>91.25%</td>
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<tr>
<td>Mixed</td>
<td>6.25%</td>
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<tr>
<td>Non-European</td>
<td>2.5%</td>
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**Highest maternal educational qualification**

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<tr>
<td>Low</td>
<td>38.75%</td>
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<tr>
<td>Middle</td>
<td>40%</td>
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<tr>
<td>High</td>
<td>20%</td>
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<tr>
<td>Unknown</td>
<td>1.25%</td>
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**Family income**

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<tr>
<td>&lt;= £19,999</td>
<td>16.25%</td>
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<tr>
<td>£20,000-£39,000</td>
<td>27.5%</td>
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<tr>
<td>£40,000 - £59,000</td>
<td>18.75%</td>
</tr>
<tr>
<td>&gt;=£60,000</td>
<td>25%</td>
</tr>
<tr>
<td>Unknown</td>
<td>12.5%</td>
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Presence of the deletion was confirmed for all children with 22q11.2DS (87.5% of families were recruited from medical genetics clinics and for the remaining 12.5% medical records were made available by the families).

**8.4.3 Ethical approval**

Informed written consent was obtained prior to recruitment from the carers of the children and recruitment was carried out in agreement with protocols approved by the appropriate research National Health System (NHS) ethics and R&D committees.
8.4.4 *Psychopathology*

Psychopathology was assessed with the Child and Adolescent Psychiatric Assessment (CAPA) (Angold, Prendergast et al. 1995) by means of semi-structured interview with the primary caregiver. In the section of the interview dealing with PEs, initial screening questions probed for any evidence of perceptual disorders or hallucinations, delusions or psychotic abnormalities of thought processes. If these screening questions were answered affirmatively, the interviewer continued with more detailed questions about the nature of possible PEs. Specifically, questions are included regarding content and location of auditory, olfactory and tactile hallucinations. Questions were asked about thought insertion and broadcast, thought echo and withdrawal and delusional thinking. Phenomena not coded as PEs included hypnagogic/pompic hallucinations, eidetic imagery, elaborated fantasies, imaginary companions, illusions, hallucinations occurring as part of a seizure or clouded sensorium, spots/stripes before the eyes and sensory changes associated with headaches. PEs were also assessed by children’s self-reports using the same instrument. All interviews were conducted by trained psychologists, who were supervised by a consultant Child and Adolescent Psychiatrist. Interviews were audio-taped for monitoring and assignment of DSM-IV-TR diagnoses by the Child and Adolescent Psychiatrist. I did not regard ADHD, ODD, Pervasive Developmental Disorder (PDD) and mood and anxiety disorders as mutually exclusive diagnoses.

Autistic behavior was assessed using the Social Communication Questionnaire (SCQ) (Rutter, Bailey et al. 2003) which was completed by the primary caregiver. The SCQ has been suggested as a valid first-screen of ASD in a population cohort of school-aged children with ASD (Chandler, Charman et al. 2007). Total scores can range from 0 to 39. The cut-off of 15 which is suggestive of ASD (Chandler, Charman et al. 2007) was used in my analyses.
With regards to psychotropic medication, one child with 22q11.2DS and one sibling were receiving Ritalin for ADHD.

8.4.5 Cognition

Neuro-cognitive assessments were conducted by trained raters. An estimate of general intelligence (IQ) was obtained by administering the Wechsler Abbreviated Scale of Intelligence (WASI; 4 subtests) (Wechsler 1999). Processing speed was assessed with the Reaction Time (RTI) (child mode) test; attention and vigilance were assessed with the Match to Sample Visual Search (MTS) and the Rapid Visual Information Processing (RVP) (child mode) tests, from the CANTAB (Cambridge Neuropsychological Automated Battery eclipse version3 software) (CANTAB 2006); executive function was assessed with the Spatial Working Memory (SWM) and the Stockings of Cambridge (SOC) tests from the CANTAB. To obtain a global assessment of executive function we also administered the Wisconsin Card Sorting Test (WSCT) (Heaton, Chelune et al. 1993). Each of these tests provided data for normative comparisons which I utilized in order to establish how the cognitive performance of the children with 22q11.2DS and their siblings was in comparison to the general population. A description of the cognitive tests is given in table 25.
<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Cognitive test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing speed</td>
<td>RTI</td>
<td>For this task, the child needs to hold down a press pad button until a yellow spot appears in the screen. Then, the child needs to release the press pad button and touch the spot on the screen, neither too soon nor too late.</td>
</tr>
<tr>
<td>Attention/Vigilance</td>
<td>MTS</td>
<td>An abstract pattern, comprised of four colours appears in a red square in the centre of the screen. After a short delay, a varying number of similar patterns appears around the edge of the screen. Only one of these matches exactly the pattern shown in the red box and the child needs to indicate which one it is by touching it.</td>
</tr>
<tr>
<td></td>
<td>RVPA</td>
<td>A white box appears in the centre of the screen within which a number of numbers, from 2 to 9 appear in a pseudo-random order. The child needs to detect a target sequence (3-5-7) and use the press-pad every time they detect it.</td>
</tr>
<tr>
<td>Executive function</td>
<td>SWM</td>
<td>A number of boxes is displayed in the screen. The child needs to find one blue token by touching each of the boxes and use them to complete an empty column on the right hand side of the screen. Once it is found in one of the boxes then the token will not appear in the same box again.</td>
</tr>
<tr>
<td></td>
<td>SOC</td>
<td>There are two displays in the screen that contain three coloured balls that seem as if they are held in socks. The child needs to copy the pattern shown in the upper display by using the balls in the lower display.</td>
</tr>
<tr>
<td></td>
<td>WCST</td>
<td>This test is comprised of four stimulus cards and 64 response cards that show figures of different forms (crosses, stars, triangles, circles), different numbers of figures (one, two, three, four) and colours (red, blue, yellow, green). The four stimulus card are placed before the child and the child is then given the response cards and is asked to match each response card with one of the four stimulus cards whichever way he or she think it matches. The child is told only whether they are right or wrong but they are not told the sorting principle. Once they have made a specified number of correct responses to the initial sorting principle (usually colour), the sorting principle changes without warning to form or number.</td>
</tr>
</tbody>
</table>
8.4.6 Genotype

Individual genotyping of rs4680 was performed by a lab technician at Cardiff University, by means of single nucleotide primer extension using the SNaPshot™ method (Life Technologies, CA, USA) according to manufacturers’ instructions, with alleles being determined following analysis on an ABI3100 sequencer.

As in Chapter 7, I only included data from white participants of European origin as the frequencies of this allele have been shown to be different in different ethnicities.

8.4.7 Data Analysis

Data analysis was conducted using STATA/IC (version 11) for Windows.

Psychopathology (Goal 1)

The prevalence of psychiatric problems in children with 22q11.2DS compared to their siblings was examined using a χ² test. Associations between age and psychiatric diagnoses were examined using Spearman’s rank correlations, while association between gender and psychiatric diagnoses were examined using χ² tests. To examine whether any of the comorbid diagnoses occurred more frequently than others χ² tests were used.

Nature and prevalence of cognitive impairments (Goal 1)

All neuro-cognitive measures were standardized to have a mean of zero and a standard deviation of one, apart from the ‘failure to maintain set’ variable of the WCST (which is
categorical) and IQ score. Apart from IQ, all other cognitive variables were not normally distributed. To compare the mean differences in cognitive performance of the children with 22q11.2DS with their siblings, t-tests or, in the case of non-normally distributed cognitive variables, Mann-Whitney U tests were conducted. Spearman rank’s correlations were used to estimate the correlations between IQ and age and IQ and gender. A t-test was used examine whether the average VIQ performance in children with 22q11.2DS was different from the average PIQ performance.

**IQ in relation to other cognitive measures and patterns of associations (Goal 1)**

Spearman’s rank correlations were used to estimate the correlations between IQ and other neurocognitive measures. A comparison of the magnitudes of the correlations of the neurocognitive tests for children with 22q11.2DS versus sibling controls was conducted following Fisher's r-to-z transformation (Cohen and Cohen 1983). A two group variance-comparison test using the sdtest command was employed to examine whether there was greater variation in neuro-cognitive performance in the children with 22q11.2DS in relation to their siblings.

**Psychopathology and cognition (Goal 2)**

In order to determine whether the differences in psychiatric diagnoses between the children with 22q11.2DS and their sibling controls could be explained by IQ, I performed the following analyses; independent regressions were performed in the total sample to determine whether the independent variable (i.e., IQ score), predicted presence of psychiatric diagnosis (0=no diagnosis, 1=present diagnosis). From these regressions odds ratios (ORs) and 95% confidence intervals (95%CIs) were derived. To facilitate interpretation of the results, IQ score was standardized to have a mean of zero and a standard deviation of 1 and recoded so
that higher score indicated worse performance. These analyses were conducted only for the psychiatric diagnoses that were significantly different between the two groups: 1. ADHD (absent (0) versus present (1)); 2. any anxiety disorder (absent (0) versus present (1)); 3. ODD (absent (0) versus present (1)); 4. ASD screening (0=SCQ score <15, 1=SCQ score>=15). To test the assumption that the relationship between IQ and psychopathology was similar in children with 22q11.2DS and siblings and that combining the two groups for subsequent analysis was therefore appropriate, homogeneity of ORs was assessed using the chi-squared test of homogeneity. ORs and 95% CIs for the associations between IQ and psychopathology after adjusting for group (22q11.2DS versus siblings) status were estimated using the Mantel-Haenszel (MH) method (Kirkwood and Sterne 2003).

Mediation analysis was also conducted to examine the extent to which IQ score mediated the associations between group status and psychiatric diagnosis. Figure 7 (mediation model) shows a graphic representation of the model tested. The binary_mediation command was used to estimate the indirect effect of IQ on psychopathology, using the product of coefficients approach, which is considered to have good power and low type I error (MacKinnon, Lockwood et al. 2002). Standard errors and biased-corrected confidence intervals were also calculated using bootstrapping with 5000 replications. I repeated these analyses to explore whether the other cognitive measures mediated the relation between the deletion and psychiatric problems. To limit the number of statistical comparisons I examined only the primary scores of these cognitive tests (i.e., MTS total correct; RTI 5-choice reaction time; RVP A'; SOC problems solved in minimum moves; SWM between errors score; and number of perseverative errors). Correction for multiple testing was conducted in the exploratory analyses using the bitesti command in STATA that shows the probability of a result given the number of tests.
**COMT** in relation to the psychiatric and cognitive impairments (Goal 3)

In order to examine whether **COMT** was associated with the psychiatric problems of the children with 22q11.2DS, χ² tests were used. To examine associations of **COMT** with cognitive deficits, I analyzed mean differences between the groups (Val versus Met hemizygosity group) using t-tests or alternatively if the variables were not normally distributed, Mann-Whitney U tests.

### 8.5 Results

#### 8.5.1 Psychopathology (Goal 1)

More than half (54%) of the children with 22q11.2DS met diagnostic criteria for one or more DSM-IV-TR psychiatric disorders, compared to 10% of the siblings (p<0.001, table 26). Children with 22q11.2DS exhibited higher rates of ADHD, ODD and anxiety disorders in relation to their siblings. Furthermore, more children with 22q11.2DS (26%) met the cut-off for probable ASD diagnosis in relation to their siblings (5%).
Table 26 DSM-IV-TR derived diagnoses in children with 22q11.2DS and their siblings

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Children with 22q11.2DS</th>
<th></th>
<th>Siblings</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%) 95% CI</td>
<td>n (%) 95% CI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any diagnosis</td>
<td>43(54.5) 44 64</td>
<td>4(10.5) 6 19</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>21(26.3) 18 36</td>
<td>2(5.3) 2 11</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized Anxiety Disorder</td>
<td>5(6.3) 2 13</td>
<td>0 0 4</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panic disorder without agoraphobia</td>
<td>1(1.3) 0 6</td>
<td>0 0 4</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panic disorder with agoraphobia</td>
<td>2(2.5) 0 9</td>
<td>0 0 4</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agoraphobia without history of panic disorder</td>
<td>1(1.3)a 0 6</td>
<td>0 0 4</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific phobia</td>
<td>12(15.2)a 9 24</td>
<td>1(2.6) 0 9</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social phobia</td>
<td>14(17.5) 11 27</td>
<td>2(5.3) 2 11</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCD</td>
<td>3(3.8) 1 10</td>
<td>0 0 4</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separation Anxiety Disorder</td>
<td>2(9.1) 4 16</td>
<td>0 0 4</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>32(40.5)a 40 60</td>
<td>2(5.3) 2 11</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ODD</td>
<td>15(18.8) 12 28</td>
<td>0a 0 4</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tic disorder</td>
<td>6(7.5) 4 15</td>
<td>1(2.7)a 0 9</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>2(2.5) 0 9</td>
<td>0 0 4</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective mutism</td>
<td>1(1.3) 0 6</td>
<td>0 0 4</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening</td>
<td>20(26.0) 18 36</td>
<td>2(5.3) 2 11</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** OCD=obsessive compulsive disorder; ADHD = attention-deficit/hyperactivity disorder; ODD = oppositional defiant disorder

**Notes:** *a insufficient information to confirm diagnosis for one participant, bASD scoring >=15 on the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)*

Psychiatric diagnoses were not related to age or gender (coded as 0=males and 1=females) in children with 22q11.2DS (table 27).
High rates of comorbidity were observed for children with 22q11.2DS (figure 9): 12 (37.5%) of children with ADHD also had at least one anxiety disorder; 12 (37.5%) had ODD; and 12 (41.2%) screened positive for probable ASD. Among children with an anxiety disorder, 7 (33.3%) also had ODD and 11 (52.4%) screened positive for probable ASD. Among children with ODD, 6 (30%) also screened positive for probable ASD. None of the comorbid diagnoses occurred more frequently than others (p>0.2).

<table>
<thead>
<tr>
<th>Psychiatric diagnosis</th>
<th>rho (Age)</th>
<th>rho (Gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any diagnoses</td>
<td>-0.01</td>
<td>-0.06</td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>ADHD</td>
<td>-0.02</td>
<td>-0.09</td>
</tr>
<tr>
<td>ODD</td>
<td>0.05</td>
<td>-0.03</td>
</tr>
<tr>
<td>ASD screening(a)</td>
<td>0.16</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADHD = Attention-Deficit/Hyperactivity Disorder; ODD = Oppositional Defiant Disorder

**Notes:** *scoring >=15 on the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)
Overall, PEs (parent and self-report combined) were reported by 10% (95%CIs 5% to 18%) of children with 22q11.2DS, and 8% (95%CIs 4% to 15%) of their siblings. Parents reported PEs for 5% (95%CIs 2% to 11%) of the children with 22q11.2DS and none of their siblings, while 6% (95%CIs 2% to 13%) of the children with 22q11.2DS and 8% (95%CIs 4% to 15%) of their siblings self-reported PEs. Parental and child reports were in agreement for only one child (who had 22q11.2DS).

---

6 This figure was done in R with the help of Dr Davy Kavanagh from the Biostatistics and Bioinformatics Unit of Cardiff University.
### 8.5.2 Nature and prevalence of cognitive impairments (Goal 1)

Children with 22q11.2DS had a lower estimated total IQ and performed worse than their siblings on all cognitive tests (figure 10 and table 28). The IQ distribution of the children with 22q11.2DS was shifted over 30 points to the left compared to the sibling controls (figure 10).

**Figure 10 IQ distributions and psychopathology of children with 22q11.2DS and their siblings**

30.6 % of the children with 22q11.2 had mild learning disability (IQ range 53-69), 30.6 % had a borderline IQ score (70-79) and 38.9 % had an average IQ score (81-109). Older children with 22q11.2DS had lower IQs than younger children (r=-0.32, p=0.01) but this was
not the case for siblings ($r=-0.09$, $p=0.61$). No gender differences were found for IQ for either group. There were no differences between VIQ and PIQ for children with 22q11.2DS ($t=-0.24$, $p=0.82$). 22% of children with 22q11.2DS had higher VIQ ($\geq 10$ points) than PIQ, but equally 22% had higher PIQ ($\geq 10$ points) than VIQ. Children with 22q11.2DS were also found to perform worse than their siblings on all other neuro-cognitive tests (table 28).
Table 28 Descriptive statistics of standardized cognitive performance scores in children with 22q11.2DS and their siblings

<table>
<thead>
<tr>
<th>Cognitive measures</th>
<th>22q11.2DS children</th>
<th>Norms better than</th>
<th>N</th>
<th>Siblings mean (SD)</th>
<th>Norms better than</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean (SD)</td>
<td>5%</td>
<td>n</td>
<td>mean (SD)</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>WASI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>72</td>
<td>76.76</td>
<td>13.0</td>
<td>36</td>
<td>108.56</td>
<td>15.2</td>
<td>70%</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>72</td>
<td>78.49</td>
<td>14.2</td>
<td>36</td>
<td>107.11</td>
<td>13.3</td>
<td>68%</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>74</td>
<td>78.84</td>
<td>12.4</td>
<td>35</td>
<td>108.63</td>
<td>16.7</td>
<td>70%</td>
</tr>
<tr>
<td>CANTAB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>72</td>
<td>-0.24</td>
<td>1.1</td>
<td>n/a</td>
<td>0.48</td>
<td>0.5</td>
<td>n/a</td>
</tr>
<tr>
<td>RTI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-choice movement time</td>
<td>70</td>
<td>-0.16</td>
<td>1.1</td>
<td>65%</td>
<td>0.33</td>
<td>0.5</td>
<td>90%</td>
</tr>
<tr>
<td>5-choice reaction time</td>
<td>70</td>
<td>-0.21</td>
<td>1.1</td>
<td>35%</td>
<td>0.44</td>
<td>0.4</td>
<td>70%</td>
</tr>
<tr>
<td>RVPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP A'</td>
<td>67</td>
<td>-0.23</td>
<td>1.1</td>
<td>5%</td>
<td>0.47</td>
<td>0.4</td>
<td>30%</td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean initial thinking time (5 moves)</td>
<td>65</td>
<td>-0.02</td>
<td>1.2</td>
<td>75%</td>
<td>0.04</td>
<td>0.5</td>
<td>80%</td>
</tr>
<tr>
<td>Mean subsequent thinking time (5 moves)</td>
<td>65</td>
<td>-0.16</td>
<td>1.0</td>
<td>15%</td>
<td>0.29</td>
<td>1.0</td>
<td>35%</td>
</tr>
<tr>
<td>Problems solved in minimum moves</td>
<td>69</td>
<td>-0.32</td>
<td>0.9</td>
<td>5%</td>
<td>0.67</td>
<td>0.9</td>
<td>50%</td>
</tr>
<tr>
<td>SWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between errors</td>
<td>74</td>
<td>-0.30</td>
<td>0.9</td>
<td>30%</td>
<td>0.61</td>
<td>1.0</td>
<td>50%</td>
</tr>
<tr>
<td>Strategy</td>
<td>74</td>
<td>-0.19</td>
<td>0.9</td>
<td>40%</td>
<td>0.41</td>
<td>1.1</td>
<td>60%</td>
</tr>
<tr>
<td>WCST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>71</td>
<td>-0.31</td>
<td>1.1</td>
<td>21%</td>
<td>0.59</td>
<td>0.5</td>
<td>86%</td>
</tr>
<tr>
<td>Nonperseverative errors</td>
<td>71</td>
<td>-0.21</td>
<td>1.1</td>
<td>45%</td>
<td>0.39</td>
<td>0.5</td>
<td>68%</td>
</tr>
<tr>
<td>Failure to maintain set (%)</td>
<td>71</td>
<td>-0.21</td>
<td>1.1</td>
<td>45%</td>
<td>0.39</td>
<td>0.5</td>
<td>68%</td>
</tr>
<tr>
<td>0 time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>63.4</td>
<td>58.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonperseverative errors</td>
<td>23.9</td>
<td>30.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 times</td>
<td>12.7</td>
<td>11.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Abbreviations:** WASI=Wechsler Abbreviated Scale of Intelligence; CANTAB=Cambridge Neuropsychological Test Automated Battery; MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=Rapid Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=Wisconsin Card Sorting Test

**Notes:** 6 children with 22q11.2DS and 3 siblings did not complete any cognitive assessment, 3 children with 22q11.2DS did not do the WCST, 2 children with 22q11.2DS were mute and therefore did not complete the WASI, other missing scores were either due to the child’s cognitive or behavioural issues.
8.5.3 IQ in relation to the other cognitive measures and patterns of associations (Goal 1)

Correlation analysis (table 29) indicated that the CANTAB measures and the WCST did tap into neuro-cognitive domains that were independent of IQ in children with 22q11.2DS.

Table 29 Associations between IQ score and the other neuro-cognitive measures in children with 22q11.2DS

<table>
<thead>
<tr>
<th>Cognitive measure</th>
<th>IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rho</td>
</tr>
<tr>
<td><strong>CANTAB</strong></td>
<td></td>
</tr>
<tr>
<td>MTS</td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>RTI</strong></td>
<td></td>
</tr>
<tr>
<td>5-choice movement time</td>
<td>0.12</td>
</tr>
<tr>
<td>5-choice reaction time</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>RVPA</strong></td>
<td></td>
</tr>
<tr>
<td>RVP A’</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>SOC</strong></td>
<td></td>
</tr>
<tr>
<td>Mean initial thinking time</td>
<td>-0.04</td>
</tr>
<tr>
<td>Mean subsequent thinking time</td>
<td>0.08</td>
</tr>
<tr>
<td>Problems solved in minimum moves</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>SWM</strong></td>
<td></td>
</tr>
<tr>
<td>Between errors</td>
<td>0.35</td>
</tr>
<tr>
<td>Strategy</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>WCST</strong></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>0.25</td>
</tr>
<tr>
<td>Nonperseverative errors</td>
<td>-0.13</td>
</tr>
<tr>
<td>Failure to maintain set</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

**Abbreviations:** WASI=Wechsler Abbreviated Scale of Intelligence; CANTAB=Cambridge Neuropsychological Test Automated Battery; MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=Rapid Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=Wisconsin Card Sorting Test
As a sensitivity analysis, I reran the analyses comparing cognitive performance in children with 22q11.2DS and sibling controls, including only children with 22q11.2DS with IQ>70 and results remained substantially the same, suggesting the findings were not driven by the subgroup with mild learning disability (table 30).
Table 30 Descriptive statistics of standardized cognitive performance scores in children with 22q11.2DS and their siblings when IQ>70

<table>
<thead>
<tr>
<th>Cognitive measures</th>
<th>22q11.2DS children</th>
<th>Siblings</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean (SD)</td>
<td>n</td>
<td>mean (SD)</td>
</tr>
<tr>
<td>WASI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>48</td>
<td>83.6 (9.7)</td>
<td>36</td>
<td>108.6 (15.2)</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>48</td>
<td>85.7 (11.0)</td>
<td>36</td>
<td>107.1 (13.3)</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>48</td>
<td>84.7 (10.4)</td>
<td>35</td>
<td>108.6 (16.7)</td>
</tr>
<tr>
<td>CANTAB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>47</td>
<td>-0.03 (0.90)</td>
<td>39</td>
<td>0.48 (0.496)</td>
</tr>
<tr>
<td>RTI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-choice movement time</td>
<td>45</td>
<td>0.02 (0.65)</td>
<td>33</td>
<td>0.33 (0.48)</td>
</tr>
<tr>
<td>5-choice reaction time</td>
<td>45</td>
<td>-0.06 (0.87)</td>
<td>33</td>
<td>0.44 (0.38)</td>
</tr>
<tr>
<td>RVPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP A'</td>
<td>45</td>
<td>-0.17 (1.25)</td>
<td>32</td>
<td>0.47 (0.39)</td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean initial thinking time (5 moves)</td>
<td>45</td>
<td>-0.08 (1.39)</td>
<td>34</td>
<td>0.04 (0.45)</td>
</tr>
<tr>
<td>Mean subsequent thinking time (5 moves)</td>
<td>45</td>
<td>-0.27 (1.06)</td>
<td>34</td>
<td>0.29 (0.97)</td>
</tr>
<tr>
<td>Problems solved in minimum moves</td>
<td>47</td>
<td>-0.23 (0.95)</td>
<td>34</td>
<td>0.67 (0.87)</td>
</tr>
<tr>
<td>SWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between errors</td>
<td>47</td>
<td>-0.09 (0.83)</td>
<td>36</td>
<td>0.61 (1.03)</td>
</tr>
<tr>
<td>Strategy</td>
<td>47</td>
<td>-0.04 (0.85)</td>
<td>36</td>
<td>0.41 (1.10)</td>
</tr>
<tr>
<td>WCST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>47</td>
<td>-0.10 (0.86)</td>
<td>36</td>
<td>0.59 (0.48)</td>
</tr>
<tr>
<td>Nonperseverative errors</td>
<td>47</td>
<td>-0.25 (1.02)</td>
<td>36</td>
<td>0.39 (0.50)</td>
</tr>
<tr>
<td>Failure to maintain set (%)</td>
<td>47</td>
<td>-0.10 (0.86)</td>
<td>36</td>
<td>-4.29 (0.048)</td>
</tr>
<tr>
<td>0 time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1 times</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** WASI=Wechsler Abbreviated Scale of Intelligence; CANTAB=Cambridge Neuropsychological Test Automated Battery; MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=Rapid Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=Wisconsin Card Sorting Test
The pattern of associations between the neuro-cognitive tests ranged from low to modest in strength (table 31) indicating that these cognitive tests do measure different cognitive abilities in both groups of children. Although the correlations between the tests tended to be somewhat higher for children with 22q11.2DS (range 0.15 to 0.55) compared to sibling controls (-0.17 to 0.44), formal comparison indicated the magnitude of associations was higher in children with 22q11.2DS for only 2 out of 15 correlations: RVPA’ test with both the RTI test and perseverative errors of the WCST. The RTI test showed the highest correlations not only with the measures within the attention domain (e.g., RVPA’ test) but also with all the measures of executive function (e.g., SOC test) for children with 22q11.2DS.
Table 31 Associations between primary neuro-cognitive scores

<table>
<thead>
<tr>
<th>Cognitive measure</th>
<th>MTS 22q11.2DS Siblings</th>
<th>RTI 22q11.2DS Siblings</th>
<th>RVPA’ 22q11.2DS Siblings</th>
<th>SOC 22q11.2DS Siblings</th>
<th>SWM 22q11.2DS Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTS</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTI</td>
<td>0.28</td>
<td>0.07</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RVPA’</td>
<td>0.33</td>
<td>-0.07</td>
<td>0.55</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>SOC</td>
<td>0.18</td>
<td>-0.12</td>
<td>0.35</td>
<td>0.35</td>
<td>0.28</td>
</tr>
<tr>
<td>SWM</td>
<td>0.15</td>
<td>-0.17</td>
<td>0.26</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>WCST</td>
<td>0.16</td>
<td>0.02</td>
<td>0.25</td>
<td>0.09</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Abbreviations:** MTS=Match to Sample Visual Search (total correct); RT=Reaction Time (5-choice reaction time); RVPA=Rapid Visual Information Processing (RVP A’); SOC=Stockings of Cambridge (problems solved in minimum moves); SWM=Spatial Working Memory (between errors); WCST=Wisconsin Card Sorting Test (perseverative errors).

**Notes:** Figures in *italic* type reflect differences in magnitude of the correlations between the two groups, both at the level of p<.05. Figures in *bold* signify statistical significance.
Finally, there were larger standard deviations for most cognitive tests indicating greater variation in the cognitive performance of children with 22q11.2DS in relation to their siblings, with the exception of IQ and SWM tests (table 32).
Table 32 Results from two-group variance-comparison test in standardized cognitive performance between children with 22q11.2DS and their siblings

<table>
<thead>
<tr>
<th>Cognitive measures</th>
<th>Children with 22q11.2DS</th>
<th>Siblings</th>
<th>SD</th>
<th>SD</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>13.0</td>
<td>15.0</td>
<td>1.36</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>14.2</td>
<td>13.1</td>
<td>0.88</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance IQ</td>
<td>12.4</td>
<td>16.6</td>
<td>1.84</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CANTAB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTS</td>
<td>1.1</td>
<td>0.5</td>
<td>0.17</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTI</td>
<td>1.1</td>
<td>0.5</td>
<td>0.18</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-choice movement time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-choice reaction time</td>
<td>1.1</td>
<td>0.4</td>
<td>0.11</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP A’</td>
<td>1.1</td>
<td>0.4</td>
<td>0.12</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean initial thinking time (5 moves)</td>
<td>1.2</td>
<td>0.4</td>
<td>0.14</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean subsequent thinking time (5 moves)</td>
<td>1.0</td>
<td>1.0</td>
<td>0.97</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems solved in minimum moves</td>
<td>0.9</td>
<td>0.9</td>
<td>0.94</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between errors</td>
<td>1.0</td>
<td>0.9</td>
<td>1.47</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strategy</td>
<td>1.1</td>
<td>0.9</td>
<td>1.52</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>1.1</td>
<td>0.5</td>
<td>0.20</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonperseverative errors</td>
<td>1.1</td>
<td>0.5</td>
<td>0.20</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** WASI=Wechsler Abbreviated Scale of Intelligence; CANTAB=Cambridge Neuropsychological Test Automated Battery; MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=Rapid Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=Wisconsin Card Sorting Test

**Notes:** 6 children with 22q11.2DS and 3 siblings did not complete any cognitive assessment, 3 children with 22q11.2DS did not do the WCST, 2 children with 22q11.2DS were mute and therefore did not complete the WASI, other missing scores were either due to the child’s cognitive or behavioural issues.
8.5.4 Psychopathology and cognition (Goal 2)

Initial analyses in the total sample of children with 22q11.2DS and their siblings showed a relationship between IQ and risk for psychopathology (i.e., ADHD diagnosis, any anxiety disorder, and ASD screening) (table 33). To be more exact, lower IQ was associated with higher risk of psychopathology. In analyses stratified by group status, however, there was no evidence of association between IQ and psychopathology either in children with 22q11.2DS or their siblings.
Table 33 The relation between IQ and psychiatric diagnoses before and after adjustment for group status

<table>
<thead>
<tr>
<th>Psychiatric diagnosis</th>
<th>n</th>
<th>Non-adjusted ORs (95%CI)</th>
<th>Children with 22q11.2DS</th>
<th>Siblings</th>
<th>Adjusted ORs (95%CI)</th>
<th>Stratified ORs (95%)</th>
<th>Stratified ORs (95%)</th>
<th>Homogeneity of ORs</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>107</td>
<td>1.80</td>
<td>1.12</td>
<td>2.90</td>
<td>0.66</td>
<td>1.39</td>
<td>0.24</td>
<td>10.96</td>
<td>0.74</td>
<td>0.37</td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>108</td>
<td>1.90</td>
<td>1.08</td>
<td>3.35</td>
<td>1.40</td>
<td>3.15</td>
<td>0.62</td>
<td>3.35</td>
<td>0.47</td>
<td>0.07</td>
</tr>
<tr>
<td>ASD screening</td>
<td>106</td>
<td>1.86</td>
<td>1.04</td>
<td>3.32</td>
<td>1.35</td>
<td>3.12</td>
<td>0.58</td>
<td>3.12</td>
<td>0.47</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADHD = Attention-Deficit/Hyperactivity Disorder; ODD = Oppositional Defiant Disorder

**Notes:** *scoring >=15 in the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis).

IQ score has been standardized to have a mean of zero and a standard deviation of one and recoded so that high score indicates worse performance. Adjusted ORs are ORs adjusted for group status.
In the mediation analyses, with IQ as the mediator, the indirect effects via IQ (figure 7, mediation model, paths a and b) were considerably weaker than the direct effects between group status and psychopathology (path c) suggesting that psychiatric disorder in 22q11.2DS is unlikely to result indirectly, through impaired IQ (table 34).

Table 34 Direct and indirect effects via IQ of group status (22q11.2DS status vs. control status) on psychiatric diagnoses

<table>
<thead>
<tr>
<th>Psychiatric diagnosis</th>
<th>Indirect effect (mediated via IQ) (95%CI)</th>
<th>Direct effect (95%CI)</th>
<th>Total effect (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>-0.10 -0.35 0.13</td>
<td>0.66 0.31 0.93</td>
<td>0.56 0.35 0.70</td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>0.07 -0.17 0.27</td>
<td>0.38 0.01 0.66</td>
<td>0.44 0.20 0.63</td>
</tr>
<tr>
<td>ASD screening(^a)</td>
<td>0.05 -0.25 0.32</td>
<td>0.39 0.00 0.70</td>
<td>0.44 0.17 0.62</td>
</tr>
</tbody>
</table>

*Abbreviations:* ADHD = Attention-Deficit/Hyperactivity Disorder; ODD = Oppositional Defiant Disorder

*Notes:* \(^a\)scoring >=15 in the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)

As an exploratory analysis, regression analyses were conducted for other cognitive measures (table 35). Results showed an association between ADHD and the MTS (an indicator for attention) for the siblings, an association between ADHD and RVPA’ (an indicator of attention) for the whole sample, an association between ASD and SOC (an indicator of executive function) for the children with 22q11.2DS, and finally an association between ADHD and SWM (an indicator of working memory) for the whole sample.
Table 35 The relation between IQ and psychiatric diagnoses before and after adjustment for group status

<table>
<thead>
<tr>
<th>Cognitive test &amp; Psychiatric diagnosis</th>
<th>N</th>
<th>Non-adjusted ORs (95%CI)</th>
<th>Children with 22q11.2DS Stratified ORs (95%)</th>
<th>Siblings Stratified ORs (95%)</th>
<th>Adjusted ORs (95%CI)</th>
<th>Homogeneity of ORs</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stratified ORs (95%)</td>
<td>Stratified ORs (95%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>107</td>
<td>1.23 0.83 1.82</td>
<td>0.87 0.57 1.33</td>
<td>40.8 1.84 909.1</td>
<td>0.93 0.62 1.42</td>
<td>5.80 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>108</td>
<td>0.93 0.57 1.53</td>
<td>0.78 0.49 1.26</td>
<td>0.27 0.01 6.01</td>
<td>0.77 0.48 1.22</td>
<td>0.44 0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06</td>
<td>1.12 0.71 1.77</td>
<td>0.92 0.57 1.49</td>
<td>0.59 0.03 13.00</td>
<td>0.91 0.57 1.46</td>
<td>0.08 0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTI</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>102</td>
<td>1.40 0.93 2.11</td>
<td>1.15 0.75 1.75</td>
<td>0.39 0.01 16.00</td>
<td>1.13 0.75 1.72</td>
<td>0.32 0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>103</td>
<td>0.92 0.55 1.55</td>
<td>0.81 0.51 1.30</td>
<td>0.17 0.00 6.82</td>
<td>0.79 0.49 1.26</td>
<td>0.69 0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101</td>
<td>0.97 0.58 1.61</td>
<td>0.86 0.53 1.39</td>
<td>0.06 0.00 2.5</td>
<td>0.82 0.51 1.32</td>
<td>1.92 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>98</td>
<td>2.51 1.25 5.03</td>
<td>1.49 0.97 2.30</td>
<td>0.14 0.00 5.26</td>
<td>1.45 0.94 2.22</td>
<td>1.62 0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>99</td>
<td>1.16 0.75 1.77</td>
<td>1.02 0.63 1.64</td>
<td>0.11 0.00 4.12</td>
<td>0.98 0.61 1.57</td>
<td>1.42 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97</td>
<td>1.59 0.92 2.78</td>
<td>1.42 0.87 2.31</td>
<td>0.35 0.01 13.0</td>
<td>1.39 0.86 2.24</td>
<td>0.57 0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>102</td>
<td>1.44 0.92 2.56</td>
<td>0.88 0.52 1.50</td>
<td>1.99 0.39 10.27</td>
<td>0.95 0.58 1.58</td>
<td>0.85 0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>103</td>
<td>1.56 0.92 2.63</td>
<td>0.97 0.54 1.75</td>
<td>5.01 0.97 25.86</td>
<td>1.17 0.67 2.03</td>
<td>3.41 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101</td>
<td>0.89 0.55 1.47</td>
<td>0.51 0.28 0.93</td>
<td>1.99 0.39 10.27</td>
<td>0.60 0.34 1.06</td>
<td>2.33 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>109</td>
<td>1.79 1.12 2.87</td>
<td>1.21 0.70 2.08</td>
<td>2.11 0.53 8.44</td>
<td>1.30 0.78 2.16</td>
<td>0.54 0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>110</td>
<td>1.43 0.86 2.36</td>
<td>1.07 0.58 1.95</td>
<td>1.25 0.31 5.02</td>
<td>1.09 0.63 1.90</td>
<td>0.04 0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108</td>
<td>1.45 0.87 2.41</td>
<td>1.10 0.60 2.03</td>
<td>1.22 0.31 4.87</td>
<td>1.12 0.64 1.96</td>
<td>0.02 0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>106</td>
<td>1.03 0.69 1.55</td>
<td>0.67 0.43 1.04</td>
<td>2.19 0.11 43.1</td>
<td>0.68 0.44 1.06</td>
<td>0.60 0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety disorder</td>
<td>107</td>
<td>1.25 0.81 1.93</td>
<td>0.92 0.57 1.50</td>
<td>4.69 0.23 90.5</td>
<td>0.96 0.59 1.55</td>
<td>1.09 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASD screening&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105</td>
<td>1.47 0.95 2.27</td>
<td>1.22 0.75 2.00</td>
<td>0.30 0.02 5.96</td>
<td>1.18 0.73 1.92</td>
<td>0.82 0.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=RAPID Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=WISCONSIN Card Sorting Test; ADHD = Attention-Deficit/Hyperactivity Disorder

Notes: <sup>a</sup> scoring >=15 in the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)
For these associations, further mediation analyses were conducted (table 36). It is noteworthy that with regards to the association between ADHD and MTS in the siblings, there was evidence of heterogeneity of the odds ratios and therefore I did not conduct mediation analysis for this association. Mediation analyses only showed an indirect effect for the SOC task component problems solved in minimum moves (-12, 95%CI: -0.27 to -0.008) and ASD (table 36). However, given the number of tests in the exploratory analysis (n=21), this effect did not survive correction for multiple testing because none of the associations were at p <0.003 level.
Table 36 Direct and indirect effects via IQ of group status (22q11.2DS status vs. control status) on psychiatric diagnoses

<table>
<thead>
<tr>
<th></th>
<th>Indirect effect (95%CI)</th>
<th>Direct effect (95%CI)</th>
<th>Total effect (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD through RVPA</td>
<td>0.08 -0.01 0.19</td>
<td>0.44 0.22 0.66</td>
<td>0.52 0.31 0.69</td>
</tr>
<tr>
<td>ASD screening through SOC</td>
<td>-0.12 -0.25 -0.009</td>
<td>0.52 0.19 0.77</td>
<td>0.40 0.10 0.60</td>
</tr>
<tr>
<td>ADHD through SWM</td>
<td>0.05 -0.04 0.16</td>
<td>0.50 0.21 0.67</td>
<td>0.55 0.34 0.69</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADHD = Attention-Deficit/Hyperactivity Disorder; ODD = Oppositional Defiant Disorder; RVPA = Rapid Visual Information Processing; SOC = Stockings of Cambridge; SWM = Spatial Working Memory

**Notes:** a scoring >=15 in the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)
8.5.5 **COMT in relation to the psychiatric and cognitive impairments (Goal 3)**

No evidence was found for associations between *COMT* (rs4680) and the cognitive measures or psychiatric diagnoses in children with 22q11.2DS (table 37).
Table 37 Associations between COMT and i) psychiatric diagnoses and ii) cognitive measures in children with 22q11.2DS

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Val</th>
<th>Met</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (%males)</td>
<td>38.7</td>
<td>56.8</td>
<td>2.20</td>
<td>0.14</td>
</tr>
<tr>
<td>Age</td>
<td>31</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Anxiety Disorder</td>
<td>6(35.3)</td>
<td>11(64.7)</td>
<td>0.97</td>
<td>0.33</td>
</tr>
<tr>
<td>ADHD</td>
<td>12(44.4)</td>
<td>15(55.6)</td>
<td>0.02</td>
<td>0.88</td>
</tr>
<tr>
<td>ODD</td>
<td>6(50)</td>
<td>6(50)</td>
<td>0.11</td>
<td>0.74</td>
</tr>
<tr>
<td>ASD screening</td>
<td>9(56.3)</td>
<td>7(43.8)</td>
<td>1.30</td>
<td>0.25</td>
</tr>
<tr>
<td>Cognitive measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WASI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>28</td>
<td>33</td>
<td>0.12</td>
<td>0.90</td>
</tr>
<tr>
<td>CANTAB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>26</td>
<td>35</td>
<td>1.00</td>
<td>0.32</td>
</tr>
<tr>
<td>RTI</td>
<td>27</td>
<td>33</td>
<td>-0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>RVPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP A'</td>
<td>26</td>
<td>32</td>
<td>-0.45</td>
<td>0.65</td>
</tr>
<tr>
<td>SOC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems solved in minimum moves</td>
<td>27</td>
<td>32</td>
<td>-0.78</td>
<td>0.43</td>
</tr>
<tr>
<td>SWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between errors</td>
<td>27</td>
<td>36</td>
<td>-1.05</td>
<td>0.29</td>
</tr>
<tr>
<td>WCST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perseverative errors</td>
<td>28</td>
<td>33</td>
<td>0.24</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Abbreviations:** WASI=Wechsler Abbreviated Scale of Intelligence; CANTAB=Cambridge Neuropsychological Test Automated Battery; MTS=Match to Sample Visual Search; RT=Reaction Time; RVPA=Rapid Visual Information Processing; SOC=Stockings of Cambridge; SWM=Spatial Working Memory; WCST=Wisco nsin Card Sorting Test; ADHD = Attention-Deficit/Hyperactivity Disorder; ODD = Oppositional Defiant Disorder

**Notes:** aScoring >=15 in the Social Communication Questionnaire (suggestive of Autism Spectrum Disorder diagnosis)
8.6 Discussion

Psychopathology

More than half (54%) of children with 22q11.2DS met diagnostic criteria for one or more DSM-IV-TR psychiatric disorders. They had higher rates of ADHD, ASD, ODD and anxiety disorders compared to sibling controls. These rates are broadly similar to those reported in previous studies of children with 22q11.2DS (e.g., (Green, Gothelf et al. 2009)) and population studies of children with intellectual disabilities (Barkley 1990, Dekker and Koot 2003, Emerson 2003) indicating that in childhood, 22q11.2DS does not seem to be related to a specific psychiatric phenotype (Karayiorgou, Simon et al. 2010), at least as captured by current diagnostic systems (Antshel, Faraone et al. 2007). It also suggests that these disorders can share underlying pathogenic mechanisms with intellectual disability. Comorbidity was also substantial, providing further evidence that the effect of the deletion is widespread across a range of neurodevelopmental syndromes. This is in keeping with previous studies of 22q11.2DS (e.g., (Jansen, Duijff et al. 2007)), studies of rare CNVs and single gene disorders which similarly tend to be associated with increased risk of many different types of psychopathology (e.g., (Miller, Shen et al. 2009)).

The frequency of PEs was similar between children with 22q11.2DS and their siblings and similar to rates reported in the general population (as shown in Chapter 6), indicating that at this age children with 22q11.2Ds are too young to exhibit (or adequately describe) the higher rates of PEs robustly seen in late adolescence and adulthood. Interestingly, parent and child reports were in agreement only for one child with 22q11.2DS and none of the siblings. For
the siblings, in particular, PEs were only self-reported. This could be because children are not willing to share these symptoms with their parents or due to difficulties associated with obtaining reliable data on PEs in children of this age.

**Cognition**

The findings demonstrate that the IQ distribution of the children with 22q11.2DS is shifted over 30 points to the left in relation to their siblings. This is in agreement with previous studies in 22q11.2DS (e.g., (Hooper, Curtiss et al. 2013)) as well as the literature on single gene disorders (e.g., fragile X (Tranfaglia 2011)) and other neurodevelopmental syndromes (e.g., ASD and ADHD (Langley, Martin et al. 2011)). Even though performance on the neuro-cognitive tests was generally impaired in the entire sample of children with 22q11.2DS, it was also variable. That is, performance on IQ was unrelated to performance on most cognitive tests, a finding in accordance with reports in other neurodevelopmental disorders (e.g., ADHD (Rommelse, Altink et al. 2008)). Furthermore, amongst the neuro-cognitive tests, performance in one domain did not represent a good indicator of performance in another domain (as evidenced by modest correlation coefficients). However, for children with 22q11.2DS performance on the Rapid Visual Information Processing test (an indicator of attention) was more strongly associated with performance on the Reaction Time test (an indicator of processing speed) as well as with the number of perseverative errors from the WCST (an indicator of executive function) than for their siblings.

Generally, the 5-choice Reaction Time score (an indicator of processing speed) showed the highest correlations (0.25 to 0.55) for children with 22q11.2DS, not only with measures within the domain of attention, but also with all measures of executive function. This is in accordance with the existing literature that underlines the relevance and importance of
processing speed for both typical and atypical development. This is also in line with the results in Chapter 6, where amongst a range of cognitive tests, processing speed was found to best predict PEs in children from the general population. However, the small number of children exhibiting PEs in this study did not allow testing for correlations between the processing speed test and PEs.

Furthermore, children with 22q11.2DS had greater variation in cognitive performance in relation to their siblings, a finding in agreement with a previous report in 22q11.2DS (Rockers, Ousley et al. 2009). This variation could be due to the deletion having widespread effects in cognitive performance. However, the possibility of other confounding or mediating factors, such as more attention or behavioural problems in children with 22q11.2DS in relation to their siblings, which might have influenced their performance in the cognitive tests, cannot be excluded. Moreover, I did not find evidence of better verbal than non-verbal abilities, in line with some (e.g., (Lewandowski, Shashi et al. 2007)) but not all previous studies (e.g., (De Smedt, Devriendt et al. 2007)). Moreover, though previous studies have shown discrepancies between VIQ and PIQ (usually between 8 to 10 points (e.g., (Moss, Batshaw et al. 1999))), the number of children with better VIQ than PIQ (>=10 points) in our study was similar to the number with better PIQ than VIQ (>=10 points). Therefore this study does not support the hypothesis that 22q11.2DS is specifically associated with Non-Verbal Learning Disability.

Lack of associations between cognitive impairment and psychopathology

There has been disagreement on whether there is a relationship between IQ and psychopathology with some studies reporting relationships (e.g., (De Smedt, Devriendt et al.
2007)) while others do not (e.g., (Antshel, Fremont et al. 2006)). In line with my predictions, there was no relation between psychopathology and IQ in children with 22q11.2DS.

The availability of a sample of siblings in which the same assessments were conducted as in the children with 22q11.2DS, in combination with a relatively large sample size allowed the first mediation analysis for this syndrome to be conducted. The findings showed that psychopathology in 22q11.2DS is not explained by indirect effects, mediated by the children’s intellectual impairment.

I also explored the associations with other neuro-cognitive measures and results indicated only an indirect effect of the deletion to probable ASD diagnosis through the Stockings of Cambridge (an indicator of planning) test. This effect, however, was in the opposite direction from what I expected and therefore, given the exploratory nature of the analysis, I interpreted this result as lack of evidence for a mediated effect of the deletion through cognition.

The absence of an association between cognitive impairment and psychopathology as well as the absence of an indirect effect of the deletion on psychopathology through cognitive function, might seem contrary to my findings in Chapters 6 and 7 where there was evidence of associations between cognitive function and PEs and evidence of an indirect effect of COMT on PEs through processing speed and attention. I will discuss this in the General Discussion as well as how the dimensional model of psychosis can accommodate these controversies.

\textit{COMT}
Contrary to my initial hypothesis, I did not find evidence for a relationship between rs4680 and prefrontal cognition or psychopathology in children with 22q11.2DS. Different sample sizes, age ranges and assessment measures compared to the previous studies might account for discrepancies. Low statistical power is also a very likely explanation. Moreover, it should be noted that the functional implications of COMT haploinsufficiency are largely unknown and that the relationship to cognition and psychopathology is likely to be complex. As stated in Chapter 7, research has shown the existence of additional risk variants within the COMT region that influence mRNA expression (Bray, Buckland et al. 2003) and translation (Nackley, Shabalina et al. 2006) as well as enzyme activity (Chen, Lipska et al. 2004). These SNPs, however, were not available at the time of my analysis.

Furthermore, the transition from childhood to adolescence is characterized by an increase in brain dopamine levels during adolescence. Therefore, it is likely that these associations might change over time. Indeed, results from a longitudinal study of children with 22q11.2DS that were followed into late adolescence showed that the Met allele was associated with decline in verbal IQ and language scores and more severe PEs, in adolescents and young adults, but not in children (Gothelf, Eliez et al. 2005). However, given that single SNPs associations with phenotypes are expected to be of small effect size in addition to the small study sample (n=31) it is very likely that this finding reflects a Type I error.

Theoretical implications

These findings raise a number of important issues. First, it is not yet known how the deletion can manifest itself in such a clinically variable manner. Various genetic factors could contribute to this variability, including the combined effect of reduced genetic dosage along with background genetic variation, in the 22q11.2 region, and elsewhere in the genome, as
well as positional effects (Williams 2011). For example, it has been hypothesized that dopamine dysregulation resulting from reduced gene dosage arising from the deletion could lead to brain disconnectivity and in turn to predisposition to the cognitive and psychiatric deficits for a subgroup of individuals with 22q11.2DS (Karayiorgou, Simon et al. 2010). Moreover, it is likely that environmental exposures also play a role.

These findings suggest that the 22q11.2 deletion has pleiotropic effects on IQ and psychopathology (O'Donovan, Kirov et al. 2008). In other words, the deletion has largely independent effects on IQ and risk of psychopathology. This implies that the associations seen more generally between impaired IQ and psychopathology might reflect the fact that cognitive impairments index genetic risk factors for psychiatric syndromes rather than that impaired cognition mediates the effects of risk.

Clinical implications

The results indicate that the prevalence of psychopathology in children with 22q11.2DS is high, with over half of young patients meeting DSM-IV-TR criteria. The prevalent diagnoses, which also often occur comorbidly in children with 22q11.2DS, include ADHD, anxiety disorders, ODD and high rates of autistic traits. The prevalence of intellectual disability is also high with 31% of children with the deletion having mild learning disability. Moreover, children with 22q11.2DS seem to exhibit a non-specific cognitive profile with generally impaired cognitive performance, which is more variable than in the non-deleted population (as indicated by larger standard deviations in the performance of children with 22q11.2DS in relation to their siblings).
Furthermore, these findings underline the potential implications of childhood 22q11.2DS for parents and health services and indicate the importance of early detection and treatment. The finding that psychopathology is not mediated by the children’s intellectual impairment suggests that, while cognitive remediation might have benefits on neuro-cognitive function, this is unlikely to ameliorate the mechanisms underlying psychopathology in children with 22q11.2DS.

These findings also provide no support for the suggestion that psychopathology in 22q11.2DS is a non-specific consequence of intellectual impairment. Rather, they are consistent with the conclusion that the mechanisms by which psychopathology is produced in 22q11.2DS might resemble those operating in more typical cases, and that 22q11.2DS might serve as a model in which mechanisms of childhood psychopathology might be studied.

**Strengths and limitations**

This the one of largest studies to date to include children with 22q11.2DS with a relatively narrow age range as well as controls, and one of the few studies to obtain detailed psychiatric as well as neuro-cognitive phenotypic information. Moreover, this is the first study to use mediation analysis to further examine the relationships between IQ and psychopathology. Given that children with 22q11.2DS may have a risk of developing schizophrenia in adulthood in the order of 30% (Murphy, Jones et al. 1999, Bassett, Chow et al. 2005), follow-up of these children (which is currently undertaken by the PIs on the ECHO study) will allow us to identify risk and protective factors for schizophrenia and to study potential mechanisms and markers of high risk that are unconfounded by reverse causation and medication effects.
On the other hand, even though this study is one of the largest to date, low statistical power could be an explanation for the non-significant results. Also, even though the age range of the children is narrow in comparison to previous studies in children with 22q11.2DS, the possibility that significant developmental changes during that period might have influenced our findings cannot be excluded. Finally, the deletion status of the siblings is not confirmed. Nevertheless, this is more likely to have underestimated the associations.

**Conclusions**

I set out to determine the prevalence and nature of psychopathology and neuro-cognitive dysfunction, and their inter-relationship, in children with 22q11.2DS. There were high rates of psychopathology and impairments across a wide range of neuro-cognitive functions as compared to sibling controls. I found no evidence that the presence of psychopathology is correlated with neuro-cognitive impairment and it would appear that the former is not mediated by the latter. These findings have important clinical and theoretical implications. Further work is required to determine whether these findings can be extended to older age groups with 22q11.2DS, other CNVs and other high-risk samples.

In the following section I will provide an overview of the findings of this thesis and discuss their overall theoretical and clinical implications as well as avenues for future research.
Chapter 9 Discussion of Thesis Findings

9.1 Overview

Schizophrenia is a disabling lifelong disorder and a leading cause of suffering for patients and their families. The limited understanding of its pathophysiology has restricted interventions to treat the positive symptoms while the negative and cognitive symptoms remain untreated, hindering the improvement of the patient’s functioning and quality of life. Elucidating the neurodevelopmental trajectory of schizophrenia but also addressing the cognitive impairments of the disorder are essential steps towards its early diagnosis and prevention.

Cohort studies have been used to study schizophrenia but these often rely on historical records and have limited data of interest since the sources of information are mostly indirect and not intended to be used in research on understanding schizophrenia. This is why studying high-risk populations is beneficial. Moreover, given that schizophrenia appears to be a neurodevelopmental disorder, examining children at increased risk could provide us with a better understanding of the processes that take place early in the development of psychosis.

This thesis set out to examine whether and how cognition is related to psychopathology in children that are at increased risk of schizophrenia as well as the possible roles of COMT variation in these relationships. Examining these relationships could also inform us on the way genetic risk influences brain and behaviour.
In order to explore these questions I have studied two different samples.

The first sample came from the large population-based Avon Longitudinal Study of Parents and Children (ALSPAC) where I compared children reporting psychotic experiences (PEs) with children who do not (Chapter 6). I conducted a longitudinal analysis to examine the relationships between \textit{a priori} selected key cognitive domains and PEs. The results provided evidence that processing speed and attention are related to greater risk of PEs in children, with processing speed being a key cognitive feature. Furthermore, within this population the relationships between cognition and later occurrence of PEs were similar to those that have previously been reported between cognition and schizophrenia.

In Chapter 7 I examined whether COMT, an enzyme that degrades dopamine and has been considered a plausible candidate for schizophrenia and cognitive deficits, is associated with PEs indirectly (mediated) through its effect on processing speed and attention. My hypothesis was that there may be an indirect association between genetic variation in \textit{COMT} and PEs, where \textit{COMT} is associated with worse processing speed and attention which subsequently may increase risk of PEs. The rationale for this study is based on my findings as reported in the preceding Chapter 6. My underlying hypothesis was that if the association between PEs and impairments in processing speed and attention is causal, one possible explanation would be an increase in false prediction errors in individuals at increased risk. That is, disturbed brain connectivity could potentially lead to false prediction errors through its effect on processing speed and attention. Given that dopamine has been shown to alter brain connectivity, it could be that genetic variation in \textit{COMT} could lead to PEs through affecting processing speed and attention. Taking into account the literature indicating that \textit{COMT} may also be associated with anxiety disorders, with the low activity alleles/haplotypes showing a relationship with both better cognition and higher risk of anxiety disorders, I also examined
whether there was an indirect effect of COMT on PEs through anxiety disorders. The findings showed that COMT was indirectly associated with PEs through processing speed, IQ and attention, but there was no evidence for a total effect of COMT on PEs or an indirect effect through anxiety disorders.

The second sample comprised children with 22q11.2DS (Chapter 8). I examined the nature and prevalence of psychopathology and cognitive dysfunction in this sample and their siblings and also explored to what extent risk of psychopathology that is associated with the deletion may be influenced indirectly by the children’s intellectual impairment. There were high rates of psychopathology and impairments across a wide range of cognitive functions in children with 22q11.2DS when compared with sibling controls. I found no evidence that the presence of psychopathology was correlated with cognitive dysfunction and also found no evidence for an indirect association between the deletion and the risk of psychopathology through cognitive function. Finally, I also examined whether genetic variation in COMT was associated with the cognitive and psychiatric problems experienced by children with 22q11.2DS. There was no evidence that COMT variation played a role in these problems in children with 22q11.2DS.

### 9.2 Bringing the findings together

The approach I have used in this thesis (i.e., studying samples at increased risk from a birth-cohort study and a cross-sectional study on children with 22q11.2DS) has potential advantages. Even though cohort studies provide the remarkable advantage of offering a lifelong perspective and a statistically powerful design, they are not suited for the study of
rare syndromes, like 22q11.2DS. Therefore, both study designs can offer unique frames of reference and combining them can help obtain a better perspective.

It was not my intention to directly compare these two samples, because they were assessed with different instruments measuring different outcomes under different research designs. Rather, my intention was to obtain a better understanding of the relations between cognition and psychopathology in groups at increased risk. Moreover, even though both samples are at increased risk of developing schizophrenia, they differ; children with 22q11.2DS constitute a clinical population with a much higher risk of developing schizophrenia than children with PEs from the general population and also a population with multiple and severe physical, cognitive and psychiatric impairments resulting from haploinsufficiency of approximately 40 genes, including \textit{COMT}. Hence, if we assume that there is ‘a continuum of genetic and environmentally induced neurodevelopmental impairment’ (p. 174, (Owen, O’Donovan et al. 2011)) it is more likely that children with 22q11.2DS are at the more extreme end of the spectrum in relation to children with PEs from the general population.

Moreover, I was not able to look at the relationships between cognition and PEs in children with 22q11.2DS. This will be possible in the follow-up of these children (which is currently undertaken by the PIs on the ECHO study) where reports of PEs are likely to increase. Only one study has examined whether impaired cognitive function predicts risk of PEs in individuals with 22q11.2DS (Schneider, Schaer et al. 2013). To be more exact, 104 individuals with 22q11.2DS (mean age: 14.83 (7.53), age range: 6 to 44 years old) were followed up after a period of 3.86 years. At time 2, 56 patients (mean age: 16.6 (6.73)) had positive symptoms while 7 of them were also receiving psychotropic medication. No associations were found between impaired cognitive function at time 1 and higher risk of PEs.
at time 2. However, it was not examined whether associations were attenuated by the use of psychotropic medication.

Several studies looking at relations between cognitive function and psychosis in 22q11.2DS have not found associations either (e.g., (Murphy, Jones et al. 1999, Bassett, Chow et al. 2005, Monks, Niarchou et al. 2013). For example, a study including 83 adults with 22q11.2DS (mean age: 31 (10.6), age range: 17 to 63) of whom 24 were diagnosed with a psychotic disorder, did not find evidence for associations with IQ (Monks, Niarchou et al. 2013).

In my view, the different position of the samples (i.e., individuals with PEs from the general population versus individuals with 22q11.2DS) on this continuum of risk could be an explanation for the inconsistent findings. The findings are in accordance with one of the most prominent current theories of schizophrenia; the dysconnection theory which has received support from several streams of evidence (for a review see: (Stephan, Friston et al. 2009)).

According to the dysconnection theory, the symptoms of schizophrenia arise due to an abnormal functional integration among brain regions in schizophrenia which is caused by impairments in synaptic plasticity (Stephan, Friston et al. 2009, Adams, Stephan et al. 2013, Lakhan, Caro et al. 2013). Central to this theory is the impaired N- Methyl-D-aspartate receptor (NMDAR) mediated synaptic plasticity that is caused by abnormal regulation of NMDAR by dopamine, serotonin or acetylcholine. It remains, however, unclear to what extent abnormal connectivity precedes the risk of schizophrenia. A recent study on 30 individuals with 22q11.2DS and 30 healthy controls that employed whole brain tractography found a significant decrease in brain connectivity in 22q11.2DS which is in accordance with the dysconnection theory (Ottet, Schaefer et al. 2013). The fact that in the study by (Schneider,
Schaer et al. 2013) cognition was not related to PEs in individuals with 22q11.2DS could be due to disturbed connectivity in the brain systems supporting these functions to the extent that they become independent from each other. While there have not been any studies comparing children with 22q11.2DS with children with PEs from the general population, it is likely that brain connectivity is more disturbed in children with 22q11.2DS than it is in children with PEs. This could be the reason why the associations that we see in children with PEs are not present in children with 22q11.2DS.

On the other hand, it could also be that the mechanisms by which psychosis occurs in individuals with 22q11.2DS are different from these in individuals with PEs from the general population. Similar phenotypes could arise from small deviations in factors that occur very early in development, such as reduced gene dosage and defective myelination with widespread effects in brain structure and connectivity during development (Karayiorgou, Simon et al. 2010). For example, studies in children with Williams Syndrome (a syndrome caused by a deletion in chromosomal region 7q.11.2) have shown that even when behavioural performance is within the normal range, it can arise from different underlying processes in relation to children from the general population (Karmiloff-Smith, Grant et al. 1997). This could be extended to individuals with 22q11.2DS; for example, even though PEs are reported by children with 22q11.2DS and children from the general population, it remains to be examined whether the underlying mechanisms giving rise to this phenotype are also similar.

In addition to these interpretations other possible explanations and limitations that I have discussed in the previous Chapters also need to be taken into account.

For instance, low statistical power could explain why the associations between cognition and PEs found in Chapter 6 are not found in 22q11.2DS studies. Moreover, with regards to the
reported association between processing speed and PEs, I cannot exclude the possibility this is a chance finding, or the possibility of reverse causation; PEs were only assessed at one time point and it could be that PEs at an earlier time point, although difficult to assess at children younger than 8 years old, have played a role in the defective processing speed.

9.3 Research implications

Overall the findings from the studies on the ALSPAC birth cohort have important theoretical and clinical implications. They are in accordance to the neurodevelopmental hypothesis of schizophrenia as they suggest that the mechanisms mediating the association between impaired cognition and schizophrenia also mediate the association between impaired cognition and PEs.

To be more specific, the study looking at cognition in relation to PEs (Chapter 6) is the first study examining in depth the relations between cognitive domains that have been previously found to be associated with schizophrenia and PEs in a large population based study of children. It is also the first study showing that defective processing speed is associated with higher risk of PEs in children. Processing speed has been regarded as the ‘most sensitive construct to cerebral damage’ and one of the ‘most basic operations of the human mind’ (p.272, (DeLuca 2008)). Studies have shown that it can be beneficial to provide training to improve processing speed in older populations and further research is needed to examine this intervention in other clinical populations and children (Ball and Vance 2008). Thus, these findings provide a better understanding of the deficits that place children at higher risk of developing schizophrenia and can inform potential intervention strategies.
The study looking at the total and indirect effects of COMT on PEs through cognitive function and anxiety disorders in children (Chapter 7) is the first study to examine whether COMT SNPs and haplotypes other than rs4680 (Barnett, Heron et al. 2007) are associated with processing speed in children, as well as the first study that examined the associations between COMT and anxiety disorders in children. Furthermore, it is also the first study that examined possible indirect effects of COMT on PEs through cognitive function and anxiety disorders in children. The finding that processing speed is associated with COMT SNPs and haplotypes indicates this gene can have a widespread effect in the brain. Moreover, the existence of an indirect effect of COMT on PEs through processing speed and attention indicates a potential mechanism underlying the emergence of PEs in children as well as provides further support that processing speed and attention may be useful as potential endophenotypes in schizophrenia.

With regards to the study in children with 22q11.2DS (Chapter 8), this is one of the largest studies to date with children with 22q11.2DS of relatively narrow age range with a control sample of siblings, close in age. In addition, this is one of the few studies that conducted detailed psychiatric and neuro-cognitive phenotyping in all children and used mediation analysis to try to understand more about the mechanisms through which the 22q11.2 deletion is associated with increased psychopathology.

The findings of this study have several important implications. They are consistent with the deletion having pleiotropic effects on cognition and psychopathology as well as with the conclusion that psychopathology in 22q11.2DS is not a result of intellectual impairment. They also show that children with 22q11.2DS have a range of cognitive impairments and psychopathology which further highlights the need for early detection and treatment. Moreover, these results suggest that cognitive remediation is unlikely to improve mechanisms
that underlie the risk of psychopathology in 22q11.2DS, even though more research is needed to replicate these findings in a larger sample and examine potential effects of more specific cognitive deficits on psychiatric phenotypes.

9.4 Future directions

One of the key findings of this thesis was the prominent role of processing speed in increasing the risk of PEs in children and the implication it may lie on the pathway between genetic variation in COMT and PEs. Moreover, processing speed was also associated with a range of other cognitive functions in children with 22q11.2DS underlying the importance of this cognitive construct in both typical and atypical development.

It is worth mentioning that processing speed has also been associated with other disorders such as normal aging, chronic fatigue syndrome, dementia, traumatic-brain injury, HIV symptomatic, multiple sclerosis, systemic lupus erythematosus, depression, reading disability and mental retardation (for a review see: (DeLuca 2008)). Despite all this evidence as well as findings showing that it impacts upon other cognitive processes, an integrative model of processing speed still does not exist. Current knowledge is limited to the conclusion that processing speed is ‘some sort of global, biologically driven mechanism that limits the speed with which information is processed’ (p. 272, (DeLuca 2008)). Therefore, further studies are needed to identify its biological substrates and the ways it drives development in other cognitive functions.
The indirect effect of *COMT* on PEs in children through processing speed, IQ and attention represents another key finding. This could reflect a causal effect between *COMT* and PEs that might help elucidate the pathophysiology of schizophrenia with potential implications for intervention. Therefore, further research is needed to replicate these findings and put them in a neurobiological context.

With regards to 22q11.2DS, examining this syndrome provides a great opportunity to understand the mechanisms that take place in schizophrenia, especially given the fact that 22q11.2DS can be diagnosed in utero thus providing a lifespan perspective on the precursors of schizophrenia. One of the major limitations in the field, however, is the limited sample sizes and the fact that the several research groups have used different psychometric instruments. Recently, a research initiative involving 22 institutions worldwide has been successful in obtaining NIMH funding. This International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome (22q11.2DS) aims to disentangle the aforementioned issues by using an integrative genomic and phenotypic approach. Cardiff University is one of the five Lead Centres and I have been fortunate to have had the opportunity to be part of this initiative and I look forward to contributing to this research effort in the future.

The findings of the ECHO study have also indicated that 22q11.2DS does not seem to be related to a specific psychiatric phenotype in childhood. Rather it seems that the effect of the deletion is widespread across a range of neurodevelopmental syndromes. This is relevant to the issue of classification and categorization of psychiatric disorders (also see Chapter 1.1). It could be that the current diagnostic systems fail to capture the 22q11.2DS childhood psychiatric phenotype. This classification issue and proposed future directions have been eloquently described by Steven Hyman (Hyman 2010). One of the aims of the International
22q11.2DS Consortium is also to investigate psychopathology at multiple levels adopting an integrative approach that combines genomic and neuropsychiatric paradigms.

Finally, another future direction is the study of children with other CNVs associated with high risk of schizophrenia. A number of studies have reported that CNVs in regions 1q21.1, 15q11.2, 15q13.3, occur more frequently in patients with schizophrenia than controls (Stefansson, Rujescu et al. 2008). Little is currently known about these newly identified CNVs. For example, 1q21.1 Deletion Syndrome has been associated with cardiac abnormalities, developmental delay and a range of psychiatric disorders (Mefford, Sharp et al. 2008). Other CNVs like 16p11.2 Deletion Syndrome have also been associated with high rates of learning disability and psychiatric problems (George, Taylor et al. 2012, Zufferey, Sherr et al. 2012). These syndromes have only recently been recognized and with the rapid advances in genetic laboratory techniques more CNVs associated with psychopathology might be discovered. Understanding the implications of these CNVs for different psychiatric disorders and cognitive impairments and elucidating their associations throughout development will inform early prevention and treatment of schizophrenia.

9.5 Conclusions

Schizophrenia is a debilitating mental health disorder and integrative approaches are needed to disentangle the largely unknown underlying pathophysiology. In this thesis I explored cognition and psychopathology and the role of the COMT genotype in children at increased risk of schizophrenia with the aim of making a contribution to our understanding of the processes that take place early in the development of psychosis. The findings of this thesis
have potentially important theoretical and clinical implications and they also show that using
different research designs can widen the scope of interpretation. Such an approach of utilizing
different designs to investigate specific aims can help future studies in high risk samples to
improve our understanding of aetiological mechanisms underlying schizophrenia.
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