Examining the Clinical and Genetic Overlap of Attention Deficit Hyperactivity Disorder and Autism Spectrum Disorder Traits

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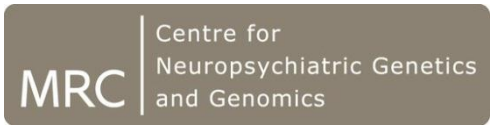

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Thesis Summary

Attention deficit hyperactivity disorder (ADHD) is a common and impairing neurodevelopmental disorder, which frequently co-occurs with autism spectrum disorder (ASD). Both disorders are highly heritable and recent studies report a substantial degree of overlap in genetic risks for ADHD and ASD. The overall objective of this thesis is to examine the clinical co-occurrence and shared genetic susceptibility of these conditions, as well as of related developmental problems.

First, the presentation of ASD traits is examined in a clinical sample of children diagnosed with ADHD. This is followed by an assessment of whether the presence of ASD traits in children with ADHD is associated with additional cognitive or developmental difficulties. Lastly, it is investigated whether common genetic risk variants which are associated with clinically-diagnosed ADHD are also associated with ADHD traits, ASD-like social-communication difficulties and neurocognitive abilities (i.e. IQ, working memory, inhibitory control and facial emotion recognition) in children from a general population sample.

The results show that ASD traits split into separate, albeit correlated dimensions of social-communication difficulties and restrictive, repetitive behaviours (RRBs) in children with ADHD. They also suggest that there may be some overlap of RRBs and hyperactive-impulsive ADHD symptoms. Increasing levels of ASD traits in children with ADHD are found to index more ADHD symptoms, as well as lower cognitive abilities and a greater likelihood of developmental difficulties. Finally, the results demonstrate that common genetic risk variants relevant to ADHD diagnosis are associated with ADHD and social-communication problems as well as cognitive difficulties (lower IQ and working memory abilities) in children in the general population.

This thesis extends our understanding of the clinical importance of assessing ASD in the context of ADHD. Furthermore, the findings demonstrate that common genetic risk variants for childhood ADHD are also relevant to other neurodevelopmental and cognitive outcomes in the general population.

Publications resulting from work in this thesis

Martin, J., Hamshere, M., Stergiakouli, E., O'Donovan, M. C., & Thapar, A. (2014). Neurocognitive abilities in the general population and polygenic risk scores for attention deficit hyperactivity disorder. *Journal of Child Psychology and Psychiatry (Epub ahead of print])*. doi:10.1111/jcpp.12336

Martin, J., Hamshere, M. L., Stergiakouli, E., O'Donovan, M. C., & Thapar, A. (2014). Genetic Risk for Attention Deficit Hyperactivity Disorder Contributes to Neurodevelopmental Traits in the General Population. *Biological Psychiatry*, *76*(8), 664-671.

Cooper, M.*, **Martin, J.*,** Langley, K., Hamshere, M., & Thapar, A. (* joint 1st authors). (2014). Autistic traits in children with ADHD index clinical and cognitive problems. *European Child and Adolescent Psychiatry*, 23(1), 23-34.

Martin, J., Hamshere, M. L., O'Donovan, M. C., Rutter, M., & Thapar, A. (2013). Factor structure of autistic traits in children with ADHD. *Journal of Autism and Developmental Disorders*, 44(1), 204-215.

Additional, related publications to which I have contributed

Martin J., O'Donovan M. C., Thapar A., Langley K., Williams N. (In Press). The relative contribution of common and rare genetic variants to ADHD. *Translational Psychiatry*.

Martin, J., Cooper, M., Hamshere, M., Pocklington, A., Scherer, S., Kent, L., Gill, M., Owen, M., Williams, N., O'Donovan, M., Thapar, A., Holmans, P. (2014). Biological overlap of Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder: Evidence from copy number variants. *Journal of the American Academy of Child & Adolescent Psychiatry*, 53, 761-770.e26

Hamshere, M. L., Langley, K., **Martin, J.,** Agha, S. S., Stergiakouli, E., Anney, R. J., Buitelaar, J., et al. (2013). High loading of polygenic risk for ADHD in children with comorbid aggression. *American Journal of Psychiatry*, 170(8), 909-916.

Ahuja, A.*, **Martin, J.*,** Langley, K., & Thapar, A. (2013). (* joint 1st authors). Intellectual disability in children with ADHD. *Journal of Pediatrics*, 163(3), 890-895.e1.

Hamshere, M. L., Stergiakouli, E., Langley, K., **Martin, J.,** Holmans, P., Kent, L., Owen, M. J., et al. (2013). A shared polygenic contribution between childhood ADHD and adult schizophrenia. *The British Journal of Psychiatry*, 203(2), 107-111.

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Chapter 1

Background Literature

1.1 Introduction

The overall aim of this thesis is to inform our understanding of the presentation and aetiology of attention deficit hyperactivity disorder (ADHD) by examining its clinical and genetic relationship with autism spectrum disorder (ASD) and other early developmental and cognitive problems. This chapter will first consider important diagnostic and clinical features of ADHD and ASD. This will be followed by a discussion of the clinical overlap and similarities in neurocognitive and developmental deficits associated with ADHD and ASD. The focus will then turn to examining the heritability, genetic architecture (i.e. the number, frequencies and effect sizes of risk variants) and genetic overlap of the two conditions. Finally, limitations of current research will be discussed and the specific aims of the thesis will be outlined.

1.2 Definitions & diagnoses

ADHD is a childhood-onset neurodevelopmental condition characterised by developmentally inappropriate levels of inattention, hyperactivity and impulsivity. The recently released Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) has seen several reasonably subtle changes to diagnostic recommendations and criteria for ADHD, although the core deficits characterising this condition remain largely comparable to those in the DSM-IV. ADHD is also known as Hyperkinetic Disorder in the ICD-10 (the International Classification of Diseases), which is currently under revision. The diagnostic criteria for ADHD and Hyperkinetic Disorder can be found in Table 1.1 below. Given the developmental nature of ADHD, the diagnostic criteria stipulate early onset of core features, i.e. prior to age 12 years (age 7 years in ICD-10 and previously in DSM-IV). Although associated with early childhood, ADHD symptoms often persist into adolescence and adulthood and are associated with risk for other psychiatric problems and poor outcomes (Barkley

et al., 2006; Langley et al., 2010; Mannuzza et al., 1993, 1998; Yoshimasu et al., 2012). Longitudinal studies suggest that ADHD symptoms, particularly those of hyperactivity and impulsivity, can decrease somewhat over time, though rates of full remission are not high (Hill & Schoener, 1996; Lahey et al., 2005). DSM-IV included three diagnostic subtypes of ADHD: predominantly inattentive presentation, predominantly hyperactive-impulsive presentation and combined presentation. However, the developmental stability of these subtypes is rather poor, with the mix of inattentive and hyperactive-impulsive symptoms in affected children fluctuating over time (Willcutt et al., 2012). The DSM-5 no longer considers these as 'subtypes' but rather allows for descriptive presentation specifiers for a diagnosis of ADHD, corresponding to each of the DSM-IV subtypes.

Historically, it has been recommended in diagnostic manuals (e.g. ICD-10 & DSM-IV) that ADHD could not be diagnosed in an individual with an existing diagnosis of a 'pervasive developmental disorder', now known as 'autism spectrum disorder' (ASD) in DSM-5. However, the past decade has seen an abundance of evidence from research studies disputing this exclusion (discussed below), which has resulted in the removal of this restriction in the DSM-5. The core criteria of ASD are distinct to those of ADHD (see Table 1.2). ASD is characterised by difficulties in social communication and social interaction, as well as restrictive, repetitive or stereotyped patterns of behaviours, interests or activities. The DSM-5 has seen the merging of a number of supposed subtypes of ASD (i.e. classical autism, Asperger's syndrome and 'pervasive developmental disorder-not otherwise specified'). The DSM-5 also introduced a new diagnostic category called 'Social Communication Disorder', which is likely to apply to individuals not meeting full criteria for ASD, by virtue of absence of clear restrictive and repetitive behaviours. It is unclear though whether this separate diagnostic category is substantially different from the social communication and social interaction domain of ASD (Skuse, 2012). Moreover, there is a lack of supportive evidence for this new diagnostic category, suggesting that social communication and pragmatic language impairments may be best considered as a dimension of symptoms (Norbury, 2014).

Table 1.1: DSM-5 and ICD-10 Diagnostic Criteria for ADHD

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Table 1.2: DSM-IV and DSM-5 Diagnostic Criteria for ASD

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1.3 Dimensionality

Whilst clinical diagnoses are defined categorically, psychopathology can also be viewed dimensionally, with the diagnosed condition seen as the extreme end of a continuous distribution of traits in the general population. There is evidence that ADHD symptoms are continuously distributed in children from the general population (Rodriguez et al., 2007). Similarly, ASD traits have also been shown to be continuously distributed in population samples (Constantino & Todd, 2003; Posserud et al., 2006). The lack of a discontinuity at the severe end of distributions of ADHD and ASD traits indicates that there is likely to be no clear, definitive point where sub-threshold levels of these traits abruptly become clinically meaningful and indicative of disorder.

Importantly, clinical diagnoses are not simply defined by the presence of a certain amount of symptoms, but also by the additional impairment in daily functioning attributable to these symptoms and other features (e.g. pervasiveness of ADHD symptoms across multiple settings). Indeed, children with sub-threshold ADHD symptoms (i.e. not meeting full criteria for ADHD) frequently have comorbid problems and present with impairment of functioning as compared with controls (Balázs & Keresztény, 2014; Hong et al., 2014). There is little doubt that categorical clinical diagnoses are meaningful and important for decisions regarding treatment. However, utilising categorical diagnoses for research, with the aim of understanding the biological underpinnings of these disorders and informing classification, is arguably less valuable and even somewhat circular. As such, considering these conditions as distributions of continuous traits may be a valuable research approach. In recent years, the National Institute for Mental Health developed a novel framework called the Research Domain Criteria (RDoC) for the purpose of researching the aetiology and nature of mental health problems (Casey et al., 2013; Insel et al., 2010). The RDoC framework relies on a dimensional cross-diagnostic approach to investigate underlying biology of mental health across the life span. The hope is that research using this framework will help to develop a new, more biologically-oriented classification system for mental health problems.

Multiple dimensions

ADHD has long been considered to consist of two distinct but correlated symptom domains; these are inattentive and hyperactive-impulsive symptoms. Numerous studies, including factor analyses, have supported the division of ADHD into these two separate, albeit strongly related domains (Willcutt et al., 2012). Historically, ASD symptoms have been divided into a triad of impairments, with deficits of communication, social-interaction problems and restrictive-repetitive behaviours (RRBs) considered separately. Although the different ASD behaviours occur together more than expected by chance, correlations between them are only moderate, suggesting that the sub-domains are to some extent clinically separable (Happé & Ronald, 2008; Ronald et al., 2006b). Factor analyses of autistic traits in clinical ASD and community samples using a variety of ASD measurement tools generally indicate that multiple factors account for the observed covariance structure of ASD symptoms and traits (Happé & Ronald, 2008; Mandy & Skuse, 2008). Likely due to differences in study design (e.g. the types of measures used to assess ASD), there is little agreement in terms of the specific factors and their composition. However, nearly all factor analytic studies derive at least one factor related to social-communication features and a separate factor related to 'non-social' behaviour or RRBs (Mandy & Skuse, 2008). In DSM-5, ASD is now considered to be divisible into the two dimensions of social-communication difficulties and RRBs. As such, ADHD and ASD can each be conceptualised as consisting of two related core dimensions of behavioural traits.

1.4 Prevalence

ADHD and ASD are two of the most common neurodevelopmental disorders. Reliable prevalence rates are difficult to measure accurately due to methodological heterogeneity across geographical location and across time. One meta-analysis of 102 studies has estimated the world-wide point prevalence of childhood ADHD to be 5.3% (Polanczyk et al., 2007), though there is wide geographical variability. More recent meta-analyses of studies using only DSM-IV diagnostic criteria

suggest that the prevalence rate of ADHD is between 5.9-7.1%, depending on source of informant (Willcutt, 2012). Prevalence of ADHD in the UK has been estimated at about 2.2% for any DSM-IV ADHD diagnosis (Ford et al., 2003) and 1.4% for ICD-10 Hyperkinetic Disorders (Meltzer et al., 2000). ASD has been estimated to affect about 0.3-1.0% of children (Elsabbagh et al., 2012; Rutter, 2005). Although estimates of prevalence of both ADHD and ASD appear to have increased in the past few decades (Elsabbagh et al., 2012; Visser et al., 2010), a number of factors are likely to account for this. A recent meta-regression of 135 ADHD prevalence studies suggests that after accounting for heterogeneity of study methods, prevalence rates have not increased significantly over the last three decades (Polanczyk et al., 2014). A review of prevalence studies of ASD suggests that changing diagnostic classifications, under-powered studies and methodological heterogeneity may account for rising prevalence rates (Fisch, 2012). In general, it would seem that changes in diagnostic criteria and growing recognition and awareness, particularly of more mild forms of the conditions, may largely account for apparently growing prevalence rates of ADHD and ASD in clinic populations.

Gender

One important observation with regards to prevalence of childhood ADHD and ASD is that both conditions have a significantly higher rate of occurrence in males than females. Depending on the sample type, the male:female ratio of ADHD is about 3-7:1 (Lahey et al., 1994; Polanczyk et al., 2007) and for ASD this ratio is about 3-8:1 (Baird et al., 2006; Keen & Ward, 2004). In children with ADHD, this gender difference is particularly noticeable in children ascertained from clinics relative to community samples (Gershon, 2002), possibly due to higher levels of comorbid behavioural problems in males, resulting in higher levels of clinical referrals in males (Levy et al., 2005). On the other hand, the gender discrepancy in ASD is higher in populations of "high-functioning" children (i.e. those who have fewer cognitive difficulties), as compared with children with ASD who have comorbid intellectual disability (Fombonne, 2003).

1.5 Phenotypic overlap of ADHD and ASD

Symptom co-occurrence

As mentioned earlier, a fairly recent diagnostic development is that the DSM-5 now allows for a joint diagnosis of both ADHD and ASD in one individual. This change from previous diagnostic manuals was prompted by numerous studies which highlighted the high rates of co-occurrence of these conditions. This observed overlap questioned the assumption made in the DSM-IV and ICD-10 that the presence of ADHD traits in ASD could be accounted for by core ASD symptoms. Some have argued that although it is plausible that certain ADHD behaviours (e.g. excessive motor activity or inattention to social stimuli) may be explained by the presence of ASD symptoms (i.e. frequent repetitive motor behaviours or social difficulties), many ADHD symptoms in children with ASD cannot easily be explained by the ASD symptoms (Reiersen, 2011). Furthermore, the clinical characteristics (in terms of symptom types and onset) of ADHD symptoms are similar in children with ADHD with and without ASD (Frazier et al., 2001). As such, the examination of ADHD symptoms in the context of ASD, and vice versa, is an important and growing research area.

Indeed, studies examining the prevalence of comorbid conditions in children diagnosed with ADHD show that ASD is one of the most commonly co-occurring disorders and more generally, that children with ADHD have elevated levels of sub-clinical ASD symptoms as compared with control children (Grzadzinski et al., 2011; Kochhar et al., 2011; Kotte et al., 2013; Mulligan et al., 2009a; Nijmeijer et al., 2009; Rommelse et al., 2009). Similarly, studies of children with ASD show high rates of clinically meaningful ADHD symptoms (Gadow et al., 2006; Ghaziuddin et al., 1998; Goldstein & Schwebach, 2004; Simonoff et al., 2008; Thede & Coolidge, 2007; Yerys et al., 2009b; Yoshida & Uchiyama, 2004). Estimates suggest that between 20-50% of children with ADHD meet diagnostic criteria for ASD and 30-80% of children with ASD meet criteria for ADHD (Rommelse et al., 2010).

Overlap of ADHD and ASD symptoms has also been noted in general population and twin samples (Lichtenstein et al., 2010; Reiersen et al., 2007; Ronald et al., 2008). Furthermore, longitudinal trajectory analysis in the general population shows that children with persistently high levels of ADHD traits tend to also have persistently high levels of social-communication difficulties (St Pourcain et al., 2011). Longitudinal analyses in twins also highlight that ADHD and ASD traits show associations between the ages of 8 and 12 years, with ADHD possibly predicting ASD more strongly than ASD predicting later ADHD (Taylor et al., 2012). There is also some suggestion that ADHD and ASD diagnoses are unstable over time, with some children meeting criteria for ASD in early childhood but developing a symptom profile meeting only ADHD criteria at a later age (Fein et al., 2005).

Factor structure

Interestingly, the co-occurrence of ADHD with ASD symptoms has been demonstrated to occur within all three of the DSM-IV ASD sub-domains, though restrictive-repetitive behaviours have been found to be less frequent than social and communication deficits in children with ADHD (Rommelse et al., 2011). Although it is generally accepted that ASD splits into multiple dimensions (as discussed in section 1.3), it is not clear whether the presence of ADHD affects the nature of ASD symptoms. Furthermore, it is not clear whether any individual ADHD and ASD symptoms are especially likely to co-occur in a clinical sample of children diagnosed with these conditions. One factor analysis of ADHD and ASD symptoms in a community sample of children suggested that the core diagnostic criteria of ADHD and ASD are distinct in terms of factor loadings (i.e. ADHD and ASD symptoms load on separate sets of factors; Ghanizadeh, 2010). However, given prevalence rates of 5% and less in general population samples (Polanczyk et al., 2007; Rutter, 2005), it is not known whether there is a different pattern of clustering of ADHD and ASD symptoms at the more extreme end of the distribution of these traits. These questions will be addressed in chapter 3 of this thesis.

Clinical meaning

Another important question about the co-occurrence of ADHD and ASD traits seen in clinical and community samples is whether the severity of ASD traits indexes a different clinical profile of ADHD and associated developmental and cognitive features. One study has suggested that children with ADHD with high levels of ASD traits may be more likely to have the more severe, DSM-IV combined subtype of ADHD than children with ADHD with low levels of ASD traits (Grzadzinski et al., 2011). Similarly, ASD trait scores within a clinical ADHD sample have been found to be associated with higher levels of hyperactive-impulsive symptoms, with possible association with more inattentive ADHD symptoms (Kröger et al., 2011). Conversely, studies in children with ASD suggest that co-occurring ADHD symptoms also index a more severe profile of ASD symptoms (Gadow et al., 2006; Holtmann et al., 2007; Yerys et al., 2009b), although some do not find this pattern of results (Ghaziuddin et al., 2010). On the whole, the results suggest that the co-occurrence of ADHD and ASD is of clinical significance.

Co-occurring psychopathology

ADHD and ASD not only frequently co-occur, but both conditions are commonly also associated with other forms of psychopathology. ADHD is associated with especially high rates of oppositional defiant disorder (ODD) and conduct disorder (CD), as well as anxiety, depression, Tourette's syndrome and other psychiatric disorders (Biederman et al., 1991; Larson et al., 2011; Rommelse et al., 2009). Children with ASD also frequently display disruptive behaviours (i.e. ODD and CD), anxiety and mood problems (de Bruin et al., 2007; Mukaddes & Fateh, 2010; Simonoff et al., 2008; Thede & Coolidge, 2007).

A comparison of comorbid psychopathology in children with ADHD or ASD suggests that rates of oppositional, conduct and depression problems are similar, although children with ASD have higher rates of anxiety problems (van Steensel et al., 2013). Studies also suggest that increasing levels of ASD traits in children with ADHD may index higher rates of comorbid ODD and CD (Mulligan et al.,

2009a), although smaller studies do not find this effect (Grzadzinski et al., 2011; Kröger et al., 2011). Similarly, children with co-occurring ADHD and ASD seem to have higher rates of comorbid ODD, CD, anxiety and depression problems than children with ASD-only (Gadow et al., 2006; Guttmann-Steinmetz et al., 2009, 2010; Holtmann et al., 2007; Yerys et al., 2009b). Taken as a whole, the evidence suggests that co-occurring ADHD and ASD traits in children with either condition appear to index higher levels of comorbid psychopathology.

Associated neurocognitive & developmental difficulties

General cognitive ability

Children with ADHD & ASD show similar deficits in a number of neurocognitive domains, as well as impairments with respect to other developmental domains (e.g. motor coordination). ADHD and ASD commonly co-occur with intellectual disability (ID) and both conditions tend to be associated with lower general cognitive ability (e.g. IQ) than in typically developing individuals (Dykens & Hodapp, 2001; Frazier et al., 2004; Matson & Shoemaker, 2009; Russell et al., 2014; Voigt et al., 2006). This relationship is so strong that it has been argued that it is conceptually problematic to match groups of children with ADHD or other neurodevelopmental problems on IQ, when comparing against typically developing controls, or to co-vary for IQ in such comparisons (Dennis et al., 2009). Such methods may introduce biases and remove important variance related to the phenotypes of interest, potentially resulting in misleading findings. The common exclusion of children with IQ<70 from studies of ADHD and ASD also limits the clinical representativeness of such samples and may reduce the power of analyses involving IQ. For instance, although there is some suggestion that variation in IQ is not associated with the presence of ASD traits in children with ADHD (Grzadzinski et al., 2011; Kochhar et al., 2011) or ADHD traits in children with ASD (Gadow et al., 2006; Guttmann-Steinmetz et al., 2009), the exclusion of children with low IQs is an important limitation of these studies which needs to be addressed.

Executive functioning

In addition to an association with general cognitive difficulties, ADHD and ASD have also been associated with deficits in executive functioning (EF), which is a broad umbrella term for a number of related higher-order cognitive processes involved in self-regulation and mental control. Amongst these processes are working memory (the part of short-term memory involved in conscious processing of information), response inhibition (the process of suppressing inappropriate or unnecessary actions), interference control (the ability to selectively inhibit processing of irrelevant information), planning (the cognitive processes involved in formulating and selecting a sequence of thoughts or actions to reach a goal) and cognitive flexibility (the mental ability to switch between different concepts in response to changing situational demands). Given how broad the concept of EF is, it is useful to conceptualise EF in terms of these individual domains rather than as a single entity for the purposes of research. Many studies exploring EF cognitive processes in children with ADHD and ASD suggest that these deficits may act as risk factors. Another way of considering the role of EF in neurodevelopment is to view 'intact' or good EF abilities as a protective factor, which compensates for atypical functioning of other neural systems in children at genetic risk for ADHD or ASD (Johnson, 2012). As yet, the nature of the association between EF deficits and neurodevelopmental problems is unclear.

A review and meta-analysis of EF deficits in ADHD, across a large number of participants in 97 studies, found an overall moderate effect size (Willcutt et al., 2008). This meta-analysis found that the strongest and most consistent associations with ADHD came from measures of response inhibition, working memory, vigilance and planning. However, although consistent group differences between children with and without ADHD emerge in these EF domains when multiple studies are pooled, heterogeneity within ADHD needs to be considered. Indeed, only about half of the children in an ADHD sample can be reasonably classified as "impaired" on any given EF measure relative to controls (Nigg et al., 2005). It has also been suggested that variability in EF abilities may be used to find more homogenous subtypes of ADHD by clustering children according to their EF

profiles (Roberts et al., 2013). However, it is important to note that cognitive difficulties in ADHD may fluctuate, for example depending on the level of incentive offered during testing (Kuntsi et al., 2009).

In ASD, a review and meta-analysis of EF studies found medium to large effect sizes for difficulties in planning, cognitive flexibility, inhibition, working memory, vigilance and fluency (Willcutt et al., 2008). Studies comparing children with a primary diagnosis of ADHD or ASD have had mixed results, with some detecting differences and some similarities in any given EF sub-domain (Rommelse et al., 2011). One review has suggested that there may be a "double dissociation" in ADHD and ASD in terms of EF, with ASD being more strongly associated with problems in cognitive flexibility and planning and ADHD being more strongly associated with deficits in sustained attention and response inhibition (Gargaro et al., 2011). However, the evidence for such a double dissociation is inconclusive and another review of EF studies concludes that in general, EF deficits in various sub-domains (e.g. response inhibition, working memory, vigilance) are qualitatively similar in both conditions, although they can vary in degree of severity (Rommelse et al., 2011). Nevertheless, this review also highlights the lack of sufficient research examining the relationship of EF difficulties to traits of both phenotypes; studies to date are small, with inconsistencies in methodology, inclusion criteria and the specific EF sub-domains tested, making it difficult to make any firm conclusions.

Social cognition

Social cognition is the ability to reason about another person's emotions and thoughts and encompasses the perception of facial expressions, affective prosody (emotional tone of language) and body posture, as well as theory of mind, empathy and humour processing (Uekermann et al., 2010). The majority of studies investigating social cognitive abilities of children with ADHD and ASD have focused on theory of mind (ToM), which is the ability to ascribe mental states to others, as well as on facial emotion recognition abilities (Demopoulos et al., 2013).

ToM difficulties, have long been implicated as a core feature of ASD in affected children (Baron-Cohen, 1989, 2000). However, ToM abilities appear to be largely unimpaired in ADHD (Geurts et al., 2010; Uekermann et al., 2010). Moreover, in children with ASD and co-occurring ADHD symptoms, ToM difficulties appear to be associated with ASD, not ADHD symptoms (Ames & White, 2011). This literature suggests that ToM deficits are primarily implicated in ASD and not ADHD.

On the other hand, difficulties with facial emotion recognition appear to be associated with both conditions. Reviews find somewhat mixed results for facial emotion recognition abilities in children with ADHD, but generally studies indicate some difficulties in ADHD relative to controls (Collin et al., 2013; Uekermann et al., 2010). A recent meta-analysis of 48 studies found that facial emotion recognition difficulties were significantly associated with ASD (Uljarevic & Hamilton, 2012), although another review highlights that findings are inconsistent and suggests that many individuals with ASD develop compensatory mechanisms in this domain (Harms et al., 2010). Indeed, a recent population study has suggested that although females with traits of social-communication difficulties perform relatively well on facial emotion recognition tasks as compared with males with equivalent levels of these traits, both genders struggle on an unfamiliar task of recognising emotions from abstract social motion cues (Kothari et al., 2013). As such, although facial emotion recognition difficulties are related to ASD, they may not affect all individuals equally.

The few studies comparing children diagnosed with ADHD or ASD in terms of emotion recognition abilities are somewhat inconsistent. One small study suggested that children with ADHD and those with co-occurring ADHD and ASD show more pronounced difficulties in facial emotion recognition than children with ASD only (Sinzig et al., 2008b). However, a recent, larger study reported that children with ASD perform significantly worse than those with ADHD on tasks of both facial and vocal emotion processing (Demopoulos et al., 2013). Although it would seem that both ADHD and ASD are associated to some extent with deficits in facial emotion recognition, there is some suggestion that children with ASD have specific abnormalities in gaze processing when viewing

faces, whereas those with ADHD may have early visual attention problems (Tye et al., 2013). It is important to note that there is variability within children with ADHD and ASD, with not all children showing deficits in facial emotion recognition. Furthermore, difficulties in the domains of general cognition, executive functioning and social cognition are not unique to ADHD and ASD, but rather these deficits are also associated with other neurodevelopmental and psychiatric disorders, including anxiety, tic, depressive and psychotic disorders (David et al., 2008; Fett et al., 2011; Willcutt et al., 2008).

Other developmental difficulties

Children with ADHD and ASD also frequently present with comorbid learning (e.g. reading or mathematical) difficulties (Capano et al., 2008; Dykman & Ackerman, 1991; Jones et al., 2009; Mayes & Calhoun, 2006), motor difficulties, such as developmental co-ordination disorder (Blondis, 1999; Fournier et al., 2010; Lingam et al., 2010), and sensory difficulties (Cheung & Siu, 2009; Dunn & Bennett, 2002; Kern et al., 2007; Mangeot et al., 2001; Yochman et al., 2004), the latter of which have been newly added to the DSM-5 diagnostic criteria for ASD. There is some evidence that autistic traits in children with ADHD are associated with higher rates of motor and language problems (Mulligan et al., 2009a; Reiersen et al., 2008b). However, further studies are necessary to confirm these findings and clarify the specifics of the relationship between ADHD and ASD traits with developmental and cognitive deficits. This topic will be examined in chapter 4.

1.6 Genetic risk factors

Family studies

There is considerable evidence from family, twin and adoption studies highlighting the importance of genetic risk factors for ADHD and ASD. These studies examine similarities in the psychiatric outcomes of individuals who differ in their degree of biological relatedness and amount of shared environment. Studies consistently show that parents and siblings of clinically-referred children with ADHD have increased rates of ADHD and higher rates of sub-threshold symptoms of ADHD, as

compared with healthy controls (Biederman, 2005). Indeed, the presence of parental ADHD in a sample of children diagnosed with ADHD is associated with more severe levels of ADHD in the children, as well as other adverse clinical outcomes (Agha et al., 2013).

ASD family studies also find that relatives of affected children show elevated levels of social, communication and learning difficulties compared to controls, referred to as the 'broader autism phenotype' (Bailey et al., 1998; Constantino et al., 2006; Pickles et al., 2000). Likewise, comparing broader autism phenotype traits in biological and non-biological relatives (e.g. adoptive or step-parents of probands with ASD) confirms the familial nature of ASD (Szatmari et al., 2000). A comparison of parents of multiple affected children (commonly referred to as "multiplex" families) to those with no more than one affected child (i.e. "simplex" families) finds higher levels of ASD traits in the former (Bernier et al., 2011), suggesting that there may be some manifestations of ASD that are more familial (i.e. with higher genetic loading) than others. Although family studies indicate that ADHD and ASD are both highly familial, unlike twin and adoption studies, such studies cannot fully disentangle the relative importance of genetic and environmental influences on these conditions.

Twin and adoption studies

The twin study design, on the other hand, has been widely used to examine the role of genetic and environmental influences on the manifestation of ADHD and ASD. In this design, concordance rates for a disorder are compared in monozygotic (identical; MZ) and dizygotic (non-identical; DZ) twins. Twin studies make the assumption that MZ twins share 100% of their genome, whereas DZ twins share 50% of their genome (equivalent to the proportion of the genome shared on average by typical siblings) and that both sets of twins, assuming they are raised together, share the same environment, to the same extent. Structural equation modelling can be used to estimate the proportion of genetic, shared environmental and unique environmental influences on phenotype. Importantly, this method will capture both inherited genetic variants (i.e. those likely to be present

in other family members), as well as spontaneously-occurring or *de novo* germline mutations, which occurred prior to splitting of the zygote in the identical twins. Additionally, contributions from gene-environment correlations and gene-gene interactions may be captured by the heritability estimates.

Twin studies have shown that ADHD and ASD are both highly heritable. A review of 20 clinical and community twin studies of ADHD estimated the mean heritability for ADHD to be approximately 76%, with the environment playing only a modest role (Faraone et al., 2005). With regards to ASD, a recent review of more than 30 twin studies highlighted the consistent finding of high heritability in both narrowly- and broadly-defined ASD samples, as well as in general population samples, with the majority of heritability estimates ranging from 60-90% (Ronald & Hoekstra, 2011). This review also reported that non-shared environmental influences (a term that includes any measurement error) may play a moderate role in ASD, but that there are only modest effects, if any, of shared environmental influences.

The similar heritability rates in clinical and community samples indicate that ADHD and ASD in the population are as heritable as clinically-defined ADHD and ASD. This further suggests that ADHD and ASD are likely to be extreme ends of continuously distributed traits in the population. Moreover, heritability rates for ADHD and ASD tend to be comparable when calculated using different categorical cut-offs (i.e. screening or more stringent diagnostic cut-off points) or as a continuous distribution of symptom scores, in a given twin sample (Larsson et al., 2011; Levy et al., 1997; Lundström et al., 2012; Robinson et al., 2011; Ronald et al., 2006a). However, one study found that heritability rates for continuously assessed ADHD and ASD traits are a little lower than for categorical diagnoses, though with overlapping confidence intervals (Anckarsäter et al., 2012). On the whole, these studies imply that genetic risk variants relevant to ADHD and ASD diagnosis may also be important for manifestation of symptoms at the milder ends of the distributions of ADHD and ASD traits.

Twin studies have also been used to estimate heritability rates separately for the core dimensions of ADHD and ASD. For ADHD, estimates of heritability for inattentive and hyperactive-impulsive symptoms range from 59-79% and 55-88%, respectively (Greven et al., 2011; Larsson et al., 2006; McLoughlin et al., 2007). Bivariate genetic analyses show substantial co-heritability of the two ADHD dimensions, as well as some level of genetic heterogeneity and specificity, with about 55-62% of genetic influences shared (Greven et al., 2011; McLoughlin et al., 2007). Likewise, communication deficits, social interaction deficits and restrictive repetitive behaviours all show high heritability (71-77%), but with moderate genetic correlations (23-53%), suggesting that the three DSM-IV sub-domains are also genetically separable to some extent (Ronald et al., 2006b). The genetic heterogeneity of the core ADHD and ASD dimensions suggests that it may be worthwhile to consider these separately in molecular genetic studies. Further work is needed to evaluate whether this is a valuable approach.

There are several limitations of the assumptions made by twin analyses. Firstly, MZ twins may not be 100% genetically identical as a result of post-germline somatic mutations. Phenotypes of MZ twins may also differ based on epigenetic changes throughout development, which may affect gene expression. Furthermore, it is possible that MZ twins share more aspects of their rearing environment, than do DZ twins. Moreover, given the higher incidence of prematurity and low birth weight in twins (Luke & Keith, 1992), they may not be an entirely representative subgroup of the population. The interpretation of the exact heritability estimates from twin studies is also complicated somewhat by the fact that ASD, and to a lesser extent ADHD, have relatively low prevalence in population samples of twins, which may reduce statistical power to accurately estimate the proportion of genetic risk factors involved in these disorders.

Adoption studies also contribute evidence to the importance of genetic risk factors for ADHD. Studies show that biological parents of children with ADHD are more likely to be diagnosed with or have elevated traits of ADHD than adoptive parents or control adults (Alberts-Corush et al., 1986;

Sprich et al., 2000). Because of the many practical difficulties with conducting adoption studies, the sample sizes of these studies have tended to be low. Also, adopted children who are at genetic risk for ADHD are also likely to have been exposed to potential prenatal adversity as well as early postnatal environmental risk factors prior to adoption. This is because their biological parents are likely to have had ADHD or other psychiatric problems themselves, thereby passing on this genetic risk as well as affecting the early environment.

However, despite the limitations of each design, family, adoption and twin studies provide consistent and robust evidence to suggest that ADHD and ASD are both familial and highly heritable. This is regardless of whether these conditions are assessed categorically or continuously, or whether they are considered as unitary constructs or multiple dimensions. Given this high heritability, numerous molecular genetic studies have investigated and begun to shed some light on the genetic architecture (i.e. the number, frequencies and effect sizes of risk variants) and underlying biology of these conditions.

Molecular genetic studies

Common genetic variants

Early molecular genetic studies investigating the source of the high heritability rates of ADHD and ASD focused on linkage analyses to try to identify regions of interest and association studies of candidate genes to try to identify specific genes involved in these disorders. However, the results of these studies are limited in identifying specific genetic risk variants. A meta-analysis of seven independent linkage studies of ADHD reported one region (16q23.1) of genome-wide significant linkage (Zhou et al., 2008). A comprehensive review of linkage studies in ASD reported only two loci (17q11-21 and 7q) which have been replicated at genome-wide significant levels (Abrahams & Geschwind, 2008). The scarcity of significant results in linkage studies is likely, at least in part, due to the small effect sizes of risk genes and the small sample sizes of these studies.

Although the pathogenesis of these disorders is unknown, candidate gene studies endeavoured to use a hypothesis-driven approach to select genes to study (e.g. based on evidence from the pharmacological treatment of ADHD) and compared allele frequencies in cases and controls. However, the sample sizes in these studies have been relatively small and the results are fairly inconsistent. Although reviews and meta-analyses point to converging evidence from human studies and mouse models for some of the most well-studied candidate genes, estimated effect sizes are rather small (Abrahams & Geschwind, 2008; Gizer et al., 2009). For example, a meta-analysis of 18 candidate genes for ADHD found significant associations for the following 6 genes: *DAT1*, *DRD4*, *DRD5*, *5HTT*, *HTR1B*, and *SNAP25* (Gizer et al., 2009). However, the effect sizes reported were modest (odds ratios ranging from 1.12-1.33). On the whole, these early studies demonstrated that the genetic variants contributing to ADHD and ASD are likely to have quite small effects and that larger samples are needed to have sufficient power to detect true risk variants.

More recent improvements in genotyping technology have given rise to genome-wide association studies (GWAS), which typically use regression analyses to compare allele frequencies in affected and unrelated, unaffected individuals (i.e. cases vs. controls). An alternative to this case-control approach is to compare allele frequencies in affected offspring and both parents; in this situation, unaffected parents are considered to be "pseudo-controls", with the non-transmitted genotypes used as the control data (Mick et al., 2010).

GWAS examine variants across the whole genome in a hypothesis-free manner with regards to the disease pathophysiology. Standard GWAS chips tend to genotype about 500,000-1,000,000 single nucleotide polymorphisms (SNPs). These genotyped SNPs capture a large proportion of human common genetic variation (typically with >1% minor allele frequency), owing to the fact that genotyped markers are often highly correlated with many untyped markers. This correlation is known as linkage disequilibrium, which is a term that refers to the non-random association of alleles across multiple sites in the genome. Testing the genome-wide burden of so many SNPs for

association with disease results in a high multiple testing burden. To account for this, the generally accepted threshold for a SNP to be considered genome-wide significant is $p<5 \times 10^{-8}$ (Dudbridge & Gusnanto, 2008).

Several GWAS of childhood ADHD have been published to date, with no SNPs yet crossing this threshold for significance (Hinney et al., 2011; Mick et al., 2010; Neale et al., 2008, 2010b; Stergiakouli et al., 2012; Yang et al., 2013). The largest published ADHD GWAS, a meta-analysis of 4 samples using data from a total of 2,064 parent-offspring trios, 896 cases and 2,455 controls, also revealed no significant individual SNPs (Neale et al., 2010a). This meta-analysis was the product of an international collaboration of multiple research groups, known as the Psychiatric Genomics Consortium (PGC). The PGC is in the process of substantially increasing the sample size of genotyped ADHD cases and controls to enable a much larger meta-analysis of ADHD GWAS to be performed.

There have also been several publications of ASD GWAS, which collectively report only 2 SNPs at the level of genome-wide significance and another 7 near this significance threshold (Anney et al., 2010, 2012; Ma et al., 2009; Wang et al., 2009; Weiss et al., 2009). Sample sizes in these studies have likely been too small for detecting genome-wide significant risk variants, regardless of the minor allele frequencies of the SNPs. The largest of these studies to date consisted of 2,705 parent-offspring trios (Anney et al., 2012).

Recent successes from the PGC schizophrenia research group demonstrate that much larger sample sizes than are currently available for ADHD and ASD will be needed to detect true genome-wide significant variants. For example, one of the earlier collaborative schizophrenia GWAS found 7 genome-wide significant SNPs using a total sample of 17,836 cases and 33,859 controls (Ripke et al., 2011), while the most recent schizophrenia GWAS meta-analysis reported 128 independent genome-wide significant SNPs based on a total analysis of 36,989 cases and 113,075 controls (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). It is clear that

psychiatric conditions such as ADHD, ASD or schizophrenia, are complex, with hundreds or even thousands of genetic variants with small effects likely to play a role. Larger collaborative samples of ADHD and ASD cases will be necessary for the discovery of specific common genetic variants and to determine whether the genetic architecture of these disorders is similar.

A number of studies have used the method of 'polygenic risk score analysis', sometimes also referred to as 'polygenic risk profiling', to harness the information provided by available GWAS (Purcell et al., 2009). Using this method, a pre-specified p-value threshold is used to select alleles more common in cases than controls from the discovery GWAS sample. This set of 'risk' alleles is then used to calculate a 'polygenic risk score' for each individual in an independent set of cases and controls. Thus, thousands of genetic variants, most showing only very weak association with the phenotype of interest, are used *en masse* to summarise each individual's burden of common genetic risk variants for that phenotype. A recent analysis using this method demonstrates that children clinically diagnosed with ADHD (N=452) have significantly higher polygenic risk scores for ADHD (based on an independent discovery sample) than controls (N=5,081), although with a very modest effect size (Hamshere et al., 2013a). Likewise, polygenic risk score analysis in an ASD sample (N=1,301), using an independent clinical ASD discovery GWAS (N=1,404), shows that children with ASD have higher polygenic risk scores than pseudo-controls (i.e. the non-transmitted parental genotypes), with only a very small effect size (Anney et al., 2012). Thus, these studies suggest that despite the small sample sizes, GWAS analyses are detecting some true SNP associations and collectively, these variants do play an important, if not necessarily large, role in risk for ADHD and ASD. However, effect sizes are dependent on sample sizes and in analyses of low sample size, predictive ability tends to be better with larger SNP sets (i.e. using less stringent p-value thresholds) (Wray et al., 2014).

A recent method called 'genomic-relationship-matrix restricted maximum likelihood' (GREML) using the software Genome-wide Complex Trait Analysis (GCTA) (Yang et al., 2011) has been used

to further demonstrate the relevance of common genetic variants to ADHD and ASD. This method allows for the estimation of heritability attributable to markers on GWAS SNP arrays (or indexed by linkage disequilibrium). This estimate of heritability is sometimes called 'SNP-chip' or 'narrow-sense' heritability. This method considers the genetic similarity of each pair of cases and each pair of control individuals and determines whether these comparisons are on average higher for cases than controls (Lee et al., 2013). A recent international collaborative study undertaken by the PGC estimated the heritability from SNPs to be 28% for ADHD and 17% for ASD (Lee et al., 2013). A genome-wide analysis of Han Chinese children estimated 42% heritability for ADHD from SNPs, which although higher than the estimate from the PGC sample, was not significantly different in a direct comparison (Yang et al., 2013).

One study has shown that SNP heritability estimates for ASD differ depending on ascertainment of the sample; simplex ASD cases (i.e. those with no affected first degree relatives) showed SNP heritability estimates of 40-50%, while multiplex cases (i.e. those with one or more affected relatives) showed a higher heritability estimate, of 65.5% (Klei et al., 2012). This is consistent with the expectation that there is higher genetic loading in multiplex families and that spontaneous rare mutations and other non-inherited genetic risk factors are likely to play a more important role in non-familial ASD. Another recent study used the GREML method in a Swedish population sample of individuals with ASD and unaffected controls and estimated the heritability for ASD based on SNPs to be approximately 49% (Gaugler et al., 2014). Both of these studies also reported that liability variants are distributed across the genome, with heritability estimates for each chromosome correlated with the length of the chromosome (Gaugler et al., 2014; Klei et al., 2012).

The SNP-chip heritability estimates of these studies are substantially higher than those reported by the PGC analysis (17%). This discrepancy may be a result of the PGC analysis employing pseudo-controls to estimate SNP heritability (Lee et al., 2013), given that SNP heritability estimates from pseudo-controls based on parents of ASD children are considerable (38%) (Klei et al., 2012).

Although one of the benefits of using pseudo-controls based on the parent-offspring trio design is reducing biases from population stratification, this design may be less sensitive than the case-control design for estimating SNP heritability.

Population stratification is an important issue to consider, as the GREML method may conceivably be sensitive to even minor differences in case and control ancestry. Typical use of a strict identity-by-descent quality control filter for removing marginally-related individuals and the fitting of principal components derived from SNP data as covariates will correct for population stratification effects to some extent (Lee et al., 2013). Furthermore, as with all case-control genetic analyses, it is important to ensure that the cases and controls come from as close and comparable a genetic background as possible, otherwise SNP heritability estimates could be inflated.

Overall, these recent GREML studies indicate that a certain proportion of estimated heritability from twin studies can be attributable to additive, common genetic variants. However, SNP heritability estimates are substantially lower than heritability estimates based on twin studies. This is likely because unlike twin studies, the GREML method does not consider the role of rare, *de novo* and non-additive genetic variation and the analysis is restricted to the common variants tagged on genotyping arrays. A direct comparison of the twin and GREML methods could provide insight into the relative proportion of common genetic variants in disease risk. However, a recent comparison of these methods in a population sample of twin children reports no significant genetic influence of SNPs to heritability estimates using the GREML method for behavioural problems, including ADHD and ASD traits (Trzaskowski et al., 2013). This result is inconsistent with the studies in clinical populations discussed above (Klei et al., 2012; Lee et al., 2013; Yang et al., 2013). It is not clear why this might be but it could be related to the lower power of a non-clinical community sample, where only a small proportion of the children will have had clinically significant behavioural problems (Faraone, 2013). However, similar analyses in a different population sample found significant SNP-chip heritability estimates for social-communication problems; about 18% for pragmatic language

scores and 24% for parent-rated social cognition scores at age 8 years (St Pourcain et al., 2013, 2014). Although further work is needed to clarify the full extent of the contribution of common genetic variants to ADHD and ASD, particularly at the lower end of the severity spectrum (i.e. ADHD and ASD traits in community samples), it is clear from GWAS data that common variants do play a significant role in both of these conditions.

Studies of rare genetic mutations

There is also support in the literature for the involvement of rare genetic variants in ADHD and ASD. It has been known for many years that numerous genetic syndromes (with known genetic risks) are associated with either or both of these conditions. Some of the more common examples of such syndromes include Fragile X syndrome, Velo-cardio-facial syndrome (VCFS), Tuberous Sclerosis complex, Smith-Lemli-Opitz syndrome and Williams-Beuren syndrome (Lo-Castro et al., 2011; Weiss, 2009). These syndromes are frequently also accompanied by intellectual disability and multiple other medical and physical issues. However, individuals with these syndromes do not always manifest ADHD or ASD; in other words, the known genetic risk variants for these syndromes have incomplete penetrance for behavioural phenotypes. Moreover, collectively, these conditions are very rare and cannot account for more than a small proportion of ADHD and ASD cases.

More recent genome-wide scans have implicated the role of copy number variants (CNVs), which are sub-microscopic deletions and duplications of sections of DNA. In ADHD, several studies report an overall burden of large (>100kb or >500kb) and rare (<1% population frequency) CNVs in children with ADHD, when compared with population controls (Williams et al., 2010, 2012; Yang et al., 2013). Although others have reported no overall burden of CNVs in ADHD cases relative to controls (Elia et al., 2010; Lionel et al., 2011), this may be because of differences in consideration of length and rarity of analysed CNVs. For example, Lionel and colleagues (2011) looked at CNVs>20kb in length, and defined "rare" to mean not overlapping more than 50% of a control CNV, whereas Elia and colleagues (2010) do not report restricting CNVs in this way for the burden analysis. This is an

important methodological distinction because larger and rarer CNVs have better concordance across different genotyping chips, are determined with greater accuracy and are more robustly associated with neurodevelopmental disorders (Williams et al., 2010). One important point to note is that only a small proportion of affected children have such large deletions or duplications and only a handful of specific loci (e.g. duplications at 15q13.3 and 16p13.11) have been especially enriched in ADHD cases relative to controls (Williams et al., 2010, 2012).

There have been many more studies implicating CNVs in children with ASD. Multiple studies have shown that there is a higher global burden of rare CNVs (of various size) in ASD as compared with population controls (Glessner et al., 2009; Levy et al., 2011; Marshall et al., 2008; Pinto et al., 2010). Several studies have highlighted the particular relevance of *de novo* CNVs (Sanders et al., 2011), especially in children from simplex families (Marshall et al., 2008; Sebat et al., 2007). Other studies highlight that a 'double hit' of two large CNVs is more common in children with ASD than in controls (Girirajan et al., 2012) and that females with ASD are more likely than males to be affected by a large, particularly disruptive (i.e. affecting more genes) *de novo* CNV (Gilman et al., 2011; Levy et al., 2011). Furthermore, other chromosomal abnormalities such as inversions, insertions and translocations are also likely to play a role in ASD (Talkowski et al., 2012). Thus, the evidence to date strongly implicates the role of CNVs in the aetiology of both ADHD and ASD.

Recent technological advances in genomics have led to studies involving sequencing entire exomes, at ever decreasing costs. Although four recent moderately large exome sequencing studies do not report an excess burden of rare single nucleotide mutations in children with ASD relative to their unaffected family members, the studies have revealed some interesting first results (Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012). In particular, *de novo* mutations identified in children with ASD were more likely to be nonsense or non-synonymous mutations than those present in unaffected siblings (Iossifov et al., 2012; Neale et al., 2012; Sanders et al., 2012). Nonsense mutations are point mutations that are predicted to result in non-functional proteins (e.g.

because an early translation codon is introduced, resulting in protein truncation). These types of mutations are thus potentially more likely to play a causal role in ASD. Numerous much smaller sequencing studies, some targeting only certain genes in a hypothesis-driven manner, have also yielded preliminary results of rare mutations, many *de novo*, thereby implicating dozens of putative candidate risk genes (Devlin & Scherer, 2012). However, given the rarity of individual point mutations, ASD sequencing studies are not yet sufficiently large to conclusively implicate specific candidate genes in disease risk for ASD. To date, there have been no published studies of exome sequencing of large groups of ADHD families. However, future large exome sequencing and also whole genome sequencing studies may yield further insights into specific rare genetic mutations and the biological mechanisms that they disrupt in these conditions.

Biological pathway analyses

As discussed above, numerous genetic studies of ADHD and ASD have implicated a range of genetic variants on a spectrum of frequencies, from very rare mutations to common SNPs. However, sample sizes in these studies are currently underpowered to reliably detect specific causal variants. An alternative method of examining the underlying biology of these conditions is to use information from gene annotation databases to determine whether genetic variants associated (often only weakly) with these conditions cluster together in functional biological pathways or networks.

In ADHD, one such study identified a number of CNVs disrupting a network of glutamate receptor genes (Elia et al., 2012). Another study combined information from published ADHD GWAS findings with pathway analysis and a literature search to implicate a network of genes linked to 'neurite outgrowth' functions (Poelmans et al., 2011). A pathway analysis combining information from GWAS SNPs and rare CNVs detected thirteen biological pathways (including ones related to cholesterol as well as development of the central nervous system), which were enriched for both types of variants, suggesting that both common and rare variants disrupt some similar biological processes in ADHD (Stergiakouli et al., 2012). Others have explored candidate biological pathways

chosen *a priori* in relation to phenotype and found that clusters of genes related to serotonin, dopamine, noradrenaline, and neurite outgrowth moderate the severity of hyperactive-impulsive symptoms in children with ADHD (Bralten et al., 2013).

In ASD, pathway analyses of rare CNV data implicate networks of genes related to synapse development and maintenance, axon targeting, neuron motility, neurotransmission, cellular proliferation, projection and motility, and GTPase/Ras signalling (Gilman et al., 2011; Guilmatre et al., 2009; Pinto et al., 2010). One study used data from gene expression networks and found that common and rare variants which have previously been implicated in ASD converge on networks of genes involved in synaptic and neuronal plasticity (Ben-David & Shifman, 2012). Exome sequencing shows that many of the identified *de novo* mutations in ASD cases have related functions; many of them occur in highly interconnected protein networks and show a high degree of connectivity to previously reported genes (Neale et al., 2012; O'Roak et al., 2012). Thus, pathway analyses are beginning to shed light on the types of biological pathways that are being disrupted by common and rare variants in ADHD and ASD.

1.7 Genetic overlap of ADHD and ASD

As has been discussed thus far, there is substantial evidence for the clinical co-occurrence of ADHD and ASD (see section 1.5) and for the high heritability of both conditions (see section 1.6). Moreover, as will be discussed next, family and twin studies show that these conditions also share genetic aetiology. Two family studies report that siblings of children with ADHD have elevated ASD scores relative to controls, although this effect may be limited to male siblings (Mulligan et al., 2009a; Nijmeijer et al., 2009). A more recent family study reported elevated levels of both ADHD and ASD symptoms in the unaffected siblings of both ADHD and ASD probands (Oerlemans et al., 2014). A recent population study found that a diagnosis of ADHD in the mother predicted a 6-fold risk for ADHD and a 2.5-fold risk for ASD in offspring, highlighting the important role of shared familial transmission of genetic risk for ADHD and ASD (Musser et al., 2014). Bivariate analyses in a

UK community twin sample (N=6,099 twin pairs) show that ADHD and ASD traits at age 8 years are moderately co-heritable, with a genetic correlation of about 0.56-0.57 in the entire population sample and about 0.62-0.72 at the quantitative extremes of ADHD and ASD trait distributions (Ronald et al., 2008). A follow-up study using this sample further showed that childhood ADHD and ASD traits at age 8 years are also moderately genetically correlated with ADHD and ASD traits at age 12 years, with the strongest genetic overlap of ADHD traits at age 8 and ASD communication difficulties at age 12 (Taylor et al., 2012). A study of Australian young adult twins (N=284 pairs) confirmed that ADHD and ASD traits were moderately genetically correlated in young adults (Reiersen et al., 2008a). A large Swedish population twin study (N=8,429 twin pairs) estimated that about 60% of the genetic variation in liability for ASD at a diagnostic level was shared with ADHD (Lichtenstein et al., 2010). Thus, a number of family and twin studies highlight that ADHD and ASD share genetic aetiology, both at a clinical/diagnostic level, as well as in the full distribution of traits in the population.

Several molecular genetic studies have recently shed some light on possible sources of the co-heritability of ADHD and ASD. Three genome-wide studies have found that large, rare CNVs associated with ADHD are enriched for loci which have previously been reported in ASD (Lionel et al., 2011; Williams et al., 2010, 2012). Furthermore, a pathway analysis of CNVs in ADHD and ASD cases shows a significant overlap in biological pathways disrupted by large, rare CNVs, even when physically overlapping CNV loci are excluded from the analysis (Martin et al, 2014). Thus, these studies suggest that large, rare CNVs may contribute to some of the observed overlap in genetic aetiology of ADHD and ASD. However, as mentioned earlier, these variants are quite rare and therefore other types of genetic variants are also likely to be important.

Given that GWAS studies have thus far not robustly implicated many specific common variants (SNPs) in ADHD or ASD, it is too early to conclude from these studies whether individual variants contribute to the co-heritability of these phenotypes. Two recent cross-disorder international

collaboration studies from the PGC have used GWAS data to explore whether common genetic variants from ADHD, ASD and three adult psychiatric conditions (schizophrenia, bipolar disorder and major depressive disorder) overlap. Both of these studies utilised the same datasets, including the largest currently available published datasets for ADHD and ASD (Anney et al., 2012; Neale et al., 2010a). The first study, which assessed whether polygenic risk scores based on each discovery GWAS could be used to predict case status in the other dataset, found no significant overlap of common genetic variants for ADHD and ASD for all p-value selection thresholds (Smoller et al., 2013). The second study used the method of GREML and found that the co-heritability estimate (based on common variants) for ADHD and ASD did not differ from zero (Lee et al., 2013). In contrast, both of these studies report significant cross-disorder overlaps for comparisons of other pairs of disorders (e.g. schizophrenia and bipolar disorder).

Although these results are surprising given the high co-heritability estimates of twin studies, the results need to be interpreted in light of several key methodological considerations. Firstly, the sample sizes of the most recent published ADHD and ASD GWAS are much smaller than those available for the other PGC disorders (schizophrenia, bipolar disorder and major depressive disorder). Furthermore, the use of a trio design for the entire sample in the ASD dataset and some of the samples in the ADHD GWAS may have weakened the power to detect associated risk variants. As discussed in section 1.6, such a design makes use of pseudo-controls, which assumes that the non-transmitted parental DNA is equivalent to that of unaffected controls. As mentioned earlier, such an assumption may be flawed (Klei et al., 2012). As such, it is too early to determine whether common variants play a major role in the observed co-heritability of ADHD and ASD. This issue will be examined in chapters 5 and 6.

Genetic overlap with other disorders and traits

The high heritability and shared genetic aetiology of ADHD and ASD is by no means unique to this pair of disorders. Other neurodevelopmental disorders (e.g. developmental coordination disorder or

specific language impairment) are also highly heritable (Bishop et al., 2008; Martin et al., 2006), as are cognitive abilities such as executive functioning and IQ (Ando et al., 2001; Anokhin et al., 2003; Deary et al., 2009; Kuntsi et al., 2006). A large Swedish population twin study found that there is a substantial amount of shared genetic risk across ASD and learning disorders, developmental coordination disorder and tic disorders, although this was not quite as high as the overlap seen for ADHD and ASD, mentioned earlier (Lichtenstein et al., 2010). Modest to high overlap in genetic aetiology can also be seen between ADHD and developmental coordination disorder (Martin et al., 2006). Twin studies also suggest that lower IQ is highly co-heritable with ADHD traits and diagnosis (Kuntsi et al., 2004) and modestly to highly co-heritable with ASD (Hoekstra et al., 2010; Nishiyama et al., 2009). Furthermore, shared genetic risks have also been reported by family and molecular genetic studies for childhood ADHD and ASD with adult psychiatric conditions such as schizophrenia and bipolar disorder (Faraone et al., 2012; Hamshere et al., 2013b; Sullivan et al., 2012). Indeed, it would seem that overlap in genetic risks is common across most child and adult psychiatric conditions (Doherty & Owen, 2014). As such, studying the nature of the shared genetic aetiology of ADHD, ASD and related cognitive and developmental difficulties may have broader implications for understanding genetic risk factors for human psychopathology across the lifespan. Moreover, studying associated cognitive and developmental problems may have the potential to further characterise these conditions and to increase the power of genetic analyses by considering the clinical heterogeneity of ADHD and ASD.

1.8 Summary & limitations of existing literature

In summary, ADHD and ASD are neurodevelopmental/psychiatric disorders, which show similar patterns of comorbidity and associated cognitive deficits and similarly high levels of heritability. They overlap clinically and share a considerable proportion of their genetic aetiology. It is abundantly clear from molecular genetic studies that these complex psychiatric conditions are multifactorial, with hundreds or even thousands of genetic risk variants likely to be involved (Anney

et al., 2012; Hamshere et al., 2013a; Klei et al., 2012; Lee et al., 2013; Yang et al., 2013). Genetic studies have implicated variants on a spectrum of frequencies, both those that are inherited and those occurring *de novo*. Although environmental factors are also likely to play an important role, twin studies suggest that genetic risk factors account for a high proportion of the aetiology of these conditions. Given that they are both complex psychiatric conditions, there are likely to be many causal (genetic and environmental) routes for manifesting ADHD or ASD.

It is also important to note that ADHD and ASD show a high degree of clinical phenotypic heterogeneity. Although rates of co-occurrence are high, not all children with ADHD will have ASD and vice versa. Similarly, children with either or both conditions vary in the severity of their phenotype and in terms of presence of comorbid psychopathology and co-occurring cognitive and developmental difficulties. Although it is clear that large datasets are necessary for gene discovery (e.g. through GWAS or exome/genome sequencing), there is also a need for further investigation of the clinical heterogeneity of these phenotypes in relation to genetic risk factors.

Despite growing clinical recognition that ADHD and ASD are related and can both be assessed in a child presenting with developmental problems, there are many gaps in our understanding of how these conditions relate to one another clinically. For example, it is not clear whether ASD symptoms can be accurately assessed in children with ADHD and whether the meaning of these symptoms is similar to that in children without ADHD. Also, the extent of the clinical impact of ASD symptoms in children with ADHD on other early developmental and cognitive outcomes is as yet unknown.

Furthermore, the source of the high co-heritability and shared genetic aetiology of ADHD and ASD needs to be further explored. Although overlap in CNV loci appears to contribute to this observed co-heritability, such CNVs are rare and cannot fully account for the findings of twin studies. Several recent studies of common SNPs suggest that there is no clear overlap but these studies utilised relatively small and underpowered clinical samples, with a considerable proportion of the samples using pseudo-controls. Given the dimensional nature of ADHD, additional analyses assessing ADHD

and ASD as quantitative traits in relation to common genetic variants may shed more light on whether these variants play a role in the genetic overlap reported by twin and family studies. Similarly, research should consider whether common genetic variants which may play a role in clinical ADHD or ASD are also relevant to cognitive difficulties associated with ADHD and ASD.

1.9 Thesis aims & hypotheses

This thesis is divided into two parts. The first objective is to examine the presentation of ASD traits in the context of an ADHD diagnosis, using a clinical sample. The aims are to:

1. Determine whether the factor structure of ASD traits in a clinical sample of children with ADHD is similar to that found in samples of children with ASD and children from the general population. It is hypothesised that social-communication deficits and restrictive-repetitive behaviours will be accounted for by separate, albeit correlated, factors. (Chapter 3)
2. Determine whether ADHD symptoms and ASD traits in a clinical sample of children with ADHD overlap or are distinct from one another, i.e. whether in a combined factor analysis, ADHD symptoms and ASD traits are accounted for by separate factors or not. (Chapter 3)
3. Determine whether ASD traits in children with ADHD index a more severe profile of cognitive difficulties and delayed development. (Chapter 4)

The second objective is to determine whether common genetic risk variants for clinically-diagnosed ADHD are relevant to ADHD and ASD traits and related neurocognitive abilities in a general population sample. The specific aims are to:

1. Determine whether common genetic risk variants relevant to clinically diagnosed ADHD are associated with traits of ADHD and ASD in children from the general population. (Chapter 5)
2. Determine whether common genetic risk variants relevant to ADHD diagnosis are associated with neurocognitive difficulties (i.e. general cognition, executive functioning and social cognition). (Chapter 6)

The next chapter will provide a general description of the two samples used for addressing the above aims. Chapters 3 and 4 will address the first objective and chapters 5 and 6 will address the second objective. Chapter 7 will conclude the thesis with a more general discussion of the implications of the findings, methodological considerations, strengths and limitations and suggestions for future work.

Chapter 2

Description of Samples

This chapter describes in detail the samples used in this thesis. It also gives an overview of the measures, with some background information on their psychometric properties. Details of study-specific inclusion criteria, additional details of measures and data analysis procedures are presented in the relevant chapters. The analyses are based on two samples. Chapters 3 and 4 utilise data from a clinical sample of children diagnosed with ADHD, whereas chapters 5 and 6 are based on data from children taking part in the Avon Longitudinal Study of Parents and Children (ALSPAC), a general population cohort sample.

2.1 Clinical sample of children diagnosed with ADHD

Recruitment & Procedures

Children aged 5 to 18 years with a confirmed or suspected clinical diagnosis of ADHD were recruited through Child and Adolescent Psychiatry or Paediatric out-patient clinics in Wales and across the United Kingdom for a study of genetic and environmental influences on ADHD. Clinicians completed a brief referral form with the parents of the study child. The form contained information on contact details of the family, the name and position of the referrer and the clinical diagnoses of the child. Once referred to the study, the parents were contacted by telephone either by an administrator or one of the research psychologists on the study team. A screening questionnaire was completed over the telephone in order to explain the study briefly and to determine eligibility of the child (see below for study inclusion and exclusion criteria).

If no cause for exclusion was noted during the telephone screening interview, a time was arranged with the parents for the study team to visit the family's home in order to carry out the assessments. The family was also given the alternative option to come to the University Hospital of Wales, Cardiff, for the assessments, with travel expenses covered (only N=3 chose this option). A

questionnaire pack with information sheets was sent in advance of the visit and collected or completed at the visit. Two trained psychology graduate research assistants visited the family, as arranged. One of the researchers interviewed the parent(s), assisted with questionnaire completion and obtained a DNA sample from biological parents. Meanwhile, the second researcher administered the cognitive assessments and obtained a blood or saliva sample from the child. Assessments lasted approximately 2-3 hours.

Approval for the study was obtained from the North West England and Wales Multicentre Research Ethics Committees. Written informed consent to participate was obtained from parents and children aged 16 years and older and assent was gained from children under 16 years of age. Families were provided with a thank-you payment in the form of a high street voucher for £15. Newsletters were sent at the end of the study to inform participants about the key results of the research.

Inclusion & exclusion criteria

Information obtained at the time of referral, during the screening telephone interview and at the home visit was used to determine inclusion in the study. The main inclusion criterion was the presence of a diagnosis of DSM-IV or DSM-III-R ADHD, confirmed by research diagnostic interview (Angold et al., 1995). Exclusion criteria were a known clinician's diagnosis of ASD, schizophrenia, Tourette's syndrome, bipolar disorder, history of epilepsy, brain damage or any other neurological or genetic disorder. Presence of Tourette's syndrome or bipolar disorder was assessed using the research diagnostic interview (Angold et al., 1995). Presence of intellectual disability (ID; IQ<70) was not an exclusion criterion for the study; however, for sensitivity, the main analyses in chapters 3 and 4 are repeated without this group of children. These inclusion criteria resulted in an overall sample size of N=1,132 children, recruited over the course of several related grants.

Phenotypic measures

ADHD & clinical comorbidities

Research diagnoses of ADHD and psychiatric comorbidities were ascertained using the parent version of the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 1995), a semi-structured diagnostic interview. The CAPA includes items that allow investigators to generate both symptom counts and categorical diagnoses of the presence of psychiatric disorders. Parents were asked about the presence of each of the 18 ADHD symptoms from the DSM-IV/ICD-10 and about impairment of functioning. Please refer back to Table 1.1 for details of ADHD diagnostic criteria. The total number of symptoms was summed for inattentive and hyperactive-impulsive traits separately (out of 9 each), as well as for total ADHD traits (out of 18). Confirmation of pervasiveness of ADHD symptoms and impairment across settings, which is necessary for a DSM-IV diagnosis, was assessed through questionnaires sent to the child's school (DuPaul and Conner's teacher rating scale (Conners et al., 1998; DuPaul, 1981)) or the Child ADHD Teacher Telephone Interview (ChATTI (Holmes et al., 2004)). CAPA interviews were undertaken by trained psychologists and were audiotaped. Cases were discussed weekly with the supervising child and adolescent psychiatrist (Professor Anita Thapar). Inter-rater reliability for ADHD diagnostic subtype, assessed using 60 cases, was perfect (κ=1.0).

Comorbid psychiatric disorders assessed using the CAPA were DSM-IV oppositional defiant disorder (ODD), conduct disorder (CD), anxiety disorders (generalised anxiety disorder, social anxiety or separation anxiety) and depression. As CD was the primary outcome for the original study, an inter-rater reliability estimate was calculated for parent-rated CD symptoms; inter-rater reliability was very good (κ =0.98). Children aged 12 years and over also completed the child version of the CAPA (Angold & Costello, 1995), assessing only these comorbid problems (but not ADHD). In accordance with previous research, a symptom was considered as present if either the parent or child reported it (Langley et al., 2010). Symptom totals and diagnoses were calculated according to DSM-IV.

ASD traits

ASD traits were assessed using the Social Communication Questionnaire (SCQ), formerly known as the Autism Screening Questionnaire (Berument et al., 1999; Rutter et al., 2003). The SCQ is a validated 40-item parent-rated questionnaire, based on the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994). Parents responded to each question by marking 'yes' or 'no' on the form. Items were re-coded to indicate presence or absence of the autistic behaviour. A total score of autistic traits was then calculated. Item 1 is a language screening item and was not included in calculating the overall total. A score of 15 or greater has been suggested to indicate clinically-relevant levels of autistic traits (Berument et al., 1999). This measure is unavailable for children recruited at the beginning of the study, as it was introduced into the protocol partway through the study. SCQ data were available for N=821 children. See Appendix 2.1 for a list of all SCQ items.

Cognitive assessments

Parents were asked to withhold stimulant medication from their child for 24 hours prior to testing. However, this was not enforced strictly and a proportion of the parents chose to give their child their usual medication (N=634 (66.7%) of parents complied).

Full-scale IQ was assessed using the Wechsler Intelligence Scale for Children, version III (WISC-III; N=387) or IV (WISC-IV; N=620), using all 10 subtests (Wechsler, 1992, 2003). The WISC-III was based on the following four components: Verbal Comprehension Factor, Freedom from Distractibility Factor, Perceptual Organisation Factor and Processing Speed Factor. The WISC-IV, an updated version of the WISC-III, was based on the following four indices: Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index and Processing Speed. The Digit Span subtest of the WISC-IV is a measure of verbal working memory. The task requires the child to repeat lists of digits in a) the presented order and b) reverse order. It has been used in previous research to assess this domain of functioning in children with ADHD and ASD (Gau & Shang, 2010; Poirier et al., 2011).

The Wechsler Objective Reading Dimensions (WORD) (Wechsler, 1993) was administered to assess basic reading, spelling and reading comprehension abilities. Due to time restrictions, this measure was only administered in children up to the age of 12 years. The Intra-Extra Dimensional Shift task from the British Cambridge Neuropsychological Battery (CANTAB) (Cambridge Cognition, 1996) was administered as a measure of executive functioning (set shifting, visual discrimination and attentional flexibility). This computer-based task requires the child to learn to discriminate between two shapes, while ignoring an additional dimension (white lines) overlaying the target shapes. This is followed by a test of whether the child can learn to shift attention to the previously irrelevant dimension. This task is based on the Wisconsin Card Sorting Task (WCST) (Berg, 1948).

Other parent-completed questionnaires

Parents were asked general questions about their child's speech and motor development at the interview; these were: "was your child talking by age 2?", "has your child ever had speech therapy?", "was your child walking by 18 months?", and "is your child clumsy? –not at all, –just a little, –pretty much, –very much". Children who were not talking by age 2 and/or had had speech therapy were classed as positive for this broad measure of language problems. Children who were not walking by 18 months and/or were clumsy "pretty much" or "very much" were classed as positive for this broad measure of motor problems.

Family annual income, parental employment status and parental educational attainment were assessed by parental questionnaire. Low income was defined as annual family income <£20,000 (equivalent ~US$32,000), and low educational attainment was defined as having left school without qualifications (GCSE or equivalent) at age 16 years. Socioeconomic status (SES) was classified by the occupation of the main family wage earner using the UK Standard Occupation Classification (Office of National Statistics, 2001). Three SES categories were defined (low: unskilled workers/unemployed; medium: manual and non-manual skilled/partially skilled workers; high: professional and managerial workers). Parents were also asked about their ethnicity as well as that

of the children's biological grandparents. Children were classed as "British" if both parents and all 4 grandparents were British and "non-British" if at least one parent or grandparent was of non-British ethnicity.

Table 2.1: Characteristics & demographics of the clinical ADHD sample

		N	%
DSM-IV ADHD diagnosis subtype	Combined	605	73.7
	Inattentive	48	5.8
	Hyperactive-Impulsive	80	9.7
	DSM-III-R only	85	10.4
	Unknown*	3	0.4
ODD/CD	ODD	354	43.6
	CD	147	18.1
	Neither ODD or CD	311	38.3
Anxiety/ Depression	Any anxiety disorder	55	7.0
	Depression	5	0.6
	Both	2	0.3
	Neither	724	92.1
Family socioeconomic status	Low	375	50.4
	Medium	277	37.2
	High	92	12.4
Family income	Low	325	35.9
	Medium-high	182	64.1
Parental education	No GCSEs	152	28.3
	GCSEs or higher	386	71.7
Ethnicity	British	737	93.9
	Non-British	48	6.1

* Missing data points mean diagnostic subtype cannot be reliably determined, although sufficient symptoms for several possible subtypes are present; N.B. Numbers do not always add up to total N=821 owing to missing data; ODD: oppositional defiant disorder; CD: conduct disorder

Clinical ADHD sample demographics and characteristics

As this thesis focuses on ADHD and ASD, the clinical sample used for the analyses consisted of those children who met inclusion criteria to the above study who also had SCQ data available

(N=821). Of these, N=124 children were female (15.1%). The children were aged on average 10.3 years old (SD=2.9 years, range=5-18 years). Table 2.1 shows the breakdown of DSM-IV research diagnoses in the sample, as well as socio-demographic characteristics of the children. The mean IQ in the sample was 83.9 (SD=13.7, range=43-124). Figure 2.1 shows the distribution of IQ scores. There were N=97 children (12.6%) with intellectual disability as assessed by IQ<70.

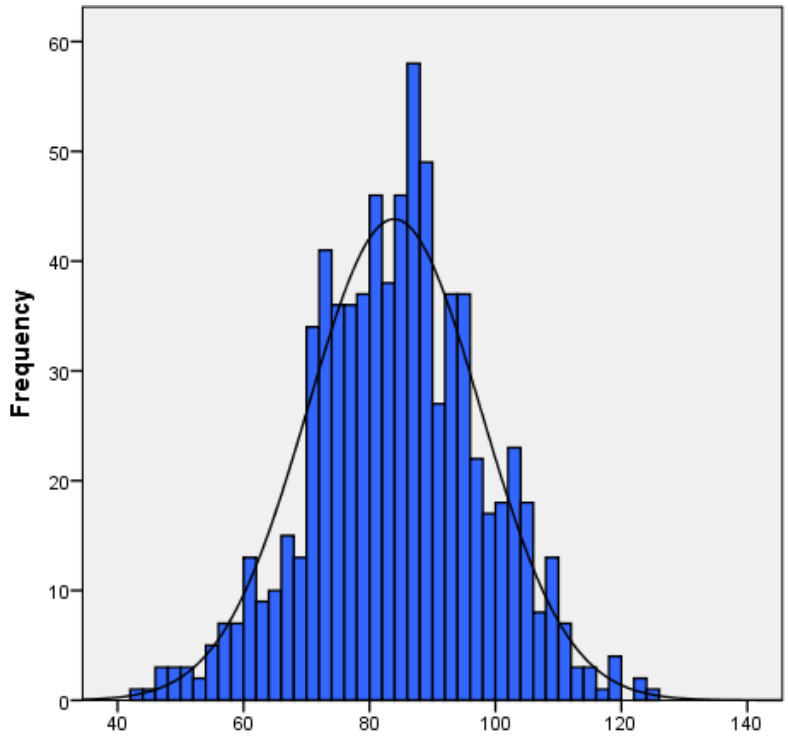

Figure 2.1: Distribution of IQ in the clinical ADHD sample

Genetic data

A proportion (49%; N=559) of the whole ADHD sample of N=1,132 children were genotyped for the purpose of a genome-wide association study (Stergiakouli et al., 2012). DNA from children meeting DSM-IV ADHD criteria, assessed using the same diagnostic interview, was also available from collaborators in St. Andrews, Scotland (N=44) and Dublin, Ireland (N=196). DNA for all ADHD cases (N=799 total) was extracted from saliva or peripheral blood samples and the samples were genotyped with the Illumina (San Diego, CA, USA) Human660W-Quad BeadChip. Control genetic data were obtained from the Wellcome Trust Case-Control Consortium-Phase 2 (WTCCC2) (The

Wellcome Trust Case Control Consortium, 2007). This dataset comprised 3,000 individuals from the 1958 British Birth Cohort and 3,000 individuals from the UK Blood Services collection. Combining these two samples for use as comparison subjects in genetic association studies using UK case samples has been validated (The Wellcome Trust Case Control Consortium, 2007). Control DNA samples were genotyped by WTCCC2 using the Illumina Human1.2M BeadChip. BeadStudio (version 2.0) was used to assign genotypes.

Quality control procedures are described in detail in the GWAS publication (Stergiakouli et al., 2012) and a brief description of these procedures can be found in Appendix 2.2. After quality control, genome-wide data were available for N=727 case subjects and N=5,081 control subjects for 502,702 autosomal SNPs. The results of this published GWAS were used for the purpose of generating polygenic risk scores in the general population sample for analyses presented in chapters 5 and 6.

2.2 Children from the general population

Recruitment & Procedures

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a large, well-characterised longitudinal dataset (Boyd et al., 2013; Fraser et al., 2013). ALSPAC originally recruited N=14,541 pregnant women resident in Avon, England, with expected delivery dates between April 1, 1991, and December 31, 1992. Of these pregnancies, N=13,988 children were alive at 1 year of age. An additional 713 children who would have been eligible but whose mothers did not enrol during pregnancy were enrolled after age 7, resulting in a total sample of N=14,701 children alive at age 1 year. The study website (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/) contains details of all available data through a fully searchable data dictionary. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and Local Research Ethics Committees. The analyses presented in this thesis were approved by the ALSPAC Executive Committee (project reference B1342).

Data collection in this sample occurred at numerous time-points through a variety of means and is still ongoing. The data used in this thesis were primarily obtained through parent-completed postal questionnaires regarding the study child, as well as from direct cognitive assessments of the child at several 'Focus' clinics.

Inclusion & exclusion criteria

Children from triplet and quadruplet births were excluded from the analysed sample. Furthermore, only one child from twin births was included in the analysis due to genotyping quality control procedures (see section on Genetic data below for further details). Details on inclusion criteria for analyses in chapters 5 and 6 are presented in sections 5.3 and 6.3. Briefly, only children who had both genetic data after quality control and phenotype data were included. Phenotype data for the ADHD and ASD-related measures were considered as available if children had <30% missing items. Children with intellectual disability (IQ<70) or diagnosed ADHD or ASD were included in the analyses.

Phenotypic measures

ADHD & ASD-related measures

Data on ADHD traits were collected using the parent-rated Development and Well-Being Assessment (DAWBA), when participants were aged approximately 7 years and 7 months old (Goodman et al., 2008). The DAWBA is a semi-structured assessment package that generates DSM-IV diagnoses and symptom scores of childhood psychopathology. Scores were calculated for inattentive and hyperactive-impulsive traits separately, as well as a score for total ADHD traits. The DAWBA was also completed by parents when children were at the approximate age of 10 years and 8 months. Total and subtype ADHD scores were also calculated at this age.

Social-communication traits were assessed using the Social and Communication Disorders Checklist (SCDC), which was administered at the same time as the DAWBA ADHD measures (Skuse et al., 2005) and the pragmatic language subscales of the Children's Communication Checklist

(CCC), which parents completed when children were at the approximate age of 9 years and 7 months (Bishop, 1998). A quantitative measure of restricted, repetitive behaviours was not available. Both the CCC and the SCDC have been shown to have good predictive reliability for a clinical diagnosis of ASD in the ALSPAC sample (Steer et al., 2010). The CCC shows good reliability (0.80), internal consistency (0.80-0.87) and validity for language problems (Bishop, 1998) and the SCDC shows good internal consistency (0.93), high test-retest reliability (0.81) and validity for a diagnosis of ASD (Skuse et al., 2005). The SCDC assesses social cognition and understanding, whereas the CCC pragmatic language subscales measure the ability to use language in a social context. Please see Appendices 2.3 and 2.4 for a list of the items making up the SCDC and the CCC pragmatic language subscales. Previous research has shown that children with ADHD or ASD have lower pragmatic language ability scores than typically developing controls, but those with ASD have lower scores than those with ADHD (Geurts et al., 2010). The CCC data were used to calculate a pragmatic language total score, based on the following five sub-scales: inappropriate initiation, coherence, stereotyped conversation, conversational context and conversational rapport. A total score for social cognition, as assessed with the SCDC, was also calculated.

Information was also available on categorical DSM-IV ADHD diagnoses based on the full set of DAWBA items at approximately age 7 years. Data on ASD diagnoses were available based on clinical records, utilising a clinician's diagnosis of ASD (Williams et al., 2008).

Neurocognitive measures

As part of the on-going ALSPAC longitudinal study, families were invited to attend a 'Focus' clinic when the children were at the approximate age of 8.5 years. A number of neuropsychological tests were carried out with the children during this clinic.

A short form of the WISC-III assessment was employed, with alternate items from all 10 subtests administered (Wechsler, 1992). This test was used to obtain an estimate of verbal IQ, performance IQ and full scale IQ. The WISC-III Digit Span task was administered in addition to the above 10

subtests. This task is nearly identical to the WISC-IV subtest of the same name (described earlier in section 2.1) and provides a measure of verbal working memory.

Attentional inhibitory control was assessed using the Opposite Worlds task from the Tests of Everyday Attention for Children battery (Robertson et al., 1996). The first part of the task (the Same Worlds control condition) requires the child to read a list of consecutive numbers comprising "1"s and "2"s. The second part of the task (the Opposite Worlds experimental condition) requires the child to inhibit the predominant response by saying "two" when presented with the number one and vice versa. The mean time taken to complete the Same Worlds trials was subtracted from the mean time for the Opposite Worlds trials to obtain a measure of attentional inhibitory control.

Facial emotion recognition was assessed using a computerised version of the faces subtest of the Diagnostic Analysis of Nonverbal Accuracy (DANVA) (Nowicki & Duke, 1994). Children were presented with 24 children's faces and were asked to state which of four emotions (happy, sad, angry or fearful) the face was showing. Half of the faces had high emotional intensity (the easy condition) and half of the faces had low emotional intensity (the hard condition). A sum of the total number of errors made was calculated to give a measure of facial emotion recognition difficulties.

Families were also invited to attend another 'Focus' clinic when the children were at the approximate age of approximately 10.5 years. At this time, the children completed the Counting Span Task, a measure of working memory. In this task, children needed to count red dots on a screen of blue and red dots, while trying to recall the number of red dots on previously presented screens. This task provides a 'global score' measure, corresponding to the number of correct trials.

Other measures

For assessments of social background, questionnaires were used to assess social class based on parental occupations, parental educational level and household ownership status. As with the clinical ADHD sample described in section 2.1, three SES categories were defined (low: unskilled workers/unemployed; medium: manual and non-manual skilled/partially skilled workers; high:

professional and managerial workers) based on maternal and paternal reported occupations. Families were also split into whether the parents reported owning their house/having a mortgage or not. Maternal education levels were split into the binary variable of whether mothers had attained a) A-levels or higher education or b) only vocational or GCSE-level education.

Genetic data

A total of 9,912 ALSPAC children were genotyped using the Illumina HumanHap550 quad genome-wide SNP genotyping platform by 23andMe subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, USA. Quality control procedures were performed by ALSPAC researchers and are described in Appendix 2.2. After quality control, genome-wide data were available for N=8,231 of the children. The dataset contained 500,527 SNPs.

An additional step in quality control was undertaken because of the nature of the genetic analyses performed in chapters 5 and 6, which involved using the ADHD clinical sample as a discovery sample for analyses (see chapters 5 and 6 for details). To ensure that there were no related individuals in both samples, an identity by descent (IBD) analysis was conducted by Dr Evie Stergiakouli, a co-author on the publications resulting from chapters 5 and 6, across the clinical ADHD sample and the ALSPAC sample, using PLINK (Purcell et al., 2007). This was deemed necessary because a large proportion of the children in the discovery clinical ADHD sample (N=559; 70%) and all of the target ALSPAC sample were recruited from a similar geographical region (Wales and Southwest England) and are of a similar age. Individuals in ALSPAC who showed IBD≥12% (equivalent to the average biological relatedness of first cousins) in relation to any individual in the clinical ADHD sample (N=2) were removed from analyses, leaving a final sample of N=8,229 children in ALSPAC.

Sample demographics and characteristics

This sample of ALSPAC children with genetic data available after quality control (N=8,229) was used for analyses in chapters 5 and 6. Of these children, N=4,015 (48.8%) were female. Table 2.2

shows the breakdown of ADHD and ASD diagnoses in the sample, as well as socio-demographic characteristics of the children. The mean IQ in the sample was 104.8 (SD=16.4). Figure 2.2 shows the distribution of IQ scores. There were N=86 children (1.6%) with intellectual disability (IQ<70).

Table 2.2: Characteristics & demographics of the ALSPAC sample

		N	%
DSM-IV ADHD or Clinical ASD diagnosis	ADHD	105	1.8
	ASD	35	0.6
	Both ADHD & ASD	8	0.1
	Neither	5,585	97.4
Socioeconomic status	Low	40	0.6
	Medium	2,845	40.6
	High	4,117	58.8
House-owner	No	1,581	20.9
	Yes	5,987	79.1
Maternal education	GCSEs/vocational	1,859	25.1
	A-level or higher	5,541	74.9

N.B. Numbers do not add up to total N=8,229 owing to missing data

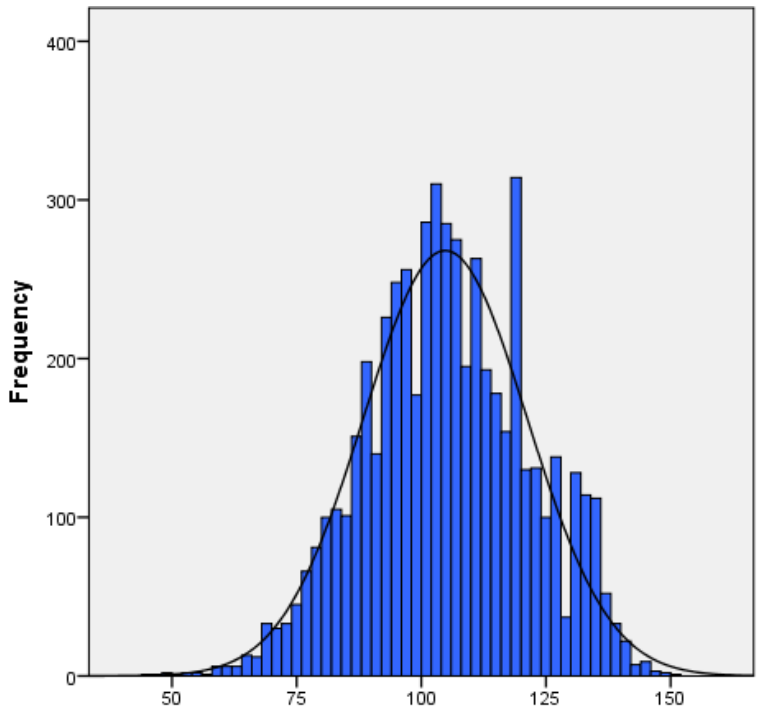

Figure 2.2: Distribution of IQ in the ALSPAC sample

2.3 Brief notes regarding measures and sample sizes

Measures

Similar measures across the two samples were utilised for analyses, wherever possible. Table 2.3 below summarises the developmental and cognitive domains of interest along with corresponding measures in the two samples.

Table 2.3: List of behavioural and cognitive measures available in both samples

Domain	Clinical ADHD sample	ALSPAC sample
ADHD: inattentive & hyperactive-impulsive	CAPA	DAWBA
ASD: Social-communication	SCQ	CCC/SCDC
ASD: RRBs	SCQ	Unavailable
IQ	WISC-IV full-scale	WISC-III abbreviated
Working memory	WISC-IV Digit Span	WISC-III Digit Span & Counting Span Task
Other executive functioning	IED CANTAB (set-shifting)	Opposite Worlds task (attentional inhibitory control)
Facial emotion recognition	Unavailable	DANVA
Reading	WORD	Not examined

CAPA: Child and Adolescent Psychiatric Assessment; DAWBA: Development and Well-Being Assessment; SCQ: Social Communication Questionnaire; CCC: Children's Communication Checklist; SCDC: Social and Communication Disorders Checklist; RRBs: restrictive and repetitive behaviours; WISC: Wechsler Intelligence Scale for Children; IED: Intra-extra dimensional shift task; CANTAB: Cambridge neuropsychological test automated battery; DANVA: Diagnostic Analysis of Nonverbal Accuracy; WORD: Wechsler Objective Reading Dimensions.

Sample sizes

The analyses in chapters 3 and 4 are based on the clinical sample of children diagnosed with ADHD, whereas chapters 5 and 6 are based on data from children taking part in ALSPAC. Although multiple chapters are based on the same samples, there is some variability in which individuals are included in each analysis, due to missing data.

Chapter 3

Factor Structure of Autistic Traits in Children with ADHD

The work presented in this chapter has been published:

***Martin, J.**, Hamshere, M. L., O'Donovan, M. C., Rutter, M., & Thapar, A. (2013). Factor structure of autistic traits in children with ADHD. Journal of Autism and Developmental Disorders, 44(1), 204–15.*

The published article has been edited for this chapter in order to include additional results (previously available as supplementary materials) and to reduce the amount of repetition across chapter 2 and section 3.3 in this chapter. Please note that there is some repetition of content in section 3.2 and the thesis Introduction (chapter 1).

Please see http://link.springer.com/article/10.1007/s10803-013-1865-0 for the full published article.

3.1 Summary

ADHD and ASD often co-occur. Factor analyses of ASD traits in children with and without ASD indicate the presence of social and restrictive-repetitive behaviour (RRB) factors. This study used exploratory factor analyses to determine the structure of ASD traits (assessed using the Social Communication Questionnaire) in children with ADHD. Distinct factors were observed for 'social' and 'rigidity' traits, corresponding to previous factor analyses in clinical ASD and population samples. This indicates that the split between social-communication and RRB dimensions is unaffected by a diagnosis of ADHD in children. Moreover, the results also suggest that there is some overlap across hyperactive-impulsive symptoms and RRB traits in children with ADHD, which merits further investigation.

3.2 Introduction

ADHD and ASD show a high rate of symptom overlap, with a substantial proportion of individuals with one of the conditions also meeting diagnostic criteria for the other (Rommelse et al., 2010). Although historically, diagnostic manuals have not allowed for a joint diagnosis of ADHD and ASD, with an ASD diagnosis trumping and excluding a diagnosis of ADHD (DSM-IV & ICD-10), the observed co-occurrence has prompted changes to these diagnostic exclusions for the DSM-5. Overlap of the two conditions is found at the diagnostic level and at the level of symptoms below diagnostic thresholds in referred children and in the general population (Grzadzinski et al., 2011; Reiersen et al., 2007; Rommelse et al., 2010; Ronald et al., 2008). Moreover, given the high heritability of both conditions (Faraone et al., 2005; Freitag, 2006), it is of note that family and twin studies show co-heritability (Lichtenstein et al., 2010; Lundström et al., 2011; Mulligan et al., 2009a; Nijmeijer et al., 2009; Taylor et al., 2012).

In recent years, interest has been growing in exploring the overlap of ADHD and ASD, in terms of associated clinical comorbidities, neuropsychological deficits (e.g. executive functioning) and shared genetic susceptibility. Given the time and cost of performing in depth interviews and observational diagnostic assessments of ASD such as the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1989, 1994), many studies have relied on questionnaires to measure autistic traits. The Social Communication Questionnaire (SCQ; formerly known as the Autism Screening Questionnaire, ASQ) (Berument et al., 1999; Rutter et al., 2003) is a parent-rated questionnaire assessing ASD behaviours. It is based on the ADI-R (Lord et al., 1994) and has been found to agree well with it on a diagnostic level, although only adequately on an item-by-item basis (Bishop & Norbury, 2002). The SCQ has been widely used as a screening tool or quantitative measure of autistic traits in samples of children with suspected ASD (Eaves et al., 2006), children with ADHD (Kochhar et al., 2011; Kröger et al., 2011; Mulligan et al., 2009a) or other psychopathology (Pine et al., 2008; Towbin et al., 2005) and also in

the general population (Mulligan et al., 2009b). Children diagnosed with ADHD score higher on the SCQ than their unaffected siblings or typically developing controls (Kochhar et al., 2011; Mulligan et al., 2009a).

Like the ADI-R, the SCQ can be divided into subscales for the three DSM-IV and ICD-10 diagnostic sub-domains of autistic symptoms: social-interaction deficits, communication problems and restrictive-repetitive behaviours (RRBs). There is a growing body of evidence suggesting that dividing ASD symptoms into these sub-domains is meaningful (Happé & Ronald, 2008); although the behaviours occur together frequently, both phenotypic and genetic correlations between them are only moderate, suggesting that the three sub-domains are to some extent clinically and genetically separable (Ronald et al., 2006a, 2006b). Nevertheless, the sub-domains appear to cluster more than expected by chance (Ronald et al., 2006b). In a recent population-based twin sample, the authors tested whether different molecular genetic variants, assessed genome-wide, predicted social and 'non-social' (i.e. RRB) traits, separately (Ronald et al., 2010a). The study did not find evidence of these clinical domains being separate at a molecular genetic level, although only 1-2 genome-wide genetic variants were found to be nominally associated with either sub-domain (without allowing for multiple testing), and these did not replicate in an independent clinical sample of children diagnosed with ASD. These non-significant results are arguably due to the study's relatively small sample size for a genome-wide association study that requires multiple testing (ranging from N=372-436 in the high and low trait comparison groups), making it unclear whether the same genetic variants are involved in these sub-domains. Therefore, more familial and molecular genetic studies in larger samples are needed to clarify the extent to which these social and RRB sub-domains are aetiologically related. Interestingly, the overlap of ADHD with ASD symptoms has been demonstrated to occur within all three of these sub-domains, though RRBs have been found to be less frequent than social and communication deficits in children with ADHD (Rommelse et al., 2011).

Factor analyses of autistic traits in clinical ASD and community samples using a variety of ASD measurement tools generally indicate that multiple factors account for the observed covariance structure of ASD symptoms and traits (Happé & Ronald, 2008; Mandy & Skuse, 2008). Likely due to differences in study design, there is little agreement in terms of the specific factors and their composition. However, nearly all factor analytic studies derive at least one factor related to social-communication features and a separate factor related to 'non-social' behaviour or RRBs (Mandy & Skuse, 2008). To date, there has been no published factor analysis of autistic traits in a group of children with ADHD and it is not yet known whether the presence of ADHD affects the nature of autistic symptoms in some manner.

The main aim of this study was thus to explore the structure of autistic traits (as measured by the SCQ) in a clinical sample of children with ADHD, to determine whether this structure was similar to that found in samples of children with ASD and those from the general population. It was hypothesised that social-communication traits and RRBs would be accounted for by separate, albeit correlated, factors.

A secondary aim was to explore the relationship between ADHD symptoms and ASD traits in a combined exploratory factor analysis. One previous study suggests that the core diagnostic criteria of ADHD and ASD do not overlap (i.e. ADHD and ASD symptoms load on separate sets of factors) in a general population sample of school children (Ghanizadeh, 2010). However, although deemed as common conditions, ADHD and ASD have prevalence rates of less than 5% in general population samples, with wide variability in reported prevalence rates in different geographic regions (Polanczyk et al., 2007; Rutter, 2005). Therefore, it would be valuable to determine whether there is any overlap in ADHD and ASD symptomatology in a clinical sample of children diagnosed with ADHD (i.e. a group of children who have higher rates of individual symptom presence than the average child in the population).

3.3 Method

Sample

The sample used for this analysis was the clinical sample of children with ADHD. Recruitment procedures, general inclusion criteria and an overview of the measures used are detailed in section 2.1. For the current analyses, the sample (N=821) consisted of children with data on the measure of autistic traits (the SCQ).

Measures

The CAPA, a semi-structured interview conducted with parents (Angold et al., 1995), was used to assess the presence or absence of each of the 18 ADHD symptoms from the DSM-IV/ICD-10. Parents also completed the SCQ (Berument et al., 1999; Rutter et al., 2003). The language screening question (item 1) was omitted from analysis, leaving 39 items related to autistic traits. These items were divided into the three sub-domains of ASD symptoms, as defined by the diagnostic symptoms stipulated by the DSM-IV and ICD-10; there were 20 items classed as 'social-interaction deficits', 10 as 'communication deficits', 8 as 'restricted and repetitive behaviours (RRBs)' and one item (item 18) was unclassified. See Appendix 2.1 for a list of all SCQ items as well as sub-domain classification. Full-scale IQ was assessed with the WISC-III or WISC-IV (Wechsler, 1992, 2003), using all subtests.

Factor analysis

To test the main hypothesis, an exploratory factor analysis (EFA) was performed on the 39 SCQ items and the results were compared with a previous factor analysis of the SCQ in a sample of children with ASD and other psychiatric problems (Berument et al., 1999). The secondary aim of the study was addressed by adding in the 18 ADHD symptoms into the EFA model; this analysis is henceforth referred to as the combined SCQ-ADHD analysis.

Cases with any missing data in either analysis were excluded (SCQ analysis: 97 with 1/39, 52 with 2/39 and 112 with >2/39 items missing; combined SCQ-ADHD analysis: 117 with 1/57, 55 with 2/57

and 115 with >2/57 items missing), leaving a complete data set of N=560 for the SCQ analysis, and N=534 for the combined SCQ-ADHD analysis. Children included in the analyses did not differ from those excluded (for having missing data) in terms of gender, age at assessment, family social status, the severity of their ADHD symptoms or presence of oppositional defiant disorder, conduct disorder or anxiety (p>0.05). They did differ in terms of IQ, with children with missing data (SCQ analysis: mean IQ=82.4; SCQ-ADHD analysis: mean IQ=82.3) having lower IQ (SCQ analysis: t(df)=-2.0(752), p=0.045; SCQ-ADHD analysis: t(df)=-2.3(752), p=0.019) than those with no missing items (SCQ analysis: mean IQ=84.6; SCQ-ADHD analysis: mean IQ=84.7).

All variables were dichotomous (symptom present/absent). For each analysis, bivariate associations were calculated as tetrachoric correlations. The tetrachoric correlation matrices were not positive definite, so a smoothing algorithm was applied to the correlation matrix (using the R command: tetrachoric ("data", smooth=T)). Visual inspection of the matrices showed extremely high correlation (tetrachoric correlation=0.95) of SCQ items 24 "when he/she was 4-5 did he/she nod his/her head to mean *yes*?" and 25 "when he/she was 4-5 did he/she shake his/her head to mean *no*?", therefore item 25 was dropped from further analyses.

For each analysis, the correlation matrix was analysed using exploratory factor analysis (EFA) to find the minimum residual (minres) solution. This solution was deemed most appropriate given that the assumption of multivariate normality was not fulfilled due to dichotomous variables and the tetrachoric correlation matrices being not positive definite. Choice of number of factors was based on a combination of theory (based on previous literature) and points of inflection on the scree plots. These methods were used in conjunction to maximise variance explained, while maintaining a parsimonious and theory-driven approach towards conceptualising the target constructs. For plausible alternative solutions, patterns of correlations across the factor scores were also examined to ascertain the pattern of association across the derived measured factors. The EFA solution was rotated using an oblique rotation (oblimin) as non-independence of the underlying factors was

hypothesised and observed (Matsunaga, 2010). For ease of interpretation, factor loadings below 0.2 are not shown. Factor analyses were performed in R.

Factor scores

To test whether IQ, age, gender or presence of intellectual disability (ID; IQ<70) had any effects on the results, factor scores were calculated for each analysis. For each factor, a weighted average score for each individual was calculated using all the loadings from the pattern matrices as weights. Pearson correlations were calculated for each factor score with age and IQ. *T*-tests were used to compare factor scores in 1) boys relative to girls and 2) children with ID relative to those without ID.

3.4 Results

ADHD sample

The following sample description is based on the N=560 children with sufficient data for the SCQ analysis. The children were aged 5-18 years (mean=10.4, SD=3.0) and 87 (15.5%) were female. 405 (72.3%) children met criteria for the DSM-IV combined ADHD subtype, 28 (5.0%) had the inattentive subtype, 60 (10.7%) had the hyperactive-impulsive subtype and 67 (12.0%) met criteria for DSM-III-R ADHD. 63 (11.9%) of the children had comorbid ID (IQ<70), 234 (44.5%) had oppositional defiant disorder, 103 (18.6%) had conduct disorder, 42 (7.8%) had anxiety and 3 (0.6%) had depression.

The total number of ADHD symptoms ranged from 7-18 with a mean of 15.1 (SD=2.5). SCQ item scores ranged from 0-35 (mean=12.8, SD=6.7). The prevalence of individual ADHD symptoms ranged from 69.9%-96.6%. The prevalence of individual SCQ items ranged from 7.9%-67.3%.

SCQ analysis

The scree plot for the EFA of the SCQ items showed two points of inflection: occurring after 3 and 8 factors, explaining 43.8% and 62.2% of the variance, respectively. A 3-factor solution was chosen on the basis of this information combined with theory (the DSM-IV & ICD-10 distinguish between three

sub-domains of ASD and the majority of previous factor analytic studies suggest that three factors are meaningful (Mandy & Skuse, 2008)). Factor 1 was modestly correlated with factors 2 and 3 but factors 2 and 3 were uncorrelated (see Table 3.1). Table 3.2 shows the pattern matrix of loadings for the rotated solution, indicating which items load highly on each ASD DSM-IV sub-domain.

Table 3.1: Factor correlations for both EFA analyses

		SCQ EFA			SCQ-ADHD EFA	
		factor 1 – social	factor 2 – rigidity	factor 3 – non-verbal communication	factor 1 – social	factor 2– rigidity/ hyperactivity
SCQ EFA	factor 2 – rigidity	0.43*				
	factor 3 – non-verbal communication	0.37*	-0.05			
SCQ-ADHD EFA	factor 1 – social	0.98*	0.39*	0.54*		
	factor 2 – rigidity/ hyperactivity	0.47*	0.97*	-0.16*	0.39*	
	factor 3 – inattentiveness	0.13*	0.17*	-0.12*	0.11*	0.24*

* Correlation is significant at the 0.01 level (2-tailed)

Factor 1 was comprised of items regarding social-interaction and communication skills and was labelled the 'social' factor. Similarly to a previous factor analysis of the SCQ in children with ASD and other psychiatric problems (Berument et al., 1999), this 'social' factor is comprised primarily of a similar set of social-interaction items. Factor 2 in the current analysis was comprised of all the RRB items as well as a few of the social and communication items and was labelled as the 'rigidity' factor. The majority of items comprising the 'rigidity' factor were all of those that constituted two separate factors labelled 'abnormal language' and 'stereotyped behaviour' in the previous EFA of the SCQ in children with ASD and other psychiatric problems (Berument et al., 1999). Factor 3 contained items to do with gesturing and was labelled the 'non-verbal communication' factor. In the factor analysis by Berument and colleagues (1999), these items clustered with the majority of the social-interaction items in the 'social' factor.

Table 3.2: Pattern matrix of loadings for factor analysis of SCQ items

SCQ item	DSM-IV sub-domains	Factor 1 – social	Factor 2 – rigidity	Factor 3 – non-verbal communication
34- joins in social games (4/5)	COM	0.78		
37- positive response to other children (4/5)	SOC	0.77		
39- plays imaginative games with others (4/5)	COM	0.77		
36- interest in other children (4/5)	SOC	0.76		
27- reciprocates smiles (4/5)	SOC	0.73		
30- wants others to join in (4/5)	SOC	0.69		
29- shares things (4/5)	SOC	0.67		
40- plays cooperatively (4/5)	SOC	0.67		
38- attention without name called (4/5)	SOC	0.66		
33- range of facial expressions (4/5)	SOC	0.63		
35- pretend play (4/5)	COM	0.61		0.25
31- comforts others (4/5)	SOC	0.60		
28- shows things to engage interest (4/5)	SOC	0.57		0.30
26- looks at faces (4/5)	SOC	0.45	0.28	
2- talks to be friendly	COM	0.30		0.23
10- appropriate facial expressions	SOC	0.21		
8- repeats things exactly	COM		0.81	
4- uses odd phrases	COM		0.80	
17- repetitive complicated movements	RRB		0.72	
13- interested in parts not whole	RRB		0.72	
7- invents words/phrases	COM		0.68	
16- unusual movements	RRB		0.65	
5- inappropriate questions	SOC		0.61	
9- has rituals	RRB		0.55	
11- uses other's hand as tool	SOC		0.54	
12- unusual interests	RRB		0.54	
15- unusual sensory interests	RRB		0.54	
6- pronoun reversal	COM		0.50	
14- unusually intense interests	RRB		0.48	
18- self-injures	-	0.20	0.43	
19- always carries specific object around	RRB		0.37	
3- can have a conversation	COM	0.30	0.31	0.27
20- has friends	SOC		0.22	
32- uses gestures with sounds/words (4/5)	SOC			0.71
24- nods head (4/5)	SOC			0.67
22- points to show things (4/5)	SOC	0.27		0.66
23- uses gestures (4/5)	SOC			0.66
21- copies people's actions (4/5)	COM	0.29		0.40

COM: Communication sub-domain; SOC: Social-interaction sub-domain; RRB: Restrictive and repetitive behaviours sub-domain. To aid interpretation, loadings of <0.2 are not presented.

Combined SCQ-ADHD analysis

Next, the 18 ADHD symptoms and 38 SCQ items were analysed together. The scree plot showed two points of inflection: occurring after 3 and 5 factors, explaining 35.3% and 44.6% of the variance, respectively. The range of Pearson factor inter-correlation coefficients for the 3-factor solution (see Table 3.1) was lower than that for the 5-factor solution (r=-0.08-0.46, p=0.23-p<0.001), indicating greater coherence between the derived factors of the 3-factor solution relative to the 5-factor solution. On the basis of this information and the 'parsimony principle' (Kline, 2010) a 3-factor solution was chosen. All three factors were modestly correlated (see Table 3.1). The rotated factor loadings are shown in Table 3.3, indicating which items load on which ASD and ADHD sub-domains (based on DSM-IV). Factor 1 was very similar to the 'social' factor of the SCQ analysis, as can be seen both by its composition and correlation coefficient of 0.98 with this factor (see Table 3.1). Likewise, factor 2 of the combined SCQ-ADHD analysis was very similar to the 'rigidity' factor of the SCQ analysis (correlation coefficient of 0.97), with the addition of the majority of the hyperactive-impulsive ADHD symptoms. Factor 3 comprised the inattentive ADHD symptoms and one of the hyperactive symptoms. These factors were labelled 'social', 'rigidity/hyperactivity' and 'inattentiveness', respectively.

It can be seen from Table 3.1 that all the factors from the SCQ analysis are significantly correlated with those from the combined SCQ-ADHD analysis. The majority are positively correlated, with the exception of the SCQ 'non-verbal communication' factor with the SCQ-ADHD 'rigidity/hyperactivity' and 'inattentiveness' factors (those comprising ADHD symptoms), which were negatively correlated.

Table 3.3: Pattern matrix of loadings for factor analysis of SCQ items and ADHD symptoms

	DSM-IV sub-domains	Factor 1 – social	Factor 2 – rigidity/ hyperactivity	Factor 3 – inattentiveness
SCQ 30- wants others to join in (4/5)	SOC	0.75		
SCQ 39- plays imaginative games with others (4/5)	COM	0.73		
SCQ 34- joins in social games (4/5)	COM	0.72		
SCQ 27- reciprocates smiles (4/5)	SOC	0.70		
SCQ 28- shows things to engage interest (4/5)	SOC	0.69		
SCQ 35- pretend play (4/5)	COM	0.69		
SCQ 29- shares things (4/5)	SOC	0.64		
SCQ 22- points to show things (4/5)	SOC	0.63		
SCQ 36- interest in other children (4/5)	SOC	0.62	0.25	
SCQ 31- comforts others (4/5)	SOC	0.62		
SCQ 37- positive response to other children (4/5)	SOC	0.61	0.29	
SCQ 38- attention without name called (4/5)	SOC	0.58	0.22	
SCQ 40- plays cooperatively (4/5)	SOC	0.57	0.28	
SCQ 33- range of facial expressions (4/5)	SOC	0.56		
SCQ 26- looks at faces (4/5)	SOC	0.50	0.28	
SCQ 32- uses gestures with sounds/words (4/5)	SOC	0.50		
SCQ 21- copies people's actions (4/5)	COM	0.46		
SCQ 3- can have a conversation	COM	0.42	0.28	
SCQ 2- talks to be friendly	COM	0.41		
SCQ 24- nods head (4/5)	SOC	0.34		
SCQ 20- has friends	SOC	0.30		
SCQ 23- uses gestures (4/5)	SOC	0.22		
SCQ 10- appropriate facial expressions	SOC	0.21		
SCQ 13- interested in parts not whole	RRB		0.74	
SCQ 8- repeats things exactly	COM		0.69	
SCQ 17- repetitive complicated movements	RRB		0.66	
SCQ 4- uses odd phrases	COM		0.66	
SCQ 7- invents words/phrases	COM		0.65	
SCQ 11- uses other's hand as tool	SOC		0.63	
SCQ 16- unusual movements	RRB		0.62	
SCQ 12- unusual interests	RRB		0.58	
SCQ 9- has rituals	RRB		0.56	
SCQ 15- unusual sensory interests	RRB		0.56	
SCQ 18- self-injures	-		0.51	
ADHD IMP 4- talking excessively	IMP		0.50	
SCQ 5- inappropriate questions	SOC		0.49	
SCQ 14- unusually intense interests	RRB		0.49	
ADHD HYP 3- running excessively	HYP		0.47	
SCQ 6- pronoun reversal	COM		0.41	
ADHD HYP 5- often noisy	HYP		0.39	0.36
ADHD HYP 4- on the go	HYP		0.37	

SCQ 19- always carries specific object around	RRB	0.37	
ADHD HYP 1- fidgeting	HYP	0.37	0.29
ADHD IMP 1- waiting for turn	IMP	0.32	0.27
ADHD IMP 2- blurting out answers	IMP	0.26	
ADHD IMP 3- interrupting	IMP	0.23	0.23
ADHD IN 1- sustaining attention	INA		0.74
ADHD IN 7- making careless mistakes	INA		0.72
ADHD IN 2- following instructions	INA		0.71
ADHD IN 8- forgetful	INA		0.69
ADHD IN 9- organisational difficulty	INA		0.65
ADHD IN 3- avoiding mental effort	INA		0.62
ADHD IN 6- not listening	INA		0.60
ADHD IN 4- easily distracted	INA		0.55
ADHD IN 5- losing things	INA		0.44
ADHD HYP 2- remaining seated	HYP	0.29	0.41

COM: Communication sub-domain; SOC: Social-interaction sub-domain; RRB: Restrictive and repetitive behaviours sub-domain; IMP: Impulsive symptoms; HYP: Hyperactive symptoms; INA: Inattentive symptoms. To aid interpretation, loadings of <0.2 are not presented.

Correlates of factor scores

Correlation results for age and IQ with the factors are displayed in Table 3.4. Age at assessment was negatively correlated with the 'rigidity' factor of the SCQ analysis and the 'rigidity/hyperactivity' factor of the combined SCQ-ADHD analysis and positively correlated with the 'non-verbal communication' factor of the SCQ analysis. In other words, younger children showed higher rates of RRBs and hyperactive-impulsive behaviours, whereas older children showed more of some of the social-communication ASD traits. The factor scores from both analyses were negatively correlated with IQ ($p<0.05$), except for the 'inattentiveness' factor from the combined SCQ-ADHD analysis, which only showed a trend towards a negative correlation ($p=0.09$).

Table 3.4: Factor score correlations with age and IQ

Factor		Age	IQ
SCQ EFA	factor 1 – social	0.01	-0.21**
	factor 2 – rigidity	-0.13**	-0.12**
	factor 3 – non-verbal communication	0.12**	-0.13**
SCQ-ADHD EFA	factor 1 – social	0.05	-0.21**
	factor 2 – rigidity/ hyperactivity	-0.20**	-0.10*
	factor 3 – inattentiveness	-0.03	-0.08

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Children with ID (IQ<70) had higher factor scores for the first two factors of either analysis ($p<0.05$), i.e. the 'social', 'rigidity' and 'rigidity/hyperactivity' factors. These results are displayed in Table 3.5. In terms of gender, boys tended to have higher scores than girls ($p<0.05$) on the 'social' factor of the SCQ analysis. There were no gender differences for the other factors.

Table 3.5: Association of factor scores with gender and presence of ID

Factor		Gender	Mean(SD)	t	p	ID	Mean(SD)	t	p
SCQ EFA	factor 1 – social	F	0.25(0.19)	-2.18	0.03	No ID	0.28(0.22)	-3.95	3.7×10^{-5}
		M	0.30(0.23)			ID	0.40(0.25)		
	factor 2 – rigidity	F	0.40(0.22)	0.25	0.80	No ID	0.37(0.24)	-2.81	0.01
		M	0.39(0.25)			ID	0.46(0.27)		
	factor 3 – non-verbal communication	F	0.32(0.28)	-0.25	0.80	No ID	0.32(0.28)	-1.51	0.13
		M	0.33(0.29)			ID	0.38(0.32)		
SCQ-ADHD EFA	factor 1 – social	F	0.24(0.20)	-1.61	0.11	No ID	0.26(0.21)	-3.49	1.2×10^{-4}
		M	0.28(0.22)			ID	0.37(0.23)		
	factor 2 – rigidity/ hyperactivity	F	0.50(0.19)	-0.39	0.70	No ID	0.49(0.21)	-2.44	0.01
		M	0.51(0.22)			ID	0.57(0.23)		
	factor 3 – inattentiveness	F	0.89(0.18)	0.54	0.59	No ID	0.88(0.19)	-1.36	0.17
		M	0.88(0.19)			ID	0.91(0.17)		

ID: intellectual disability (IQ<70)

3.5 Discussion

In children with ADHD, the results of the SCQ factor analysis indicate that items corresponding to DSM-IV social deficits and RRBs constitute mainly separate factors, with various items for DSM-IV communication deficits clustering with one or the other factor. In comparison to a previous factor analysis of the SCQ in children with ASD and other psychiatric problems (Berument et al., 1999), the largest factor is comprised of social-interaction items, whereas items concerning RRBs constitute a separate factor, which in this previous EFA were sub-divided into two separate factors labelled 'abnormal language' and 'stereotyped behaviour'. Although there are differences in which specific items are included in the different factors in the current analysis compared to the one by Berument and colleagues (1999), the similarities are striking.

Moreover, the results suggest that the factor structure of autistic traits (measured by the SCQ) in children with ADHD is comparable to previous reports of factor analyses of autistic measures in children with ASD and the general population showing separate social and 'non-social' or RRB factors (Happé & Ronald, 2008; Mandy & Skuse, 2008). It is important to note though, that despite these separate clusters of items, these factors (in particular the 'social' and 'rigidity' factors) are correlated, indicating that they are not independent of each other. Although the symptoms reported here are not strictly at diagnostic levels, these results also support the growing body of evidence for changing ASD diagnostic criteria from a triad (social-interaction deficits, communication problems and RRBs) to a dyad (social-communication deficits and RRBs) of symptom types in the DSM-5 (Frazier et al., 2012; Mandy et al., 2012).

The second analysis, which explored whether ADHD symptoms and SCQ items group together or form separate factors, found that the two factors of items of social-communication deficits from the SCQ factor analysis (i.e. the 'social' and 'non-verbal communication' factors) combined into a single 'social' factor and the hyperactive-impulsive symptoms tended to group with RRB items in a 'rigidity/hyperactivity' factor, with a separate factor for inattentive ADHD symptoms. Although

there are minor differences in which items load more strongly on the specific factors in this analysis compared to the analysis of the SCQ items only, the division of the ASD traits into 'social' and 'rigidity' factors appears unaffected by including ADHD symptoms in the analysis.

The observed division of the ADHD symptoms into separate factors of hyperactive-impulsive and inattentive symptoms is well-supported in the ADHD literature (Willcutt et al., 2012). Indeed, as with social-communication symptoms relative to RRBs (Ronald et al., 2006a, 2010a, 2006b), there is evidence suggesting that hyperactive-impulsive and inattentive symptoms show some level of genetic heterogeneity and specificity in addition to substantial genetic overlap (Greven et al., 2011). It is of particular interest that the hyperactive-impulsive ADHD items clustered with the RRB items and one can only speculate as to why this might be the case. This finding requires further investigation and replication in other samples.

There is growing evidence that ADHD and ASD are each the extreme end of a continuum rather than being distinct categories (Constantino & Todd, 2003; Larsson et al., 2011; Levy et al., 1997). Given this, the division of ADHD into two dimensional scales of inattentive and hyperactive-impulsive symptoms and of ASD into social-communication and RRB dimensions has important implications for classification of developmental problems, investigating the aetiology of these traits and understanding the high heritability and co-heritability of the two disorders (Faraone et al., 2005; Freitag, 2006; Lichtenstein et al., 2010).

Although a 3-factor solution for the combined SCQ-ADHD analysis is believed to be the optimal solution, an alternative was to choose a less parsimonious 5-factor solution (see Appendix 3.1). This solution bears many similarities to the main analyses. The primary factor is composed of a highly similar set of items to the SCQ 'social' and the SCQ-ADHD 3-factor 'social' factors and is very highly correlated with these (Pearson correlation coefficients of 0.99 and 0.98, respectively). Similarly, the second factor of the 5-factor SCQ-ADHD solution is very highly correlated with the 'rigidity' SCQ factor (0.995) and the 'rigidity/hyperactivity' factor (0.98) of the SCQ-ADHD 3-factor analysis. The

third factor appears to correspond to the 'inattentiveness' factor of the SCQ-ADHD 3-factor analysis (correlation=0.98). The fourth factor contains primarily hyperactive and impulsive ADHD symptoms and the fifth factor corresponds to the 'non-verbal communication' factor of the SCQ analysis (correlation=0.96). The key point about the 5-factor solution is that the ASD items come out separately to the ADHD symptoms (i.e. it has three factors of ASD items corresponding to the SCQ analysis and two separate factors for ADHD symptoms). Such a solution is in line with the previous exploratory factor analysis of core ADHD and ASD diagnostic criteria in a population sample of school children (Ghanizadeh, 2010). However, it is important to consider competing factor solutions and further studies are needed to clarify the extent of the overlap of RRBs and hyperactive-impulsive ADHD symptoms. It would also be worth exploring the factor structure of ADHD and ASD symptoms in children diagnosed with ASD. One small study (N=65) has attempted to do this, and although they found distinct factors for ASD and ADHD, they do not consider competing models and provide no clear justification for the choice of a 2-factor solution (Ghanizadeh, 2012).

Although children with ID (IQ<70) tend to be excluded from studies of ADHD and sometimes also of ASD, these children were included in the present analyses (N=63). Given the high association of lower IQ and higher rates of ID in these neurodevelopmental conditions (Frazier et al., 2004; Voigt et al., 2006), IQ is not statistically separable from neurodevelopmental problems (Dennis et al., 2009) and the deliberate recruitment of children without ID may bias representativeness of such samples. Indeed, analysis of factor scores in relation to IQ showed that the factor scores were negatively correlated with IQ, indicating that children with the most severe symptom profiles were likely to score lower on the IQ test. There is also evidence that children with ADHD and comorbid ID do not differ in their ADHD profile to those with ADHD without ID (Ahuja et al., 2013; Antshel et al., 2006). A complete re-analysis of the data excluding the children with ID shows no marked differences to the pattern of observed results (see Appendices 3.2 and 3.3).

Given that ADHD and ASD are developmental conditions, it is not surprising that some of the factor scores showed associations with age. There appeared to be no effect for age for the primary 'social' factors, whereas the 'rigidity' and 'rigidity/hyperactivity' factor scores decreased with age and the 'non-verbal communication' factor scores increased with age. Although it is well-established that hyperactivity and impulsivity decrease with age (Willcutt et al., 2012), it is less clear why older children would struggle more on the items comprising the 'non-verbal communication' factor, unless this is related to parental recall of items from when the children were aged 4-5 years old.

In terms of gender, boys had higher scores than girls on the 'social' factor of the SCQ analysis but boys and girls did not differ on the other factor scores. Given that there is a high ratio of boys to girls in samples of children with ADHD and ASD, it is reasonable that boys with ADHD are more likely to have higher ASD scores than girls, although it is unclear why this is the case only for the social difficulties. Limiting the analysis to boys-only makes no difference to the pattern of observed results (see Appendices 3.4 and 3.5).

The results of this study need to be considered in light of several limitations. Data on ADHD and ASD were derived from different types of instruments. Whilst ADHD symptoms were measured using a diagnostic interview completed with parents (Angold et al., 1995), ASD traits were measured using a questionnaire measure (Rutter et al., 2003). Future studies will need to examine whether the pattern of results is affected by the type of instrument used.

Although the relatively large sample size was a strength of the current study, it was not sufficiently large enough to analyse stratified age groups. Also, parental recall of their children's behaviour (particularly at ages 4-5) might have been different for adolescents. The findings of this study relate to clinic children diagnosed with ADHD and it is possible that the observed association of hyperactive-impulsive items with RRBs might not be evident in children with lower levels of these traits, as has been suggested by one other study (Ghanizadeh, 2010).

However, despite these caveats, the study contributes novel findings to the growing body of literature exploring the overlap of ADHD and ASD. The results highlight that there are distinct dimensions of social-communication difficulties and RRBs in children diagnosed with ADHD, supporting such a finding in children with ASD and in the general population (Mandy & Skuse, 2008). This finding further implies that the presence or absence of ADHD does not affect the manifestation of social-communication difficulties and RRBs in children. The results also support the switch from a triad to a dyad of diagnostic symptom dimensions in the DSM-5 (Frazier et al., 2012; Mandy et al., 2012). The suggestion that hyperactive-impulsive traits may be linked with RRBs is an intriguing one and requires replication and further study, both in clinically referred children and in the general population.

Chapter 4

Autistic Traits in Children with ADHD Index Cognitive and Developmental Problems

The work presented in this chapter forms part of a published research article.

Cooper, M., **Martin, J.***, Langley, K., Hamshere, M., & Thapar, A. (* joint 1st authors). (2014). Autistic traits in children with ADHD index clinical and cognitive problems. European Child and Adolescent Psychiatry, 23(1), 23–34.*

Additional outcomes (i.e. clinical comorbidities) were included in the original publication; these formed a chapter of Miriam Cooper's PhD thesis, a co-author on the publication, and as such are not included here. Please note there is some repetition of background information in section 4.2 that has already been presented in section 3.2 and in chapter 1.

Please see http://link.springer.com/article/10.1007/s00787-013-0398-6 for the full published article:

4.1 Summary

Traits of ASD occur frequently in ADHD, but the significance of their presence is not fully understood. The analysis in this chapter aimed to determine whether higher levels of autistic traits, as measured by the Social Communication Questionnaire (SCQ), index cognitive or developmental problems in a large clinical sample of children with ADHD (N=711). Regression analyses examined associations of SCQ scores with core ADHD features and cognitive and developmental problems. For outcomes showing an association with total SCQ score, secondary analyses determined levels of differential association of the two DSM-5 ASD sub-domains (social-communication difficulties

and restrictive, repetitive behaviours). Results suggest that ASD traits within ADHD are associated with more ADHD symptoms, lower IQ, more working memory deficits and presence of general language and motor delays. The associations with cognitive and developmental problems persisted after accounting for ADHD severity, suggesting that ASD traits independently index the severity of these impairments in the context of ADHD. Secondary analyses indicate unique contributions of the social-communication sub-domain to the associations with IQ and working memory. They also show that repetitive behaviours independently predict hyperactive-impulsive symptoms and motor delays. It would be worthwhile for clinicians to consider levels of social-communication difficulties and repetitive behaviours in children with ADHD who do not meet diagnostic criteria for ASD, as these appear to index higher levels of phenotypic complexity.

4.2 Introduction

ADHD and ASD are early-onset neurodevelopmental disorders, which frequently co-occur. High rates of symptom overlap have been shown both in clinical samples (Ghaziuddin et al., 1998; Stahlberg et al., 2004; Thede & Coolidge, 2007; Yoshida & Uchiyama, 2004) and general population samples (Lichtenstein et al., 2010; Reiersen et al., 2008a; Ronald et al., 2008; Simonoff et al., 2008). The two conditions also share many co-occurring impairments in a number of developmental and cognitive domains. There is strong comorbidity of both disorders with intellectual disability (ID) and they are associated with lower IQ (Dykens & Hodapp, 2001; Frazier et al., 2004; Matson & Shoemaker, 2009; Voigt et al., 2006). They are also associated with reading difficulties (Dykman & Ackerman, 1991; Jones et al., 2009; Maughan & Carroll, 2006) and motor problems, such as developmental co-ordination disorder (Blondis, 1999; Fournier et al., 2010). Specific speech and language problems and general language delay, core features in ASD, are frequently seen in those with ADHD (Bruce et al., 2006; Hagberg et al., 2010). Furthermore, both disorders are associated with deficits in executive functioning, including response inhibition, working memory and planning abilities (Willcutt et al., 2008).

There has been a recent shift in thinking of ADHD and ASD as conditions that cannot be diagnosed in one individual (DSM-IV and ICD-10) to a rising awareness of the strong relationship between these disorders, both in terms of research and clinical practice (DSM-5). In light of this, there have been a number of attempts to examine the phenotypic similarities between children with a primary diagnosis of ADHD or ASD and children meeting diagnostic criteria or having a high level of traits of both conditions (Frazier et al., 2001; Grzadzinski et al., 2011; Kochhar et al., 2011; Kröger et al., 2011). The focus of this chapter is on the presence of elevated ASD traits in children with ADHD.

Research suggests that the profile of ADHD symptoms in affected children appears fairly similar, regardless of the presence of ASD traits (Frazier et al., 2001; Grzadzinski et al., 2011). However, there is some indication that children with ADHD who have higher levels of ASD traits may be more likely to have the more severe, DSM-IV combined, subtype of ADHD (Grzadzinski et al., 2011) and also more hyperactive-impulsive symptoms, though it is unclear whether inattentive symptoms are higher (Kröger et al., 2011).

In terms of general cognitive ability, the presence of ASD traits in children with ADHD does not appear to be associated with IQ (Grzadzinski et al., 2011; Kochhar et al., 2011). However, the two studies so far excluded children with IQ<70 and therefore the full extent of impairments in IQ in children with ADHD and co-occurring ASD traits has not been fully explored. A recent review of the few studies examining deficits in various executive functioning (EF) sub-domains in children with ADHD and ASD suggests that executive functioning deficits appear qualitatively similar in both conditions, but can vary in degree of severity (Rommelse et al., 2011). However, this review also highlights the lack of sufficient research directly comparing children with traits of both phenotypes, relative to those with only one phenotype; studies to date are small, with inconsistencies in methodology, inclusion criteria and the specific executive functioning sub-domains tested, making it difficult to draw any firm conclusions. In terms of other developmental domains, there is some evidence that ASD traits in children with ADHD are associated with higher rates of motor and

language problems (Mulligan et al., 2009a). Further studies are necessary to confirm these findings and clarify the specifics of the relationship between ASD traits and cognitive and developmental deficits in children with ADHD.

Rationale for current study

The aim of this study was to determine whether ASD traits (assessed as a continuous measure) in a sample of children with ADHD index a more severe cognitive and developmental profile. It was hypothesised that the presence of an increasing number of ASD symptoms in those with ADHD will be associated with lower IQ, lower reading ability, more executive functioning problems and a greater rate of language and motor problems. Although traditionally regarded as clustering very strongly, the sub-domains of ASD (deficits in social-communication problems and restrictive and repetitive behaviours) have been suggested to dissociate, both in terms of clinical presentation and in terms of underlying genetic aetiology (Happé & Ronald, 2008). As such, for any outcomes showing an overall association with total ASD trait scores, exploratory analyses were used to determine whether these sub-domains of ASD symptoms show differential association (i.e. to determine whether either sub-domain is independently associated with the outcome, over and above the other sub-domain).

4.3 Method

Sample

This analysis used data from the clinical sample of children with ADHD, described in detail in section 2.1. Please refer back to that section for recruitment procedures, general inclusion criteria and an overview of the measures used. Children were eligible for inclusion in the present analysis if they had a current diagnosis of DSM-III-R or DSM-IV ADHD with complete subtype data available and sufficient ASD trait data for analysis (N=711). There were 113 females (15.9%) in the sample and the children were aged between 5-18 years (mean=10.3, SD=2.9).

Measures

Research diagnoses of ADHD and symptom counts were ascertained using the parent-rated CAPA (Angold et al., 1995), a semi-structured interview. Symptom totals were calculated for inattentive and hyperactive-impulsive traits (out of 9 each), as well as total ADHD traits (out of 18).

ASD traits were analysed as a continuous measure using the parent-rated Social Communication Questionnaire (SCQ) (Berument et al., 1999; Rutter et al., 2003). Item 1 is a language screening item and was omitted from the total score. For consistency and comparability, where parents answered 'no' to this item and consequently omitted the following seven questions, these children were not included in analyses (N=20). The remaining items were summed to give a total out of 39. The scores were examined as a continuous variable as there is evidence that autistic traits are continuously distributed throughout the population (Constantino & Todd, 2003).

The 39 items were also divided into the two DSM-5 sub-domains of ASD symptoms, as suggested by previous research, including the factor analyses reported in chapter 3 of this thesis (Happé & Ronald, 2008; Mandy & Skuse, 2008; Martin et al., 2013). There were 8 items classed as 'restricted and repetitive behaviours (RRBs)' and 30 items were classed as 'social-communication deficits'. Item 18 (regarding self-harm) was unclassified as it is not a part of the diagnostic criteria for ASD. Please refer to Appendix 2.1 for a list of all SCQ items and sub-domain classification. Totals for each sub-domain were calculated based on these items.

To account for missing questionnaire items in calculating the total SCQ score and sub-domains, a prorated score was calculated for children with 10% or fewer missing items, where the fraction of endorsed items out of those completed was multiplied by the total number of items. Children with >10% missing on either sub-domain or the total score were omitted from analyses (N=107). Group comparisons showed that these children with excessive missing data had lower IQ (mean=81.5) than the children included in the final sample (mean=84.3, N=711; t(df)=2.1(143.2), p=0.033),

although they did not differ in terms of gender, age at assessment, family socioeconomic status (SES) or severity of their ADHD symptoms (all $p>0.05$).

Cognitive assessments were performed by trained psychologists. Parents were asked to withhold stimulant medication from their child for 24 hours prior to testing. The WISC-III or WISC-IV (Wechsler, 1992, 2003) was used to assess full-scale IQ, using all subtests. The WISC-IV Digit Span subtest is a measure of verbal working memory and was also considered separately. The Wechsler Objective Reading Dimensions (WORD) (Wechsler, 1993) was used to assess reading, spelling and reading comprehension abilities in children up to the age of 12 years. The intra-extra dimensional shift (IED) task from the Cambridge Neuropsychological Battery (CANTAB) (Cambridge Cognition, 1996) was used as a measure of executive functioning (visual discrimination, set shifting and attentional flexibility). The measure used was whether the child had successfully completed stage 8, the crucial stage at which the extra-dimensional shift first occurs; this variable measures ability to shift attention to a previously irrelevant feature (known as set-shifting), akin to a change in category in the Wisconsin Card Sorting task (WCST) (Berg, 1948) and has been found to be particularly impaired in un-medicated children with ADHD (Kempton et al., 1999; Rhodes et al., 2005) and in children with ASD (Hughes et al., 1994; Yerys et al., 2009a) relative to controls.

Parents were also asked general questions about their child's speech and motor development at the interview. Children who were not talking by age 2 and/or had had speech therapy were classed as positive for this broad measure of language problems. Children who were not walking by 18 months and/or were clumsy "pretty much" or "very much" were classed as positive for this broad measure of motor problems.

Family annual income, parental employment status and parental educational attainment were assessed by parental questionnaire. Low income was defined as annual family income <£20,000, and low educational attainment was defined as having left school without qualifications (GCSE or equivalent) at age 16 years. SES was classified by the occupation of the main family wage earner in

the family. Three SES categories were defined, with the medium and high categories merged to give binary categories of low SES or otherwise.

Data analyses

All analyses were performed using SPSS version 18. All tests were two-tailed. Where a variable was not normally distributed, the scores were transformed (ln x+1) to make them normally distributed, and analyses were run on transformed scores. First, the effect of putative covariates was assessed using univariate logistic and linear regression analyses. Next, logistic and linear regression analyses were used to determine the associations of total SCQ score (predictor variable) with cognitive and developmental characteristics (outcomes) of the ADHD sample. The primary model analysed unadjusted associations between SCQ scores and outcomes in the full sample. Next, IQ, age, gender and family SES were included as covariates to determine to what extent observed associations were explained by other variables; children with any one of these covariates missing (N=112) could not be included in this model. Finally, severity of ADHD symptoms was added as a further covariate to determine whether ADHD symptom levels contributed to the observed associations. However, IQ was not included as a covariate in the analysis of the WISC-IV Digit Span subtest in view of this subtest being a component of full-scale IQ. Correction for multiple testing was administered using Bonferroni; alpha was set at p=0.006 (0.05/8) for 8 tests performed (6 tests of cognitive outcomes and 2 tests of developmental outcomes).

Exploratory analyses

As a secondary test of IQ abilities, the four WISC-IV indices were entered simultaneously into one multivariate regression model, to determine the level of each index's unique contribution to explaining the variance in SCQ scores (i.e. to determine whether any of the indices are independently associated with the outcome over and above the other indices).

For outcome variables that showed an association with overall SCQ score, secondary analyses were performed on the two SCQ sub-domain scores (social-communication deficits and RRBs), to

determine whether scores on either sub-domain predicted a greater association with the outcome variables above and beyond shared variance with the other sub-domain. For each outcome variable, covariates (age, IQ, gender and family SES) and the two SCQ sub-domain scores were entered into a single multivariate model to test for association with the outcomes. As these sub-domain analyses were exploratory and secondary, the same threshold for multiple testing ($p<0.006$) was used when interpreting the results.

Sensitivity analyses

Although children with ID are generally excluded from ADHD research studies, they were included in the current sample. To determine whether their inclusion affected the pattern of results, the main analyses were repeated without these cases (N=85).

Furthermore, the sensitivity of the observed results to the use of a continuous measure of ASD traits was tested by dividing the sample into groups based on the screening cut-off threshold used in the literature (Berument et al., 1999) and comparing children with total SCQ<15 (N=433) to those with SCQ score ≥15 (N=278).

4.4 Results

Total SCQ scores and IQ scores were normally distributed and IQ ranged between 43-124 (mean=84.3, SD=14.0), which included 85 children with an IQ<70. Figure 4.1 displays the frequency distribution of SCQ scores across the sample. Table 4.1 shows general sample characteristics, including mean total SCQ by several key descriptive and diagnostic variables.

Covariates

Total SCQ scores were associated with lower full-scale IQ ($\beta=-0.21$, $p=5.7x10^{-8}$), greater rate of low family income [odds ratio (OR)=1.04, 95% confidence intervals (CI)=1.01-1.07, p=0.006] and low family SES (OR=1.04, 95% CI=1.01-1.06, p=0.003). There was no association of SCQ and child's age at assessment ($\beta=-0.04$, p=0.31), gender (OR=1.01, 95% CI=0.98-1.04, p=0.54) or parental education

(OR=1.00, 95% CI=0.97-1.03, p=0.91). In terms of ADHD profile, SCQ scores were positively associated with the number of inattentive (β=0.12, p=0.001), hyperactive-impulsive (β=0.21, $p=1.1x10^{-08}$) and total ADHD (β=0.21, $p=1.4x10^{-8}$) symptoms.

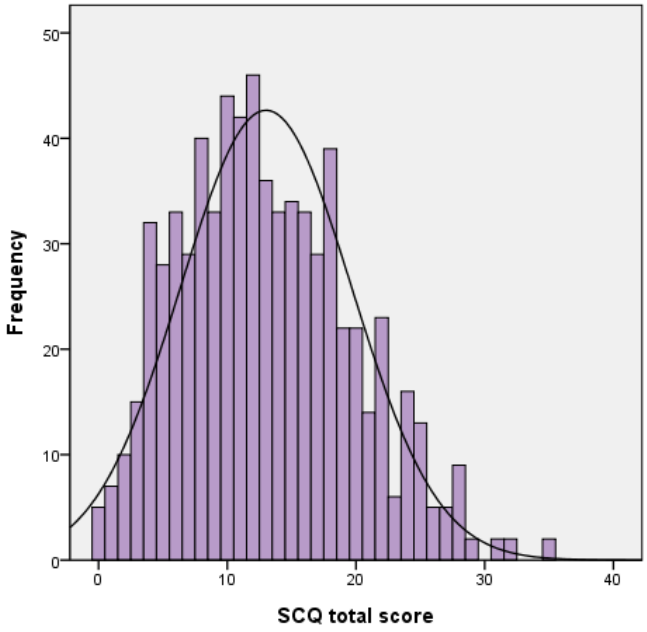

Figure 4.1: Distribution of total Social Communication Questionnaire scores in the sample

Table 4.1: Sample characteristics in relation to Social Communication Questionnaire scores

Variable		N	%	SCQ mean(SD)
Gender	Male	598	84.1	13.1(6.7)
	Female	113	15.9	12.7(6.3)
Socioeconomic status	Low	332	51.5	13.7(6.7)
	Medium-high	313	48.5	12.1(6.6)
Family income	Low income	285	63.3	13.7(7.2)
	Medium-high income	165	36.7	11.8(6.6)
Parental education	No GCSEs	128	27.1	13.0(6.9)
	GCSEs or higher	345	72.9	13.1(7.0)
ADHD diagnosis subtype	DSM-IV Combined	519	73.0	13.7(6.7)
	DSM-IV Inattentive	43	6.0	9.9(6.2)
	DSM-IV Hyperactive-Impulsive	73	10.3	11.4(6.9)
	DSM-III-R only	76	10.7	11.8(5.6)
Presence of ID	No ID (IQ≥70)	583	82.0	12.5(6.5)
	ID (IQ<70)	85	12.0	15.3(7.2)
	Unknown (no IQ data)	43	6.0	15.4(7.0)
Total sample		**711**	**100**	**13.0(6.6)**

ID: intellectual disability

Main analyses

Results of regression analyses of SCQ scores with cognitive and developmental outcomes are displayed in Table 4.2. Full-scale IQ was negatively associated with SCQ score (all three models). A multivariate regression model explored the relative contributions of the four indices of the WISC-IV to this association. There were no unique contributions of Verbal Comprehension Index, Perceptual Reasoning Index or Processing Speed ($p>0.05$). However, there was a trend towards an association between higher SCQ scores and a lower Working Memory Index, above and beyond contributions shared with the other three WISC-IV indices (unadjusted analysis: $\beta=-0.14$, $p=0.014$; adjusted for all covariates: $\beta=-0.17$, $p=0.0029$). An analysis of the Digit Span subtest, a measure of working memory, found that lower scores were significantly predicted by higher SCQ scores, a finding that persisted after adjusting for covariates.

Successful completion of the extra-dimensional shift stage of the CANTAB IED task was not found to be associated with SCQ scores. There was also no association of SCQ scores with WORD Basic Reading, Spelling or Reading Comprehension, after accounting for multiple testing. Higher SCQ scores were associated with a greater likelihood of motor problems and general language problems. This pattern of results was consistent after co-varying for gender, age, SES, IQ and ADHD severity.

Table 4.2: Associations of Social Communication Questionnaire scores with cognitive and developmental outcomes

Outcome variable	Unadjusted (max N=711)				Adjusted [a] (max N=599)	Adjusted [b] (max N=599)
	B (Std. Error)	β	OR (95% CI)	p-value	p-value	p-value
WISC-IV full-scale IQ	-0.45 (0.08)	-0.21		$5.7x10^{-8}$	$1.1x10^{-6}$ [c]	$1.6x10^{-6}$ [c]
WISC-IV Digit Span subtest	-0.07 (0.02)	-0.17		$8.6x10^{-5}$	$1.7x10^{-4}$ [c]	$5.4x10^{-4}$ [c]
WORD Basic Reading	-0.05 (0.02)	-0.12		0.020	0.99 [d]	0.78 [d]
WORD Spelling [e]	-4.47 (2.10)	-0.11		0.034	0.95 [d]	0.68 [d]
WORD Reading Comprehension	-0.03 (0.02)	-0.07		0.17	0.18 [d]	0.13 [d]
CANTAB IED completed stage 8			1.00 (0.97-1.04)	0.76	0.33	0.43
Motor problems			1.09 (1.06-1.12)	$2.6x10^{-9}$	$5.2x10^{-9}$	$6.7x10^{-7}$
Language problems			1.07 (1.04-1.10)	$3.9x10^{-6}$	$7.8x10^{-6}$	$2.7x10^{-3}$

WISC-IV: Wechsler Intelligence Scale for Children version IV; WORD: Wechsler Objective Reading Dimensions; CANTAB IED: intra-extra dimensional shift task from the Cambridge Neuropsychological Battery

Statistical threshold to account for multiple testing: p-value < 0.006; direction of effect across models is consistent unless otherwise stated

[a] Adjusted for IQ, age, gender and family socio-economic status

[b] Adjusted for IQ, age, gender, family socio-economic status and ADHD severity

[c] Not corrected for IQ

[d] Direction of effect opposite to that in in the unadjusted analysis

[e] Transformed

Secondary analysis of SCQ sub-domains

For each of the outcome variables showing a significant association with total SCQ score, additional tests were performed to determine whether either of the SCQ sub-domains (social-communication deficits or RRBs) showed an independent contribution to the observed association. Results are shown in Table 4.3. After adjusting for covariates and accounting for multiple testing, it was found that social-communication problems were associated with lower IQ and working memory (both in terms of the Working Memory Index and the specific scores on the Digit Span subtest) above and beyond variance shared with RRBs. Furthermore, RRBs independently predicted presence of motor problems. Social-communication problems were also nominally associated with language problems, although this result was non-significant after correcting for multiple testing.

Given that ADHD symptoms were also associated with total SCQ score, the independent contributions of the SCQ sub-domains to these symptoms were also examined. After accounting for age, gender, family SES and IQ, neither SCQ sub-domain showed an independent association with inattentive traits (social-communication: β=0.08, p=0.069; RRBs: β=0.04, p=0.34). On the other hand, RRBs were found to be independently associated with hyperactive-impulsive symptoms (β=0.15, p=0.00054), while social-communication traits were not (β=0.07, p=0.10).

Sensitivity analyses

Excluding children with ID (N=85) did not alter the pattern of the observed results (see Table 4.4). Similarly, splitting the sample into two groups based on SCQ score (using the threshold of ≥15; N=433 with SCQ<15 and N=278 with SCQ≥15) gives the same pattern of results and direction of effects as reported for the continuous analyses (see Table 4.5).

Table 4.3: Secondary analyses – multivariate regressions of ASD sub-domains in relation to significant outcomes

Outcome variable	Sub-domain	B (Std. Error)	β	OR (95% CI)	p-value
WISC-IV full-scale IQ [a]	S-C	-0.58 (0.11)	-0.22		2.9×10^{-7}
	RRBs	0.18 (0.29)	0.03		0.55
WISC-IV WMI [a]	S-C	-0.55 (0.13)	-0.22		2.9×10^{-5}
	RRBs	-0.07 (0.35)	-0.01		0.84
WISC-IV Digit Span [a]	S-C	-0.10 (0.03)	-0.19		2.3×10^{-4}
	RRBs	0.04 (0.07)	0.03		0.54
Motor problems	S-C			1.04 (1.00-1.09)	0.056
	RRBs			1.26 (1.12-1.41)	7.7×10^{-5}
Language problems	S-C			1.06 (1.01-1.11)	0.021
	RRBs			1.05 (0.93-1.17)	0.43

Analyses adjusted for IQ, age, gender, family socio-economic status and ADHD severity. [a] not corrected for IQ

Statistical threshold to account for multiple testing: p-value < 0.006

WISC-IV: Wechsler Intelligence Scale for Children version IV; WMI: Working Memory Index; S-C: Social-communication; RRBs: Restrictive and repetitive behaviours

Table 4.4: Associations of Social Communication Questionnaire with cognitive and developmental outcomes, excluding those with ID (N=85)

Outcome variable	Unadjusted (max N=626)				Adjusted [a] (max N=514)	Adjusted [b] (max N=514)
	B (Std. Error)	β	OR (95% CI)	p-value	p-value	p-value
WISC-IV full-scale IQ	-0.26 (0.07)	-0.15		$3.2x10^{-4}$	0.002 [c]	0.0022 [c]
WISC-IV Digit Span subtest	-0.04 (0.02)	-0.11		0.018	0.044 [c]	0.035 [c]
WORD Basic Reading	-0.04 (0.02)	-0.10		0.05	0.83 [d]	0.99 [d]
WORD Spelling [e]	-3.46 (2.19)	-0.08		0.11	0.91 [d]	0.84 [d]
WORD Reading Comprehension	-0.02 (0.02)	-0.05		0.32	0.32 [d]	0.27 [d]
CANTAB IED completed stage 8			0.98 (1.06-0.98)	0.23	0.32	0.23
Motor problems			1.06 (1.13-1.06)	$5.7x10^{-8}$	$4.9x10^{-6}$	$8.8x10^{-5}$
Language problems			1.03 (1.10-1.03)	$1.4x10^{-4}$	$7.3x10^{-3}$	0.012

WISC-IV: Wechsler Intelligence Scale for Children version IV; WORD: Wechsler Objective Reading Dimensions; CANTAB IED: intra-extra dimensional shift task from the Cambridge Neuropsychological Battery

Statistical threshold to account for multiple testing: p-value < 0.006; direction of effect across models is consistent unless otherwise stated

[a] Adjusted for IQ, age, gender and family socio-economic status

[b] Adjusted for IQ, age, gender, family socio-economic status and ADHD severity

[c] Not corrected for IQ

[d] Direction of effect opposite to that in in the unadjusted analysis

[e] Transformed

Table 4.5: Social Communication Questionnaire score as a binary predictor of cognitive and developmental outcomes

Outcome variable	Unadjusted (max N=711)				Adjusted [a] (max N=599)	Adjusted [b] (max N=599)
	B (Std. Error)	β	OR (95% CI)	p-value	p-value	p-value
WISC-IV full-scale IQ	-5.76 (1.11)	-0.20		$2.6x10^{-7}$	$1.3x10^{-5}$ [c]	$1.9x10^{-5}$ [c]
WISC-IV Digit Span subtest	-0.92 (0.26)	-0.16		$3.6x10^{-4}$	$7.3x10^{-4}$ [c]	$6.8x10^{-4}$ [c]
WORD Basic Reading	0.00 (0.00)	-0.02		0.62	0.067 [d]	0.044 [d]
WORD Spelling [e]	-0.10 (0.16)	-0.03		0.55	0.15 [d]	0.091 [d]
WORD Reading Comprehension	0.00 (0.00)	-0.01		0.79	0.025 [d]	0.019 [d]
CANTAB IED completed stage 8			0.51 (1.40-0.51)	0.51	0.84	0.72
Motor problems			1.49 (3.10-1.49)	$4.3x10^{-5}$	$1.3x10^{-3}$	$4.5x10^{-3}$
Language problems			1.54 (3.29-1.54)	$3.1x10^{-5}$	$2.3x10^{-3}$	$2.9x10^{-3}$

WISC-IV: Wechsler Intelligence Scale for Children version IV; WORD: Wechsler Objective Reading Dimensions; CANTAB IED: intra-extra dimensional shift task from the Cambridge Neuropsychological Battery

Statistical threshold to account for multiple testing: p-value < 0.006; direction of effect across models is consistent unless otherwise stated

[a] Adjusted for IQ, age, gender and family socio-economic status

[b] Adjusted for IQ, age, gender, family socio-economic status and ADHD severity

[c] Not corrected for IQ

[d] Direction of effect opposite to that in in the unadjusted analysis

[e] Transformed

4.5 Discussion

The aim of the analyses in this chapter was to determine whether, in children with a diagnosis of ADHD, ASD traits index severity of phenotype in terms of cognitive and developmental features. As measured using the SCQ, ASD traits in this sample (mean=13.0, SD=6.6) were lower than previously reported in children ascertained primarily in terms of a diagnosis of ASD (mean=22.3 (Berument et al., 1999)). This is consistent with what would be expected given that this sample is comprised of children with ADHD and those with a clinician's diagnosis of ASD were not included. Furthermore, this mean is higher than previously reported in typically developing children (mean=3.89, SD=2.77 (Mulligan et al., 2009a); mean=2.8, SD=2.1 (Kochhar et al., 2011)) and somewhat higher than reported for other ADHD samples (mean=8.5, SD=6.2 (Mulligan et al., 2009a); mean=11.6, SD=5.5 (Kochhar et al., 2011)). Although not a direct comparison, the higher SCQ traits in the current sample relative to previously reported scores in controls are consistent with the many previous observations of high levels of comorbidity between ADHD and ASD (Ghaziuddin et al., 1998; Lichtenstein et al., 2010; Reiersen et al., 2008a; Ronald et al., 2008; Simonoff et al., 2008; Stahlberg et al., 2004; Thede & Coolidge, 2007; Yoshida & Uchiyama, 2004).

As predicted, the results suggest that ASD traits within children with ADHD (even when known ASD cases are not included) are associated with a more severe phenotype in terms of several cognitive and developmental features. Firstly, in line with previous research, higher SCQ scores predicted greater severity of ADHD symptoms (Grzadzinski et al., 2011; Kröger et al., 2011). The results also showed that higher SCQ scores were associated with lower cognitive ability (specifically, lower IQ and impairments in working memory) and a greater rate of general developmental (motor and language) problems. This pattern of results persisted even after taking into account potential confounding effects of age at assessment, IQ (where appropriate), gender and family socioeconomic status. Results were also not found to be driven by severity of ADHD itself. These results lend strength to the assertion that ASD symptoms are independently associated with these

cognitive and developmental difficulties, rather than simply being a proxy marker of ADHD symptom severity.

Although more working memory deficits were predicted by increasing SCQ scores, there was no association between SCQ scores and whether children were able to successfully complete the extra-dimensional shift component of the IED CANTAB task, a further measure of executive functioning. Although previous studies have shown that un-medicated children with ADHD and children with ASD perform less well on this aspect of the IED task than controls (Hughes et al., 1994; Kempton et al., 1999; Rhodes et al., 2005; Yerys et al., 2009a), the current results suggest that ASD traits in ADHD do not index a greater deficit in set-shifting. A previous study utilising the IED task indicates that children with a joint diagnosis of ADHD and ASD may struggle more on this task than children with ASD without ADHD but the study detected no differences in the performance of children with ADHD without ASD compared to the other groups, except that they took longer to complete the task (Sinzig et al., 2008a). Executive functioning is a broad construct and the current results suggest that different aspects of executive functioning (e.g. working memory and set-shifting) need to be considered separately, as they may show differential association with ASD traits in the context of ADHD.

Although no associations were found between reading and spelling and SCQ scores, the WORD was only assessed in children between 5-11 years old (N=375). Similarly, the CANTAB IED task was only available for a proportion of the children (N=252), which may have reduced the power to detect potential effects. The measures of general motor and language development were broad screening questions indicative of developmental problems rather than detailed validated measures, which precluded the assessment of more subtle associations of ASD symptoms with general development.

Secondary analyses were performed to determine the relative contributions of the two SCQ sub-domains (social-communication deficits and RRBs) to observed main associations. It was found that

the sub-domain of social-communication was driving the main associations of SCQ with IQ and working memory. This finding is plausible, given that performance on a cognitive task such as the WISC-IV, which is administered by a psychologist, requires the ability to communicate successfully in order to understand and comply with task instructions. Associations were also observed between the sub-domain of RRBs and hyperactive-impulsive symptoms, as well as motor problems. The results suggest that RRBs in children with ADHD, as assessed by the SCQ, may index a more general dysfunction of motor processes (i.e. hyperactivity, impulsivity and motor problems in the form of late onset walking and clumsiness). These results also support the observed overlap of hyperactive-impulsive symptoms and RRBs reported in chapter 3 of this thesis (Martin et al., 2013), although the analyses were performed in the same sample and therefore would benefit from replication and further exploration in other large samples of children with ADHD.

The independent associations of social-communication difficulties and RRBs with several of the cognitive and developmental outcomes further support research which suggests that these dimensions are distinct (Happé & Ronald, 2008). Previous research has shown that symptoms in both sub-domains contribute to the higher SCQ scores in children with ADHD, when compared to their unaffected siblings and typically developing controls (Mulligan et al., 2009a), although a smaller study found that this was only the case for social-communication problems but not RRBs, when compared with typically developing controls (Kochhar et al., 2011).

An alternative approach to investigating the effects of comorbidity between ASD and ADHD is to assess whether a sample of children ascertained primarily in terms of a diagnosis of ASD shows similarly elevated comorbid and cognitive problems. Studies generally find that children with ADHD+ASD have elevated ASD symptomatology and lower IQ scores than children with ASD-only (Gadow et al., 2006; Guttmann-Steinmetz et al., 2009, 2010; Holtmann et al., 2007; Yerys et al., 2009b), although others do not (Frazier et al., 2001; Ghaziuddin et al., 2010). Thus it would seem

that, in general, ADHD problems in children with ASD also index a more severe phenotype and cognitive problems, lending further support to the removal of the diagnostic exclusion in DSM-5.

It is not clear whether the SCQ has validity for ASD traits in ADHD in the same way that it does in those with ASD and the general population, although that could easily also apply to assessment of other forms of comorbid psychopathology in ADHD (e.g. conduct disorder). For instance, the item on "complicated movements of the body" (part of the sub-domain of RRBs) may be interpreted as referring to straightforward hyperactivity, an idea that has been proposed by others in general with regards to parental reports of children's behaviour (Kochhar et al., 2011; Reiersen, 2011). An analysis of the properties of the SCQ in a sample of young people 'at risk' for ASD found that 25% of those who screened false-positive for ASD went on to receive a diagnosis of ADHD or hyperkinetic disorder (Chandler et al., 2007), but to date it has not been examined in detail as to whether the SCQ has good discriminant validity of ASD symptoms in a population with ADHD specifically. However, as shown in the factor analysis presented in chapter 3 of this thesis, the structure of the SCQ is similar in children with ADHD to that found in children with ASD or in the general population, with separate dimensions of social-communication deficits and RRBs (Martin et al., 2013).

Given that children with ADHD and ASD show higher rates of ID, children were not excluded on the basis of IQ so as not to lose clinically valuable information. Likely for historical reasons (Still, 1902), children with ID have generally been excluded from ADHD research studies but not from ASD research. It is important to note that the pattern of the results remained the same when ID cases (N=85) were excluded, although the strength of the associations between SCQ scores with IQ and working memory decreased below the p-value threshold for multiple testing. This suggests that previous studies (Grzadzinski et al., 2011; Kochhar et al., 2011) may have not found an association between lower IQ and presence of ASD traits in children with ADHD because of the exclusion of children with IQ<70.

Some of the strengths of this analysis include the large sample size, assessment of ADHD using the parent CAPA, a semi-structured interview, and a comprehensive assessment of cognition. This analysis assessed ADHD using DSM-IV criteria that require excluding cases of ASD and therefore children with a clinician's diagnosis of ASD were not included. Although the continuous analysis of SCQ scores can be viewed as a strength of the analytical approach, a limitation of the analysis is that ASD scores were not assessed using standardised diagnostic tools such as the ADI-R (Lord et al., 1994) or the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 1989). Other studies of ASD traits in ADHD have excluded children over a certain threshold of ASD problems who are then confirmed as having ASD on subsequent assessment, for example using the Parental Account of Children's Symptoms (Mulligan et al., 2009a). Thus, it is likely that some of the children with particularly high SCQ scores in this sample would have a clinical diagnosis of an ASD if assessed using standardised measures. It is worth noting that analysing the data using the SCQ as a binary variable (i.e. dividing the children into groups based on the screening cut-off threshold used in the literature (Berument et al., 1999)) and thus comparing children with total SCQ<15 to those with SCQ score ≥15, gives the same pattern of results and direction of effects as reported for the continuous analyses.

A further limitation of the analysis is that although parents were asked to withhold stimulant medication for 24 hours prior to cognitive testing, a small proportion of parents (14.4%) reported not following these instructions. However, this information could not be reliably used as a covariate when examining the association of SCQ scores with the cognitive tests due to the high variability of the types and dosages of medication the children took and, perhaps more importantly, the variability in time of day the assessments took place (ranging from morning to evening).

Finally, a major limitation of this cross-sectional data is that without longitudinal follow-up it is not possible to ascertain a causal direction of the observed associations between SCQ scores and the cognitive and developmental outcomes.

Clinical implications and conclusions

Overall, the findings suggest that in children with ADHD, the presence of ASD traits (which are not at a diagnostic level) indexes a more impaired phenotype, encompassing not only severity of ADHD, but also general cognitive and working memory deficits, as well as motor and language developmental delays. These findings corroborate and extend previous research (Grzadzinski et al., 2011; Kröger et al., 2011; Mulligan et al., 2009a) and would benefit from subsequent replication with other large samples. Ideally, a set of controls also assessed on all measures could be included in future studies.

With the changes that DSM-5 have brought and ICD-11 is set to bring, clinical thinking and practice are already moving beyond the restriction of precluding a dual diagnosis of ADHD and ASD. These results further support this change and highlight the need for clinicians to acknowledge the strong overlap of these neurodevelopmental traits in children. Clinicians may also need to consider levels of social-communication difficulties and repetitive behaviours in children with ADHD who do not meet diagnostic criteria for ASD, as these index higher levels of cognitive and developmental problems. The presence of undiagnosed deficits in social-communication abilities may also have implications for the effectiveness of medication and behavioural strategies aimed at ameliorating ADHD symptoms. Addressing ASD symptoms in children with ADHD may have the potential to improve the effect of ADHD interventions and their associated impairment.

Chapter 5

Genetic Risk for ADHD Contributes to Neurodevelopmental Traits in the General Population

The work presented in this chapter has been published:

Martin, J., Hamshere, M. L., Stergiakouli, E., O'Donovan, M. C., & Thapar, A. (2014). Genetic Risk for Attention Deficit Hyperactivity Disorder Contributes to Neurodevelopmental Traits in the General Population. Biological Psychiatry, 76(8), 664-671.

The published article has been edited for this chapter in order to include additional results and reduce the amount of repetition across chapter 2 and section 5.3. Please note however that this chapter covers background information already dealt with in previous chapters.

Please see http://www.biologicalpsychiatryjournal.com/article/S0006-3223(14)00108-5/abstract for the full published article.

5.1 Summary

ADHD can be viewed as the extreme end of traits in the general population. Epidemiological and twin studies also suggest that ADHD frequently co-occurs with and shares genetic susceptibility with ASD. The aims of this chapter were to determine whether a composite of common molecular genetic variants, previously found to be associated with clinically-diagnosed ADHD, predicts ADHD and ASD-related traits in the general population. Polygenic risk scores were calculated in the Avon Longitudinal Study of Parents and Children (ALSPAC) population sample (N=8,229), based on a discovery case-control genome-wide association study of childhood ADHD. Regression analyses were used to assess whether polygenic scores predicted ADHD traits and also ASD-related

measures (pragmatic language abilities and social cognition) in ALSPAC. Polygenic risk scores were also compared in males and females endorsing any (≥1) ADHD item (N=3,623). ADHD polygenic risk scores showed a positive association with ADHD traits (hyperactive-impulsive: p=0.0039; inattentive: p=0.037). ADHD polygenic risk was also negatively associated with pragmatic language abilities (p=0.037), but not with social cognition (p=0.43). In children with a rating ≥1 for ADHD traits, females had a higher polygenic risk score than males (p=0.003). These findings provide molecular genetic evidence that risk alleles for the categorical disorder of ADHD influence hyperactive-impulsive and attentional traits in the general population. The results further suggest that common genetic variation that contributes to ADHD diagnosis may also influence ASD-related traits, which at their extreme are a characteristic feature of ASD.

5.2 Introduction

ADHD is a highly heritable neurodevelopmental disorder. The disorder occurs more frequently in males, with a male:female ratio of about 3-7:1 (Lahey et al., 1994; Polanczyk et al., 2007). Similarly to other common disorders, the genetic architecture of ADHD is complex, with rare and common variants involved (Stergiakouli et al., 2012). Whilst clinical diagnoses are defined categorically, ADHD psychopathology can also be viewed dimensionally, with inattentive and hyperactive-impulsive symptoms distributed continuously in the general population (Rodriguez et al., 2007). Twin and epidemiological studies find that heritability estimates for dimensional ADHD are similar across a variety of cut-off points (Larsson et al., 2011; Levy et al., 1997). This indicates that genetic factors act throughout the full distribution of ADHD symptoms. However, the postulated relationship between dimensional measures of ADHD in the population and clinical diagnoses has not yet been confirmed at the level of molecular genetics.

In recent years, it has become clear that the boundaries between different neurodevelopmental and psychiatric disorders are not clear cut, as exemplified by the observed clinical and genetic overlap between ADHD and other disorders. Rates of co-occurrence are especially high for ADHD and ASD (Rommelse et al., 2010). Studies of children with clinical diagnoses have found that large (>500kb),

rare (<1% frequency) copy number variants (CNVs) in ADHD show significant overlap with CNV loci previously implicated in ASD (Williams et al., 2010, 2012), although a recent collaborative cross-phenotype analysis found no clear common genetic overlap in diagnosed ADHD and ASD cases (Smoller et al., 2013). ASD can also be viewed dimensionally (Constantino & Todd, 2003) and twin studies find that ADHD and ASD traits share common genetic influences in the general population, as well as at the quantitative extreme (Lichtenstein et al., 2010; Lundström et al., 2011; Polderman et al., 2012; Reiersen et al., 2007; Ronald et al., 2010b, 2008; Taylor et al., 2012). This suggests that genetic variants associated with ADHD diagnosis might also contribute to population variation in ASD-related trait measures.

Previous research suggests that children clinically diagnosed with ADHD (N=452) differ from controls (N=5,081) on the basis of a polygenic risk score, an aggregate score of thousands of common alleles of very small effect which together index genetic risk for ADHD (Hamshere et al., 2013a). In this chapter, I test the hypothesis that, *en masse,* common genetic variants that confer risk for a clinical ADHD diagnosis are associated with ADHD traits in the general population. Moreover, given the established clinical and genetic overlap between ADHD and ASD (Lichtenstein et al., 2010; Reiersen et al., 2007; Ronald et al., 2008), I undertake analysis of the secondary hypothesis that, *en masse,* ADHD common genetic variants are also associated with ASD-related/social-communication traits in the general population.

5.3 Method

Target population sample – ALSPAC

This analysis used data from the Avon Longitudinal Study of Parents and Children (ALSPAC), described in detail in section 2.2. Please refer back to that section for recruitment procedures, general inclusion criteria and an overview of the measures used. Children were eligible for inclusion in the present analysis if they had genome-wide data following quality control and available phenotype data (N=5,495-5,661, depending on the measure).

Phenotypic measures

Data on ADHD traits were collected when participants were aged approximately 7 years and 7 months old, using the parent Development and Well-Being Assessment (DAWBA) (Goodman et al., 2008). For each ADHD item, parents marked boxes to say whether their child showed the behaviour; these were coded: 0 for "no", 1 for "a little more than others" and 2 for "a lot more than others". A total ADHD trait score was calculated by summing these responses to give a possible range of 0-36. Given the robust evidence that inattentive and hyperactive-impulsive ADHD traits are separable symptom dimensions (Willcutt et al., 2012) (see also chapter 3), scores were also calculated for these ADHD trait dimensions separately (with a possible range of 0-18 each).

Social-communication traits were assessed using the Social and Communication Disorders Checklist (SCDC) (Skuse et al., 2005) and the pragmatic language scales of the Children's Communication Checklist (CCC) (Bishop, 1998). A quantitative measure of restricted, repetitive behaviours was not available. Both the CCC and the SCDC have been shown to have good predictive reliability for a clinical diagnosis of ASD in the ALSPAC sample (Steer et al., 2010). The SCDC assesses social cognition and understanding, whereas the CCC pragmatic language scales measure ability to use language in a social context.

The SCDC was assessed at the same time as the DAWBA ADHD measures. Parents were asked to judge how much 12 descriptions applied to their child's behaviour (see Appendix 2.3 for the list of items). The responses were coded: 0 for "not at all true", 1 for "sometimes true" and 2 for "very/often true". A total SCDC score was calculated by summing these responses (with a possible range of 0-24).

An abridged version of the CCC was used to assess language abilities at the approximate age of 9 years and 7 months. Parents were asked to rate whether statements about their child were "certainly true", "somewhat true" or "not true", which were coded as 0, 1 and 2, respectively (see Appendix 2.4 for the list of items). The following sub-scales were summed to generate a pragmatic language abilities score: inappropriate initiation, coherence, stereotyped conversation,

conversational context and conversational rapport. Sub-scale scores were based on 6-8 items each (see Appendix 2.4 for details). The pragmatic language total score was obtained for children with data available for each subscale. As the CCC measures language abilities, lower scores suggest pragmatic language deficits.

Information on DSM-IV ADHD diagnoses is available based on the DAWBA at approximately age 7. Data on ASD diagnoses are available based on clinical records, utilising a clinician's diagnosis of ASD (Williams et al., 2008). Scores on measures with <30% missing items were mean-imputed.

Genetic data

Quality control (QC) procedures for the sample are detailed in section 2.2 and Appendix 2.2. After QC, genome-wide data were available for 500,527 SNPs for N=8,229 of the children in ALSPAC, of whom N=4,213 (51.2%) were male.

Discovery clinical sample for generating ADHD polygenic risk scores

The analytic method described by the International Schizophrenia Consortium (ISC) (Purcell et al., 2009) was used to identify ADHD risk alleles in a discovery GWAS from which polygenic risk scores were derived in the ALSPAC individuals. A published GWAS (Stergiakouli et al., 2012) of British and Irish clinic children with a confirmed DSM-IV research diagnosis of ADHD (N=727) and population controls (N=5,081) was used as the primary discovery sample to define risk alleles. This clinical sample was selected as the primary discovery sample as it is similar to the ALSPAC general population in ethnicity and underwent similar diagnostic assessment procedures. The ascertainment of DNA samples, QC procedures and GWAS results have been described in section 2.1 and Appendix 2.2. This discovery GWAS was based on 502,702 SNPs after QC.

Calculation of polygenic risk scores

The analysis was confined to autosomal SNPs. SNPs in relative linkage equilibrium (i.e. relatively independent of one another) in the ALSPAC genome-wide data were selected using a sliding window of 200 SNPs, moving it along the genome 5 SNPs at a time and dropping a SNP when the

pair-wise estimate of linkage disequilibrium (R^2) exceeded 0.2, using the command (--indep-pairwise 200 5 0.2) in the software PLINK (Purcell et al., 2007), giving a list of 101,200 SNPs. Corresponding p-values, associated risk alleles and odds ratios were identified for the selected SNPs in the GWAS discovery sample, if available. In line with previous studies (Hamshere et al., 2011, 2013a; Purcell et al., 2009; Ripke et al., 2011; Sklar et al., 2011), alleles that were more common in cases than controls for SNPs showing evidence of association at the very relaxed threshold of p<0.5 in the discovery sample were considered risk alleles. These identified alleles were used to calculate a polygenic risk score for each individual in ALSPAC, corresponding to the sum of the number of score alleles (weighted by odds ratio) across the set of SNPs, using the PLINK command (--score), with imputation of missing genotypes in PLINK (Purcell et al., 2007). The ADHD polygenic risk scores were based on the 49,595 SNPs that were present in both sets of SNPs (i.e. in the pruned set of 101,200 SNPs in the ALSPAC sample and in the set of 258,531 SNPs with p<0.5 in the clinical ADHD sample). Scores were normally distributed in the ALSPAC sample (N=8,229). Polygenic risk scores were also calculated at a variety of other p-value thresholds (p<1, p<0.4, p<0.3, p<0.2, p<0.1, p<0.05, p<0.01) to test the sensitivity of observed results. Please see Table 5.1 for the number of SNPs at each threshold. The polygenic risk scores were standardised using z-score transformations (i.e. the mean of the sample was subtracted from each score and this was divided by the standard deviation).

Table 5.1: Number of SNPs from the clinical ADHD discovery sample mapped to alleles & used to calculate polygenic risk scores at each threshold

p-value threshold	# of SNPs
p<1	96,554
p<0.5	49,595
p<0.4	40,060
p<0.3	30,542
p<0.2	20,746
p<0.1	10,687
p<0.05	5,417
p<0.01	1,193

Data analysis strategy

In the ALSPAC sample, children with ADHD or ASD diagnoses were compared with each other and with the remainder of the sample on ADHD, SCDC and CCC traits, using *t*-tests. Females and males were also compared. Analyses were conducted on the 8,229 ALSPAC children with full genetic data available after all quality control.

Due to a strongly negatively-skewed distribution of the CCC pragmatic language data, variables were transformed (ln x + 1) to make them normally distributed and linear regression analyses were performed to test for association with ADHD polygenic risk scores. ADHD and SCDC traits were highly positively-skewed, contained an excess of zero values and could not be transformed to normality (see Figure 5.1 for raw variable distributions). Analysing such data using standard linear regressions may yield biased estimates of parameters and increases Type I & II error rates (Karazsia & van Dulmen, 2008; Zuur et al., 2009). The distribution of data was better explained by a negative binomial than a Poisson distribution of simulated data with the same mean and N (see Figure 5.2). Therefore, these data were analysed using zero-inflated negative binomial (ZINB) regression models. Gender was included as a covariate in all regression models.

The ZINB model consists of two sub-models that allow for a distribution with an inflated number of individuals with values of zero: a) logistic regression model of an unobserved dichotomous outcome to predict who has a score=0 and who has a score>0 and b) negative binomial model of the continuous outcome in those having a score≥0. Likelihood ratio tests were used to determine an overall p-value for each ZINB model in comparison to a null model, which included gender but not polygenic risk score. ZINB analyses were performed using Mplus version 7 (Muthén & Muthén, 1998).

For each association test, the amount of variance explained was calculated as the difference of Nagelkerke pseudo-R^2 in the full model, as compared with the null model. Given the non-independence of the outcome variables, all results are interpreted using a significance threshold of $p<0.05$.

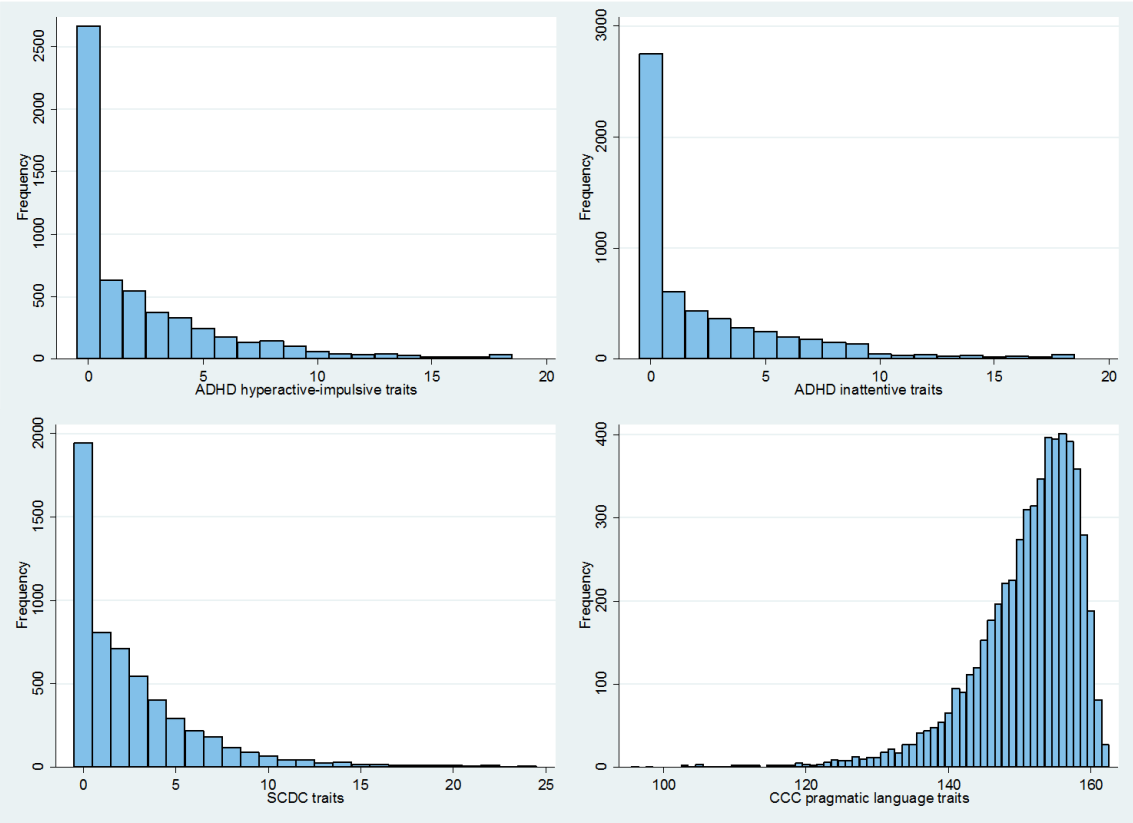

Figure 5.1: Histograms of ADHD & social-communication traits in the ALSPAC sample

ADHD: attention deficit hyperactivity disorder; SCDC: Social and Communication Disorders Checklist; CCC: Children's Communication Checklist

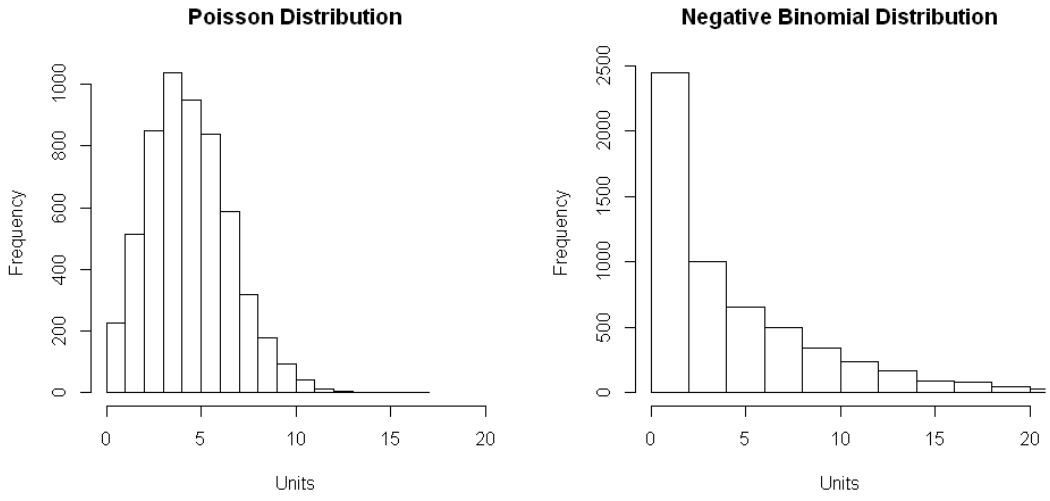

Figure 5.2: Simulated data showing Poisson and negative binomial distributions

Simulations of an expected Poisson distribution (left) and a negative binomial distribution (right) in R, using the sample size (N=5,661) and mean (4.9) of the total ADHD traits in the ALSPAC sample.

Given that previous analysis of polygenic risk scores for ADHD in a clinical sample of children with ADHD showed that females had higher polygenic risk scores than males (Hamshere et al., 2013a), a *t*-test was used to test whether polygenic risk scores in children rating positive for any (≥1) ADHD trait in the target sample were significantly higher in females than males.

Where significant associations were observed, secondary analyses were performed to determine whether the same associations could be detected for ADHD traits at a later time point (approximate age 10 years and 8 months). Replication was sought using a second ADHD GWAS discovery sample, that of the Psychiatric Genomics Consortium (PGC) (Neale et al., 2010a). This sample contained 2,064 trios, 896 cases and 2,455 control individuals from four individual studies. A total of 54 cases (2% of the cases in this second discovery sample) overlapped with the main discovery sample but could not be removed as only the summary statistics were available for this analysis. The polygenic risk scores derived from this replication discovery sample were based on 47,226 SNPs.

5.4 Results

Sample phenotypic characteristics

Figure 5.3 presents descriptive statistics of the trait measures in children with no ADHD/ASD (N=5,585), those diagnosed with ADHD (N=105), ASD (N=35) or both (N=8). Table 5.2 shows the breakdown of the sample by diagnosis, including those with missing data. In those with data for both measures, of the children with a diagnosis of ADHD, 7.1% also had a diagnosis of ASD and 36.4% of those with ASD also had ADHD; thus there were more children with both diagnoses than would be expected by chance (Chi^2=136.0, p=2.1x10^{-31}).

Table 5.2: ADHD and ASD diagnoses in the sample

		ASD			
		absent	present	missing	Total
ADHD	absent	5,585	14	13	5,612
	present	104	8	1	113
	missing	2,133	21	350	2,504
	Total	7,822	43	364	8,229

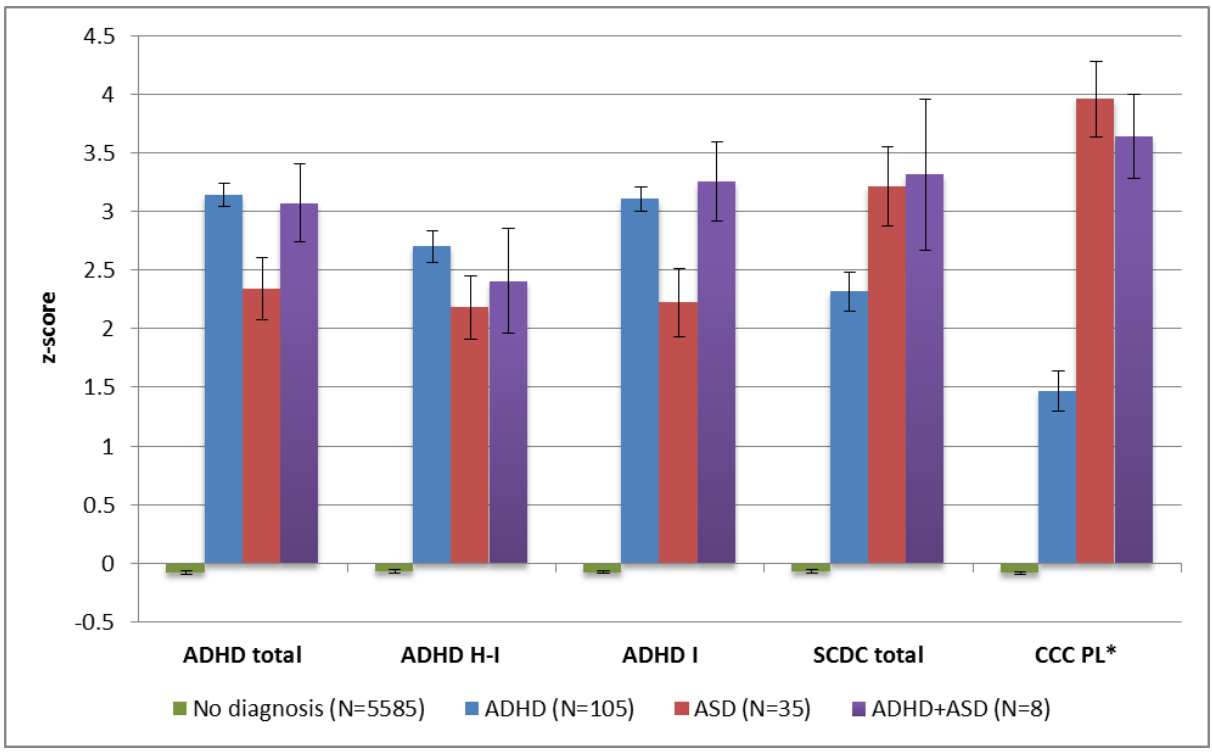

Figure 5.3: Mean z-scores of ADHD & social-communication outcomes, displayed by diagnostic group

*Scores reversed; ADHD: attention deficit hyperactivity disorder; H-I: hyperactive-impulsive; I: inattentive; SCDC: Social and Communication Disorders Checklist; CCC PL: Children's Communication Checklist pragmatic language; Error bars represent standard errors of the mean

As expected, ADHD trait levels were higher in children with a diagnosis of ASD than in those without ADHD or ASD (hyperactive-impulsive: $t(df)=13.0(5,527)$, $p=2.9x10^{-38}$; inattentive: $t(df)=13.1(5,522)$, $p=9.6x10^{-39}$). Children with ASD had lower levels of inattentive traits than children with ADHD ($t(df)=-3.5(128)$, $p=0.0006$) but did not differ significantly in terms of hyperactive-impulsive traits ($t(df)=-1.7(130)$, $p=0.092$).

Children with an ADHD diagnosis had significantly higher SCDC scores ($t(df)=26.7(5,596)$, $p=5.0x10^{-148}$) and lower CCC pragmatic language scores ($t(df)=-11.5(4,890)$, $p=5.7x10^{-30}$) than those without ADHD or ASD, but had lower SCDC scores ($t(df)=-2.5(129)$, $p=0.016$) and higher pragmatic language ability scores ($t(df)=6.2(108)$, $p=1.2x10^{-8}$) than children with ASD. The ADHD and social-communication outcomes were moderately correlated (see Table 5.3).

Table 5.3: Pearson correlation coefficients of ADHD and social-communication measures

	ADHD HI	ADHD I	ADHD total	SCDC
ADHD I	0.71			
ADHD total	0.92	0.93		
SCDC	0.65	0.58	0.66	
CCC PL	-0.51	-0.48	-0.53	-0.51

H-I: hyperactive-impulsive; I: inattentive; SCDC: Social and Communication Disorders Checklist; CCC PL: Children's Communication Checklist pragmatic language; All associations significant at p<0.001

As compared with males, females had lower scores for ADHD (hyperactive-impulsive: t(df)=-12.5(5,659), $p=2.7x10^{-35}$; inattentive: t(df)=-13.1(5,654), $p=1.9x10^{-38}$) and SCDC (t(df)=-9.5(5,651), $p=3.0x10^{-21}$) and higher CCC pragmatic language ability scores (t(df)=6.4(5,639), $p=1.3x10^{-10}$).

Polygenic risk score analysis of ADHD and ASD-related/social-communication traits

Among children with any ADHD traits (≥1; N=3,623), females had a higher polygenic risk score than males (t(df)=2.9(3,621), p=0.0033, Cohen's d=0.098). This is not attributable to an overall population difference on polygenic risk score by gender (t(df)=1.6(8,227), p=0.11). Gender was included as a covariate in all further analyses.

Results of association analyses of ADHD polygenic risk scores with the ADHD and social-communication outcomes are shown in Table 5.4. ZINB models show that ADHD polygenic risk predicted ADHD total scores (R^2=0.005, p=0.0026), hyperactive-impulsive traits (R^2=0.002, p=0.0039) and inattentive traits (R^2=0.002, p=0.037). The ZINB models indicate that the association signal comes from the zero-inflated part (part a) of the model for all ADHD outcomes.

To further explore the contribution of polygenic risk scores to ADHD trait levels, the population was split into three arbitrary groups, based on increasing trait score: children who scored zero (N=2038), those with low levels of ADHD (score=1-11; N=2817) and those with moderate-to-high levels of ADHD (score≥12; N=806). ANOVA shows a significant group difference (F(df)=4.66(2), p=0.010) and *post-hoc* tests revealed that children with no ADHD traits had a lower mean polygenic risk score than those with ADHD scores of 1-11 (p=0.022) and also those with scores ≥12 (p=0.037). The difference between the two other groups was not significant (p=0.80).

ADHD polygenic risk scores showed a significant association with lower CCC pragmatic language scores (β=-0.028, p=0.037). Exploration of whether findings were attributable to specific CCC subscales showed associations with lower scores on the 'inappropriate initiation' (β=-0.034, p=0.009) and 'conversational context' (β=-0.034, p=0.010) subscales but not with 'coherence', 'stereotyped conversation' and 'conversational rapport' (all p>0.05). No association was found between polygenic risk scores and SCDC total scores (p>0.05).

Given that polygenic risk scores were associated with both ADHD and pragmatic language, structural equation modelling (SEM) was used to determine the unique effect of polygenic risk score on each of these outcomes. SEM allows for the simultaneous assessment of relationships between multiple predictors and outcomes while accounting for correlation of outcomes. SEM with inattentive and hyperactive-impulsive ADHD traits and pragmatic language abilities as correlated outcomes confirmed that each of these variables is independently predicted by polygenic risk scores (see Figure 5.4).

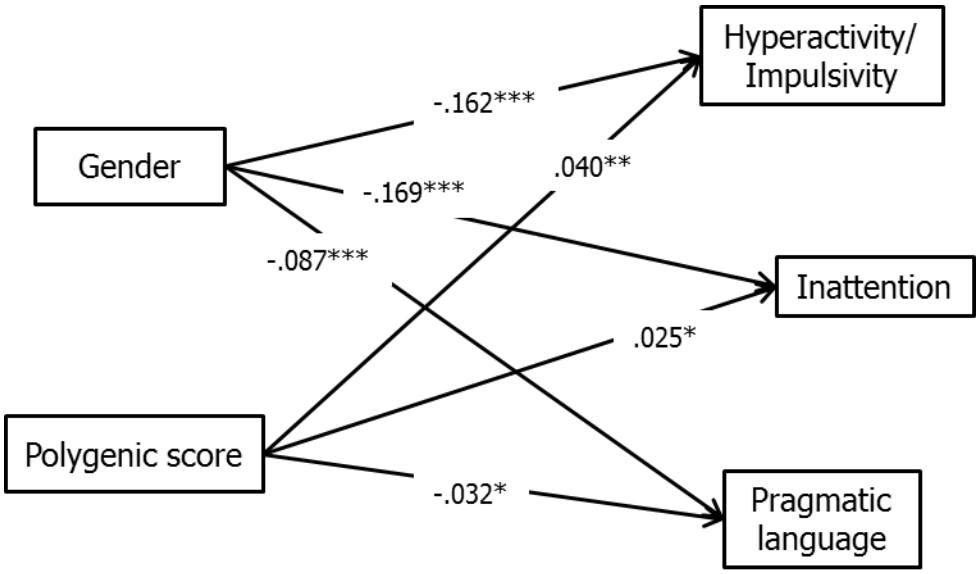

Figure 5.4: Structural equation modelling of polygenic risk scores predicting multiple correlated outcomes

Structural equation modeling was performed in Mplus version 7. The MLR model estimator was used as it provides full information maximum likelihood estimation with robust standard errors, using all available data for the model. Analysis was based on N=6,423. Standardized path coefficients are shown. Correlation coefficients and arrows are not shown. All paths were estimated and no fit statistics are available due to model saturation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

The amount of variance explained for all models was very small ($R^2 \leq 0.005$), although this estimate does not reflect the true magnitude of the genetic overlap as it is highly sensitive to discovery sample size (Purcell et al., 2009). Including the 10 EIGENSTRAT principal components as covariates in the analyses to allow for population stratification did not affect the results (see Appendix 5.1).

Testing associations at age 10

Pearson correlation coefficients showed a strong association of ADHD traits from age 7 years to age 10 years (hyperactive-impulsive traits: N=4,757, r=0.71, inattentive traits: N=4,746, r=0.68. total ADHD traits: N=4,751, r=0.74). The observed association between polygenic risk scores and ADHD (at age 7 years) could also be seen at the later time point (age 10 years) for total ADHD traits (R^2=0.004, p=0.012) and hyperactive-impulsive traits (R^2=0.003, p=0.039), with weak association with inattentive traits (R^2=0.002, p=0.055). See Table 5.5 for details. Among children with any ADHD traits at age 10 (≥1; N=3,316), females had a higher polygenic risk score than males (t=2.35, p=0.019, Cohen's d=0.082).

Replication using second discovery sample

Polygenic risk scores based on the second discovery ADHD GWAS sample (Neale et al., 2010) were not significantly associated with ADHD traits at age 7 (p>0.05) but did show an association at age 10 with total ADHD traits (R^2=0.001, p=0.019) and hyperactive-impulsive traits (R^2<0.001, p=0.018), and a weak association with inattentive traits (R^2<0.001, p=0.055); see Table 5.6. Polygenic risk scores based on the second discovery sample also showed an association with the CCC 'conversational context' subscale (β=-0.031, p=0.017) but showed no association with the CCC 'inappropriate initiation' subscale (β=-0.006, p=0.37).

In children with ADHD trait scores≥1 at age 7, there was a trend for females to have a higher polygenic risk score than males, calculated using this second discovery sample (t=1.80, p=0.071, Cohen's d=0.060). At age 10, females had significantly higher polygenic risk scores than males (t=2.18, p=0.029, Cohen's d=0.076).

Table 5.4: Associations of polygenic risk scores with ADHD and ASD-related phenotypes in ALSPAC

Outcome	N	ZINB count outcome			ZINB zero-inflated outcome			ZINB overall p	ZINB overall R^2	Linear regression*			
		β	SE	p	β	SE	p			β	SE	p	R^2
ADHD total traits	5661	0.11	0.10	0.30	-0.06	0.02	0.005	**0.0026**	**0.005**	0.032	0.013	0.013	0.001
ADHD hyperactive-impulsive traits	5661	0.15	0.13	0.24	-0.05	0.02	0.024	**0.0039**	**0.002**	0.037	0.013	0.005	0.001
ADHD inattentive traits	5656	0.05	0.13	0.71	-0.05	0.02	0.019	**0.037**	**0.002**	0.023	0.013	0.084	0.001
SCDC total score	5653	0.15	0.19	0.45	0.02	0.04	0.67	**0.43**	**<0.001**	0.012	0.013	0.35	0.0002
CCC pragmatic language score	5641	N/A								-0.028	0.013	**0.037**	**0.001**

* Linear regression results of ADHD and SCDC phenotypes included only for comparison and ease of interpretation; main results shown in bold. All analyses include gender as a covariate. ZINB: zero-inflated negative binomial; ADHD: attention deficit hyperactivity disorder; SCDC: Social and Communication Disorders Checklist; CCC: Children's Communication Checklist. Polygenic risk scores derived using a threshold of p<0.5 in the discovery sample GWAS results (see text).

Table 5.5: Secondary analysis – Associations of polygenic risk scores with ADHD at age 10 years

Outcome	N	ZINB count outcome			ZINB zero-inflated outcome			ZINB overall p	ZINB overall R^2	Linear regression*			
		β	SE	p	β	SE	p			β	SE	p	R^2
ADHD total traits	5500	-0.05	0.12	0.68	-0.06	0.02	0.003	**0.012**	**0.004**	0.087	0.086	0.31	0.0002
ADHD hyperactive-impulsive traits	5505	-0.15	0.25	0.53	-0.06	0.02	0.012	**0.039**	**0.003**	0.019	0.043	0.66	$3.4x10^{-5}$
ADHD inattentive traits	5495	0.02	0.14	0.90	-0.04	0.02	0.021	**0.055**	**0.002**	0.076	0.051	0.14	0.0004

* Linear regression results included only for comparison and ease of interpretation; main results shown in bold. All analyses include gender as a covariate. ZINB: zero-inflated negative binomial; ADHD: attention deficit hyperactivity disorder. Polygenic risk scores derived using a threshold of p<0.5 in the discovery sample GWAS results (see text).

Table 5.6: Replication analyses – Associations of polygenic risk scores based on second discovery sample with ADHD at both time points

Time	Outcome	N	ZINB count outcome			ZINB zero-inflated outcome			ZINB overall p	ZINB overall R^2	Linear regression*			
			β	SE	p	β	SE	p			β	SE	p	R^2
age 7	**ADHD total traits**	5661	0.11	0.11	0.30	-0.02	0.02	0.338	**0.20**	**0.001**	0.023	0.13	0.052	0.001
	ADHD hyperactive-impulsive traits	5661	0.05	0.10	0.58	-0.03	0.02	0.20	**0.26**	**<0.001**	0.020	0.013	0.12	0.0004
	ADHD inattentive traits	5656	0.18	0.20	0.39	-0.02	0.02	0.44	**0.17**	**<0.001**	0.027	0.013	0.043	0.001
age 10	**ADHD total traits**	5500	0.27	0.24	0.26	-0.02	0.02	0.45	**0.019**	**<0.001**	0.26	0.087	0.003	0.002
	ADHD hyperactive-impulsive traits	5505	0.30	0.39	0.44	-0.01	0.02	0.56	**0.018**	**<0.001**	0.13	0.043	0.003	0.002
	ADHD inattentive traits	5495	0.29	0.33	0.38	-0.01	0.02	0.65	**0.055**	**<0.001**	0.13	0.052	0.015	0.001

* Linear regression results included only for comparison and ease of interpretation; main results shown in bold. All analyses include gender as a covariate. ZINB: zero-inflated negative binomial; ADHD: attention deficit hyperactivity disorder. Polygenic risk scores derived using a threshold of p<0.5 in the discovery sample GWAS results (see text).

5.5 Discussion

As hypothesised, the analysis presented in this chapter found that ADHD polygenic risk scores, based on common genetic variants previously found to be associated with risk of a clinical diagnosis of ADHD (Stergiakouli et al., 2012), were also associated with ADHD traits measured at ages 7 and 10 years, in the general population. The importance of this finding is that it provides support at the level of molecular genetics for the hypothesis that ADHD represents the extreme end of traits present in the general population (Larsson et al., 2011; Levy et al., 1997). The results also support the relevance of common genetic variants to ADHD (Stergiakouli et al., 2012), extending findings by showing that they also act on non-clinical ADHD traits in a community sample.

The exploratory ANOVA results show that polygenic risk scores, which are derived from common genetic variants relevant to clinical (i.e. severe) ADHD, predicted both low and high levels of ADHD traits in the general population. The ZINB analysis suggested that the association signal between polygenic risk scores and ADHD traits originates from the zero-inflated part of the model (i.e. whether ADHD trait score was zero or non-zero). This result might be due to greater power at the lower end of ADHD traits, as progressively fewer children have higher levels of ADHD traits.

Consistent with previous literature in clinical and general population samples (Gadow et al., 2006; Lichtenstein et al., 2010; Lundström et al., 2011), children with a diagnosis of ADHD had more ASD-related/social-communication problems than those without a diagnosis of ADHD or ASD, while children with ASD had more ADHD traits than those without either diagnosis. Interestingly, although children with ADHD had higher inattentive traits than those with ASD, levels of hyperactive-impulsive traits in these two groups did not differ significantly. However, this could have been due to low power as few children in ALSPAC had a clinical ASD diagnosis.

Results of the genetic analysis also suggest that risk alleles for ADHD may contribute to phenotypic traits in the general population beyond core ADHD features. Polygenic risk scores previously found

to be associated with ADHD diagnosis were also nominally associated with pragmatic language abilities in the general population, but not with social cognition traits, as indexed by SCDC scores.

Secondary exploratory analyses suggested that the association of ADHD polygenic risk scores with pragmatic language scores was driven by scores on the 'inappropriate initiation' and 'conversational context' subscales of the CCC. Some items in the 'inappropriate initiation' subscale may tap into impulsive ADHD behaviours (in particular, the CCC item "he/she talks too much") but items in the 'conversational context' subscale (e.g. "he/she can understand sarcasm" or "he/she says things which are tactless or socially inappropriate") have no apparent link with ADHD features. Overall, the findings suggest that risk variants for ADHD may have pleiotropic effects on closely-related but conceptually different neurodevelopmental traits in the general population. These findings also support those from a twin study, which found that ADHD traits at age 8 shared genetic effects and were most associated with ASD communication difficulties, rather than ASD social difficulties or stereotyped behaviours (Taylor et al., 2012).

One possible advantage of the primary discovery ADHD sample used to derive risk alleles, over the replication sample, is its similarity to the ALSPAC cohort in terms of ancestry and geography, but nevertheless, the sample was relatively small (Stergiakouli et al., 2012). Analyses using a second, larger ADHD sample (Neale et al., 2010a) show a partial replication of the primary analysis; polygenic risk scores based on this sample predicted ADHD traits at age 10, though not at age 7. Similarly, although polygenic risk scores derived from the second ADHD dataset predicted pragmatic language problems, as assessed using the CCC 'conversational context' subscale, they did not predict variation on the CCC 'inappropriate initiation' subscale. On the whole, these replication results do suggest that the associations of ADHD polygenic risk scores with ADHD traits and pragmatic language problems are robust. However, further replication is necessary to conclusively rule out possible type I error. The results also further highlight the fact that absence of

clear individually associated loci in current GWAS studies of ADHD reflects inadequate power of the GWAS samples rather than an absence of common susceptibility variants.

Although an association between ADHD polygenic risk scores and pragmatic language abilities was found, there was no association with social cognition, as measured by the SCDC. A recent collaborative cross-phenotype analysis suggests that common GWAS variants do not contribute to the overlap in diagnoses of ADHD and ASD (Smoller et al., 2013). Nevertheless, twin evidence is consistent in finding high heritability for neurodevelopmental trait measures and in showing shared genetic influences on ADHD and ASD (Larsson et al., 2011; Levy et al., 1997; Lichtenstein et al., 2010). Thus, it is too early to discount the contribution of common variants to the overlap of ADHD and ASD, particularly in terms of continuously distributed traits. The current analysis points to a possible overlap between susceptibility to clinically diagnosed ADHD and pragmatic language difficulties at a trait level in the general population.

As expected, male children in ALSPAC had higher ADHD trait scores than females (Keen & Ward, 2004; Lichtenstein et al., 2010; Meltzer et al., 2000). However, a novel observation was that in the group of children with any ADHD symptoms at either age, females had higher polygenic risk scores than males. For polygenic risk scores based on the second discovery sample, there was a trend towards similarly higher scores in females at age 7 and significantly higher scores were observed in females at age 10 years. These results support the previous observation that in children diagnosed with ADHD, females have higher polygenic risk scores than males (Hamshere et al., 2013a). One limitation of the earlier study is that it was based on a clinical sample, so the gender difference may have reflected referral bias (i.e. referred females may have, on average, had a more severe phenotype). The present finding in an epidemiological sample argues against that, and suggests a different liability threshold for females than males, with females requiring a more extreme load of risk factors to manifest ADHD. This is consistent with non-molecular based studies; for example, one study observed that siblings of females with ADHD have more ADHD symptoms than siblings

of males with ADHD (Rhee & Waldman, 2004). Similar findings have been reported in non-identical twin children with ASD (Robinson et al., 2013).

A limitation of this analysis was that although the SCDC and CCC measures of social cognition and pragmatic language are predictive of a clinical diagnosis of ASD in the sample (Steer et al., 2010), they are not strictly measures of the specific deficits required for an ASD diagnosis. Moreover, there was no reliable quantitative measure of restrictive and repetitive behaviours available. The finding of an association between ADHD polygenic risk scores and pragmatic language deficits is potentially also relevant to the new DSM-5 category of 'Social Communication Disorder' (Skuse, 2012).

As the ALSPAC cohort is longitudinal, the sample suffers from attrition. Previous studies have determined that predictors of attrition include socioeconomic and pregnancy factors, as well as presence of behavioural difficulties, including ADHD, in the child (Wolke et al., 2009). Assuming that attrition results from the behavioural manifestation of genetic risk, resultant attrition bias is likely to reduce the correlation between risk scores and traits. Multiple imputation methods have been used previously for missing ALSPAC data but do not appear to alter association patterns (Langley et al., 2012).

Due to the relatively small ADHD GWAS discovery sample sizes, power to detect susceptibility variants is low and aggregate scores based on GWAS are likely to be based on a poor signal-to-noise ratio (Neale et al., 2010a; Stergiakouli et al., 2012). This is a possible explanation for the relatively small amount of phenotype variance explained by polygenic risk scores in the current study, estimates of explained variance in this form of analysis being strongly affected by discovery sample size. Another limitation of the current study is that a small number (N=54) of cases overlapped in both discovery samples. Although $p<0.5$ is frequently used as a threshold for calculating polygenic risk scores (Hamshere et al., 2011; Purcell et al., 2009; Ripke et al., 2011; Sklar et al., 2011), this is largely a convention established on the basis of the optimal threshold in the early study of

schizophrenia which inspired the wider application of polygenic risk score analysis (Purcell et al., 2009). As shown by modelling in that study, the optimal threshold depends on both genetic architecture and sample size and therefore other thresholds have the potential to show greater effects. A sensitivity analysis in the present study using a variety of p-value thresholds for calculating polygenic risk scores demonstrated that observed effects are fairly consistent across thresholds (see Appendix 5.2).

Conclusions

In the current study, polygenic risk previously found to be associated with clinical ADHD diagnosis predicted inattentive and hyperactive-impulsive traits in a general population sample. The study also indicates that common genetic variants associated with ADHD may also be associated with pragmatic language ability in the general population, a trait measure that is distinct from the core deficits of ADHD. The approach of testing genetic risks that contribute to dimensions that cut-across diagnostic categories, rather than using DSM diagnoses, is in line with the Research Domain Criteria (RDoC) framework (Insel et al., 2010), and is likely to be a valuable approach for future neurodevelopmental and psychiatric research. As the power of GWAS increases, this method has the potential to further explore the biological overlap of these traits.

Chapter 6

Neurocognitive Abilities in the General Population and Polygenic Risk Scores for ADHD

The work presented in this chapter has recently been accepted for publication and is now in press:

Martin, J., Hamshere, M., Stergiakouli, E., O'Donovan, M. C., & Thapar, A. (2014). Neurocognitive abilities in the general population and polygenic risk scores for attention deficit hyperactivity disorder. Journal of Child Psychology and Psychiatry (Epub ahead of print]). doi:10.1111/jcpp.12336

The journal manuscript has been edited for this chapter in order to include additional results (available as supplementary materials in the manuscript) and to reduce the amount of repetition across chapter 2 and section 6.3 in this chapter. Please also note that this chapter repeats some background information that has already been covered in previous chapters.

6.1 Summary

The genetic architecture of ADHD is complex, with rare and common variants involved. Common genetic variants (as indexed by a polygenic risk score) associated with clinical ADHD significantly predict ADHD and autistic-like behavioural traits in children from the general population, suggesting that ADHD lies at the extreme of normal trait variation (as discussed in chapter 5). ADHD and other neurodevelopmental disorders share neurocognitive difficulties in several domains (e.g. impaired cognitive ability and executive functions). In this chapter, it was predicted that ADHD polygenic risk scores derived from an independent clinical ADHD case-control genome-wide association study (GWAS) will contribute to variation in neurocognitive abilities in the general population. Children (N=6,832) from a UK population cohort, the Avon Longitudinal Study of

Parents and Children (ALSPAC) underwent neurocognitive testing. Parent-reported measures of their children's ADHD and autistic-like traits were used to construct a behavioural latent variable of "neurodevelopmental traits". Polygenic risk scores for ADHD were calculated for ALSPAC children based on findings from a clinical ADHD GWAS. Structural equation modelling was used to assess associations between ADHD polygenic risk scores and neurocognitive abilities (IQ, working memory, inhibitory control and facial emotion recognition), as well as the latent "neurodevelopmental trait" measure. The results confirmed that neurocognitive and neurodevelopmental traits are correlated in children in the general population. Polygenic risk scores for ADHD were independently associated with lower IQ (β=-0.05, $p<0.001$) and working memory performance (β=-0.034, $p=0.013$), even after accounting for the relationship with latent neurodevelopmental behavioural trait scores. No associations were found between polygenic risk scores and inhibitory control or emotion recognition ($p>0.05$). These findings suggest that common genetic variants relevant to clinically-diagnosed ADHD have pleiotropic effects on neurocognitive traits as well as behavioural dimensions in the general population. This further suggests that the well-recognised association between cognition and neurodevelopmental behavioural traits is underpinned at a biological level.

6.2 Introduction

ADHD is a highly heritable neurodevelopmental disorder (Faraone et al., 2005). It is clear that the genetic architecture of ADHD is complex, with common and rare variants involved (Neale et al., 2010a; Stergiakouli et al., 2012; Williams et al., 2010; Yang et al., 2013). ADHD is strongly associated with ASD and other neurodevelopmental disorders (Thapar et al., 2013). Twin studies also demonstrate significant co-heritability across neurodevelopmental disorders and traits, including ADHD and ASD (Lichtenstein et al., 2010; Ronald et al., 2008). These findings suggest that genetic risk variants for ADHD are likely to contribute to multiple neurodevelopmental behavioural traits and disorders. Indeed, there is an increased burden of rare copy number variants (CNVs) in ADHD,

which overlap more than expected by chance with regions implicated in other neurodevelopmental conditions, such as ASD or schizophrenia (Lionel et al., 2011; Williams et al., 2010, 2012). However, such CNVs are rare and so it is likely that other types of genetic variants also contribute to this overlap.

There is evidence that commonly occurring genetic variants play a role in risk for ADHD. First, when considered in aggregate (i.e. as a polygenic risk score), common risk alleles defined from a clinical ADHD case-control genome-wide association study (GWAS) are higher in an independent set of ADHD cases than in controls (Hamshere et al., 2013a). Second, common additive variants collectively show moderate heritability for ADHD when estimated using the method of 'genomic-relationship-matrix restricted maximum likelihood' (GREML) with genome-wide complex trait analysis (GCTA) software (Lee et al., 2013; Yang et al., 2013).

The evidence is mixed so far in terms of possible overlap of common genetic risk variants across ADHD and other disorders. One study found that common risk alleles relevant to schizophrenia are enriched in ADHD cases when compared with controls (Hamshere et al., 2013b). However, two recent cross-disorder studies from the Psychiatric Genomics Consortium, found no overlap of common variants across clinical samples of ADHD and ASD or schizophrenia (Lee et al., 2013; Smoller et al., 2013). Nevertheless, these analyses are inconclusive as the ADHD and ASD samples were substantially smaller than those available for adult disorders, which did show significant overlap of risk alleles. Furthermore, in a general population cohort, polygenic risk for ADHD, using scores derived from a clinical discovery sample, predicted ADHD and social-communication/autistic-like behavioural traits, as described in chapter 5 (Martin et al., 2014). On the whole, previous studies suggest that common genetic variants may show some pleiotropic effects on different neurodevelopmental behavioural traits, both in clinical cases and in the general population.

Neurocognitive problems

Children affected by ADHD often have multiple, prominent neurocognitive difficulties. These affect a range of domains, notably involving global cognitive ability (Frazier et al., 2004) and executive functioning, such as response inhibition and working memory (Willcutt et al., 2008), as well as aspects of social cognition, such as facial emotion recognition (Collin et al., 2013; Uekermann et al., 2010). Like ADHD, these neurocognitive domains are heritable (Ando et al., 2001; Deary et al., 2009; Rommelse et al., 2011). There is also evidence suggesting that difficulties in these domains share familial and genetic risks with ADHD (Bidwell et al., 2007; Kuntsi et al., 2004; Schachar et al., 2005). Moreover, difficulties in these domains are not unique to ADHD, but rather these deficits are also strongly associated with other neurodevelopmental disorders, including ASD and schizophrenia (Fett et al., 2011; Matson & Shoemaker, 2009; Uljarevic & Hamilton, 2012; Willcutt et al., 2008). It is not yet known whether common genetic variants relevant to clinical ADHD also contribute to neurocognitive abilities, typically assessed through task-based performance, in the general population.

The aim of the current chapter was to examine whether a polygenic risk score based on case-control GWAS findings for clinical ADHD influences general cognitive ability, executive functioning and social cognition in children from the general population. It was hypothesised that polygenic risk scores would predict lower neurocognitive abilities in these domains. A secondary aim was to test whether observed associations were independent of associations between neurocognitive abilities and neurodevelopmental behavioural traits, specifically, parent-reported ADHD and social-communication behavioural traits in this sample, expanding on the analyses presented in chapter 5 (Martin et al., 2014).

6.3 Method

Target population sample – ALSPAC

This analysis used data from the Avon Longitudinal Study of Parents and Children (ALSPAC), described in detail in section 2.2. Please refer back to that section for recruitment procedures, general inclusion criteria and an overview of the measures used. Children were eligible for inclusion in the present analysis if full data (phenotypic and genotypic) were available (up to N=5,641-6,832 children, depending on the outcome variables).

Neurocognitive task measures

ALSPAC families were invited to attend a "Focus" clinic when the children were aged approximately 8.5 years old, where they underwent neuropsychological testing. A short form of the WISC-III assessment was employed to obtain an estimate of full scale IQ (Wechsler, 1992). In addition to this, the WISC-III Digit Span task was administered to obtain a measure of verbal working memory. Cognitive inhibitory control was assessed using the Opposite Worlds task from the Tests of Everyday Attention for Children battery (Robertson et al., 1996). The mean time taken to complete the control condition (Same Worlds trials) was subtracted from the mean time for the experimental condition (Opposite Worlds trials). The resulting scores were skewed and were transformed (1/square root of score) to be normally distributed. Facial emotion recognition was assessed using a computerised version of the faces subtest of the Diagnostic Analysis of Nonverbal Accuracy (DANVA) (Nowicki & Duke, 1994). The task comprised four emotion types (happy, sad, angry or fearful) shown at high (easy condition) and low (hard condition) intensities. The total number of errors made on the 12 low emotional intensity faces (normally distributed) was used for analysis.

In addition to these four measures (IQ, working memory, cognitive inhibitory control and facial emotion recognition), the Counting Span Task, another measure of working memory, was administered at the approximate age of 10.5 years. The measure used was a computer-generated normally distributed 'global score', corresponding to the number of correct trials.

Parent-reported measures of ADHD and social-communication

Measures of ADHD and social-communication assessed at a similar time to the neurocognitive measures were included in analyses as these were previously found to be associated with ADHD polygenic risk scores, as described in chapter 5 (Martin et al., 2014). Total ADHD inattentive and hyperactive-impulsive traits were calculated by summing the relevant items from the parent Development and Well-Being Assessment (DAWBA) administered at approximately 7.5 years old (Goodman et al., 2008). Social-communication traits were assessed using the Social and Communication Disorders Checklist (SCDC) at the same age (Skuse et al., 2005), as well as the pragmatic language scales of the Children's Communication Checklist (CCC) at approximately 9.5 years (Bishop, 1998). CCC pragmatic language scores were transformed (ln x + 1) and reversed so that higher scores meant more difficulties (to ease interpretation of the results).

Genetic data

A total of 9,912 ALSPAC children were genotyped. After quality control (QC), genome-wide data for 500,527 single nucleotide polymorphisms (SNPs) were available for N=8,229 of the children, of whom N=4,213 (51.2%) were male. Details of QC procedures can be found in section 2.2 as well as in Appendix 2.2.

The results of a published GWAS of British and Irish children with a diagnosis of ADHD (N=727) and population controls (N=5,081) were utilised as the discovery sample (Stergiakouli et al., 2012). The QC procedures, ascertainment of these samples and GWAS results have been described in section 2.1 and Appendix 2.2. This GWAS was based on 502,702 SNPs after QC. Polygenic risk scores were calculated for each ALSPAC child using PLINK (Purcell et al., 2007), based on the results of the above GWAS discovery sample, as described previously; see chapter 5 (Martin et al., 2014). In brief, SNPs in approximate linkage equilibrium in the ALSPAC data were identified using PLINK, with SNPs exceeding a threshold of $R^2 \geq 0.2$ excluded. As in chapter 5, the primary tests of the hypotheses were based on risk alleles enriched (at $p<0.5$) in ADHD cases in the discovery GWAS. This list of risk

alleles and corresponding odds ratios was used to calculate a polygenic risk score for each individual in ALSPAC, corresponding to the sum of the number of score alleles (weighted by odds ratio) across the set of SNPs, using the PLINK command: --score. Polygenic risk scores were also calculated at a variety of other p-value thresholds ($p<1$, $p<0.4$, $p<0.3$, $p<0.2$, $p<0.1$, $p<0.05$, $p<0.01$) to test the sensitivity of observed results. The polygenic scores were standardised using z-score transformations.

Data analysis

Pearson correlations were used to examine associations between performance on the neurocognitive measures and parent-reported ADHD and social-communication traits. Females and males were compared in terms of neurocognitive phenotypes, using a *t*-test. The neurocognitive measures were standardised using z-score transformations. Analyses were performed using Stata version 13.0 (StataCorp, 2013).

The main analysis was conducted in several steps. First, linear regression analyses were used to test for associations between polygenic risk scores and each of the neurocognitive measures, after controlling for the effect of gender.

Next, given the results of chapter 5 of this thesis showing that ADHD polygenic risk scores predict both parent-reported ADHD and social-communication traits (Martin et al., 2014), confirmatory factor analysis was used to derive a 'neurodevelopmental difficulties' latent variable. This variable was based on parent-reported ADHD inattentive and hyperactive-impulsive traits, as well as CCC pragmatic language and SCDC social cognition scores. Goodness of fit of the latent variable model was assessed using the Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI) and Tucker-Lewis Index (TLI) fit statistics. Acceptable model fit was indicated by an RMSEA fit statistic ≤0.06 and CFI and TLI statistics >0.95 (Hu & Bentler, 1999). Multiple indices were used as this provides a more comprehensive evaluation of model fit.

As a final step, structural equation modelling (SEM) was used to test a model of ADHD polygenic risk score effects on neurocognitive measures and the latent variable of neurodevelopmental difficulties. SEM simultaneously estimates the relationships of multiple manifest/observed and latent predictor and outcome variables. For the purpose of the current analysis, the SEM models combine regression, path and factor analyses. Given that neurodevelopmental and neurocognitive measures are related, modelling the association of polygenic risk scores with both measures simultaneously can be used to determine the unique effect of polygenic scores on each of these measures. On SEM figures, directly measured (i.e. manifest) variables are represented by squares, latent variables are represented by circles, single direction arrows indicate regression paths, double direction arrows indicate correlations and numbers indicate regression and correlation coefficients.

The model estimator used was 'MLR', which is robust to non-normality and provides full information maximum likelihood estimation with robust standard errors, using all available data for each model. SEM was performed using Mplus version 7 (Muthén & Muthén, 1998). All p-values presented are two-tailed. Given the non-independence of the four neurocognitive measures and the structured analytic approach, all results are interpreted using a significance threshold of $p<0.05$.

Secondary analyses

Analyses were re-run using 10 EIGENSTRAT principal components as covariates to account for possible population stratification effects. Analyses were also re-run using polygenic risk scores calculated in ALSPAC using alternative p-value selection thresholds.

Replication analyses

Where significant associations were observed, comparable analyses were run to determine whether the same associations in the ALSPAC sample could be replicated using polygenic risk scores derived from an independent ADHD discovery sample (using the same analytic method). This second sample consisted of the published international Psychiatric Genomics Consortium (PGC) meta-analysis of ADHD case-control GWAS (Neale et al., 2010a) based on four individual studies. It

consisted of 2,064 trios, 896 cases and 2,455 control individuals and 1,206,461 SNPs after quality control.

6.4 Results

Phenotypic relationships

Table 6.1 shows Pearson correlation coefficients for associations between each neurocognitive measure and ADHD and social-communication trait scores. All correlations are significant ($p<0.001$), although coefficients are low to modest.

Out of the 4 neurocognitive measures, males had lower working memory scores (age 8.5 years: $t(df)=-6.79(5,409)$, $p=1.2x10^{-11}$; age 10.5 years: $t(df)=-2.30(5,271)$, $p=0.022$) and made more emotion recognition errors ($t(df)=4.46(5,105)$, $p=8.3x10^{-6}$) than females. They did not differ from females in terms of inhibitory control ($t(df)=-1.78(5,313)$, $p=0.075$) or IQ ($t(df)=0.68(5,513)$, $p=0.50$).

Table 6.1: Pearson correlation coefficients for the neurocognitive and neurodevelopmental measures

	IQ – 8.5 years	Working memory – 8.5 years	Working memory – 10.5 years	Inhibitory control – 8.5 years	Emotion recognition – 8.5 years
Working memory – 8.5 years	0.39 5,379				
Working memory – 10.5 years	0.38 4,584	0.42 4,506			
Inhibitory control – 8.5 years	0.21 5,236	0.11 5,133	0.17 4,446		
Emotion recognition – 8.5 years	-0.16 5,032	-0.11 4,948	-0.10 4,244	-0.09 4,825	
ADHD hyperactive-impulsive – 7.5 years	-0.16 4,590	-0.13 4,495	-0.09 4,328	-0.07 4,408	0.09 4,243
ADHD inattentive – 7.5 years	-0.21 4,585	-0.19 4,491	-0.16 4,327	-0.12 4,403	0.09 4,236
Social cognition – 7.5 years	-0.14 4,589	-0.11 4,497	-0.07 4,329	-0.05 4,406	0.10 4,241
Pragmatic language – 9.5 years	-0.25 4,665	-0.18 4,577	-0.15 4,476	-0.06 4,492	0.08 4,331

For each test, the Pearson coefficient is the top value and the sample N is the bottom value. All significant at $p<0.001$

Neurocognition and polygenic scores

After adjusting for gender, ADHD polygenic risk scores, derived from the primary discovery sample (Stergiakouli et al., 2012), predicted lower IQ (N=5,515, β=-0.05, p=9.7x10^{-5}, R^2=0.0027) and lower working memory abilities (N=5,411, β=-0.034, p=0.013, R^2=0.0011). There were no associations with inhibitory control (N=5,315, β=0.006, p=0.66) or emotion recognition (N=5,107, β=-0.004, p=0.76).

The confirmatory factor analytic/latent variable model of inattentive, hyperactive-impulsive, social-cognitive and pragmatic language traits showed moderately good fit (see Figure 6.1). Next, SEM was used to simultaneously model the associations between ADHD polygenic risk scores, the latent neurodevelopmental trait variable and the neurocognitive measures (IQ, working memory, cognitive inhibitory control and facial emotion recognition), as well as the correlations between these measures.

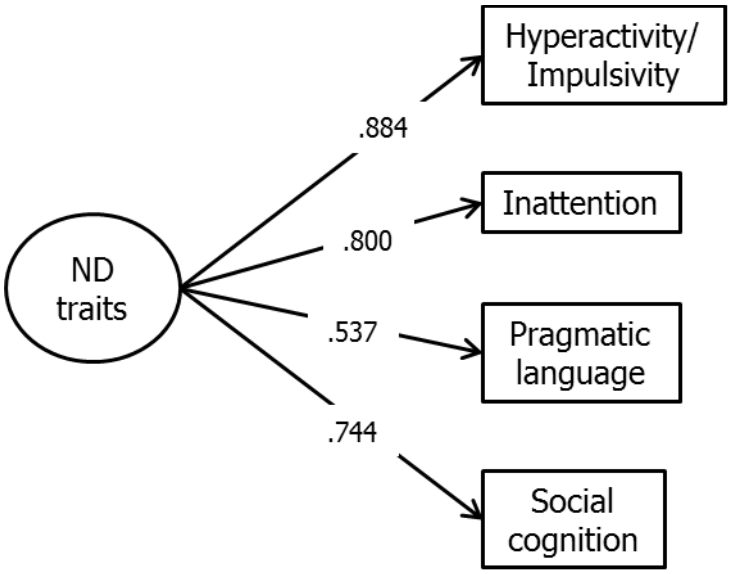

Figure 6.1: Confirmatory factor analytic/latent variable model of neurodevelopmental outcomes

ND: Neurodevelopmental; N=6,434; Goodness of fit statistics: RMSEA=0.053, CFI=0.994, TLI=0.982

Model diagrams for IQ, working memory, inhibitory control and facial emotion recognition are displayed in Figures 6.2 through 6.5, respectively. Model fit was satisfactory for all models (see figure captions). The SEM results were consistent with the linear regression analyses, with higher ADHD polygenic risk scores predicting lower IQ (β=-0.052, p<0.001, R^2=0.003) and working memory (β=-0.034, p=0.008, R^2=0.001) and no associations with inhibitory control and facial emotion recognition (p>0.05).

ADHD polygenic risk scores also predicted lower working memory abilities assessed using a different measure (i.e. the global score of the Counting Span Task), assessed at a later time point, at the approximate age of 10.5 years (β=-0.042, p=0.002, R^2= 0.0018). SEM with the neurodevelopmental difficulties latent variable showed a consistent result (β=-0.042, p=0.002, R^2=0.002); see Figure 6.6.

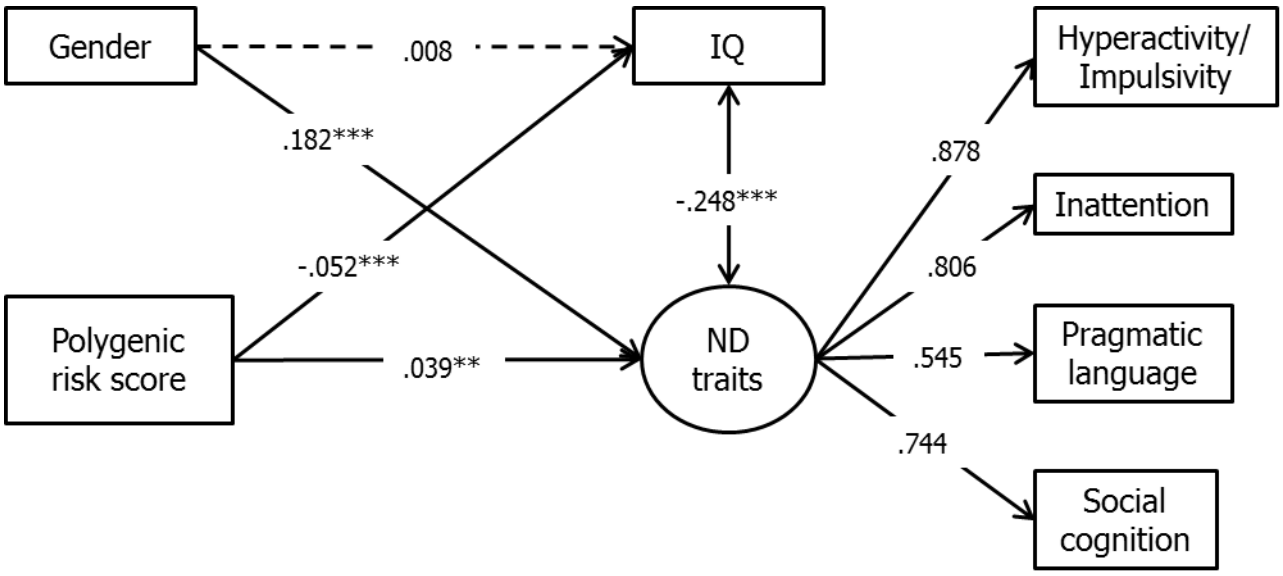

Figure 6.2: Association between polygenic risk scores with IQ

**p<0.01, ***p<0.001; ND: Neurodevelopmental; N=6,832; RMSEA=0.053, CFI=0.974, TLI=0.952

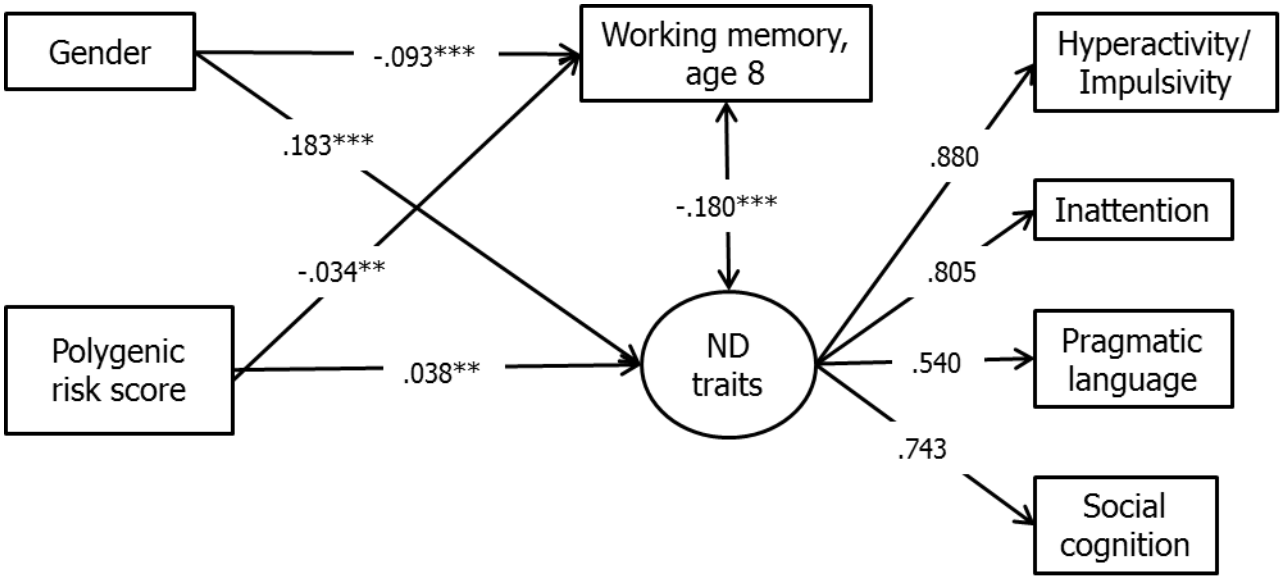

Figure 6.3: Association between polygenic risk scores with working memory at age 8 years

**p<0.01, ***p<0.001; ND: Neurodevelopmental; Working memory assessed using the Digit Span test; N=6,827; RMSEA=0.045, CFI=0.981, TLI=0.966

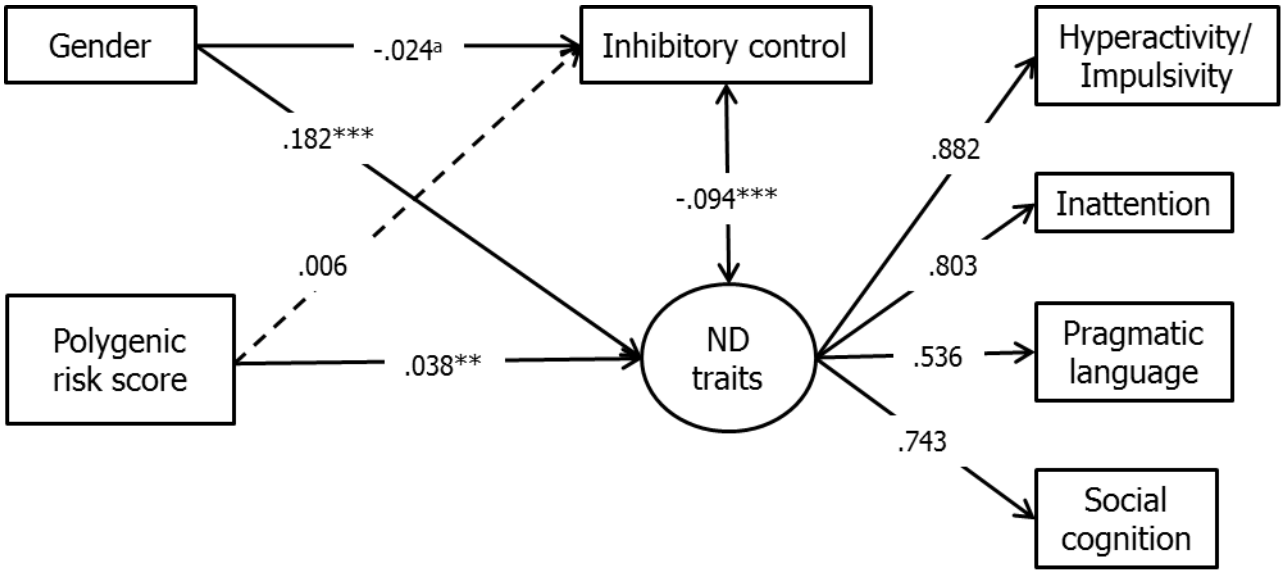

Figure 6.4: Association between polygenic risk scores with inhibitory control

[a] p<0.1, **p<0.01, ***p<0.001; ND: Neurodevelopmental; N=6,823; RMSEA=0.031, CFI=0.991, TLI=0.984

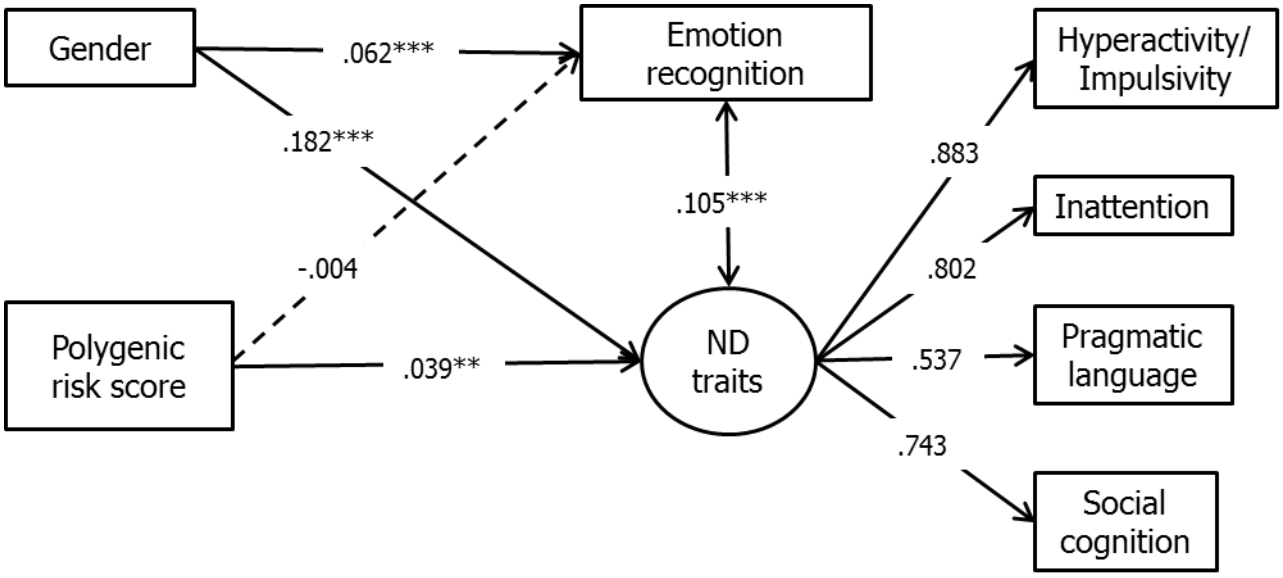

Figure 6.5: Association between polygenic risk scores with facial emotion recognition

**p<0.01, ***p<0.001; ND: Neurodevelopmental; N=6,799; RMSEA=0.026, CFI=0.993, TLI=0.988

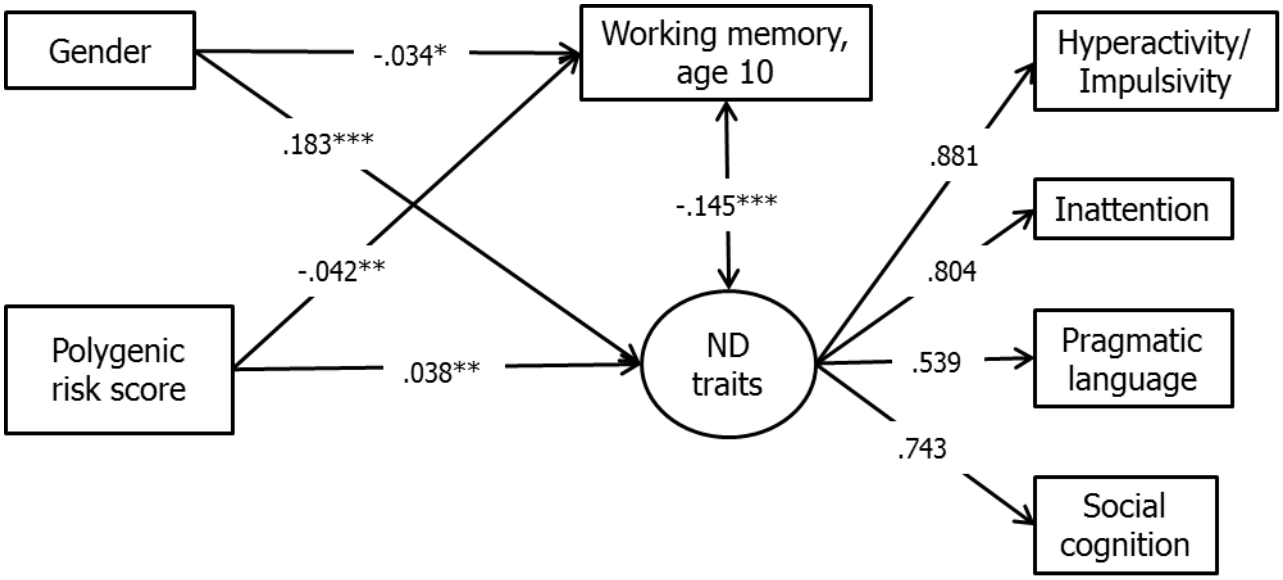

Figure 6.6: Association between polygenic scores with working memory at age 10 years

*p<0.05, **p<0.01, ***p<0.001; ND: Neurodevelopmental; Working memory assessed using the Counting Span Task; N=6,847; RMSEA=0.045, CFI=0.981, TLI=0.966

Secondary analyses

Given that polygenic risk scores were associated with both IQ and working memory and these outcomes are correlated with each other, a model with both of these outcomes was examined next. SEM including both IQ and working memory (at age 8.5 years) showed that these outcomes are both independently predicted by ADHD polygenic risk scores (see Figure 6.7).

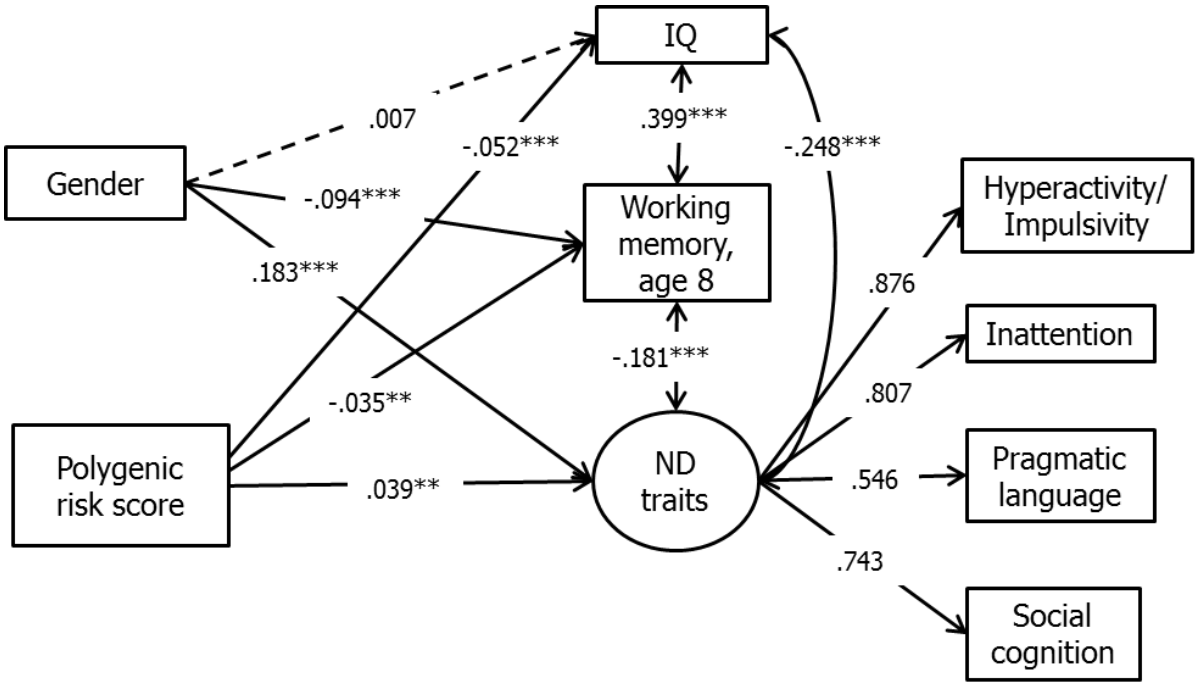

Figure 6.7: Associations between polygenic risk scores with IQ and working memory (age 8 years) as correlated outcomes

**p<0.01, ***p<0.001; ND: Neurodevelopmental; N=6,835; RMSEA=0.052, CFI=0.973, TLI=0.948

To test the sensitivity of the results with regards to inclusion of both ADHD and ASD traits in the latent neurodevelopmental trait variable, analyses were re-run with ADHD inattentive and hyperactive-impulsive traits as manifest outcome variables and omitting the measures of social-communication. The pattern of results was the same as before (see Appendix 6.1).

To look at the effect of population stratification, additional analyses co-varied for 10 EIGENSTRAT covariates; this did not affect the results (see Appendix 6.2). The pattern of results was consistent across different selection thresholds for generating the polygenic risk scores (see Figure 6.8).

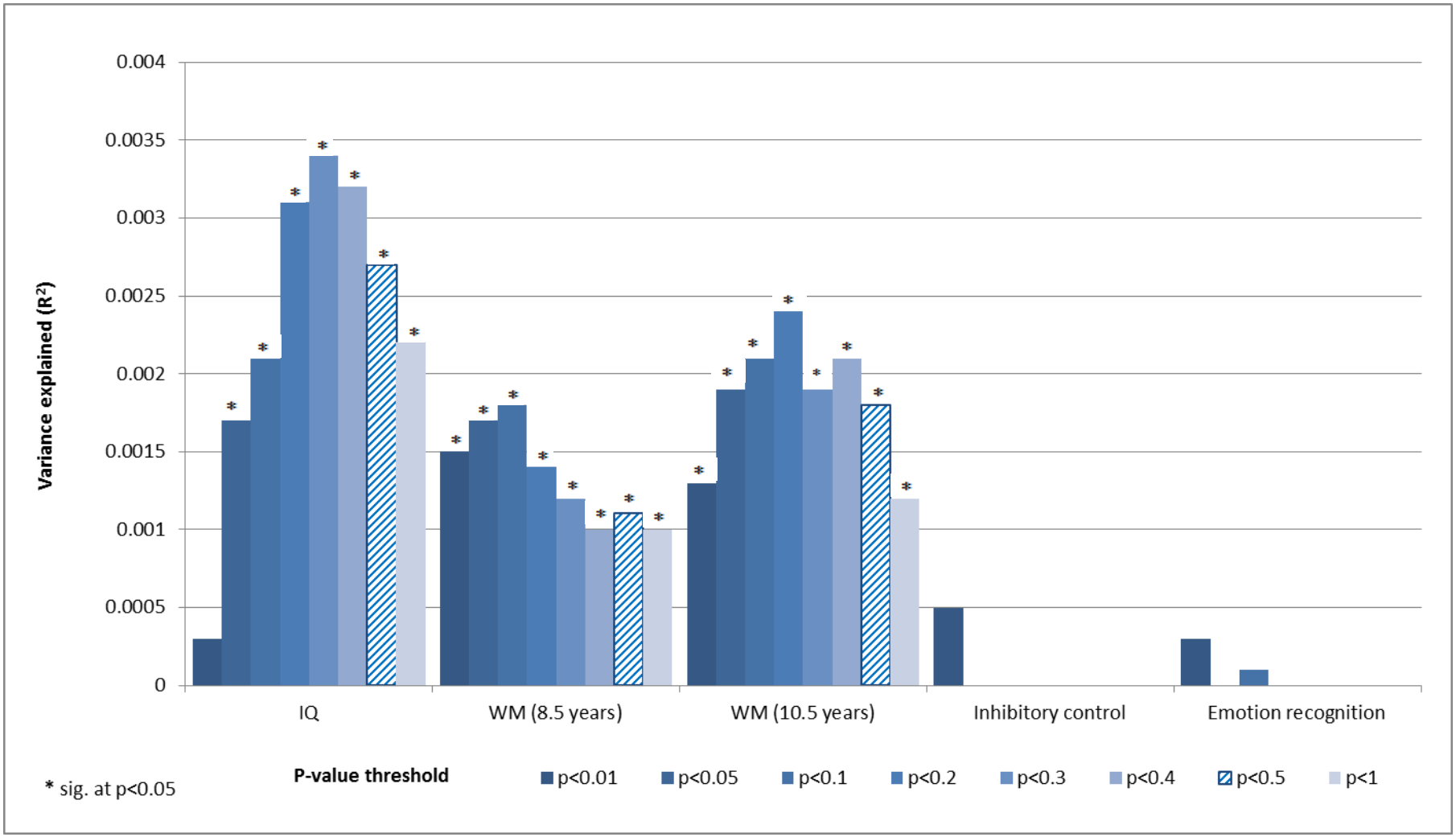

Figure 6.8: Associations of neurocognitive phenotypes with ADHD polygenic scores calculated based on the primary discovery sample, using a variety of p-value thresholds (linear regressions)

WM: working memory; Main results are based on polygenic risk scores derived using a threshold of p<0.5 (striped bars).

As mentioned before, gender was entered as a covariate for all models. When analyses were instead stratified by gender, the association between composite genetic risk scores and IQ was seen separately for both females (β=-0.060, p=0.0011, R^2=0.0038) and males (β=-0.045, p=0.023, R^2=0.0019), whereas the association with working memory at age 8 was only seen in females (β=-0.041, p=0.031, R^2=0.0017) and not males (β=-0.027, p=0.16, R^2=0.0007).

Replication analyses

Polygenic risk scores derived from the published replication discovery data (Neale et al., 2010a) also showed an association with lower IQ (β=-0.030, p=0.028, R^2=0.0009) but did not show an association with working memory at either time point (age 8: β=-0.018, p=0.18; age 10: β=-0.015, p=0.27). Analysis using SEM showed the same pattern of results (see Figures 6.9 and 6.10, as well as Appendix 6.3).

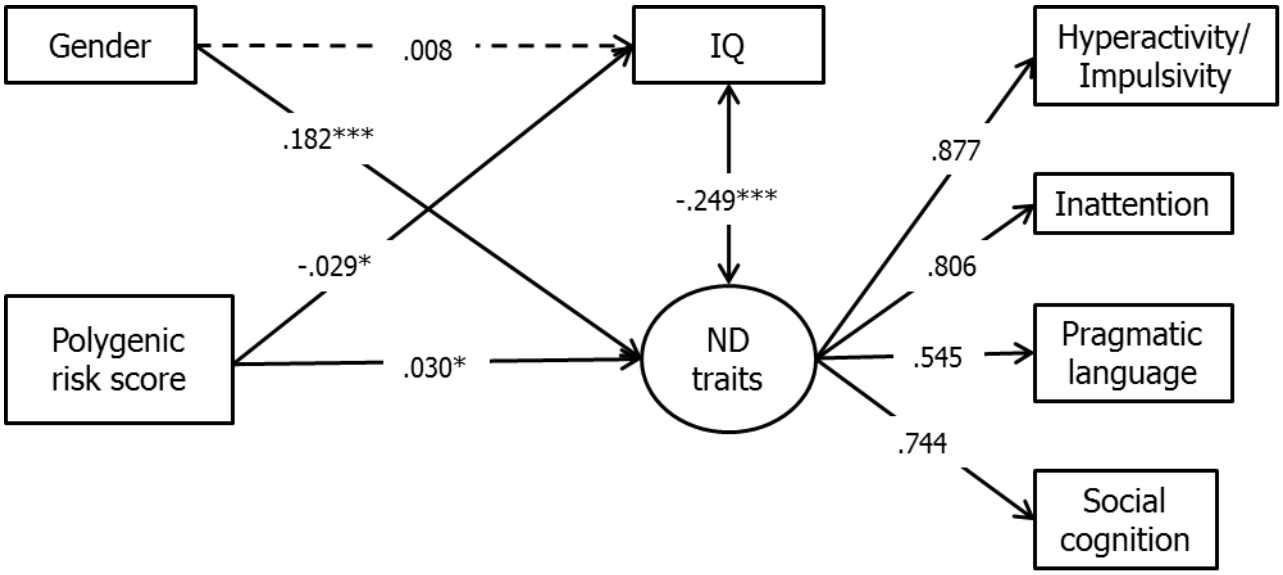

Figure 6.9: Association between polygenic risk scores (based on replication discovery sample) with IQ

*p<0.05, ***p<0.001; ND: Neurodevelopmental; N=6,832; RMSEA=0.053, CFI=0.974, TLI=0.953

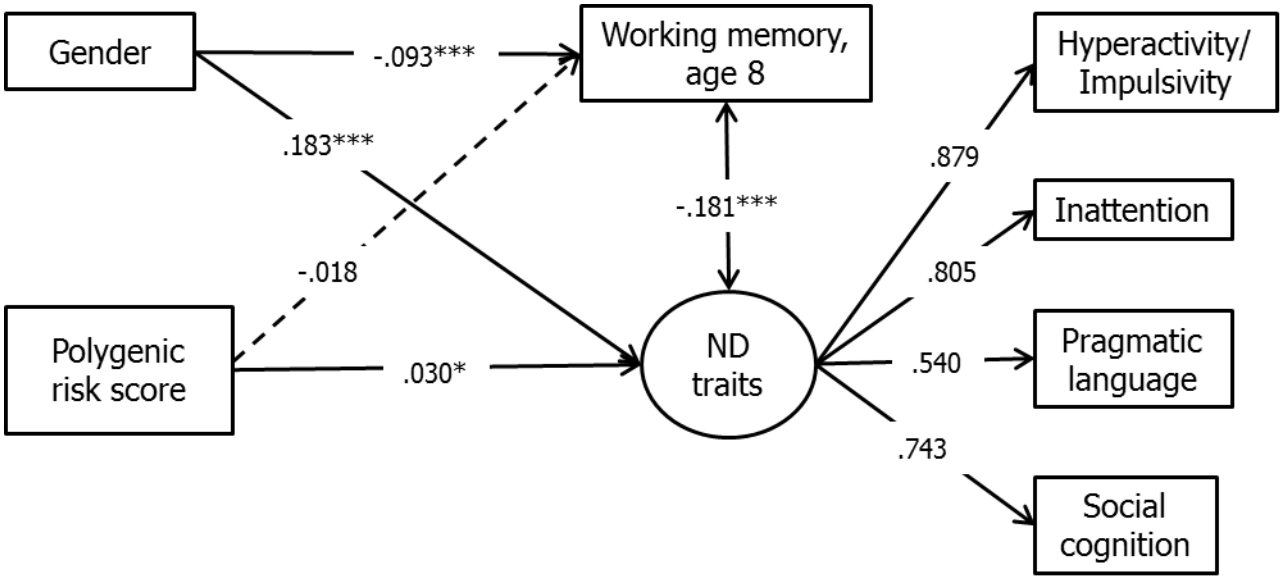

Figure 6.10: Association between polygenic risk scores (based on replication discovery sample) with working memory

*p<0.05, ***p<0.001; ND: Neurodevelopmental; Working memory assessed using the Digit Span test; N=6,827; RMSEA=0.045, CFI=0.982, TLI=0.967

6.5 Discussion

This study finds that common genetic risk variants which predict risk of clinical ADHD diagnosis and which were previously found to predict parent-reported ADHD and social-communication behavioural traits in the general population (described in chapter 5 (Martin et al., 2014)) are also associated with lower IQ and working memory in children from the general population. ADHD polygenic risk scores were not found to be associated with inhibitory control or facial emotion recognition measures. The associations of ADHD polygenic risk scores with IQ and working memory persisted even after taking into account associations with neurodevelopmental behavioural traits (ADHD and social-communication) and were robust to sensitivity testing (across different p-value selection thresholds for calculating the polygenic risk scores and when population covariates were included). These results suggest that common genetic risk variants relevant to a clinical diagnosis of ADHD may have effects on multiple neurocognitive abilities, as well as behavioural traits in the general population.

The association between ADHD polygenic risk scores and working memory was also observed with a different measure of working memory, assessed at a later time point (i.e. the Counting Span Task at age 10 years). Polygenic risk scores based on changing the allele selection thresholds were also consistently associated with both IQ and working memory problems at both time points. Additionally, the observed association between ADHD polygenic risk scores and IQ was robust to replication, using a second ADHD discovery dataset (Neale et al., 2010a). However, no association was observed with either measure of working memory using polygenic risk scores derived from the replication discovery data.

This inconsistency of results using the primary and replication discovery samples could theoretically be attributable to potential differences in clinical or cognitive profiles between the two datasets (Neale et al., 2010a; Stergiakouli et al., 2012). Alternatively, subtle differences in ancestry may have contributed to this discrepancy; the primary discovery sample is more homogenous and ancestrally more similar to the target ALSPAC sample than the replication sample, which was a meta-analysis of multiple samples.

Furthermore, the effects that are observed are very small ($R^2 \leq 0.0034$), albeit they are comparable to those reported in other studies using the polygenic risk score method (Anney et al., 2012; Hamshere et al., 2013a). Effect sizes in this type of study are affected by the relatively small ADHD GWAS discovery sample sizes, which have low power to detect susceptibility variants and thus a poor signal-to-noise ratio, making it unlikely that the current analysis reflects the true magnitude of the observed associations (Neale et al., 2010a; Stergiakouli et al., 2012). However, the possibility of a false positive finding cannot be ruled out without additional replication.

As associations were observed only between ADHD polygenic risk scores and IQ and working memory, but not inhibitory control and emotion recognition abilities, this suggests the possibility of specific pleiotropic effects. Interestingly, in clinic children diagnosed with ADHD, increasing levels of ASD traits are associated with lower IQ and more severe working memory difficulties, although

not with attentional flexibility, as described in chapter 4 (Cooper et al., 2014). The clinical study in chapter 4 suggests that IQ and working memory problems may be a marker of additional neurodevelopmental problems or phenotypic complexity in the context of an ADHD diagnosis. The current analysis builds on this finding by suggesting that genetic risks for ADHD may be relevant to lower IQ and working memory abilities in the general population, in addition to parent-reported neurodevelopmental behavioural traits examined in chapter 5 (Martin et al., 2014). One possibility is also that the Opposite Worlds task measure of inhibitory control and the DANVA measure of facial emotion recognition are inadequate measures of these neurocognitive domains. This possibility is supported by the relatively low correlation coefficients between these measures and ADHD and ASD traits (please refer back to Table 6.1).

Although in general, the correlations between the neurocognitive abilities and ADHD and social-communication traits were modest, the results from this general population sample are in keeping with findings from clinical studies showing lower IQ, working memory, inhibitory control and emotion recognition abilities in children with ADHD and other neurodevelopmental problems, such as ASD (Frazier et al., 2004; Matson & Shoemaker, 2009; Uekermann et al., 2010; Uljarevic & Hamilton, 2012; Willcutt et al., 2008). Furthermore, the magnitude of these results is consistent with the known heterogeneity of neurocognitive abilities in children with ADHD; despite group differences, when compared with controls, not all children with ADHD or other neurodevelopmental problems experience these additional deficits (Nigg et al., 2005; Willcutt et al., 2008).

The results also show that on a population level, there are average differences in neurocognitive abilities between males and females, with males showing poorer working memory and emotion recognition abilities. It is well known that neurodevelopmental problems are also more common in males than females (Keen & Ward, 2004; Lichtenstein et al., 2010). However, the reasons for these gender group differences are unclear. One theory suggests that males have a lower liability

threshold than females, which leads to them developing problems when exposed to a lower burden of risk variants. This theory has some support in terms of clinical ADHD and ASD phenotypes (Hamshere et al., 2013a; Rhee & Waldman, 2004; Robinson et al., 2013) but it is unknown whether it could also explain observed gender differences in neurocognitive domains. Further work is needed to explore the nature of these gender differences in neurocognitive abilities, particularly in the context of neurodevelopmental problems.

Given the longitudinal nature of the ALSPAC sample and the associated non-random attrition (Wolke et al., 2009), missing data were handled by using the 'full information maximum likelihood' (FIML) estimator in the SEM analyses. This approach maximised the use of all available data, in contrast to using pairwise deletion. A sensitivity test showed that restricting the SEM analyses to children who had complete data for each analysis, using listwise deletion, decreased the effect sizes but otherwise did not alter the pattern of observed results (see Appendix 6.4).

One limitation of this study is that the neurocognitive tasks assessed in the ALSPAC sample around ages 7-9 years (i.e. when the ADHD and social-communication measures were assessed), were not specifically selected for previously showing familial effects with ADHD. Previous family and twin studies have suggested that there are shared genetic effects between ADHD and measures of IQ, working memory (as assessed by the WISC Digit Span test), inhibitory control (assessed using a stop-signal reaction time task) and other cognitive measures not examined in the current study (Bidwell et al., 2007; Kuntsi et al., 2004; Schachar et al., 2005). At present it is not known whether some of the cognitive measures used in this study (i.e. the Opposite Worlds or DANVA tasks) share genetic risks with ADHD.

Conclusion

Results in this general population study indicate that a polygenic score of common genetic risk, previously found to be associated with clinical ADHD diagnosis, predicts lower general cognitive ability and lower working memory in children in the general population. These genetic variants

appear to have pleiotropic effects, predicting the presence of behavioural traits and certain aspects of neurocognitive performance in children. These results extend a growing body of literature highlighting the importance of shared molecular genetic risk factors across multiple psychiatric and psychological phenotypes.

Chapter 7

General Discussion

7.1 Summary of results

In this thesis, I first examined the presentation of ASD traits in a clinical sample of children diagnosed with ADHD. In chapter 3, an exploratory factor analysis of ASD traits suggested that social-communication difficulties and restrictive, repetitive behaviours (RRBs) are separate, albeit correlated dimensions in children with ADHD. A further exploratory factor analysis of ASD and ADHD traits in this sample indicated that there may be some overlap of hyperactive-impulsive ADHD symptoms and RRBs, whereas separate dimensions of inattentive symptoms and social-communication difficulties were observed. Analyses in chapter 4 showed that the severity of ASD traits in children with ADHD is not only associated with the severity of ADHD symptoms but also with lower cognitive and working memory abilities, as well as problems with early motor and language abilities. Additional analyses suggested that the cognitive and working memory difficulties were primarily associated with higher social-communication trait levels, while motor problems and hyperactive-impulsive symptoms were primarily associated with higher levels of RRBs. Together, the results of chapters 3 and 4 underscore the validity and importance of considering both dimensions of ASD traits in children with ADHD, as these appear to present at least partly independently of ADHD symptoms and may be markers of additional difficulties.

In the second half of this thesis, I aimed to determine whether common genetic risk variants relevant to ADHD were associated with traits of ADHD and ASD as well as neurocognitive abilities in children from the general population. The results of a case-control genome-wide association study (GWAS) of the clinical ADHD sample used for the analyses in chapters 3 and 4 and additional ADHD cases from collaborators (Stergiakouli et al., 2012) were used to derive a polygenic risk score of common genetic variants relevant to ADHD in each individual in ALSPAC. Analyses in chapter 5

showed a significant association of ADHD polygenic risk scores with behavioural traits of inattention, hyperactive-impulsive symptoms and social-communication difficulties at ages 7-10 years in the population sample. In chapter 6, these analyses were extended and results showed that polygenic risk scores are also associated with task-based neurocognitive abilities (i.e. IQ and working memory) in this same sample, even when associations with behavioural traits are taken into account. Together, chapters 5 and 6 indicate that common genetic risk variants for ADHD show a certain degree of pleiotropy and are relevant to both behavioural, parent-assessed neurodevelopmental traits as well as performance-based measures of neurocognitive abilities.

7.2 Implications of the results

This thesis adds to the growing body of literature regarding the clinical and genetic overlap of ADHD, ASD and other neurodevelopmental and cognitive domains. Firstly, the results support the growing recognition that social-communication difficulties and RRBs are separate, albeit highly related dimensions of symptoms (Mandy et al., 2012), which has recently prompted a change from a triad to a dyad classification system of ASD symptoms in the DSM-5. The results build on previous studies by suggesting that the nature of ASD traits is similar in the context of ADHD (chapter 3) to that seen in samples of children diagnosed with ASD, as well as in the general population (Mandy & Skuse, 2008; Shuster et al., 2014). Not only do ASD traits appear to split into these two correlated dimensions in children with ADHD (chapter 3), but they also appear to index different additional cognitive and developmental problems in these children (chapter 4). Thus these results also support the removal of the exclusion for ADHD in the presence of ASD in DSM-5 and highlight that it is important for clinicians to consider even sub-threshold social-communication problems and RRBs in children with ADHD.

One unresolved issue raised in this thesis is the suggestion that there may be some overlap of hyperactive-impulsive symptoms and RRBs. The exploratory factor analysis in chapter 3 supported a factor model where these symptoms clustered together. Moreover, regression analyses in chapter

4 found that continuously measured hyperactive-impulsive symptoms and RRBs were significantly associated, above and beyond the association of these traits with social-communication problems. One possible explanation for these results stems from difficulties in measuring RRBs in children with ADHD. For example, a few of the Social Communication Questionnaire (SCQ) items regarding RRBs may be interpreted by parents as asking about general excessive activity, similar to some of the hyperactive symptoms; for example the items: "Has he/she ever had any mannerisms or odd ways of moving his/her hands or fingers, such as flapping, or moving his/her fingers in front of his/her eyes?" or "Has he/she ever had any complicated movement of his/her whole body, such as spinning or repeatedly bouncing up and down?". Such methodological concerns need to be taken into consideration for future studies examining RRBs in children with ADHD. Notably, factor analyses of ADHD and ASD traits in community samples have not found this overlap of hyperactive-impulsive symptoms and RRBs (Ghanizadeh, 2010; Ronald et al., 2014). However, it remains to be seen whether these two dimensions of behaviours are particularly strongly related to one another when considered at the extreme ends of their distributions (i.e. in clinical populations).

The focus of the second half of this thesis is on molecular genetic risk factors for ADHD in relation to traits in the general population. Twin studies suggest that there is a high degree of genetic overlap for ADHD and ASD traits, both at a clinical level, as well as in the full distribution of traits in the general population (Lichtenstein et al., 2010; Reiersen et al., 2008a; Ronald et al., 2008; Taylor et al., 2012). The results of chapter 5 support these studies at a molecular genetics level, suggesting that common genetic risk variants relevant to ADHD diagnosis may also predispose to difficulties with pragmatic language abilities, which at their extreme are an element of the social-communication difficulties found in ASD. Interestingly, genetic analyses of these pragmatic language abilities in the ALSPAC sample have estimated a significant heritability rate of about 18% based solely on common, additive variants (St Pourcain et al., 2013). However, others have reported no contribution of common additive variants to behavioural problems, including ADHD and ASD traits, in a population sample of twin children (Trzaskowski et al., 2013). Further work is needed to

clarify the extent of the contribution of common additive variants to ADHD and ASD traits in the population. Additional analyses are also needed to provide further evidence for the overlap of common genetic variants for clinically-diagnosed ADHD and both clinical as well as non-clinical levels of social-communication difficulties.

The results of chapter 5 further suggest that ADHD diagnosis is the extreme end of a continuous distribution of traits in the population and that the underlying aetiology is related across this distribution. This supports findings from twin studies estimating heritability rates for ADHD using various cut-offs (Larsson et al., 2011; Levy et al., 1997). Several other, very recent studies have also examined this issue in ADHD. One study found a contribution of ADHD genetic risk variants to hyperactive-impulsive traits but not inattentive traits in a community sample (Asherson et al., 2013). A second study (Groen-Blokhuis et al., 2014) utilised a more recent (unpublished) ADHD GWAS meta-analysis as the discovery sample; this analysis was conducted by the Psychiatric Genomics Consortium (PGC) and consisted of 9 ADHD samples totalling 5,621 clinical cases and 13,589 controls. This discovery GWAS was used to calculate polygenic risk scores in a Dutch population sample of twins. Polygenic risk scores for ADHD were associated with increased levels of attention problems as measured by the Child Behaviour Checklist and the Teacher Report Form across ages 3-13 years (Groen-Blokhuis et al., 2014), lending further support to the findings in chapter 5. A further recent study has shown that polygenic risk scores derived from a GWAS of ADHD traits in the ALSPAC sample are associated with case status, as well as with increasing levels of ADHD symptoms in children diagnosed with ADHD (Stergiakouli et al., 2014). Collectively these studies suggest that the genetic architecture (at least in terms of common, additive variants) may show similarities for diagnosis and traits of ADHD. However, this is not necessarily the case for other conditions. For example, a recent study in ALSPAC using a comparable analytic approach found that common genetic risk variants relevant to a clinical diagnosis of adult schizophrenia are not associated with psychotic experiences in adolescence (Zammit et al., 2013). No studies have yet assessed whether common genetic risk variants relevant to a diagnosis of ASD contribute to

pragmatic language abilities and other measures of ASD-like traits in general population samples. It is also not known yet whether rare variants such as copy number variants (CNVs), which are relevant to clinically-diagnosed ADHD and ASD (Elia et al., 2010; Glessner et al., 2009; Levy et al., 2011; Lionel et al., 2011; Marshall et al., 2008; Pinto et al., 2010; Williams et al., 2010, 2012), also play a role in ADHD and ASD traits in the general population.

The final results chapter of this thesis (chapter 6) extends the analyses presented in chapter 5 by also examining polygenic risk scores for ADHD in relation to neurocognitive abilities in the general population. The results support models where polygenic risk for ADHD is independently associated with lower IQ, lower working memory and neurodevelopmental (ADHD and social-communication) problems in the children. These results suggest that common, additive genetic variants contribute to the shared genetic aetiology of behavioural ADHD and cognitive (e.g. IQ and working memory) traits, which has been reported by family studies (Bidwell et al., 2007; Kuntsi et al., 2004). Other researchers have also recently examined the shared genetic aetiology of cognitive problems and psychiatric conditions using a comparable method to the one reported in this thesis (chapters 5 and 6). One study found some association of polygenic risk scores for ADHD with reaction time variability in a clinical ADHD sample, though this was not found for another cognitive measure (poor inhibition, as assessed by a high rate of commission errors) or in a community sample (Asherson et al., 2013). Another study found that a polygenic risk score of common variants related to cognitive ability was significantly lower in schizophrenia cases than in population controls (Lencz et al., 2014), suggesting that there is overlap of common genetic variants predisposing to low cognitive ability and schizophrenia. The results presented in this thesis thus are some of the first to directly address the role of common, additive genetic variants in relation to behavioural neurodevelopmental traits, as well as associated neurocognitive problems. On the whole, the results demonstrate the validity of considering ADHD and ASD as continuously distributed dimensions of traits both for clinical and genetic research.

7.3 Methodological considerations

Clinical and population samples

The data used for analyses in this thesis come from two distinct samples, which have different strengths and limitations and can be used to address differing, albeit related research questions. The clinical ADHD sample has the advantage of being representative of children presenting with ADHD at child mental health services in the UK and therefore the results from analysing this sample are relevant to clinical practice in the UK. The high prevalence of co-occurring difficulties in this sample means that individual differences with regards to these difficulties can also be examined with better power. A possible disadvantage of using this sample is the low level of variability in ADHD symptoms, as all children have symptoms at diagnostic levels. On the other hand, although the general population ALSPAC sample has a low prevalence of diagnosed ADHD and ASD, it allows for the examination of ADHD and social-communication traits across the full distribution of these traits and therefore includes individuals with more subtle, sub-clinical difficulties. For example, in chapter 5, we see that polygenic risk scores for ADHD are higher even in children with low levels of ADHD traits (score of 1-11) when compared to those with no ADHD traits. Thus both of these samples have distinct advantages over each other when it comes to addressing questions regarding the clinical and genetic overlap of neurodevelopmental problems.

As mentioned in section 2.3 of chapter 2, similar phenotype measures were utilised in these samples, wherever possible. This has allowed for a certain level of consistency across these two samples. For example, the Digit Span subtest was used to measure verbal working memory in both samples. Although the WISC-IV was used in the clinical ADHD sample and the WISC-III was used in ALSPAC, this measure is virtually the same across these versions of the WISC IQ test. Similarly, ADHD symptoms were assessed in both samples using robust, standardised diagnostic instruments: the CAPA (Angold et al., 1995) in the clinical ADHD sample and the DAWBA (Goodman et al., 2008) in the ALSPAC sample. Both of these measures assess the same 18 DSM-IV/DSM-5 ADHD

symptoms in a similar manner. However, because of low prevalence of severe ADHD symptoms in the general population sample and to increase variability, the DAWBA ADHD symptoms in ALSPAC were coded: 0 for "no", 1 for "a little more than others" and 2 for "a lot more than others", with a total score being the sum of these responses for all 18 symptoms. In contrast, in the clinical ADHD sample, the CAPA ADHD symptoms were simply considered as 'present at a clinical level' or 'absent', with no middle score.

One other important difference in measures used was the use of the SCQ to measure ASD in the clinical ADHD sample, whereas a similar dedicated and comprehensive assessment tool measuring ASD was lacking in ALSPAC. However, the two measures of social-communication problems (the SCDC (Skuse et al., 2005) and CCC (Bishop, 1998)) have been shown to have good predictive reliability for a clinical diagnosis of ASD in the ALSPAC sample (Steer et al., 2010). Furthermore, these measures, particularly the pragmatic language sub-scales of the CCC, showed a good amount of variability in the ALSPAC sample, whereas more strictly measured clinical traits may have been rarer, with insufficient power for analyses. Unfortunately, there was no available quantitative measure of RRBs in ALSPAC, so the findings from the first half of the thesis regarding the possible overlap of RRBs and hyperactive-impulsive traits could not be further examined in this sample. Also, it was not possible to examine whether polygenic risk scores for ADHD were associated with RRBs in this sample.

A final consideration with regards to the use of these two samples is that the ADHD clinical sample is a cross-sectional sample spanning a fairly wide age range (5-18 years), whereas ALSPAC is a longitudinal cohort sample. Thus, no conclusions can be drawn with regards to causality in the ADHD clinical sample. Although the presence of genetic risk variants necessarily predates measurement of childhood behavioural and cognitive traits in the ALSPAC sample, it is not possible to determine on the basis of the findings of this thesis whether and what specific genetic risk variants directly contribute to the manifestation of these behavioural and cognitive problems. The

findings are preliminary and larger genetic discovery studies are needed to shed light on the pathway from genetic risk variants to complex behavioural and cognitive phenotypes.

The importance of IQ

One important issue raised several times in this thesis is that of the strong association between ADHD and lower IQ (Dykens & Hodapp, 2001; Frazier et al., 2004; Voigt et al., 2006). Given the attentional problems (such as difficulties with concentrating) that children with ADHD experience, it is highly likely that cognitive abilities assessed using standardised IQ tests are frequently underestimated in these children. Additionally, the presence of co-occurring social-communication difficulties in children with ADHD may be linked with an inability to fully understand task instructions or with a decrease in motivation to perform well. Indeed, in chapter 4 we see that social-communication difficulties in children with ADHD are associated with lower IQ. These results demonstrate that IQ may be more than just a measure of general cognitive ability but also an index of attentional, communication and other difficulties.

This further suggests that the common practice of excluding children from studies based on an arbitrary cut-off for IQ (e.g. IQ<70 or sometimes even IQ<85) may bias samples and result in research that is not representative of typical clinical samples of children with ADHD. On the other hand, it may be that diagnostic instruments do not adequately assess ADHD in children with particularly low cognitive functioning abilities. One possible solution is for researchers to more carefully take into consideration the developmental level of children when assessing ADHD and related traits. Furthermore, a study of the clinical sample of children with ADHD analysed in this thesis, which compared those with and without intellectual disability, suggests that the clinical profiles of both groups of children are quite similar, except for a greater level of conduct disorder symptoms and diagnoses in those with intellectual disability (Ahuja et al., 2013). The results presented in this thesis additionally suggest that lower IQ is not only correlated with more ASD

traits but also that it is related to common genetic risk variants for ADHD, suggesting that it may be an important marker of neurodevelopmental genetic liability.

Genetic issues

Another important issue raised by the results of this thesis is related to the genetic architecture (i.e. the number, frequencies and effect sizes of risk variants) of ADHD and other neurodevelopmental and cognitive phenotypes. The results of chapter 5 support previous research regarding the dimensionality of ADHD (Larsson et al., 2011; Levy et al., 1997), by demonstrating that the set of thousands of common genetic variants associated with clinical ADHD also play an important role at the milder end of ADHD traits in the general population. These results also support the important role of common genetic risk variants with low effect sizes in the aetiology of ADHD, which has been shown by others (Hamshere et al., 2013a; Lee et al., 2013; Stergiakouli et al., 2012).

It is important to note that rare chromosomal abnormalities, large, rare CNVs and rare single nucleotide mutations are also likely to play an important role in ADHD (Lo-Castro et al., 2011; Williams et al., 2010, 2012; Yang et al., 2013). Indeed, it appears that the presence of a CNV in children with ADHD is associated with a lower polygenic risk score of common variants, suggesting that both common and rare variants contribute independently to risk of ADHD (Martin et al., under review). The focus of the genetic analyses in this thesis has been restricted to common, additive genetic risk variants and so rare variants were not examined.

There are several important considerations regarding the method of polygenic risk score analysis of common variants. Firstly, as mentioned in chapters 5 and 6, the effect sizes or amounts of variance explained by the polygenic risk scores are very low. Whilst this is likely to be at least in part due to the low power of available ADHD GWAS for detecting true risk alleles for ADHD, the issue is more complex. Current GWAS of ADHD have thus far not found any individual significantly-associated SNPs (Hinney et al., 2011; Mick et al., 2010; Neale et al., 2008, 2010b; Stergiakouli et al., 2012; Yang et al., 2013). Polygenic score analyses suggest that despite the low power, current GWAS do

successfully detect weakly-associated risk alleles (Hamshere et al., 2013a). However, a substantial proportion of the SNPs meeting the p-value selection threshold ($p<0.5$) for inclusion in polygenic risk score analysis are likely to be false positives, resulting in a high degree of noise in the risk scores. General practice is to calculate polygenic risk scores at several different thresholds (Purcell et al., 2009) to account for the trade-off of capturing true, albeit weakly-associated, genetic risk variants versus including a high proportion of false positives, and to check the sensitivity of the results to the selection threshold that is used. As such, the robust pattern of results observed in chapters 5 and 6 following repeating analyses at differing p-value selection thresholds serves to strengthen the conclusions on the basis of these results. Analyses in other psychiatric conditions using this method demonstrate that for a low discovery sample size, predictive ability tends to be better with larger SNP sets (i.e. using less stringent p-value thresholds), whereas lower p-value selection thresholds are more optimal with increasing discovery sample size (Wray et al., 2014).

The Psychiatric Genomics Consortium (PGC), an international collaboration of researchers investigating the genetic basis of psychiatric disorders, is currently engaged in genotyping and combining ADHD case data contributed by collaborating research groups in an effort to increase the power of ADHD genetic analyses. This international effort will thus lead to a more powerful genetic discovery sample available for future analyses. One possible concern is that increasing the quantity of available samples will be associated with a decrease in overall quality of the included data and potentially will introduce factors that confound genetic effects. For example, differences in recruitment and assessment of ADHD cases from different samples may increase heterogeneity. Additionally, if individuals who are screened only minimally for ADHD are included in the sample, this could weaken the power to detect genetic risk variants associated with ADHD. Also, combining individuals with ADHD from a variety of ancestral groups needs to be mitigated through the use of a strict quality control pipeline and adjusting for population stratification effects. Although sheer numbers may help to boost the power to detect specific common genetic risk variants associated

with ADHD, there will continue to be a need to apply the findings of GWAS to smaller, well-phenotyped samples in order to address clinically-meaningful questions (Thapar & Harold, 2014).

The benefit of increasing sample sizes can be seen for other phenotypes. In particular, it is striking that GWAS of schizophrenia, which can in some ways be viewed as the flagship model for the genetic analysis of psychiatric phenotypes, also started out with detecting no genome-wide significant SNPs (Lencz et al., 2007; Sullivan et al., 2008) but have since progressed to identifying 128 SNPs significantly associated with this condition (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This suggests that increasing the sample sizes in ADHD will follow a similar pattern. However, there is no guarantee that findings for ADHD will follow this model or indeed that the genetic architecture of ADHD is similar to that for schizophrenia or other psychiatric conditions. For instance, the level of phenotypic heterogeneity of schizophrenia and ADHD may not be equivalent. Also, ADHD is much more common than schizophrenia (which has a lifetime morbid risk of about 0.7% in the population (Saha et al., 2005)). It remains to be seen whether significantly associated SNPs relevant to ADHD will be detected with larger sample sizes.

A second important issue regarding the method of polygenic score analysis in this thesis is regarding the nature of the controls used for the discovery ADHD GWAS (Neale et al., 2010a; Stergiakouli et al., 2012). The controls consisted of unscreened population samples (Stergiakouli et al., 2012) and a combination of population samples and parent-offspring trios (Neale et al., 2010a). Both of these approaches have limitations. As mentioned in section 1.6 of chapter 1, the addition of parent-offspring trios to case-control GWAS of ADHD relies on the assumption that the distribution of genetic liability for ADHD is similar in the "pseudo-controls" (i.e. the non-transmitted parental genetic data) and the population controls. However, given the familial nature of ADHD, it is highly likely that parents will themselves be carriers of genetic risk variants (transmitted and non-transmitted) relevant to ADHD. Indeed, a recent report from the PGC ADHD sample confirms that polygenic risk scores based on a large GWAS sample of self-reported ADHD cases and non-ADHD

controls ascertained from 23andMe (a personal genetics service in the USA) are significantly higher in ADHD cases than in population controls, with substantially smaller and non-significant prediction of ADHD case status when comparing polygenic scores in cases with the non-transmitted "pseudo-control" parental genotypes (Neale, 2013). Another direct assessment of this issue in ASD families also suggests that pseudo-controls in multiplex families show a substantial contribution of common genetic risk variants to estimates of SNP-chip heritability for ASD, when compared to pseudo-controls from simplex families or unaffected controls (Klei et al., 2012). Both of these studies highlight that the *non-transmitted* genetic data of parents of children with ADHD and ASD appears to contain a greater load of additive common genetic variants increasing risk for these conditions when compared to genetic data from population controls.

Another issue related to the use of parent-offspring trio samples for genetic studies concerns the potential recruitment bias of including genetic data from "intact" families, where both biological parents are not only involved in the child's life, but also willing to take part in research. Given the social and familial impairments associated with ADHD and the frequent presence of ADHD traits in parents of affected children, one might expect that intact families would consist of parents and children less severely affected by ADHD when compared to single-parent or otherwise non-intact families. Indeed, there is some suggestion in the literature that children with ADHD from non-intact families are more likely to have the more severe DSM-IV combined subtype of ADHD and comorbid problems, such as conduct disorder, than those from intact (i.e. parent-offspring trio) families (Hurtig et al., 2007; West et al., 2002). In contrast, studies examining this issue in families with an individual affected with schizophrenia have not seen such clinical differences in trios vs. non-trios (Malhotra et al., 2002; Zammit et al., 2005). In short, studies examining the parent-offspring trio design in ADHD suggest that the use of pseudo-controls based on non-transmitted parental DNA could reduce observed effect sizes in genetic studies through statistical issues as well as possible recruitment biases.

Although the main discovery GWAS of ADHD used for analyses in chapters 5 and 6 did not include parent-offspring trios (Stergiakouli et al., 2012), the second discovery sample, the ADHD meta-analysis of 4 studies, included data on 2064 parent-offspring trios (Neale et al., 2010a). This could be a potential source of the inconsistent results observed; polygenic ADHD genetic risk scores based on the replication discovery sample were not associated with ADHD traits at age 7 years (chapter 5) or with working memory difficulties at two time points (chapter 6).

The use of discovery GWAS of ADHD which rely solely on a case-control design is also not without its limitations. Population prevalence of ADHD is estimated at approximately 5% (Polanczyk et al., 2007), suggesting that about 5% of unscreened population controls may be affected with ADHD. As such, use of unscreened controls could decrease the power of detecting risk alleles relevant to ADHD. Indeed, estimates suggest that if 5% of controls actually meet the definition of cases, this would reduce power by a similar amount to reducing the overall sample size by about 10% (The Wellcome Trust Case Control Consortium, 2007). Furthermore, ADHD is much more common in males than in females and it is not yet clear whether using population controls with a sex-ratio of 1:1 is appropriate. These issues could have served to reduce the power of both of the genetic discovery samples used for the analyses in chapters 5 and 6 of this thesis.

Another issue regarding the controls of the discovery GWAS is the possibility that they differed phenotypically from the case children with ADHD beyond the presence of an ADHD diagnosis. For example, the common genetic risk variants derived from the discovery GWAS may have also indexed lower IQ and other phenotypic traits frequently seen in children with ADHD. A possible alternative interpretation of the results of chapters 5 and 6 is that the polygenic risk scores calculated in the ALSPAC sample showed association with social-communication difficulties (chapter 5) and lower IQ and working memory (chapter 6) as a result of the discovery GWAS capturing common genetic risk variants relevant to phenotypic traits beyond ADHD. Phenotype information on controls was not available for IQ or other traits so it was not possible to perform a

GWAS co-varying for the effect of other phenotypic traits. However, the association between ADHD and these other phenotypes (e.g. low IQ) is not well understood and in any case, the results of this thesis do show that common genetic risk variants which are *primarily* associated with ADHD clinical diagnosis may be relevant to other phenotypes beyond ADHD. Further work is needed to examine these findings in more detail and rule out alternative interpretations of the results.

Another consideration related to the analytical method in chapters 5 and 6 is the process of calculating polygenic risk scores in the ALSPAC children. Selection of SNPs was performed in two steps: 1) LD-pruning (linkage disequilibrium pruning) in the target sample followed by 2) selecting only those that fell below a p-value selection threshold. This was followed by weighting each of the SNPs by effect size (odds ratio of the risk allele) in the discovery GWAS and summing the number of risk alleles for each individual. There are several things to consider. Firstly, there is another commonly-used alternative to the LD-pruning method used here, known as 'clumping' (Smoller et al., 2013; Wray et al., 2014). Instead of randomly pruning out SNPs that are in high linkage-disequilibrium with those within the 200kb sliding window (LD-pruning), clumping relies on preferentially selecting SNPs within LD blocks which show a stronger association with the phenotype of interest (i.e. have a lower p-value). Clumping could thus strengthen the chance of including true positive risk SNPs in the set of SNPs used for polygenic score analysis. Using LD-pruning on the other hand, may have served to weaken the observed results of this thesis. However, clumping may result in the inclusion of multiple SNPs that are associated with the same causal variant and so may also not be the optimal strategy for this method (Wray et al., 2014). Furthermore, one recent study which compared these two methods directly, suggests that LD-pruning is actually associated with a greater amount of variance explained than when clumping is used (Groen-Blokhuis et al., 2014).

Another issue is the selection of the set of SNPs based on a statistical p-value cut-off. An alternative would be to choose SNPs which are more likely to have functional significance, for example by

limiting the SNP set to variants which are within or near genes or going a step further and selecting only SNPs that fall within specific biological pathways relevant to ADHD. In theory, selecting SNPs which are more likely to be disease-relevant could improve the proportion of true risk alleles in the set of SNPs used for calculating the risk scores. However, in practice, the small number of SNPs selected in this way and the currently small sample sizes for ADHD discovery GWAS combined with the lack of knowledge regarding relevant functional variants make it unlikely that such a method would have more power than the approach used in this thesis.

As mentioned in chapters 5 and 6, a small proportion of the cases (2% of the cases in this second discovery sample) in both discovery samples overlapped (Neale et al., 2010a; Stergiakouli et al., 2012) and could not be removed. This means that the two discovery samples were not truly independent and may have biased the results a little in favour of replication. However, given the small proportion of overlapping cases, this is unlikely to have been the main driver of the observed results.

A final issue is that the replication analyses using the second discovery GWAS (Neale et al., 2010a) followed a slightly different method to that of the analyses based on the primary discovery GWAS (Stergiakouli et al., 2012). Although the method used for calculating the polygenic risk scores in ALSPAC using these two discovery datasets was virtually identical, the available data from this second discovery sample did not contain odds ratios. Therefore, the risk alleles could not be weighted by effect size and were all treated equally when calculating the risk scores. Using such un-weighted risk alleles may have introduced more noise into the measure of genetic risk and reduced the power of these replication analyses. This is a limitation of the replication analyses, which may also account for the weaker and inconsistent results using this secondary sample.

7.4 Summary of strengths and limitations

The main strength of the analyses in this thesis is the use of the two complementary samples (the clinical ADHD sample and the population cohort ALSPAC sample), which are both large and well-

phenotyped, with a number of similar clinical and cognitive measures. The main limitations of the methods have already been discussed in detail above and in each of the results chapters. Briefly, the main limitations are as follows. The lack of a comprehensive assessment of ASD in the clinical ADHD sample made it impossible to fully take into account the presence of a clinical ASD diagnosis in these children for the analyses in chapters 3 and 4. Although the SCQ (Berument et al., 1999; Rutter et al., 2003) is a standardised screening instrument, its validity and specificity for assessing ASD traits in a sample of children with ADHD needs to be further examined, particularly given the potential overlap of the items regarding RRBs and hyperactive-impulsive behaviours found in chapter 3. Another limitation of the thesis is that the use of stimulant medication was not taken into account in the analyses of cognitive measures in chapters 4 (ADHD sample) and 6 (ALSPAC sample). Given that timing and dosage of stimulant medication is likely to affect the performance of children with ADHD on cognitive tasks, the lack of adequate covariates is a limitation of the analyses. With regards to the genetic analyses in chapters 5 and 6, the main limitation is the small discovery sample sizes, which are associated with several issues discussed in section 7.3 above. Finally, as with all research studies, both the clinical ADHD and ALSPAC datasets contain some missing data, with the ALSPAC sample additionally suffering from attrition with successive data collection time points. Thus, by ages 7-10 years old, when the measures analysed in the current thesis were collected, many of the families in the original ALSPAC cohort had dropped out of the study (of the N=8,229 with genetic data, between N=2,576-3,122 (31.3-37.9%) have missing data for variables at ages 7-10 years). Mean-imputation of the main phenotype variables was used to account for partially missing data. For the SEM analyses in chapter 6, the full-information maximum likelihood estimator was used to obtain more accurate estimates of the models. However, the missing data is a limitation of all the analyses in this thesis, which may have served to underestimate the observed effect sizes. Despite these limitations, the results of this thesis have valuable implications for our understanding of the clinical and genetic overlap of ADHD with ASD and other early developmental and cognitive difficulties.

7.5 Suggestions for future work

There are a number of possible closely-related analyses to those presented in this thesis, which have the potential to further our understanding of the overlap of ADHD, ASD, and other early developmental difficulties. First of all, examining the clinical presentation of ADHD traits in children diagnosed with ASD, using factor analyses and in relation to comorbid problems would be a complementary approach to the analyses in the first half of this thesis. It would also be worthwhile to examine whether a polygenic score of common genetic risk variants relevant to ADHD is also associated with traits of ASD and other developmental and cognitive difficulties in children with ADHD. Such an analysis was not feasible for this thesis due to the limited power of the small sample size of genotyped children with ADHD who also had data available for these measures (e.g. only N=373 with SCQ and genetic data). Larger samples are necessary to determine whether common genetic risk alleles for ADHD predispose to any other developmental problems within these children. Furthermore, complementary analyses would involve utilising a polygenic risk score of common variants associated with ASD and examining its effect on ADHD or ASD case status and other traits in a clinical context as well as in relation to these traits in the general population. Also, given the results supporting multiple dimensions in ADHD (inattentive vs. hyperactive-impulsive symptoms) and ASD (RRBs vs. social-communication difficulties), it would be worthwhile for future studies to examine whether polygenic risk scores based on quantitative GWAS of these dimensions show associations with any specific early developmental traits.

Another interesting future area of research stemming from the analyses in this thesis is that of the possible genetic aetiology of the difference in prevalence of ADHD in males and females. Results from the analyses in chapter 5 suggest that females with ADHD traits in the general population carry a higher burden of common genetic risk variants associated with clinically-diagnosed ADHD, when compared with males with ADHD traits. This gender difference was previously also found in

the clinical ADHD sample (Hamshere et al., 2013a). Future studies should aim to replicate and extend these findings.

Although this thesis has focused on the clinical and genetic overlap of ADHD and ASD, it is clear from the literature that there is a very high degree of overlap across all neurodevelopmental disorders (Dyck et al., 2011) and twin studies show that much of this overlap stems from shared underlying genetic factors (Lichtenstein et al., 2010; Martin et al., 2006). Neurodevelopmental disorders can be defined as a related cluster of disorders which are characterised by early childhood onset, relative stability across the lifespan and a greater prevalence in boys than girls. A recent large exploratory factor analysis of neurodevelopmental problems in a wide range of domains (i.e. inattention, hyperactivity-impulsivity, learning, language, social-interaction, perception, motor control, memory, planning, flexibility and tics) in a population-based twin sample (the Child and Adolescent Twin Study in Sweden) suggests that there is a general genetic factor which accounts for about a third of the phenotypic variance of these problems in the general population (Pettersson et al., 2013). This study suggests that current classification systems (i.e. the DSM-5 and ICD-10) attempt to partition into discrete entities a set of difficulties which are strongly aetiologically related. Additional clinical, family and molecular genetic studies are needed to better understand how and to what extent these early childhood disorders overlap, with a view to improving future diagnostic classifications as well as helping to treat and manage these problems.

7.6 Conclusion

The work presented in this thesis adds to the emerging body of literature highlighting the frequent co-occurrence of ADHD and ASD difficulties in children, as well as the genetic overlap of developmental and cognitive phenotypes. Results show that traits of ASD measured in children with ADHD can be split into separate clusters of social-communication difficulties and restrictive and repetitive behaviours. Additionally, these two dimensions of traits index distinct developmental and cognitive difficulties in children with ADHD. The results also show an association of ADHD

common genetic risk variants with social-communication difficulties and cognitive abilities in the general population. These results indicate that neurodevelopmental and cognitive difficulties lie on a continuous dimension in the population and are at least partially underpinned by shared genetic risk factors. Future large, collaborative genetic discovery studies are needed to pinpoint specific shared genetic risks. This research has the potential to improve the diagnosis, classification and treatment of early developmental problems, as well as our understanding of the aetiology of these conditions.

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Appendices

Appendix 2.1: The Social Communication Questionnaire

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Appendix 2.2: Quality control procedures for genetic data

Clinical ADHD sample

Individual and SNP quality control assessment was performed in PLINK by Dr Evie Stergiakouli. 800 control individuals were excluded according to WTCCC-Phase 2 recommendations. Cases and controls were excluded if they had a call rate lower than 99% or extreme (low or high) heterozygosity. Where there was evidence of relatedness (proportion of identity by descent (IBD) of more than 6%, corresponding to a genetic relationship of half first cousins or closer), one individual from each related pair was excluded. Principal component analysis was performed using EIGENSTRAT after merging the data with unrelated European, Asian and Yoruban samples from the HapMap project. After plotting the first two principal components, cases were excluded if they did not cluster with the European samples. Also, individual SNPs were excluded if they had a call rate less than 99%, had a minor allele frequency less than 1%, deviated from Hardy-Weinberg equilibrium at $p<1\times10^{-5}$, or had more than 1% discordant genotypes between the Illumina 550K and the Illumina Human 1.2M BeadChip arrays.

ALSPAC sample

Individuals were excluded from analysis on the basis of having incorrect gender assignments; minimal or excessive heterozygosity (<0.320 and >0.345 for the Sanger data and <0.310 and >0.330 for the LabCorp data); disproportionate levels of individual missingness (>3%); evidence of cryptic relatedness (>10% identity by descent (IBD)) and being of non-European ancestry (as detected by a multidimensional scaling analysis seeded with HapMap 2 individuals; EIGENSTRAT analysis

revealed no obvious population stratification and genome-wide analyses with other phenotypes indicate a low genomic inflation factor, lambda (λ)). SNPs with a minor allele frequency of <1% and call rate of <95% were removed. Furthermore, only SNPs which passed an exact test of Hardy-Weinberg equilibrium ($p > 5 \times 10^{-7}$) were considered for further use.

Appendix 2.3: The Social and Communication Disorders Checklist

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Appendix 2.4: The Children's Communication Checklist – pragmatic language subscales

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Appendix 3.1: Pattern matrix of loadings for factor analysis of SCQ items and ADHD symptoms: 5-factor solution

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
SCQ 36	0.78				
SCQ 37	0.76				
SCQ 39	0.76				
SCQ 27	0.73				
SCQ 34	0.70				
SCQ 30	0.67				0.25
SCQ 40	0.64				
SCQ 38	0.64				
SCQ 35	0.61				0.23
SCQ 31	0.60				
SCQ 29	0.60				
SCQ 33	0.60				
SCQ 28	0.47				0.47
SCQ 26	0.46	0.27			
SCQ 2	0.37				
SCQ 21	0.32				0.32
SCQ 3	0.31	0.25			0.27
SCQ 20	0.23	0.22			
SCQ 10	0.22				
SCQ 8		0.73			
SCQ 4		0.73			
SCQ 17		0.73			
SCQ 7		0.69			
SCQ 13		0.69			
SCQ 16		0.64			
SCQ 12		0.62			
SCQ 5		0.60			
SCQ 11		0.57			
SCQ 9		0.53			
SCQ 15		0.52			
SCQ 6		0.47			
SCQ 14		0.46			
SCQ 19		0.40			
SCQ 18	0.22	0.35		0.23	
ADHD IN 07			0.78		
ADHD IN 02			0.75		
ADHD IN 08			0.73		
ADHD IN 01			0.69		
ADHD IN 09			0.68		
ADHD IN 03			0.59		
ADHD IN 04		0.23	0.57		
ADHD IN 06			0.55		
ADHD IN 05			0.47		
ADHD HYP 04				0.83	0.20
ADHD HYP 03				0.76	
ADHD HYP 02			0.20	0.75	
ADHD HYP 01				0.61	
ADHD HYP 05			0.21	0.60	
ADHD IMP 01	0.29			0.47	
ADHD IMP 04		0.34		0.43	
ADHD IMP 02				0.35	

SCQ 32			0.73
SCQ 22	0.28		0.67
SCQ 23			0.61
SCQ 24			0.52
ADHD IMP 03		0.20	

SCQ: Social Communication Questionnaire; IMP: Impulsive symptoms; HYP: Hyperactive symptoms; INA: Inattentive symptoms. To aid interpretation, loadings of <0.2 are not presented.

Appendix 3.2: Pattern matrix of loadings for factor analysis of SCQ items; Children with ID excluded

SCQ item	Factor 1 – social	Factor 2 – rigidity	Factor 3 – non-verbal communication
SCQ 39	0.79		
SCQ 34	0.78		
SCQ 36	0.76		
SCQ 37	0.73		
SCQ 30	0.73		
SCQ 29	0.72		
SCQ 40	0.68		
SCQ 27	0.67		
SCQ 35	0.64		
SCQ 38	0.64		
SCQ 31	0.59		
SCQ 28	0.57		0.33
SCQ 33	0.54		
SCQ 26	0.44	0.30	
SCQ 2	0.31		0.22
SCQ 20	0.24		
SCQ 10			
SCQ 8		0.83	
SCQ 4		0.80	0.22
SCQ 17		0.71	
SCQ 13		0.71	
SCQ 7		0.68	
SCQ 16		0.65	
SCQ 5		0.60	
SCQ 15		0.57	
SCQ 9		0.53	
SCQ 6		0.50	
SCQ 12		0.50	
SCQ 11		0.49	
SCQ 14		0.46	
SCQ 18		0.41	
SCQ 3	0.21	0.38	0.31
SCQ 19		0.36	
SCQ 32			0.72
SCQ 24			0.66
SCQ 22	0.29		0.66
SCQ 23			0.63
SCQ 21	0.34		0.36

N=497; SCQ: Social Communication Questionnaire. To aid interpretation, loadings of <0.2 are not presented.

Appendix 3.3: Pattern matrix of loadings for factor analysis of SCQ items and ADHD symptoms; Children with ID excluded

	Factor 1 – social	Factor 2 – rigidity/ hyperactivity	Factor 3 – inattentiveness
SCQ 30	0.74		
SCQ 39	0.72		
SCQ 34	0.71		
SCQ 28	0.71		
SCQ 27	0.68		
SCQ 35	0.67		
SCQ 29	0.67		
SCQ 22	0.63		
SCQ 31	0.62		
SCQ 36	0.61	0.28	
SCQ 37	0.59	0.32	
SCQ 40	0.59	0.28	
SCQ 38	0.58	0.21	
SCQ 32	0.51		
SCQ 33	0.51		
SCQ 26	0.49	0.28	
SCQ 21	0.47		
SCQ 2	0.42		
SCQ 20	0.35		
SCQ 3	0.34	0.32	
SCQ 24	0.27		
SCQ 23	0.20		
SCQ 10			
SCQ 13		0.73	
SCQ 8		0.70	
SCQ 17		0.65	
SCQ 7		0.64	
SCQ 11		0.64	
SCQ 4		0.64	
SCQ 16		0.62	
SCQ 15		0.62	
SCQ 12		0.56	
SCQ 9		0.53	
ADHD IMP 04		0.50	
SCQ 5		0.49	
SCQ 18		0.49	
SCQ 14		0.46	
ADHD HYP 03		0.45	
SCQ 6		0.40	
SCQ 19		0.36	
ADHD HYP 04		0.35	
ADHD HYP 01		0.32	0.29
ADHD IMP 03		0.31	
ADHD IMP 01		0.31	0.28
ADHD IMP 02		0.27	
ADHD IN 01			0.73
ADHD IN 02			0.72
ADHD IN 08			0.71
ADHD IN 07			0.71

ADHD IN 09			0.64
ADHD IN 03			0.63
ADHD IN 06			0.59
ADHD IN 04			0.55
ADHD IN 05			0.45
ADHD HYP 02		0.25	0.42
ADHD HYP 05		0.35	0.38

N=478; SCQ: Social Communication Questionnaire; IMP: Impulsive symptoms; HYP: Hyperactive symptoms; INA: Inattentive symptoms. To aid interpretation, loadings of <0.2 are not presented.

Appendix 3.4: Pattern matrix of loadings for factor analysis of SCQ items; Females excluded

SCQ item	Factor 1 – social	Factor 2 – rigidity	Factor 3 – non-verbal communication
SCQ 39	0.81		
SCQ 36	0.81		
SCQ 37	0.80		
SCQ 34	0.76		
SCQ 40	0.72		
SCQ 27	0.71		
SCQ 33	0.68		
SCQ 38	0.67		
SCQ 30	0.65		0.21
SCQ 35	0.63		0.29
SCQ 29	0.63		
SCQ 31	0.53		0.21
SCQ 28	0.53		0.35
SCQ 26	0.41	0.30	0.21
SCQ 2	0.38		
SCQ 3	0.36	0.23	
SCQ 20	0.25		
SCQ 10	0.20		
SCQ 8		0.82	
SCQ 4		0.79	
SCQ 13		0.78	
SCQ 17		0.75	
SCQ 7		0.66	
SCQ 16		0.64	
SCQ 5		0.62	
SCQ 9		0.58	
SCQ 12		0.57	
SCQ 15		0.57	
SCQ 11		0.55	
SCQ 14		0.52	
SCQ 6		0.48	
SCQ 18		0.43	
SCQ 19		0.34	
SCQ 32			0.72
SCQ 22	0.24		0.69
SCQ 24			0.68
SCQ 23			0.66
SCQ 21	0.27		0.41

N=472; SCQ: Social Communication Questionnaire. To aid interpretation, loadings of <0.2 are not presented.

Appendix 3.5: Pattern matrix of loadings for factor analysis of SCQ items and ADHD symptoms; Females excluded

	Factor 1 – social	Factor 2 – rigidity/ hyperactivity	Factor 3 – inattentiveness
SCQ 39	0.74		
SCQ 30	0.72		
SCQ 34	0.72		
SCQ 27	0.72		
SCQ 35	0.71		
SCQ 28	0.67		
SCQ 36	0.66	0.30	
SCQ 29	0.64		
SCQ 22	0.62		
SCQ 38	0.61	0.20	
SCQ 37	0.60	0.31	
SCQ 40	0.60	0.24	
SCQ 31	0.60		
SCQ 33	0.56		
SCQ 26	0.51	0.25	
SCQ 32	0.45		
SCQ 21	0.45		
SCQ 3	0.42	0.25	
SCQ 2	0.41		
SCQ 24	0.34		
SCQ 20	0.33		
SCQ 10	0.22		
SCQ 13		0.75	
SCQ 8		0.67	
SCQ 17		0.65	
SCQ 7		0.65	
SCQ 11		0.64	
SCQ 4		0.64	
SCQ 12		0.62	
SCQ 16		0.61	
SCQ 15		0.58	
SCQ 9		0.57	
SCQ 5		0.53	
SCQ 18		0.52	
SCQ 14		0.51	
ADHD IMP 04		0.48	
ADHD HYP 03		0.43	
SCQ 6		0.43	
ADHD HYP 05		0.39	0.33
ADHD HYP 01		0.37	0.28
ADHD HYP 04		0.35	
ADHD IMP 01		0.35	
ADHD IMP 03		0.34	
SCQ 19		0.34	
ADHD IMP 02		0.28	
SCQ 23			
ADHD IN 01			0.75
ADHD IN 02			0.71
ADHD IN 07			0.68
ADHD IN 09			0.67

ADHD IN 08		0.67
ADHD IN 03		0.64
ADHD IN 04		0.58
ADHD IN 06		0.56
ADHD IN 05		0.48
ADHD HYP 02	0.35	0.41

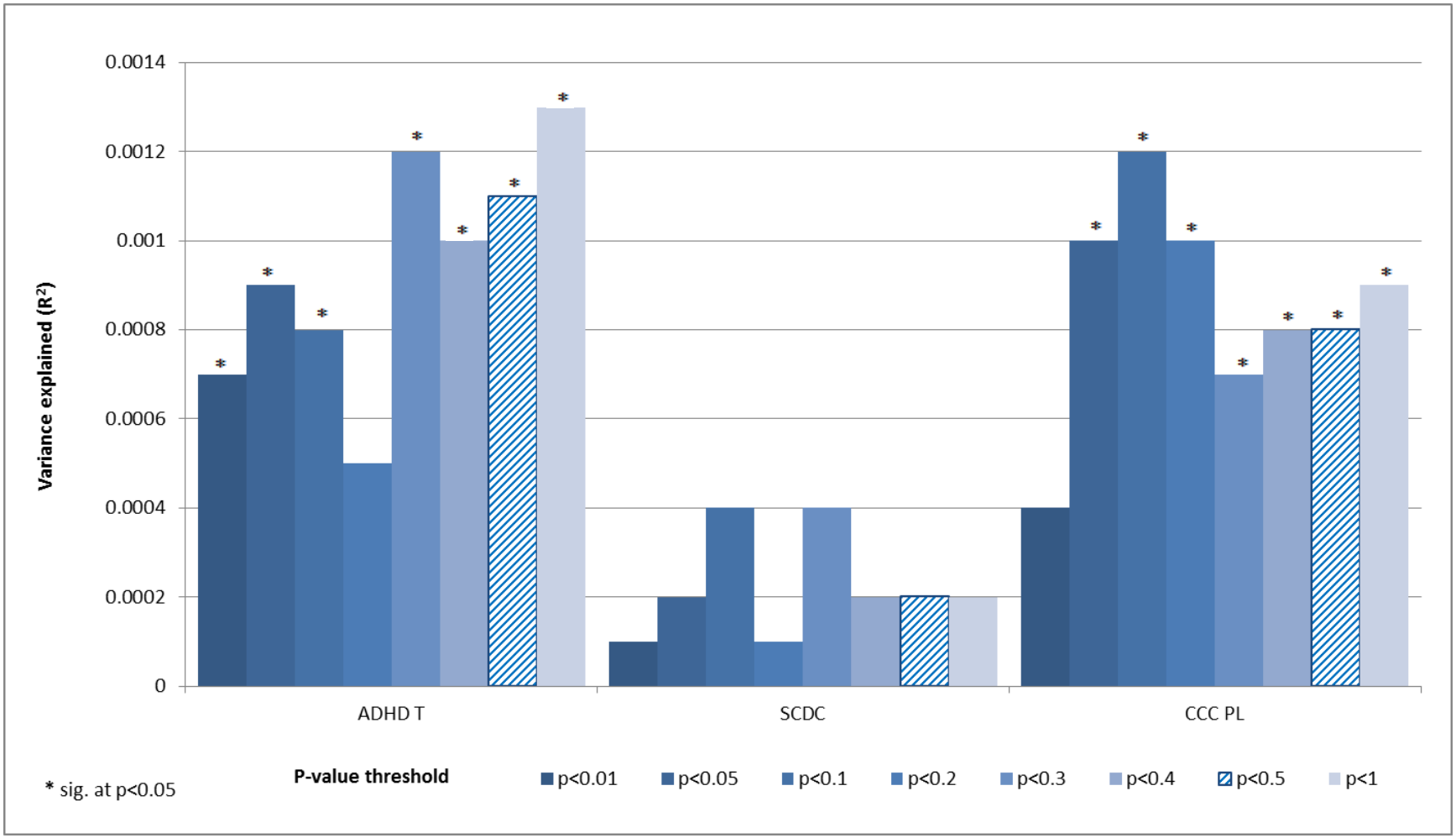
N=450; SCQ: Social Communication Questionnaire; IMP: Impulsive symptoms; HYP: Hyperactive symptoms; INA: Inattentive symptoms. To aid interpretation, loadings of <0.2 are not presented.

Appendix 5.1: Associations of polygenic score with ADHD and ASD-related phenotypes in ALSPAC

Outcome	N	ZINB count outcome			ZINB zero-inflated outcome			ZINB overall p	ZINB overall R^2	Linear regression*			
		β	SE	p	β	SE	p			β	SE	p	R^2
ADHD total traits	5661	0.12	0.10	0.25	-0.06	0.02	0.006	0.0024	0.004	0.033	0.013	0.012	0.001
ADHD hyperactive-impulsive traits	5661	0.16	0.12	0.19	-0.05	0.02	0.031	0.0034	0.002	0.037	0.013	0.005	0.0006
ADHD inattentive traits	5656	0.07	0.13	0.58	-0.04	0.02	0.025	0.037	0.003	0.023	0.013	0.075	0.001
SCDC total score	5653	0.13	0.14	0.36	0.02	0.04	0.66	0.42	<0.001	0.012	0.013	0.36	0.0001
CCC pragmatic language score	5641	N/A								-0.028	0.013	0.038	0.0008

All analyses using gender and all 10 principal components from EIGENSTRAT analyses as covariates. * Linear regression results of ADHD and SCDC phenotypes included only for ease of interpretation. ADHD, attention-deficit/hyperactivity disorder; ALSPAC, Avon Longitudinal Study of Parents and Children; ASD, autism spectrum disorder; CCC, Children's Communication Checklist; SCDC, Social and Communication Disorders Checklist; GWAS, genome-wide association study; ZINB, zero-inflated negative binomial. Polygenic scores derived using a threshold of $p<0.5$ in the discovery sample GWAS results.

Appendix 5.2: Associations of ADHD and ASD-related phenotypes with ADHD polygenic scores calculated based on the primary discovery sample, using a variety of p-value thresholds (linear regressions)



ADHD T: attention deficit hyperactivity disorder total traits; SCDC: Social and Communication Disorders Checklist; CCC PL: Children's Communication Checklist pragmatic language score. Main results are based on polygenic scores derived using a threshold of p<0.5 (striped bars).

Appendix 6.1: Associations between polygenic risk scores with ADHD traits and neurocognitive measures as correlated outcomes

Figure 6.1a

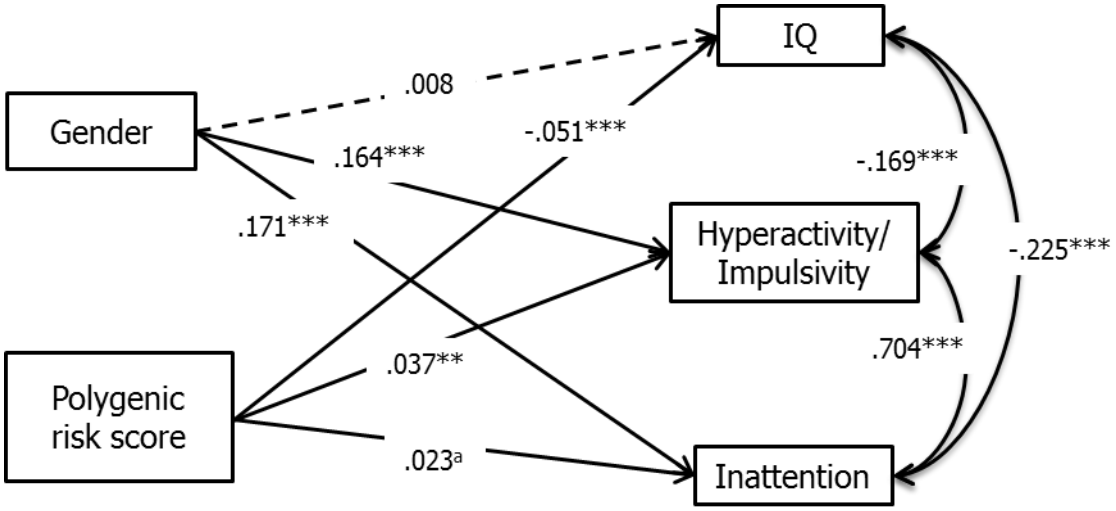


Figure 6.1b

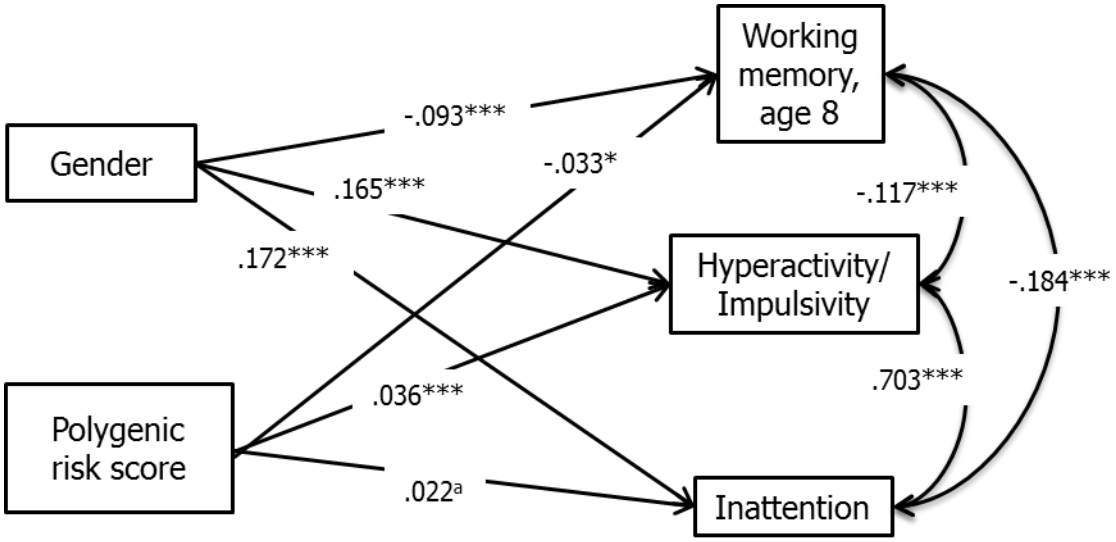


Figure 6.1c

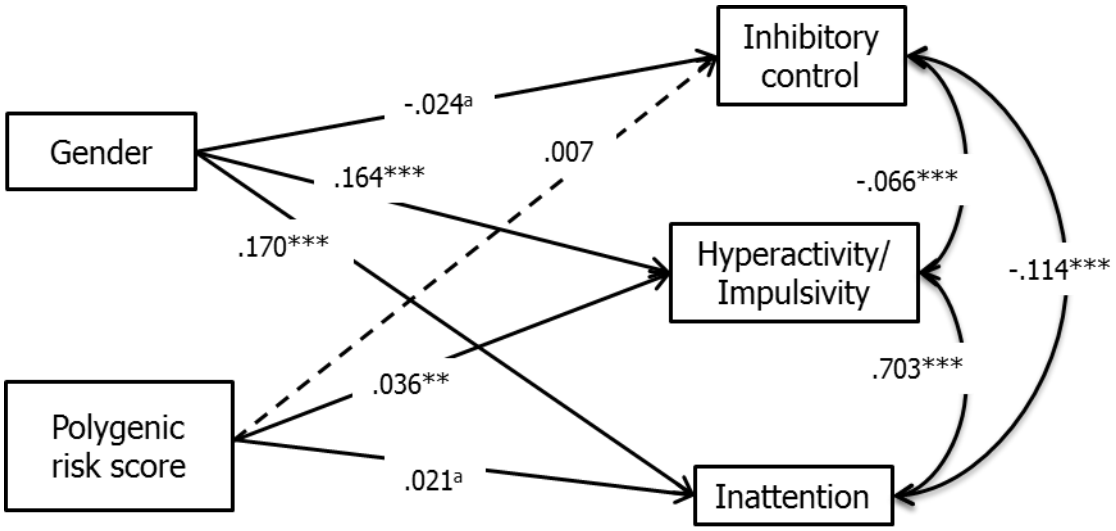


Figure 6.1d

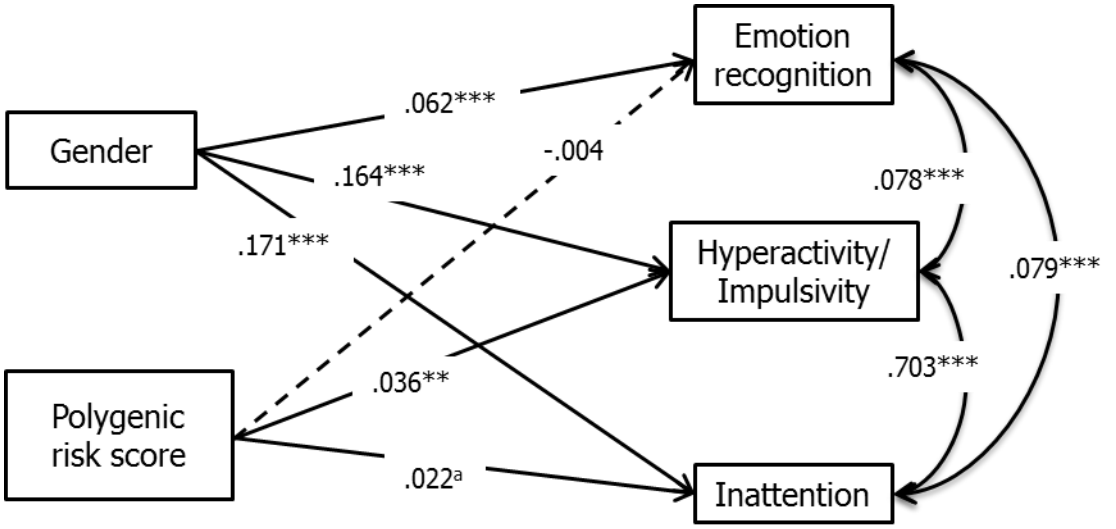
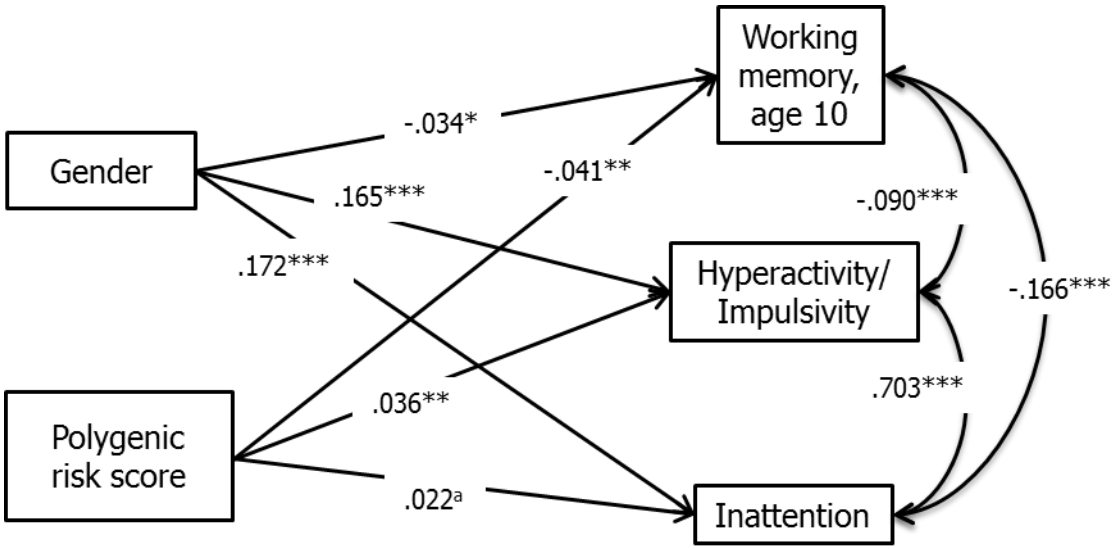


Figure 6.1e



[a] p<0.1, *p<0.05, **p<0.01, ***p<0.001; ND: Neurodevelopmental; No fit statistics available due to saturation of models

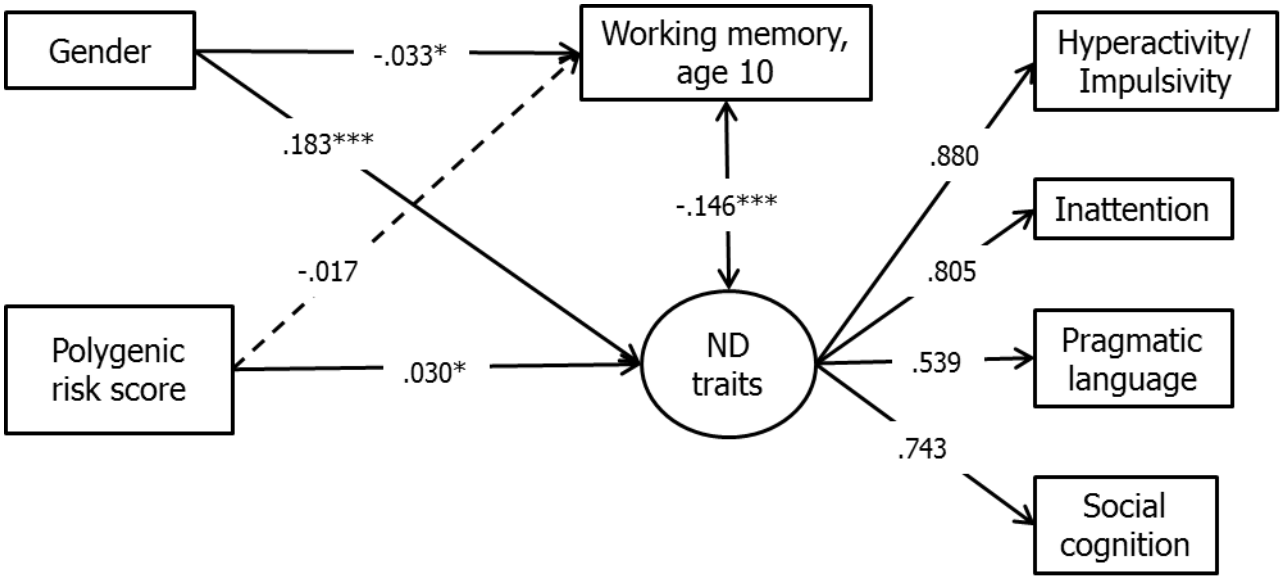
Fig. 6.1a: Association of polygenic risk scores with ADHD traits and IQ as correlated outcomes (N=6,591). Fig. 6.1b: Association of polygenic risk scores with ADHD traits and working memory, age 8.5 years (N=6,582). Fig. 6.1c: Association of polygenic risk scores with ADHD traits and inhibitory control (N=6,575). Fig. 6.1d: Association of polygenic risk scores with ADHD traits and facial emotion recognition (N=6,532). Fig. 6.1e: Association of polygenic risk scores with ADHD traits and working memory, age 10.5 years (N=6,611).

Appendix 6.2: Associations of polygenic risk scores with neurocognitive outcomes

Outcome	N	β	p	R^2
IQ	5515	-0.052	<0.001	0.0027
Working memory, age 8.5 years	5411	-0.033	0.014	0.0012
Emotion recognition	5107	-0.004	0.80	<0.0001
Inhibitory control	5315	0.005	0.72	<0.0001
Working memory, age 10.5 years	5273	-0.042	0.003	0.0017

Analyses after adjusting for 10 EIGENSTRAT covariates and gender (linear regressions)

Appendix 6.3: Association between polygenic risk scores (based on the replication discovery sample) with working memory at age 10.5 years



*p<0.05, ***p<0.001; ND: Neurodevelopmental

Association of polygenic risk scores, based on replication sample, with working memory at age 10.5 years (assessed with the Counting Span Task) (N=6,847); RMSEA=0.044, CFI=0.982, TLI=0.967

Appendix 6.4: Associations between polygenic risk scores and neurocognitive measures, using listwise deletion

Figure 6.4a

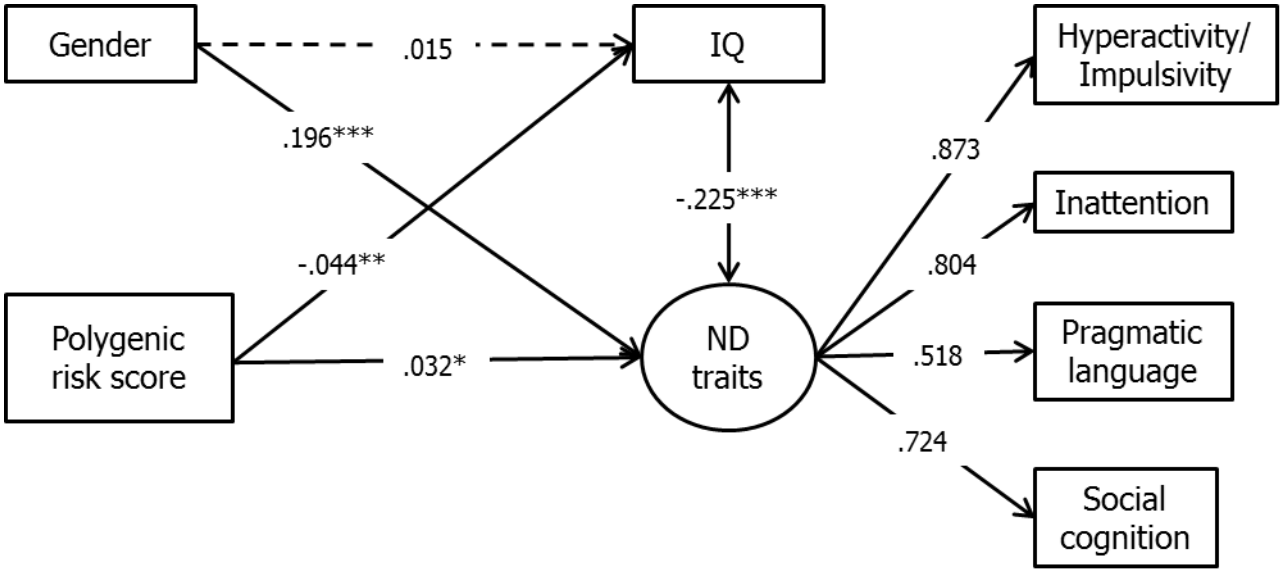


Figure 6.4b

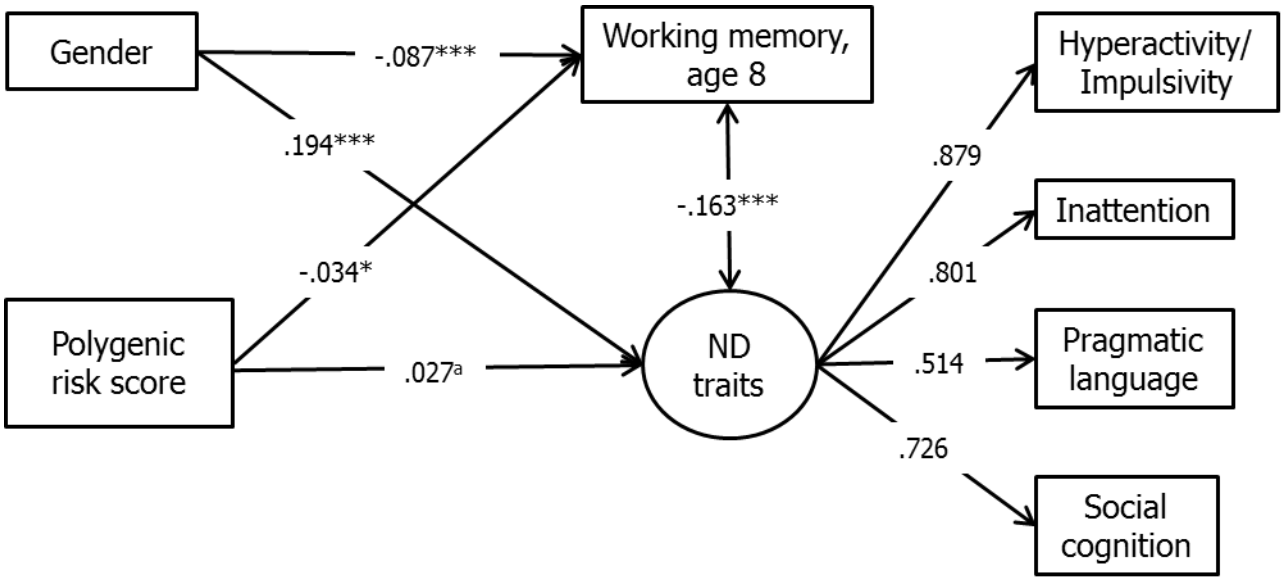


Figure 6.4c

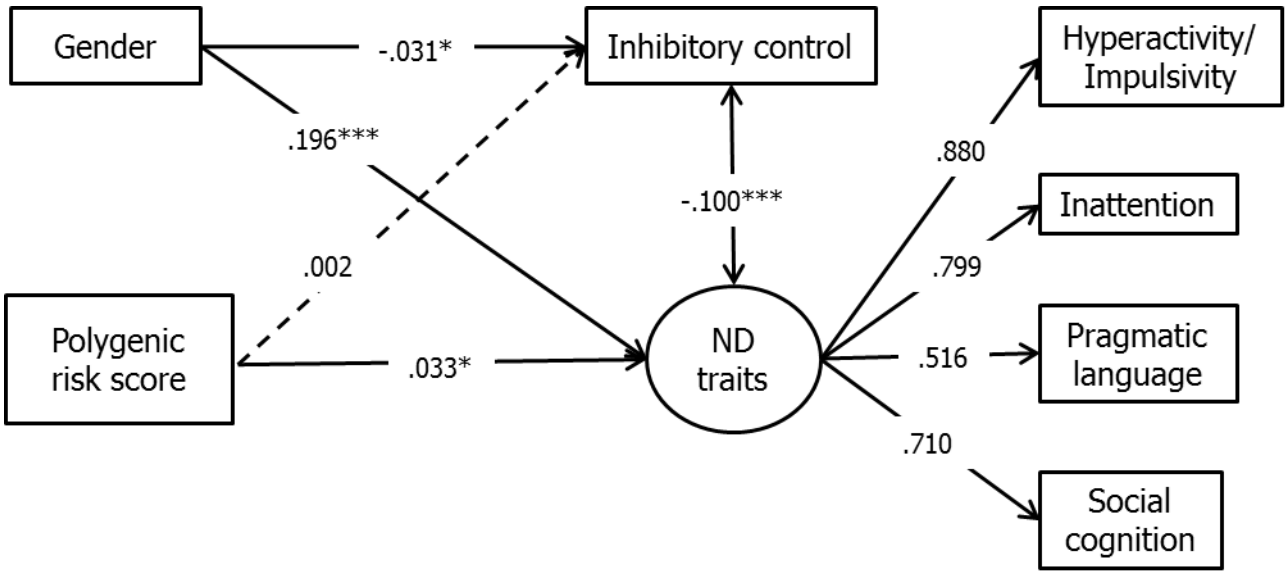


Figure 6.4d

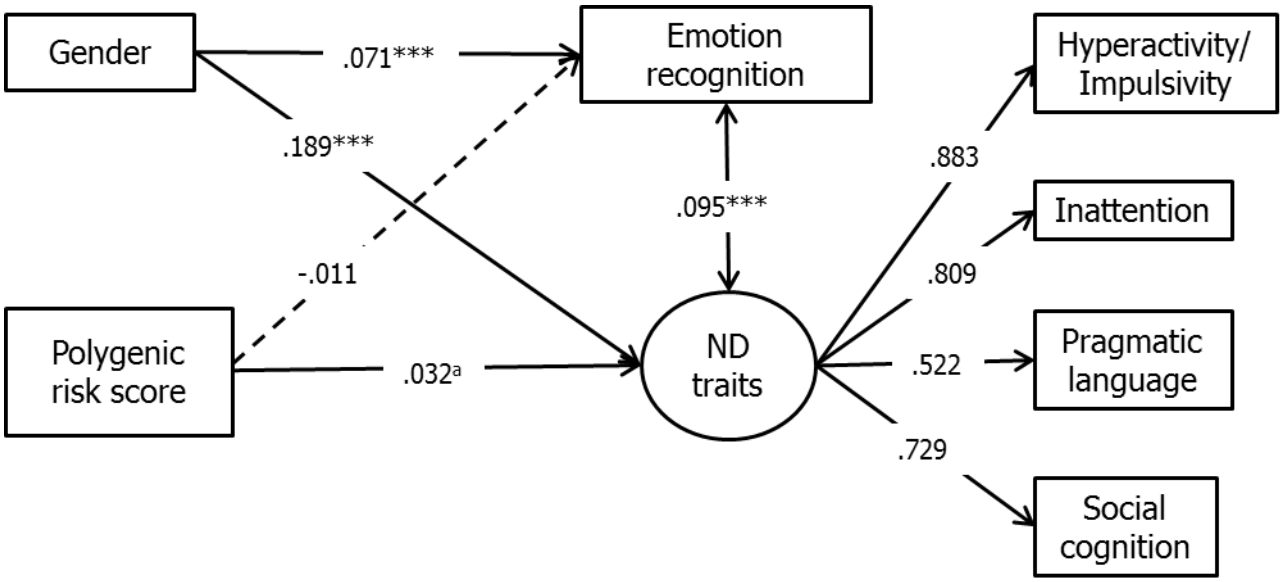
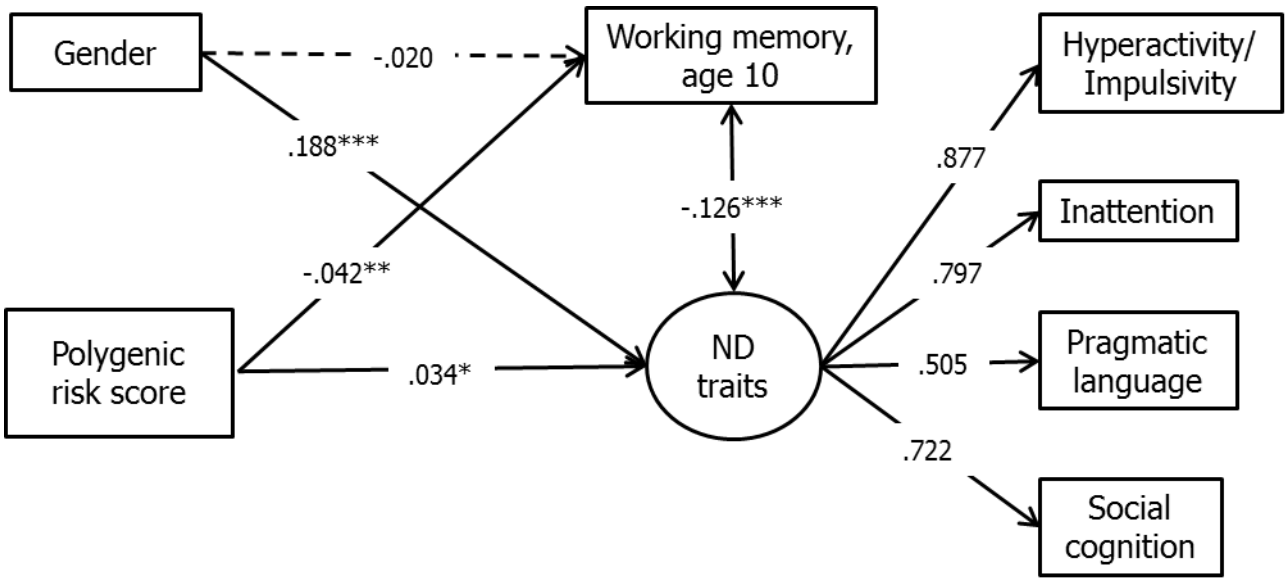


Figure 6.4e



[a] p<0.1, *p<0.05, **p<0.01, ***p<0.001; ND: Neurodevelopmental

Fig. 6.4a: Association of polygenic risk scores with ADHD traits and IQ as correlated outcomes (N=4,091); RMSEA=0.064, CFI=0.968, TLI=0.942. Fig. 6.4b: Association of polygenic risk scores with ADHD traits and working memory, age 8.5 years (N=4,010); RMSEA=0.055, CFI=0.977, TLI=0.958. Fig. 6.4c: Association of polygenic risk scores with ADHD traits and inhibitory control (N=3,928); RMSEA=0.039, CFI=0.988, TLI=0.978. Fig. 6.4d: Association of polygenic risk scores with ADHD traits and facial emotion recognition (N=3,787); RMSEA=0.031, CFI=0.993, TLI=0.986. Fig. 6.4e: Association of polygenic risk scores with ADHD traits and working memory, age 10.5 years (N=3,901); RMSEA=0.054, CFI=0.977, TLI=0.958.