SHARED AND UNIQUE UNDERPINNINGS OF ASD AND ADHD

Pre-/perinatal antecedents and cognitive deficits in the context of familial risk

Anoek Sluiter-Oerlemans

Shared and unique underpinnings of autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder (ADHD)

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Voor Dick, pap & mam

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CHAPTER 1

General introduction

GENERAL INTRODUCTION

Autism Spectrum Disorders (ASD) and Attention-Deficit/Hyperactivity Disorder (ADHD) are severely impairing, highly heritable, and highly heterogeneous neurodevelopmental disorders that manifest early in development (Lichtenstein *et al.*, 2010). ASD and ADHD are two common psychiatric disorders observed in children that frequently co-occur (APA, 2000, 2013), yet the exact causes and mechanisms underlying the single and comorbid occurrence of these disorders are poorly understood. The difficulties encountered in identifying causal factors that increase the risk of ASD or ADHD can be explained, at least in part, by the large within-disorder heterogeneity in symptom presentation, developmental course and underlying etiological mechanisms. The main aim of this thesis is to examine shared and unique mechanisms underlying ASD and ADHD by comparing pre-/perinatal antecedents and associated cognitive deficits in both disorders. An attempt is made to parse etiological heterogeneity by forming subgroups based on familial re-occurrence of the disorders. By contrasting and combining findings of ASD and ADHD, new insights can be gained into the pathophysiology of these two disorders. This might facilitate research on etiology and effective, individualized treatment for ASD and ADHD.

In the following sections, a more elaborate description is given of the clinical manifestations and associated cognitive deficits, and the genetic and early life environmental risk factors of both disorders. Then the difficulties with identifying (shared) risk factors for ASD and ADHD are discussed, followed by a description of the theoretical framework and limitations of the endophenotype model and how subgrouping based on family re-occurrence could refine this model. Finally, the specific aims as well as the outline of the thesis are presented. In the subsequent chapters (**chapters 2-7**), six empirical studies are described, addressing the specific aims of the thesis. These chapters are followed by the summary (**chapter 8**), the general discussion, the key findings, the limitations, and the directions for future research (**chapter 9**).

CLINICAL MANIFESTATIONS OF ASD AND ADHD

ASD are characterized by impairments in social interaction, deficits in verbal and non-verbal communication and by restricted or repetitive patterns of behavior and interest (APA, 2000, 2013). ASD symptoms typically manifest early in life (i.e. before age 3 years), however social deficits and behavioral patterns may not become fully manifest until social demands exceed limited capacities, and may be masked by learned strategies in later life (APA, 2000, 2013). ADHD is characterized by symptoms of hyperactivity (e.g. fidgeting, restlessness ['always on the go'], and excessive talking), impulsivity (e.g.

blurting out answers before questions have been completed, difficulty awaiting turn, and often interrupting or intruding on others), and/or inattention (e.g. forgetfulness, easily distracted, struggling to follow through on instructions, and difficulty sustaining attention) (APA, 2000, 2013). Recent prevalence rates for ASD and ADHD lie around 0.9%-1.1% and 1.4%-8%, respectively (Akinbami *et al.*, 2011, Baron-Cohen *et al.*, 2009, Blumberg *et al.*, 2013, Boyle *et al.*, 2011, Elsabbagh *et al.*, 2012, Erskine *et al.*, 2013, Kim *et al.*, 2011, Kogan *et al.*, 2009, Russell *et al.*, 2014). Both disorders affect males more than females. Large-scale populations-based studies have shown that 2-3 times more males than females are affected by ASD (Kim *et al.*, 2011). In clinical ASD samples, male-female ratio estimates range up to 4-5 times more males than females with ASD (Fombonne *et al.*, 2011). Like ASD, ADHD is more frequent in males than females. Meta-analyses in population-based samples from Europe and the United States have suggested males are 2–4 times more likely to meet full DSM-IV criteria for ADHD than females (Collin *et al.*, 2013, Verte *et al.*, 2006). In clinically referred ADHD samples, the gender ratio was about 5:1 (Boulay and Paus, 2005).

Based on diagnostic criteria, ASD and ADHD have little in common, yet ASD and ADHD appear to frequently occur together in the same child (Rommelse *et al.*, 2011, Ronald *et al.*, 2008) and in the same family (Freitag, 2007). About 30%-80% of children with ASD have symptoms that satisfy DSM-IV diagnostic criteria for ADHD (Lee and Ousley, 2006, Matson *et al.*, 2013, Nydén *et al.*, 2011, Ronald *et al.*, 2008, Simonoff *et al.*, 2008, Sinzig *et al.*, 2009), and vice versa, about 20%-50% of children diagnosed with ADHD show deficits in social interaction and communication, although in a lesser extent than can be seen in ASD (Goldstein and Schwebach, 2004, Kotte *et al.*, 2013, Mayes *et al.*, 1996, Plomin *et al.*, 2004, Ronald *et al.*, 2008). ADHD has been shown to be the second most common comorbid disorder in individuals diagnosed with ASD (Simonoff *et al.*, 2008). In clinical practice, it is sometimes difficult to differentiate between ASD and ADHD, in part due to the entanglement of symptom descriptions of both disorders. For example, inattention (a core symptom of ADHD) can easily be mistaken for social inattention (a core feature of ASD) (Rommelse *et al.*, 2010b). This might explain why a substantial proportion of children have been alternatively given a diagnosis of one or the other disorder throughout development (Fein *et al.*, 2005).

GENETIC AND ENVIRONMENTAL RISK FACTORS FOR ASD AND ADHD

ADHD and ASD are both highly heritable (Chang *et al.*, 2013, Lichtenstein *et al.*, 2010). In ASD, heritability has been estimated at > 90% for classical autism (Freitag, 2007). In ADHD, approximately 73% of the phenotypic variance is explained by heritable factors (Burt, 2009, Faraone *et al.*, 2005, Rommelse *et al.*, 2011). The genetics of ASD and ADHD are

complex. Briefly ASD literature reports that approximately 10% of individuals with ASD have an identifiable genetic etiology corresponding to known chromosomal rearrangements or single gene disorders (such as Fragile X), another 7%-10% carry monogenic forms due to de novo pathogenic mutations or copy number variances (CNVs), yet the majority of ASD cases likely stem from multifactorial underpinnings involving several to many loci and gene-gene and gene-environment interactions (Berg and Geschwind, 2012, Devlin and Scherer, 2012, Persico and Napolioni, 2013, Ruggeri *et al.*, 2014). ADHD is currently viewed as a polygenic, multifactorial disorder with (interacting) contributions from both genes and environmental factors of small effect (Williams *et al.*, 2012). To date, ADHD research has mainly focused on common genetic variants in candidate gene studies and several genes have been implicated in the etiology of ADHD, including dopaminergic (e.g. DRD4, DAT1, DRD5, COMT), noradrenergic (e.g. DBH, ADRA2A), serotonergic (e.g. 5-HTT, HTR1B, HTR2A), cholinergic (CHRNA4), and central nervous system development pathway genes (e.g. SNAP25, BDNF) (Caylak, 2012). More recently, studies report that rare genetic variants with a large effect (such as large rare copy number variations [CNVs]) may relate to ADHD etiology as well (Ben Amor *et al.*, 2005, Williams *et al.*, 2012, Williams *et al.*, 2010).

Some studies state that the high co-morbidity between ASD and ADHD might be explained by shared genetic factors (Rommelse *et al.*, 2010a). Support for this view has been found in recent twin-, family-, and linkage studies that indicate that ASD and ADHD share a portion of their heritable etiology (Lichtenstein *et al.*, 2010, Mulligan *et al.*, 2009, Nijmeijer *et al.*, 2010). That is, about 50-72% of the contributing genetic factors overlap between ASDH and ADHD (Lichtenstein *et al.*, 2010, Rommelse *et al.*, 2010b). Thus, the same variant might contribute to the risk of both disorders. This is confirmed by findings from genome-wide association studies (GWAS) that rare CNVs identified in ADHD were significantly enriched for loci implicated in ASD (Lionel *et al.*, 2011, Williams *et al.*, 2012, Williams *et al.*, 2010). Using a quantitative trait locus (QTL) approach, some overlapping susceptibility loci for ASD and ADHD were found on 7q, 12q, 15q, 16q, and 18q (Nijmeijer *et al.*, 2010). Recently, the Cross-Disorder Group of the Psychiatric Genomics Consortium reported that common single-nucleotide polymorphisms (SNPs) in regions on chromosomes 3p21 and 10q24, and within a L-type voltage-gated calcium channel subunit (CACNB2) were associated with a range of psychiatric disorders of childhood onset or adult onset, including ASD and ADHD (Cross-Disorder Group of the Psychiatric Genomics *et al.*, 2013). The overlap of genetic factors in major psychiatric disorders (including ASD and ADHD) confirms previously reported evidence of pleiotropy (i.e. when one gene has an effect on multiple phenotypes) in human complex disorders (pleiotropy might involve roughly 17% of genes that are associated with diseases or disease traits) (Grosbras *et al.*, 2005). Nevertheless, family studies also suggest some specificity in risk factors given that shared genetic variants (50-72%) do not entirely explain the

manifestation of ASD and ADHD. Thus, in addition to pleiotropic (transdiagnostic) risk factors, many genes and polymorphisms are expected to confer a liability to individual psychiatric diseases (Ozonoff *et al.*, 2011). Elucidating both shared and unique causal variants might help answer the question why one child develops ASD whereas the other child develops ADHD given shared genetic risks. All in all, these findings suggest the existence of hundreds of (pleiotropic or specific) risk genes for ASD and ADHD. It is beyond the scope of this thesis to provide a detailed overview of the genetic variants associated with either ASD or ADHD or both. For extensive reviews of the literature, we refer the interested reader to Berg and Geschwind, 2012, Caylak, 2012, Devlin and Scherer, 2012, Persico and Napolioni, 2013, Ruggeri *et al.*, 2014.

In addition to their strong genetic background, early and later life environmental factors also play an important role in susceptibility to the disorders. For example, a range of factors that adversely affect brain development during pre-/perinatal life are associated with an increase in the risk of ASD and ADHD. In meta-analyses of ASD, advanced parental age at birth, maternal prenatal medication use, gestational bleeding, diabetes, being firstborn, fetal distress, birth injury or trauma, low 5-minute APGAR score and low birth weight (< 5.5 pounds or 2,500 gram) were more frequently observed in ASD than in controls (Gardener *et al.*, 2009, 2011). Additionally, maternal infections, maternal stress, suboptimal condition of the child at birth, prematurity, smoking during pregnancy, and high birth weight more than two standard deviations above average for gestational age were also found related to ASD (Abel *et al.*, 2013, Visser *et al.*, 2013). Research on ADHD indicates that prenatal exposure to nicotine, alcohol, drugs or toxins, and maternal stress, low birth weight, low maternal age and poor maternal diet are associated with an increased likelihood of developing ADHD (Langley *et al.*, 2005, Mick *et al.*, 2002, Mill and Petronis, 2008, Thapar *et al.*, 2013, Throckmorton-Belzer *et al.*, 2009). A recent study report that advanced paternal age was associated with offspring psychiatric morbidity, possibly due to increased genetic mutations during spermatogenesis. Compared with offspring born to fathers 20 to 24 years old, offspring of fathers 45 years and older were at heightened risk of ASD and ADHD (D'Onofrio *et al.*, 2014). These findings suggest that certain pre-/perinatal insults might be associated with both disorders (such as advanced parental age, low birth weight, maternal smoking, and stress during pregnancy), however studies directly comparing the role of these pre-/perinatal risk factors in ASD and ADHD are currently lacking. It is well recognized that not all of those who are exposed to environmental adversity develop ASD or ADHD. Some early and later life environmental factors may be particularly harmful in combination with susceptibility genes through gene x environment interactions (Neuman *et al.*, 2007). This thesis will not further address gene x environment interactions in ASD and ADHD. However, it is worth mentioning it, because it highlights the enormous complexity of the etiologies with possibly hundreds of (shared or unique) risk genes, environmental risk factors and

the interaction between those in the developmental pathways leading up to ASD and/or ADHD, which poses a huge challenge to research that tries to figure out what makes an ADHD case and what makes an ASD case.

COGNITIVE DEFICITS IN ASD AND ADHD

Both ASD and ADHD have been associated with impairments in various cognitive functions. Studies of cognition in ASD have found deficits in intelligence, social cognition, executive functions (EF), and central coherence, three core cognitive domains that have each been proposed to explain the autistic phenotype (the cognitive theories of ASD are described in more detail in chapter 3) (Erskine *et al.*, 2013, Reiersen and Todd, 2008, Whiteford *et al.*, 2013). Typically, strengths in performal IQ over verbal IQ are reported in ASD cases compared to controls (Lee *et al.*, 2011). Children with ASD have difficulty with false-belief tasks, have a poorly developed Theory of Mind, and are less able to identify or respond to the emotional states of others (Uljarevic and Hamilton, 2013). Studies have also reported deficits in planning and set-shifting, theory of mind, and fluid and crystallized intelligence (Happe and Ronald, 2008, Joshi *et al.*, 2013, Pisula, 2010). Lastly, children with ASD often show a bias for parts versus wholes, resulting in an enhanced local processing style, which might explain why autistic individuals often have difficulties to extract global form/meaning, but may perform superior on tasks where a local processing is beneficial (Frith and Happe, 1994, Pellicano *et al.*, 2006).

ADHD is associated with deficits in executive functions, state regulation, reward processing, time reproduction, motor timing and motor control, and attentional control (Geurts *et al.*, 2006, Luman *et al.*, 2005, Nigg, 2005, Rommelse *et al.*, 2008a, Rommelse *et al.*, 2007a, Rommelse *et al.*, 2008b, Rommelse *et al.*, 2007b). Meta-analyses show that children with ADHD demonstrate substantial impairments in response inhibition, verbal and visuo-spatial working memory, planning and organization, set shifting, and processing speed (Willcutt *et al.*, 2005). Recent theories of ADHD emphasize the central role of attentional and executive dysfunctions, as well as impairments in affective components such as motivational processes and sensitivity to reward and reinforcement (Nigg *et al.*, 2005, Sonuga-Barke, 2005, Willcutt *et al.*, 2005). Lately, social problems in ADHD have received more research interest, and studies have reported difficulties in recognizing facial expressions (Corbett and Glidden, 2000, Da Fonseca *et al.*, 2009, Uekermann *et al.*, 2010) and affective prosody (Corbett and Glidden, 2000, Shapiro *et al.*, 1993) in children with ADHD. These findings suggest ASD and ADHD share similar functional brain abnormalities; both are associated with deficits in executive function and with difficulties in social cognition (Matson *et al.*, 2013). It is unlikely that a single theory can account for the range of neurocognitive deficits found in children with ASD and ADHD. Instead, it is

proposed that a combination of cognitive risk factors gives rise to the ASD and ADHD phenotypes (Nigg and Casey, 2005, Pellicano *et al.*, 2006), but these multiple cognitive processes might be differentially affected in ASD and ADHD.

WITHIN-DISORDER HETEROGENEITY PRESENTS A SUBSTANTIAL CHALLENGE FOR IDENTIFYING RISK FACTORS FOR ASD AND ADHD

Given their etiological complexity, involving numerous genetic and environmental risk factors and the interaction between these, the identification of relevant risk factors for ASD and ADHD has been a great challenge. Multiple causal pathways underlie the same clinical profiles (a phenomenon that has been referred to in developmental psychopathology as *equifinality* (Cicchetti and Rogosch, 1996)) and at the same time, the complex etiology may result in highly heterogeneous clinical profiles within ASD and within ADHD. This large within-disorder heterogeneity, both in symptom presentation, developmental course, and underlying etiological mechanisms, is common to both disorders (Jones and Klin, 2009) and strongly hinders research on etiology and effective treatment. For example, the identification of susceptibility genes for ASD and ADHD by linking genotype to phenotype has been troublesome, partly because of the heterogeneity at both levels (Buitelaar, 2005, Gottesman and Gould, 2003). In their hallmark paper, Gottesman and Gould describe that "models of complex genetic disorders predict a ballet choreographed interactively over time among genotype, environment, and epigenetic factors, which gives rise to a particular phenotype. Therefore, more optimally reduced measures of neuropsychiatric functioning should be more useful than behavioral "macros" in studies pursuing the biological and genetic components of psychiatric disorders" (Gottesman and Gould, 2003). Thus, the heterogeneity characteristic of both disorders on multiple levels (behavioral manifestations, associated cognitive deficits, genetic risk factors, and environmental precursors of the disorder) has spurred increasing interest in the identification of subgroups of patients possibly sharing pathophysiological underpinnings.

PARSING ETIOLOGICAL HETEROGENEITY BASED ON UNDERLYING VULNERABILITY TRAITS (ENDOPHENOTYPES)

One approach to create homogeneous subgroups of ASD or ADHD is to group patients based on their underlying endophenotypes (Gottesman and Gould, 2003, Wang *et al.*, 2012). Endophenotypes are defined as heritable vulnerability traits that increase the risk of developing a disorder (Gottesman and Gould, 2003). A variety of criteria have been

proposed to define viable and useful endophenotypes (Bearden and Freimer, 2006, Cannon and Keller, 2006, Gottesman and Gould, 2003). First, endophenotypes should be measured reliably and reproducibly. Second, an endophenotype must be associated (a) with the disease in the general population and (b) within the family. Third, the endophenotype should be heritable and familial, indicating that it must have a genetic basis and is observable in unaffected first-degree relatives of an affected individual (given that first-degree relatives are likely to carry some of the susceptibility genes for the disorder). Last, the trait should be state-independent, meaning it should become manifest in an individual whether or not the illness is active. Endophenotypes do not need to be disease-specific; endophenotypes that affect multiple disorders should be found for genetically related disorders (Cannon and Keller, 2006), such as ASD and ADHD. It has been hypothesized that endophenotypes offer a simplified approach to dissect complex traits by reducing heterogeneity (Wang *et al.*, 2012) and as such may boost the power for genetic analyses, as well as shed light on the functional outcomes of genes (Almasy and Blangero, 2001, Gottesman and Gould, 2003, Viding and Blakemore, 2007). Endophenotypes can potentially be very helpful in ASD and ADHD research, as simple, quantitative and heritable traits can be typically linked to smaller sets of underlying genes compared to complex behavioural dimensions (Kendler and Neale, 2010, Ruggeri *et al.*, 2014).

To test whether a trait meets the criteria for an endophenotype, an affected-unaffected sibling design is often applied (Almasy and Blangero, 2001, Waldman, 2005). Behavioral, cognitive, neuroanatomical, and biochemical abnormalities that are present in unaffected parents or siblings and thus shared between affected and unaffected first-degree relatives are assumed to provide an index of the multifactorial liability to the disorder (Waldman, 2005). Conversely, abnormalities that are not shared between affected and unaffected siblings may have a unique effect on the development of the disorder. This affected-unaffected siblings design has been frequently applied in ADHD research and has led to many studies documenting an increased incidence of behavioral symptoms, comorbid symptomatology, and ADHD-related cognitive deficits in unaffected family members of ADHD probands [for extensive reviews, see (Rommelse, 2008, Rommelse *et al.*, 2011)].

A similar strategy has rarely been applied in ASD research (Gizzonio *et al.*, 2014, Rommelse *et al.*, 2011, Tierney *et al.*, 2012), but given the high heritability of ASD and thus the high likelihood that unaffected relatives share some of the heritable risk factors for the disorder, it is worthwhile to pursue this approach in ASD. The few studies that have investigated potential endophenotypes for ASD report that, amongst others, hyperserotoninemia, macrocephaly, immune abnormalities, brain hypo-connectivity, weak central coherence, abnormal face processing, and impaired EF are likely candidates (Ruggeri *et al.*, 2014), though more research is clearly needed before firm conclusions can be drawn. In addition to identifying potential endophenotypes, this affected versus unaffected

sibling design is also helpful in examining the role early and later life environmental risk factors play in a disorder. When certain environmental factors are present in both affected and unaffected siblings, these factors are probably related to an overall increased risk of developing the disorder (trait factors), without a unique, determining contribution to the disorder. Vice versa, environmental risk factors (predominantly) found in affected offspring but not in unaffected offspring may have a more penetrant, possibly uniquely determining effect on the development of the disorder (state factors).

OPEN QUESTIONS AND CAVEATS OF THE ENDOPHENOTYPE DESIGN

Despite the advantages of the concept of endophenotypes, some open questions and potential caveats in relation to ASD and ADHD remain. First, a topic that is relatively underexplored is whether or not endophenotypes are specific for ASD and ADHD or shared between the disorders. Related to that, it is currently unclear whether or not the presence of comorbid symptoms of ADHD impacts on the manifestation of ASD endophenotypes and vice versa. A main cause of heterogeneity in psychiatric disease is the co-occurrence with other psychiatric disorders (such as ADHD symptoms in ASD patients and vice versa). ASD and ADHD share common etiological factors in which it is even possible that a comorbid diagnosis of ASD and ADHD represents a different nosologic entity compared to the pure forms and that the deficits of the comorbid group differ from the simple additive combination of the deficits associated with ASD and ADHD only (Rommelse *et al.*, 2011). This suggests that studies should take these comorbid symptoms into account and assess the impact of these symptoms when studying candidate endophenotypes for a given disorder. In 2011, a comprehensive review was published specifying the most promising endophenotypic measures for future genetic research targeting pleiotropic risk genes for ASD and ADHD (Rommelse *et al.*, 2011). Several cognitive domains were implicated as promising pleiotropic endophenotype candidates for ASD and ADHD (based on robust associations with at least one of the disorders, in combination with findings of comparable impairments in the other disorder, known neurobiological underpinnings, demonstrated heritability and familiality), including IQ profiles, executive functions, social cognition, motor coordination, and response variability, but only a handful of studies have pursued this issue further, including one study presented in this thesis (**chapter 3)** (Oerlemans *et al.*, 2014). Most studies did not include standardized assessment for both disorders and thus psychiatric comorbidity may have passed unnoticed and 'blurred' current findings. That is, groups described as ASD might partially be a combined ASD+ADHD group and groups described as ADHD might also include children with subthreshold or full-blown ASD (Rommelse *et al.*, 2011). With the possibility to co-diagnose ASD and ADHD in DSM-5, this will hopefully change, with

routine use of standardized measures to chart possible ADHD symptoms in individuals who are referred for possible ASD and vice versa being available for future research.

A second open question is whether these cognitive endophenotypes segregate dependently or independently within families. That is, if and to what extent multiple cognitive processes are influenced by similar familial factors. Most studies examining the viability of candidate endophenotypes have targeted one cognitive domain at a time. However, it is known from the literature that deficits in different cognitive domains are often linked (Pellicano *et al.*, 2006, Rommelse *et al.*, 2008b). It is of great relevance to gain more insight into this issue, because it could provide clues how to use these traits in genetic analyses and further knowledge on the etiological pathways of ASD and ADHD.

An important caveat of the endophenotype design is that it does not differentiate between various heritable forms of ASD and ADHD. That is, the endophenotype design is particularly useful under the assumption that psychiatric disorders are caused by multiple inherited risk factors, each increasing the susceptibility of the disorder by a small amount, that are (partly) shared between affected and unaffected relatives (Gottesman and Gould, 2003). However, it is known from literature that non-shared genetic and non-genetic causes (such as *de novo* mutations or birth complications due to high parental age) underlie a substantial amount of ASD cases (Berg and Geschwind, 2012, D'Onofrio *et al.*, 2014, Freitag, 2007) and recent studies point towards a role for rare genetic variants such as *de novo* mutations in ADHD as well (Ben Amor *et al.*, 2005, D'Onofrio *et al.*, 2013, Williams *et al.*, 2012, Williams *et al.*, 2010). This would suggest that the usefulness of the endophenotype model might be limited to cases of ASD and ADHD with a positive family history (familial cases). Without adjusting for these different forms of genetic influences, unaffected relatives with or without familial loading for the disorder will be examined as a mixed group, possibly diluting findings. A recent study in schizophrenia confirmed the idea that unaffected relatives of psychiatric patients are a heterogeneous group in itself (Quee *et al.*, 2014). The authors reported different cognitive subtypes (normal, mixed, impaired) within the unaffected sibling group and recommended that future studies should investigate underlying factors that can explain why siblings fit into certain cognitive subtypes (Quee *et al.*, 2014). We would like to argue that different modes of genetic transmission might explain why different cognitive subtypes exist within unaffected relatives, with cognitively unimpaired siblings stemming from families with non-shared (*de novo*) risk factors underlying the disorder in the proband and the cognitively impaired siblings stemming from families with shared (inherited) risk factors underlying the disorder in the proband.

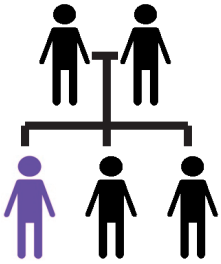
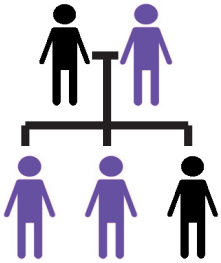
PARSING ETIOLOGIC HETEROGENEITY BASED ON FAMILY OCCURRENCE OF THE DISORDER

Distinguishing multi-incidence families (here referred to as multiplex [MPX]) from single-incidence families (here referred to as simplex [SPX]) has been shown helpful in genetic ASD research. The hypothesis is that the constellation of etiological factors differs between SPX versus MPX families; it is expected that individuals from SPX families are more likely than individuals from MPX families to develop ASD as a result of sporadic genetic and/or non-genetic causes strictly personal to the patient. This has been confirmed by studies demonstrating that a more than threefold rate of *de novo* mutations was identified in ASD SPX families (~7-10%), compared to ASD MPX families (~2-3%) or control families (~1%) (Sebat *et al.*, 2007). In contrast, members of MPX families more often exhibit ASD traits compared to members of SPX families, indicative of a more pronounced role of shared genetic predispositions (Gerdts *et al.*, 2013). See Table 1 for a brief overview of definitions, underlying assumptions and evidence for SPX-MPX stratification. Thus, subgrouping based on family re-occurence might help differentiate between families with non-shared (*de novo*) risk factors underlying the disorder in the proband (SPX) and families with shared (inherited) risk factors underlying the disorder in the proband (MPX). To our knowledge, no attempts have been made so far to test whether the usefulness of SPX-MPX stratification translates to ADHD, but with recent studies confirming the involvement of highly penetrant, rare (non-)genetic risk factors in addition to small disease-increasing effects of multiple genes, it is highly worthwhile to pursue this issue further. Of great interest is further whether SPX-MPX stratification may also be successful in the identification of shared versus unique risk factors for ASD *and* ADHD, thus combining findings from SPX and MPX ASD and ADHD families. This novel application of the SPX-MPX stratification method allows us to test whether the overlap between ASD and ADHD in affected children and their first-degree family members depends on the shared or non-shared causal mechanisms underlying the primary disorder in the family. In other words, whether the type of diagnosis (ASD or ADHD) of the proband is informative for behavioral comorbidity outcomes in these children and their unaffected relatives.

Thus, stratification into SPX and MPX families attempts to reduce etiological heterogeneity by differentiating between different forms of heritability in ASD and ADHD. Comparing SPX and MPX cases and their unaffected siblings with each other on behavioral manifestations and disorder-related cognitive deficits may unravel distinct etiological pathways for these different forms of transmission/occurrence. This may facilitate research into genetic risk factors and may help to (more) effective, individualized treatment. Additionally, SPX-MPX stratification may further our insight into the role of pre-/perinatal insults in the phenotypic manifestation and developmental course of

ASD and ADHD by differentiating between common (potentially genetic) risk factors present in multiple family members (which will be more frequent in MPX families) versus non-shared unique (or incidental) risk factors, only present in affected persons (more frequent in SPX families).

Table 1. Definition, assumptions and evidence for parsing heterogeneity based on family classification

	SIMPLEX (SPX)	**MULTIPLEX (MPX)**
Definition	Nuclear families with one affected individual	Nuclear families with two or more affected individuals
Graphical representation		
Assumptions		
Causes	The disorder may likely be caused by rare, sporadic (non-) genetic (such as *de novo* mutations or early life experiences such as pre-/perinatal insults) risk factors unique to the affected individual.	The disorder is caused by (multiple) inherited risk factors (i.e. risk genes or shared environmental factors), each increasing the susceptibility of the disorder by a small amount.
Are these causes likely shared with unaffected relatives?	No	Yes
Evidence		
ASD	A more than threefold rate of *de novo* mutations was identified in ASD SPX families (~7-10%), compared to ASD MPX families (~2-3%) or control families (~1%) (Sebat *et al.*, 2007)	Members of MPX families more often exhibit ASD traits compared to members of SPX families, indicative of a more pronounced role of shared genetic predispositions (Gerdts *et al.*, 2013) Aggregation of autistic traits is different for simplex and multiplex ASD families (Virkud *et al.*, 2009)
ADHD	Rare genetic mutations or non-shared environmental factors with a large effect are related to ADHD (Ben Amor *et al.*, 2005, Elia *et al.*, 2012, Lionel *et al.*, 2011, Williams *et al.*, 2012, Williams *et al.*, 2010) Advanced paternal age (associated with increased genetic mutations during spermatogenesis) is associated with increased risk of ADHD (D'Onofrio *et al.*, 2014)	Most ADHD studies have focused on multi-incidence families. These studies suggest that ADHD is polygenic and multifactorial with (interacting) contributions from both genes and environmental factors that increase risk for the disorder (Williams *et al.*, 2012) and is highly heritable, confirmed by findings of (sub) threshold symptoms and cognitive difficulties in unaffected relatives (Banaschewski *et al.*, 2010)

THE AIMS OF THIS THESIS

Both ASD and ADHD are clinically and etiologically heterogeneous and complex disorders. The difficulties encountered in identifying risk factors for either ASD or ADHD or both disorders could be, at least in part, explained by this large within-disorder heterogeneity. The main focus of this thesis is to examine shared and unique mechanisms underlying ASD and ADHD by comparing pre-/perinatal antecedents and associated cognitive deficits in both disorders. An attempt is made to parse etiological heterogeneity by forming subgroups based on familial re-occurrence of the disorders.

The specific aims were:

a) To identify viable cognitive endophenotypes for ASD -following ADHD research in which this method has been frequently and successfully applied-, and to test a) whether these endophenotypic candidates co-segregate within families and b) the impact of comorbid ADHD symptoms in this context (**chapters 2 and 3**).
b) To explore shared and unique underpinnings for ASD and ADHD by comparing behavioral traits, cognitive functioning and pre/perinatal antecedents between disorders and between single-, and multi-incidence families (**chapters 4-7**).
c) To examine the validity of SPX-MPX stratification as a means for identifying shared and unique underpinnings for ASD and ADHD and parsing etiological heterogeneity within disorders (**chapters 4-7**).

To this end data from two different samples of well-phenotyped groups of children and adolescents with ASD or ADHD, their unaffected siblings and their parents were analyzed.

SAMPLES

Families were recruited as part of two large family-genetic studies: the Biological Origins of Autism (BOA) study and the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study; both studies have been described in detail in previous publications which can be consulted for greater detail (e.g. van Steijn *et al.*, 2012). The BOA and IMAGE projects aim to examine the genetic, biochemical and cognitive origins of ASD and ADHD and in addition study the overlap between both disorders on these levels. Families were included if (a) they had at least one child (aged 2-20 years) with a clinical diagnosis of ASD (BOA) or if they had at least one child (aged 5-19 years) with a clinical ADHD (combined subtype) diagnosis (IMAGE), (b) at least one biological sibling (regardless of age or diagnostic status) was available and (c) at least one biological parent was willing to participate. Exclusion criteria for all families were an IQ < 60 and a diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or

Fragile-X-syndrome. Comorbid DSM-IV disorders were not excluded except for autistic disorder in the IMAGE study (other ASD subtypes were allowed). Control families were recruited via information leaflets that were sent to families living in the same geographical regions as the participating ASD families (BOA) or from primary and high schools from the same geographical regions as the participating ADHD-families (IMAGE). Selected controls were required to have no formal or suspected ASD or ADHD diagnosis.

All children and parents from both cohorts were carefully phenotyped for ASD and ADHD using validated and standardized questionnaires and diagnostic interviews, and DNA was collected for all family members. All children were also subjected to a range of neurocognitive tasks, including intelligence (estimated total, verbal and performal IQ), executive functions (set shifting, visuo-spatial and verbal working memory, and inhibition), and motor functions (speed, variability, control and timing of motor output). In the ASD cohort, social cognition (face recognition, recognition of emotional expressions, and affective prosody) was assessed as well. See Table 2 for an overview of the tasks and measures used.

In the studies described in **chapters 4-7**, case families from both cohorts were stratified into SPX and MPX families based on the number of affected individuals per family. The procedures for phenotyping and family classification are described in more detail in the chapters. Briefly, stratification was based on the primary diagnosis of recruitment, which is ASD in ASD families and ADHD in ADHD families. The presence of comorbid disorders was not taken into account. To classify as SPX, families were required to have a single affected proband, a minimum of one male sibling, and all siblings and parents of the proband unaffected by ASD or ADHD on the basis of non-clinical scores on the screening-questionnaires and/or administered diagnostic interviews. Families with siblings and parents who displayed (sub) threshold ASD and/or ADHD symptoms, in addition to the affected child, were categorized as multiplex (MPX). Families were excluded if a) only one unaffected parent from a presumed SPX family based on the number of affected children participated in this study to minimize the risk of erroneous categorization because of missing parental data, or b) if the affected proband had only female unaffected siblings to account for higher sibling recurrence risk in male siblings than female siblings.

THE STRUCTURE OF THIS THESIS

In the first two chapters we sought to identify viable cognitive endophenotypes for ASD, following ADHD research in which this method has been frequently and successfully applied. **Chapter 2** focuses on the three core cognitive domains that are proposed to underlie ASD (i.e. social cognition, executive function and central coherence), examining

Table 2. Description of the neurocognitive tasks used in this thesis

Paradigm[a]	Name task	Task components	Dependent variables
Intelligence			
Estimated IQ	Vocabulary, Similarities, Block Design, Picture Completion (WISC-III/WAIS-III)	Administered according to manual guidelines	Performal, Verbal and Total IQ
Executive function			
Inhibition	Go-NoGo [GNG] in ASD cohort and STOP task in ADHD cohort	Stimuli consisted of go-trails and no-go-trails. Children were asked to press a mouse key as quickly and accurately as possible when the go-stimulus was presented, but withhold their response to the stop-trial.	Inhibition (% false alarms - % misses) in ASD cohort and Stop signal reaction time (SSRT) in ADHD cohort
Verbal working memory	Digit Span (WISC-III/WAIS-III)	Stimuli consisted of sequences of numbers. Children were instructed to repeat a sequence in the opposite order as accurately as possible.	Maximum span backwards
Visuo-spatial working memory	Spatial Temporal Span [STS] in ASD cohort and Visuospatial Sequencing [VSS] in ADHD cohort	Stimuli consisted of nine figures presented symmetrically in a 3 by 3 square. On each trail, a sequence of figures was pointed at by a computer-driven hand. Children were then instructed to reproduce the sequence in backward order.	Percentage correct identified targets in correct order (part forward [STS and VSS] and backward [STS])
Set shifting	Response Organisation Objects [ROO] in ASD cohort and Shifting Attention Set Visual [SSV] in ADHD cohort.	The stimulus was a colored circle that was presented to the left or right of a fixation cross [ROO] or a colored square colored square that moved across a bar of ten grey squares in a random direction [SSV]. Three parts were administrated: in part 1, the stimulus was colored green and *compatible responses* were required (i.e. children were instructed to click the mouse key that corresponded to the direction in which the stimulus moved). In part 2, the stimulus was colored red and *incompatible responses* were required (i.e. children were instructed to click the response mouse button opposite to the direction of the moving stimulus). In part 3, the color of the stimulus shifted randomly between green and red and *both compatible and incompatible responses* were required. (Oerlemans *et al.*, 2013a)	Differences in percentage of errors (accuracy) between part 1 and the compatible trials of part 3
Motor function			
Variability of motor control	Baseline Speed [BS]	The stimulus was a fixation cross in the center of a computer that changed into a square at randomly varied time points. Children were asked to press a mouse key as quickly as possible after emergence of the square.	Standard deviation of reaction time in ms

Table 2. Description of the neurocognitive tasks used in this thesis (continued)

Paradigm[a]	Name task	Task components	Dependent variables
Stability of motor control	Tracking [TR]	Children were instructed to trace an invisible midline (radius 8 cm) between an outer (radius 8.5 cm) and inner (radius 7.5cm) circle with a mouse cursor, clockwise with the right hand and counter clockwise with the left hand.	Stability of motor output (standard deviation of the distances in mm with non-preferred hand)
Timing of motor control	Motor Timing [MT]	Children were asked to press a mouse button when they thought a 1-second time interval had elapsed. The start of the interval was announced by a tone. After the response, visual feedback concerning the accuracy of the response was presented on the screen. A response was corrected if it fell between the lower and upper boundary set by a dynamic tracking algorithm.	Variability in reaction time
Social cognition (only in the ASD cohort)			
Recognition of human faces	Face recognition [FR]	Stimuli consisted of color photographs of a human face with a neutral expression, were presented on a computer screen. Children were then asked to identify a target face in a display set that consisted of four faces, by clicking a mouse button	Percentage errors (accuracy) Mean reaction time (in ms)
Recognition of facial expressions	Identification of facial emotions [IFE]	Stimuli consisted of photographs of a human face, presented on a computer screen, with each photograph presenting a face with either a neutral or emotional (happy, sad, angry, fear, disgust, surprise, shame, contempt) expression. Children were asked to judge whether the presented photograph showed the target emotion or not by clicking a mouse button	Percentage errors (accuracy) Mean reaction time (in ms)
Recognition of emotion 'in voices'	Prosody [PR]	Stimuli consisted of spoken sentences with a neutral content, presented through a headphone. Sentences were spoken in a happy, sad, angry or frightened manner, with each emotion represented by twelve sentences, in random order. The children were asked to verbally identify the emotion in the voice. Response time was recorded using a headphone that acted like a voice-key response.	Percentage errors (accuracy) Mean reaction time (in ms)

WISC-III = Wechsler Intelligence scale for Children; WAIS-III = Wechsler Adult Intelligence scale

whether these domains hold potential as candidate endophenotypes and co-segregate within families based on sibling (cross-)correlations. **Chapter 3** zooms in on the social cognition domain and investigates whether recognition of facial emotion and affective prosody are viable endophenotype candidates for ASD. Subsequently, the impact of comorbid ADHD symptoms on emotion recognition in ASD is assessed.

The subsequent four chapters report on comparisons between patients and their unaffected siblings from SPX and MPX families. **Chapter 4** examines whether stratification based on family recurrence (i.e. stratification into single-incidence [simplex, SPX] and multi-incidence [multiplex, MPX] families) is a promising approach (a) for creating etiologically more homogeneous subgroups of patients, and (b) to detect overlapping and unique underpinnings for both ASD and ADHD, based on symptom presentation of ASD and ADHD traits in probands and their unaffected relatives. **Chapter 5 and 6** aim at answering whether the cognitive architecture underlying SPX and MPX ASD and ADHD families is different and useful for parsing the etiological heterogeneity of the disorders. The cognitive performance of probands with ADHD (**chapter 5**) and ASD (**chapter 6**) and their unaffected siblings from SPX and MPX families is compared with normal controls on neurocognitive tasks tapping into various cognitive domains. **Chapter 7** sets out to (a) identify the pre- and perinatal risk factors that may be associated with ASD, ADHD, or both disorders and (b) examine whether these are incidental (only found in affected offspring) or common (also present in non-affected offspring siblings), using the MPX-SPX stratification approach.

Finally, a summary of the findings is provided in **chapter 8** and in the general discussion provided in **chapter 9** we discuss key findings and limitations, and provide recommendations and directions for future research.

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CHAPTER 2

Co-segregation of social cognition, executive function and local processing style in children with ASD, their siblings and normal controls

Based on:

Oerlemans, A.M. Droste, K., Van Steijn, D.J., De Sonneville, L.M.J., Buitelaar, J.K. & Rommelse, N.N.J. (2013). Co-segregation of social cognition, executive function and local processing style in children with ASD, their siblings and normal controls.

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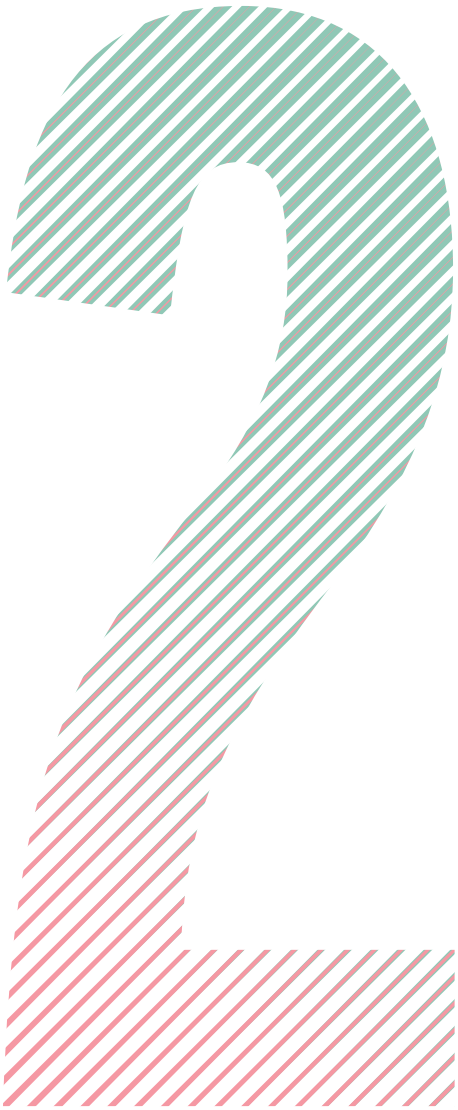

ABSTRACT

Cognitive research proposes that social cognition (SC), executive functions (EF) and local processing style (weak CC) may be fruitful areas for research into the familial-genetic underpinnings of Autism Spectrum Disorders (ASD). The performance of 140 children with ASD, 172 siblings and 127 controls on tasks measuring SC (face recognition, affective prosody, and facial emotion recognition), EF (inhibition, cognitive flexibility, and verbal working memory) and local processing style was assessed. Compelling evidence was found for the interrelatedness of SC and EF, but not local processing style, within individuals and within families, suggesting that these domains tend to co-segregate in ASD. Using the underlying shared variance of these constructs in genetic research may increase the power for detecting susceptibility genes for ASD.

INTRODUCTION

Autism spectrum disorders (ASD) are pervasive developmental disorders, characterized by impairments in social interaction and communication, and by restricted repetitive and stereotyped patterns of behavior, interests and activities (APA, 2000). Evidence from twin and family studies suggest a large genetic contribution, with heritability estimates up to 90% (see for reviews Freitag, 2007, Holt and Monaco, 2011). Twin and family studies have also shown that genetic liability for ASD is expressed in unaffected relatives of individuals with ASD through features that are milder but qualitatively similar to the defining characteristics of ASD (Losh *et al.*, 2009). This is referred to as the "broader autistic phenotype" (BAP). Exploration of the BAP might be a useful method of identifying possible etiological mechanisms that underlie ASD and of increasing the power to identify genes linked with ASD (Wong *et al.*, 2006). Research into the broader autistic phenotype has been primarily focused on the behavioral or 'phenotypic' similarities between individuals with ASD and their first-degree relatives (Bernier *et al.*, 2012, Bishop *et al.*, 2006, Losh *et al.*, 2008, Piven *et al.*, 1997, Ronald *et al.*, 2006), but lately an increasing number of studies are available on the cognitive similarities of autistic individuals and their first degree relatives in an attempt to identify heritable cognitive traits that increase the risk for developing the disorder (see for review Happe and Ronald, 2008). Examining cognitive traits in autistic individuals and relatives offers several important advantages over clinical phenotypes, because they provide insight in the causal chains of action, they aid in forming etiologically more homogeneous subgroups of patients and are hypothetically more powerful in genetic analyses (Gottesman and Gould, 2003, Rommelse *et al.*, 2011).

Most studies have focused on three core cognitive domains that have each been proposed to explain the autistic phenotype, namely impaired social cognition, executive dysfunction and 'weak central coherence' (Happe, 2003). Social cognition (SC) is a broad term that refers to the encoding, storage, retrieval and processing of information relating to other persons, and includes, among others, face and emotion recognition and Theory of Mind (ToM). Impaired recognition and understanding of emotions and impaired ToM (Baron-Cohen *et al.*, 1985) were among the first cognitive mechanisms that were claimed to explain the autistic phenotype and were remarkably successful in making predictions about the impairments in socialization, imagination and communication shown by autistic individuals (Frith and Happe, 1994). However, the failure to explain the non-social deficits as well as the 'islets of ability' (including good visuo-spatial ability, enhanced route memory and uneven IQ profile (Frith, 1989, Pellicano *et al.*, 2006)) that are often found in ASD, led to postulate two additional theories: the theory of executive dysfunction and the theory of weak central coherence. Executive functions (EF) refer to a rather broad range of component processes necessary for the control and execution

of complex behaviors and include different meta-cognitive domains such as planning, fluency, inhibition, working memory and cognitive flexibility. The executive dysfunction hypothesis is said to explain the repetitive behaviors and inflexibility in novel situations, in terms of impairments in planning, set-shifting, inhibition and working memory (see for reviews Happe and Ronald, 2008, Pisula, 2010, Rommelse *et al.*, 2011). Some discussion remains however, as to which executive functions are impaired in ASD. Previous studies have suggested that only a subset of executive processes might be impaired, with some mentioning impairments in cognitive flexibility, working memory and planning with a relative sparing of inhibitory control (Ozonoff, 1997), while others report impairments in inhibitory control with a relative intact working memory (Geurts *et al.*, 2004). The theory of weak central coherence (CC) refers to the detail-focused processing style proposed to characterize ASD (Frith, 1989, Happe and Frith, 2006). Autistic individuals often show a bias for parts versus wholes, resulting in an enhanced local processing style, which might explain why autistic individuals have often difficulty to extract global form/meaning, but may perform superior on tasks where a local processing style is beneficial (Frith and Happe, 1994). It is proposed that a combination of cognitive risk factors (e.g. poor social cognition and EF and weak CC) gives rise to the core aspects of the autistic phenotype and there is strong evidence that autistic individuals show atypicalities across multiple cognitive domains (Happe *et al.*, 2006, Pellicano *et al.*, 2006). Furthermore, previous studies highlighted functional relationships between these cognitive domains, particularly social cognition (ToM) and EF (Jarrold *et al.*, 2000, Ozonoff *et al.*, 1991, Pellicano, 2010, Pellicano *et al.*, 2006). Recently, literature has begun to document similar impairments in SC, EF and CC in unaffected first-degree relatives of individuals with ASD (Goussé *et al.*, 2009, Happe *et al.*, 2001, Happe and Frith, 2006, Losh *et al.*, 2009, Nydén *et al.*, 2011, Wallace *et al.*, 2010, Wilson *et al.*, 2010). This suggests that these cognitive domains may be fruitful areas for further research into the familial-genetic underpinnings of ASD as a continuum.

However, only a few studies have jointly evaluated *all* three domains in autistic individuals (Pellicano, 2010, Pellicano *et al.*, 2006) *and* their relatives (Goussé *et al.*, 2009, Losh *et al.*, 2009, Nydén *et al.*, 2011) and results have been inconclusive. A local processing style was found to be more common in individuals with ASD than were deficits in social cognition or EF (Pellicano *et al.*, 2006). Further and importantly, central coherence was associated with aspects of executive function, but unrelated to social cognition. Later, central coherence and executive functions skills turned out to be longitudinally predictive of theory of mind performance (an aspect of social cognition)(Pellicano, 2010), suggesting a common underlying factor to all of these impaired cognitive functions. However, it is unclear whether these findings would also apply to unaffected relatives of autistic individuals, which might not necessarily be so given that patients are at the extreme end of the ASD continuum and therefore by chance more likely to be impaired

in all three domains. Two other studies did examine the three domains in both autistic individuals *and* their unaffected relatives and reported that selective deficits in both affected and unaffected relatives appeared present regarding social cognition (Losh *et al.*, 2009) and executive function (Nydén *et al.*, 2011). However, Nydén and colleagues (2011) only examined family correlations within, but not between, cognitive domains. Losh and colleagues (2009) briefly addressed the issue of interrelatedness between autistic individuals and their first-degree relatives, but the small subsample of intact families (biologically related parents and ASD children) prevented them from conducting reliable correlation analyses. Therefore, it remains unclear if and to what extent social cognition, executive function and local (detail-focused) processing are influenced by similar familial factors. In other words, do these cognitive functions tend to co-segregate within families? Preliminary results suggest they don't. Findings suggest that cognitive impairments may decouple and segregate independently in autistic individuals and unaffected relatives (Happe and Ronald, 2008). In their review, Happé and Ronald (2008) argue that there are multiple cognitive weaknesses and strengths that account for the range of autistic behaviors and that are independent at the genetic and cognitive level (e.g. task success in one cognitive domain and task failure in another). Nevertheless, the authors stress that impairments in social cognition, executive dysfunction and weak central coherence co-occur above chance in autistic individuals. It is of great relevance to gain more insight into this issue, because it could provide clues how to use these traits in genetic analyses and further knowledge on the etiological pathways of ASD.

Therefore, the current study set out to examine whether these three domains indeed segregate independently in ASD. We administered a relatively large battery of cognitive tasks selected to assess processing within a given domain in a relatively large group of high-functioning children with ASD (N = 140 probands), their siblings (N = 172) and healthy controls (N=127). We chose three social cognition tasks, namely face recognition, facial emotion recognition and affective prosody. Most studies examine emotion recognition using facial expression cues (see Harms *et al.*, 2010), but we added a task that examined the recognition of emotions 'in voices' (affective prosody) as well, because previous studies showed ASD is strongly related to impairments in affective prosody (Golan *et al.*, 2007, Korpilahti *et al.*, 2007, Lindner and Rosen, 2006, Philip *et al.*, 2010). Furthermore, we included three executive function measures: response inhibition, cognitive flexibility and verbal working memory. Response inhibition was measured with the commonly used Go-NoGo paradigm, where children were instructed to withhold a response when the NoGo target (25% of trials) was pictured. Cognitive flexibility was assessed by administering a task that required a mixture of compatible and incompatible responses, hypothesized to require a higher level of cognitive flexibility. Verbal working memory was assessed using the Wechsler Intelligence Scale for Children, subtest Digit Span (backward condition) (Wechsler, 2002, Wechsler, 2000). Last, we included a

measure with a strong local-global component. Children who show a weak CC should be better at finding the target among similar distracter targets than children with strong CC. All selected tasks have been shown to be associated with ASD previously (Geurts *et al.*, 2004, Oerlemans *et al.*, 2014, Serra *et al.*, 2003, van der Meer *et al.*, 2012). Correlations and sibling (cross-) correlations were calculated to examine the interrelatedness of the three cognitive domains within children, as well as within families. If social cognition, executive functioning and local processing style would indeed segregate independently in ASD, we expected that there would be no significant correlations between tasks tapping the three cognitive domains within children. Furthermore, we expected that the three cognitive domains would show signs of familiality (e.g. significant correlations between scores on, for example, social cognition tasks in probands and such scores in siblings), but not of dependent segregation (e.g. no significant correlations between scores on social cognition, executive function and local processing style within the probands/ siblings or between probands and siblings).

METHOD

Participants

Families with at least one child with a clinical diagnosis of ASD (Autism, Asperger's Syndrome (AS) or PDD-NOS; APA 2000; diagnosis mostly based on Autism Diagnostic Interview - Revised (ADI-R) and Autism Diagnostic Observation Scale (ADOS) assessment) and at least one unaffected sibling were recruited as part of the Biological Origins of Autism (BOA) study at Karakter Child and Adolescent Psychiatry, University Medical Center Nijmegen, the Netherlands, which aims to examine the genetic, biochemical and cognitive origins of ASD. Control families (at least two participating biological siblings) were recruited via information leaflets that were send to families living in the same geographical regions as the participating ASD families as part of the BOA project. Controls were required to have no formal or suspected ASD diagnosis. A total of 140 ASD families consisting of 140 ASD probands and 172 siblings (145 without and 27 with ASD) and 62 control families (127 children) were included in the current study. The proband group had proportionally more males. All children were between the ages of 6 and 21 and were of European Caucasian descent. Participants were excluded if they had an IQ < 60, a diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile – X – syndrome. The study protocol was approved by the local medical-ethics committee. Written informed consent was obtained from parents and children over the age of 12 before testing.

Both the children already clinically diagnosed with ASD, their siblings and the control children were similarly screened for autism spectrum disorders using the parent and

teacher Social Communication Questionnaire ([SCQ] Berument et al., 1999). A cut-off score of > 10 for the parent version (Corsello et al., 2007) or ≥15 for the teacher version was considered as clinical. A lower cutoff was chosen for the parent reported SCQ to avoid false negatives in their undiagnosed offspring (van Steijn *et al.*, 2012). For all children scoring on either the parent or teacher rated SCQ in the clinical range (this included all children previously diagnosed with ADHD and a subsample of siblings (N=27)), a formal diagnosis of ASD was made by a certified clinician using the ADI-R (Le Couteur *et al.*, 2003). Clinically diagnosed children who did not fulfill ADI-R criteria (N=6) were excluded from the analyses along with their siblings (N=7). Siblings were regarded as unaffected if they obtained scores below cut-off on both the parent and teacher SCQ. No ADI-R was administered concerning unaffected siblings. Control children were required to obtain non-clinical scores on the parent and teacher SCQ (i.e. a raw score of < 10 and < 15 respectively) in order to be accepted in the current study.

Full-scale IQ was estimated using four subtests of the Dutch version Wechsler Intelligence Scale for children (WISC-III): Similarities, Vocabulary, Block Design and Picture Completion (Wechsler, 2002). These selected WISC-III subtests are known to correlate between .90-.95 with the Full-scale IQ (Groth-Marnat, 1997). For children older than 16 years, the Wechsler Adult Intelligence Scale (WAIS-III) was administered (Wechsler, 2000) See Table 1 for sample characteristics.

Table 1. Sample characteristics

	1. Probands N = 140		2. Siblings N = 172		3. Controls N = 127		F	Contrasts based on *p* values of <.05
	M	sd	M	sd	M	sd		
Age in years	12.4	*3.0*	12.2	*3.9*	11.0	*3.6*	6.6	1=2>3
Males (%)	82.1		47.7		42.5		30.0	1>2=3
Estimated Full Scale IQ	102.0	*13.8*	105.5	*12.9*	107.4	*12.4*	5.8	1<2=3
Estimated VIQ	99.9	*15.5*	104.8	*14.7*	108.4	*14.1*	ns	1<2=3
Estimated PIQ	104.5	*17.0*	106.7	*16.7*	106.7	*15.8*	11.2	1=2=3
SCQ parents Total score	19.2	*6.7*	5.8	*6.6*	3.1	*2.6*	313.3	1>2>3
Diagnostics (%)								
ASD	*100*		*15.7*		0			

1 = probands; 2= siblings; SCQ = Social Communication Questionnaire (total score); ASD = Autism Spectrum Disorders; VIQ = Verbal IQ ; PIQ= Performal IQ

Materials

In total, six tasks from the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville, 1999) and one subtest (Digit Span) of the Wechsler Intelligence Scale (Wechsler 2000, 2002) were selected in this study. The ANT is a computer-aided assessment battery that allows for the systematic evaluation of information processing capacities and has been proven to be a sensitive and valid tool in research into autism-related disorders. Test–retest reliability and validity of the ANT-tasks are satisfactory and have been described and illustrated elsewhere (De Sonneville, 2005, Serra *et al.*, 2003). A short description of each task is presented below. Each computer task contained an instruction trial where the examiner provided a typical item of the task, and a separate practice session. If necessary the instruction was repeated (De Sonneville, 2011). All subjects were able to perform the training items before testing. Digit Span was administered following manual guidelines (Wechsler, 2002, Wechsler, 2000). For all ANT tasks the main outcome variables were speed (mean reaction time) and accuracy (number of errors) of responses.

Social Cognition (SC)

Face Recognition

The Face Recognition (FR) task was used to measure the capacity to process social (facial) stimuli (De Sonneville, 1999). Stimuli consisted of color photographs of a human face with a neutral expression, were presented on a computer screen. Children were asked to identify a target face in a display set that consisted of four faces (see Figure 1a). The

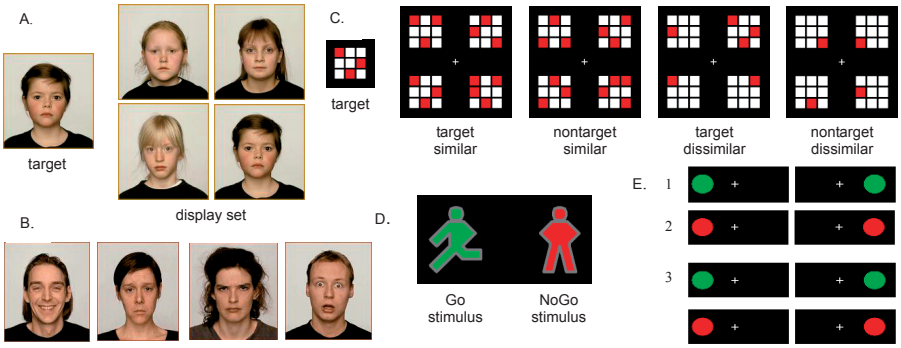

Figure 1 Examples of the stimuli used in the computerized tasks implemented in this study.
a. Example of a target face and display set in the face recognition task
b. Example of facial expressions in the facial emotion recognition task
c. Target stimulus and examples of similar and dissimilar trials in the global-local processing task
d. 'Go' and 'NoGo' stimulus in the inhibition task
e. Stimulus in the cognitive flexibility task, 1 = similar condition, 2 = dissimilar condition, 3 = mixed condition

target face was presented for 2,500 ms and then it disappeared from the screen. After 500 ms, it was followed by a display set, which remained on the screen until the child pressed a key: if the target face was present in the display set the subject was asked to click the 'yes-button' (right computer mouse button for right-handed subjects, and left computer mouse button for left-handed subjects), if the display set did not contain the target face the subject was asked to press the 'no-button' (left computer mouse button for right-handed subjects, right computer mouse button for left-handed subjects). The task consisted of 40 trials in half of which the target set contained the target face (target trials) and half of which the target face was absent. Dependent measures were speed (mean reaction time) and accuracy (number of errors).

Facial Emotion Recognition

The Identification of Facial Emotions (IFE) task was used to measure the capacity to understand facial emotional expressions (De Sonneville, 1999). Stimuli consisted of photographs of a human face, presented on a computer screen, see Figure 1b. Each photograph presented a face with either a neutral expression or one of eight different types of emotion (happiness, sadness, anger, fear, disgust, surprise, shame, contempt) and children were asked to judge whether the presented photograph showed the target emotion or not by clicking a mouse button. For the current study four target emotions were selected: happiness, sadness, anger and fear. If the presented photograph showed the target emotion the subject was asked to click the 'yes-button' , if the presented photograph did not show the target emotion, the subject had to click the 'no-button' (responses were to be given as described above). Each emotion condition consisted of 40 trials; half of which were the target emotion (requiring a 'yes' response) and half of which were a random selection of other emotions (requiring a 'no' response). Mean speed and accuracy (error) scores were calculated by averaging the scores of the four targeted emotions.

Affective Prosody

The Prosody (PR) task was administered to test the ability to recognize 'emotions in voices' (De Sonneville, 1999). Stimuli designed by Vingerhoets (Vingerhoets *et al.*, 2003) were used. They consisted of spoken sentences with a neutral content, presented through a headphone. Sentences were spoken in a happy, sad, angry or frightened manner, with each emotion represented by twelve sentences, in random order. Sentences were of approximately equal length and were articulated by two professional actors, one male and one female. The children were asked to verbally identify the emotion in the voice. Response time was recorded using a headphone that acted like a voice-key response. During listening, the emotions to be discriminated were presented on the computer screen. The task consisted

of 48 trials, 12 per emotion. A missed trial was replaced. Mean speed and accuracy (error) scores were calculated by averaging the scores of the four targeted emotions.

Executive Functions (EF)

Inhibition

The Go-NoGo (GNG) task was used to measure the capacity to inhibit a prepotent response (De Sonneville, 1999). Stimuli consisted of the "Go"-stimulus and the "NoGo"-Stimulus, as presented in Figure 1d. Children were instructed to click a mouse button as quickly and accurately as possible if the "Go"-stimulus was presented. If the "NoGo"-stimulus was presented, the subjects were instructed to withhold clicking the button. The stimulus was presented for 300 ms. The valid response window was 200-1,500 ms post onset of the stimulus. Stimuli were pseudo-randomly presented (biased condition: 56 "Go"-stimuli and 18 "NoGo" stimuli) to measure inhibition of an ongoing response. Dependent variables were measures of speed (mean reaction time "Go"-signals) and accuracy (% false alarms - % misses).

Cognitive Flexibility

The Response Organisation Objects (ROO) was used to measure cognitive flexibility (De Sonneville, 1999). The stimulus was a figure of a colored ball that was presented to the left or right of a fixation cross, as presented in Figure 1e. The color of the ball determined the required response. Three parts were administrated. In Block 1, the ball was colored green and *compatible responses* were required. Children were instructed to click the mouse button that corresponded with the orientation of the stimulus. In Block 2, the stimulus was colored red and *incompatible responses* were required. Children were instructed to click the response mouse button opposite to the orientation of the stimulus. In Block 3, the color of the stimulus shifted randomly between green and red and *both compatible and incompatible responses* were required. Cognitive flexibility was operationalized as the differences in mean reaction time (speed) and number of errors (accuracy) between part 1 and the compatible trials of part 3. The difference between both blocks lay in the fact that in Block 3, the participant did not know on forehand that a compatible response was required, whereas the participant did in Block 1.

Verbal working Memory: Digit Span Backward

The backward part of the Digit Span task (Wechsler, 2002, Wechsler, 2000) was used to measure verbal working memory. Stimuli consisted of sequences of numbers. Children were instructed to repeat a sequence in the opposite order as accurately as possible. One digit was added to the sequence if a child reproduced the sequence successfully. A maximum of 8 experimental sequences were administered, dependent on the child's

performance. The maximum span backward was used to obtain an indication of verbal working memory.

Local Processing Style

The Feature Identification (FI) task was administered to test detail-focused (local) processing (De Sonneville 1999). The stimulus was a predefined target pattern of 3 red and 6 white squares in a 3x3 matrix. After memorization of this target pattern children were asked to detect the target stimulus in a signal consisted of four patterns, as presented in Figure 1c. There were two conditions. In the first condition, the distracter patterns looked very similar to the target pattern, making the recognition of the target rather difficult. The first condition consisted of 40 trials, half of which contained the target (requiring a 'yes' response e.g. target trials) (responses were to be given as described above) and half of which required a 'no' responses (nontarget trials). In the second condition, the distracter patterns looked very dissimilar to the target pattern, which makes target detection easy. Again, this condition consisted of 40 trials, 20 of which were target trials and 20 of which were nontarget trials. The various types of trials (similar, dissimilar, target, nontarget) were presented in a random order. The target pattern was presented only at the beginning of the task and was the same for all trials. Dependent variables were the *differences* in speed (mean reaction time) and accuracy (number of errors) between the similar minus the dissimilar trials. It is expected that the similar condition represents a more local processing style and the dissimilar condition represents a global processing style. Therefore, a higher score on both dependent measures represented a more global processing style.

Procedure

Testing the children with ASD, their siblings and controls took place at Karakter Child and Adolescent Psychiatry University Centre Nijmegen and was conducted for all the children of the same family simultaneously. Stimulants were discontinued for at least 24 hours before testing and non-stimulants according to their plasma half life to allow for sufficient wash-out. Children were motivated with small breaks. The tasks described in this study were part of a broader neuropsychological assessment battery used in the BOA study. The order of administration of the tasks was counterbalanced across participants (but identical for family members).

Statistical Analyses

All analyses were carried out in SPSS version 20. All measures were subjected to a Van der Waerden transformation to normalize the measures (SPSS version 20) (Norusis, 1992) and to depict all measures on the same scale (z-scores). Some scores were mirrored, so that the scores of all variables would imply the same: higher z-scores indicated bet-

ter performances (i.e. less errors and faster response). Subsequently, a mean z-score was calculated *per* cognitive task by averaging the accuracy and speed z-scores. The following three terms were used: correlation (referring to a correlation between two variables in the same subject), sibling correlation (referring to a correlation between siblings for the same variable) and sibling cross-correlation (referring to a correlation between siblings for two different variables). Correction for multiple testing was applied using the False Discovery Rate (FDR) correction with a *q*-value of 0.05. Following Cohen's guidelines (Cohen, 1988), effect sizes for correlations were defined in terms of small (r = .10), medium (r = .30) and large effects (r = .50).

First, group differences between children with ASD and controls, corrected for age, sex and total estimated IQ, were calculated for all cognitive measures, to examine whether the selected tasks were significantly associated with ASD. Further, to test the interrelatedness of the domains within individuals, correlations were calculated between all dependent variables. These analyses were run separately for ASD probands, siblings and controls and the size of the corresponding correlations was compared between groups using the independent correlation coefficients calculation test (Preacher, 2002). Then for each ASD proband/control child, one sibling closest in gender and age was selected, and sibling correlations and sibling cross-correlations were calculated to examine the degree of familiality of all dependent measures. Results were reported with and without correction for differences in age and intellectual ability (estimated Total IQ) between siblings.

Second, a component score was calculated for social cognition and executive function by averaging the mean z-scores of the corresponding tasks. Regression analyses were conducted to examine whether the performance of the ASD probands on, for example the SC component score, could predict the performance of their siblings on the SC component score as well as on the EF component score and on local processing style. Three separate regression analyses were conducted with the SC, EF component score or the local processing style score of the probands as predictor and the three component scores of the siblings as dependent variables. Age difference and Total IQ difference between siblings were implemented in the model as well. Similar analyses were run for control sibling pairs.

RESULTS

The affective prosody recognition task was not administered to children younger than 9 years of age. Affective prosody recognition data was based on 119 ASD probands, 131 siblings and 81 controls. The percentage of missing data was random and < 1% for accuracy and speed measures of the face recognition and facial emotion recognition task, < 3% for local processing style measures, digit span, cognitive flexibility and affective

Table 2. Means, standard deviations and range (minimum-maximum scores) of the untransformed task variables for ASD probands, their siblings and controls

		1.Probands (N=140)				2.Siblings (N=172)				3. Controls (N=127)				F	Group contrasts based on *p* values <.05
		Mean	*sd*	Min	Max	Mean	*sd*	Min	Max	Mean	sd	Min	Max		
	Face Recognition														
	% Errors	13.2	*10.0*	0	52.5	12.2	*9.3*	0.0	50.0	12.2	9.2	0.0	40.0		
	Speed (in ms)	1876.2	*575.4*	950.0	4107.0	1754.6	*579.2*	724.5	3615.5	1822.3	647.3	730.5	4199.5		
	Facial ER														
SC	% Errors	13.3	*8.3*	1.9	51.3	11.4	*6.8*	0.0	37.5	12.2	7.2	1.9	32.5		
	Speed (in ms)	971.2	*212.1*	495.6	1546.3	959.8	*227.9*	564.9	1656.8	1002.7	239.6	470.5	166.2		
	Prosody														
	% Errors	28.9	*11.9*	4.2	62.5	27.8	*10.5*	6.3	62.5	29.9	12.1	6.3	62.5		
	Speed (in ms)	3458.8	*528.7*	1935.0	4548.5	3356.6	*569.3*	1902.3	4436.3	2957.6	668.1	1427.5	4399.3	**19.1**	1=2>3
	Local processing														
	% FI errors similar	15.7	*12.5*	0.0	55.0	13.7	*11.0*	0.0	62.5	13.8	10.2	0.0	50.0		
Weak CC	% FI errors dissimilar	6.9	*9.6*	0.0	57.5	5.9	*7.9*	0.0	50.0	5.6	6.1	0.0	42.5		
	FI speed similar (in ms)	2096.0	*629.3*	1081.5	3905.0	2141.5	*714.2*	973.0	4328.0	2271.8	749.7	989.5	4111.8		
	FI speed dissimilar (in ms)	1166.5	*335.6*	649.5	2039.5	1159.4	*406.2*	612.0	2599.5	1183.9	389.5	604.5	2672.0		

Table 2. Means, standard deviations and range (minimum-maximum scores) of the untransformed task variables for ASD probands, their siblings and controls (continued)

		1.Probands (N=140)				2.Siblings (N=172)				3. Controls (N=127)				F	Group contrasts based on *p* values <.05
		Mean	*sd*	Min	Max	Mean	*sd*	Min	Max	Mean	sd	Min	Max		
EF	*Inhibition*														
	% false alarms	27.7	*17.0*	0.0	83.3	24.5	*14.7*	0.0	72.2	23.4	14.1	0.0	77.8	**4.7**	1>2=3
	% misses	2.6	*3.6*	0.0	26.8	2.7	*5.8*	0.0	41.1	2.4	3.7	0.0	19.6		
	Difference % fa - % mis	25.7	*16.8*	-3.6	83.3	21.8	*14.8*	-24.4	68.7	21.1	13.5	-5.4	68.9	**3.8**	1>2=3
	Speed (in ms)	346.4	*63.5*	245.0	575.0	349.1	*73.7*	259.0	935.0	371.6	87.2	253.0	782.0	**4.6**	1=2<3
	Cognitive Flexibility														
	% Errors compatible Block 1	1.7	*2.9*	0.0	16.7	2.1	*3.8*	0.0	33.3	1.2	2.2	0.0	13.3		
	% Errors compatible Block 3	8.8	*8.2*	0.0	43.3	7.7	*8.3*	0.0	50.0	8.6	6.9	0.0	40.0		
	Speed compatible Block 1 (in ms)	359.6	*92.7*	247.0	702.0	377.8	*139.9*	248.0	1460.0	375.0	105.3	239.0	856.0		
	Speed compatible Block 3 (in ms)	723.2	*270.6*	363.0	1731.0	727.8	*304.6*	307.0	1929.0	744.7	274.6	346.0	1828.0		
	DS max span backward	4.1	*1.3*	2.0	8.0	4.0	*1.4*	0.0	8.0	3.9	1.2	0.0	7.0		

Note. 1= probands; 2 = siblings; 3 = controls; SC = social cognition; LP = local processing; EF = executive function; N errors = number of errors; FI = Feature Integration task; fa = false alarms, mis = misses; DS = Digit Span, min = minimum observed score; max = maximum observed score; ns = non significant.
Significant findings are presented in **bold.**

prosody recognition (for children aged ≥ 9 years) and 10% for response inhibition. Missing data were replaced by means of Expectation Maximization (Tabachnick and Fidell, 2001). Analyses were carried out with and without expectation maximization, which revealed similar results. Results were therefore reported with missing data replaced. All analyses were carried out with (N=172) and without ASD affected siblings (N=145) and revealed similar results. Reported results include affected siblings. Raw means and standard deviations are presented in Table 2.

Crude comparisons between children with ASD (probands and affected siblings) and controls, corrected for age, sex and total estimated IQ, revealed a significant multivariate group effect (F (7, 203) = 3.14, p = .004), indicating that children with ASD performed generally worse on the tasks used in this study compared to healthy controls. Post hoc analyses revealed significant group differences on 5/7 cognitive measures (face recognition (F (1, 292) = 22.15, p <.001), facial emotion recognition (F (1, 292) = 6.11, p = .014), affective prosody recognition (F (1, 217) = 10.50, p = .001), response inhibition (F (1, 292) = 9.87, p = .002) and verbal working memory (F (1, 297) = 8.42, p = < .004)). A marginally significant effect was found for cognitive flexibility (F (1, 292) = 3.78, p = .076).

Interrelatedness of cognitive domains

Significant *positive* correlations with medium to large effect sizes were found within the SC domain, as well as between all tasks of the SC and EF domain, as is shown in Table 3. This suggests that a worse performance on SC was related to poorer EF skills. Within the EF domain, all three tasks were positively correlated with medium to large effect sizes. Furthermore, a small *negative* correlation was found between the local processing measure and facial emotion recognition in probands, indicating that poorer facial emotion recognition skills related to a more detailed focused (local) processing style in children with ASD. This correlation was not found for unaffected siblings or controls.

About half of the correlations remained significant after correction for age and estimated Total IQ, but effect sizes for these correlations became small to medium. Verbal working memory was mostly unrelated to other EF and SC tasks after correction for age and IQ. Four of the measures revealed significantly higher correlations in controls than in ASD probands. In one of the measures, the correlation was highest for ASD probands (facial emotion recognition-local processing). However, these differences became non-significant after controlling for age and IQ, see Table 3.

Familiality of the domains

Sibling correlations were calculated for all tasks with and without correction for the age and total IQ difference between probands and siblings (Table 4). Siblings resembled each other significantly on all SC and EF tasks (with sibling correlations ranging from .21- .71, Table 4) suggesting familiality for these tasks. One of the measures revealed

Table 3. Correlations between social cognition, central coherence en executive function measures with and without correction for age and estimated Total IQ separately for 140 ASD probands, 172 siblings and 127 controls

		Social Cognition (SC)			Local processing (weak CC)	Executive Function (EF)			Contrasts based on 2-tailed *p*values <.05 as calculated with Preacher's Correlation Test (2002)
		Face R	Facial ER	Prosody		Inhibition	Cogn. flex.	WM	
		rp[a] / rs[b] / rc[c]	rp / rs / rc	rp / rs / rc	rp / rs / rc	rp / rs / rc	rp / rs / rc	rp / rs / rc	
Without correction for age and IQ									
SC	Face R	1.00	**.73/.75/.78**[1]	**.35/.53/.67**[2]	-.09/-.16/-.04	**.66/.68/.63**	**.41/.32/.48**	**.46/.48/.49**	[1] rp<rs=rc [2] rp<rc
SC	Facial ER		1.00	**.34/.52/.69**[3]	**-.22**/-.15/-.06[4]	**.59/.61/.63**	**.45/.39/.57**[5]	**.33/.43/.45**	[3] rp<rs<rc [4] rp=rs>rc [5] rs<rc
SC	Prosody			1.00	.01/-.01/-.01	**.30/.49/.51**[6]	**.22/.24/.32**	**.30/.23/.33**	[6] rp<rc
Local processing					1.00	-.18/-.12/-.12	-.-.14/.00/.21	-.18/.08/-.07	
EF	Inhibition					1.00	**.51/.39/.44**	**.48/.39/.40**	
EF	Cogn. flex.						1.00	**.30/.27/.32**	
EF	WM							1.00	
After correction for age and IQ[d]									
SC	Face R	1.00	**.56/.40/.49**	.16**/.26/.36**	-.08/-.14/-.11	**.40/.33/**.20	**.24**/.10/.18	**.23/.**17/**.22**	
SC	Facial ER		1.00	.16**/.23/.43**	**-.24**/-.17/-.13	**.33/**.18/**.28**	**.30/.24/.35**	.07/.10/.17	
SC	Prosody			1.00	.04/.05/.03	.12/.21/.26	.06/.12 /.10	.10/.00/.19	
Local Processing					1.00	-.17/-.10/-.19	-.10/.04/.20	-.04/.16/-.12	
EF	Inhibition					1.00	**.35/.22/**.20	.16/.05-.13	
EF	Cogn.Flex						1.00	.11/.10/.09	
EF	WM							1.00	

Note. Face R = face recognition; Facial ER = facial emotion recognition; Prosody = emotional prosody; Cogn. flex. = cognitive flexibility; WM = verbal working memory

[a] rp= proband correlations

[b] rs = sibling correlations

[c] rc = control correlations

[d] partial correlations corrected for age and estimated Total IQ based on 319 probands and siblings from ASD families (for prosody, correlations were based on 256 children) and 123 control children (for prosody, correlations were based on 77 control children).

Significant correlations presented in **bold after correction for multiple testing (FDR).**

Table 4. Sibling correlations and sibling cross-correlations for ASD and control families for tasks of social cognition (SC), executive functioning (EF) and local processing style (weak CC) with and without correction for the age and total IQ difference between siblings, based on 146 ASD proband – sibling pairs and 55 control sibling pairs.

		Social Cognition (SC)			Local processing (weak CC)	Executive Function (EF)			Contrasts based on 2-tailed *p* values <.05 as calculated with the Preacher Correlation Test (2002)
		Face R	Facial ER	Prosody		Inhibition	Cogn. flex.	WM	
		r^a / $r_{control}$[b]	r / $r_{control}$	r / $r_{control}$	r / $r_{control}$	r / $r_{control}$	r / $r_{control}$	r / $r_{control}$	
Without correction for age and IQ difference									
	Face R	**.38/.55**	**.34/.53**	.16/**.58**[1]	-.05/-.14	**.27**/.26	**.25**/.19	**.34**/.28	[1] r < $r_{control}$
SC	Facial ER		**.30/.52**	.19/**.47**	-.06/-.03	.11/**.38**	.18/.23	**.23**/.19	
	Prosody[c]			**.35/.56**	-.17/-.13	.14/.24	-.01/.15	**.23**/.30	
Local processing					.07/.02	.00/-.12	.13/.08	.12/-.17	
	Inhibition					.18/**.35**	**.21**/.19	**.30/.40**	
EF	Cogn. flex.						**.21**/.28	.18/.10	
	WM							**.33/.32**	
After correction for age and IQ difference[d]									
	Face R	**.51/.71**	**.47/.63**	**.30/.55**	-.06/-.03	**.36/.44**	**.22/.39**	**.29/.34**	
SC	Facial ER		**.44/.62**	.23/**.42**	-.08/.20	.11/**.58**[2]	.15/**.45**	.16/.28	[2] r < $r_{control}$
	Prosody			**.38/.64**	-.20/.07	.14/**.52**[3]	-.12/.12	.19/**.42**	[3] r < $r_{control}$
Local Processing					.08/.08	.01/-.15	.14/.12	.17/-.17	
	Inhibition					**.33/.60**[4]	**.21/.41**	**.26/.57**[5]	[4,5] r < $r_{control}$
EF	Cogn.Flex						**.20/.35**	.18/.04	
	WM							**.36**/.28	

Note. Face R = face recognition; Facial ER = facial emotion recognition; Prosody = emotional prosody; Cogn. flex. = cognitive flexibility; WM = verbal working memory
Underlined are sibling correlations.

[a] r = correlation coefficient between ASD probands and their siblings

[b] $r_{controls}$ = correlation coefficient between control sibling pairs

[c] for prosody, correlations are based on 133 ASD probands-sibling pairs and 51 control sibling pairs

[c] partial sibling cross-correlations corrected for age and estimated Total IQ based on 128 ASD probands-sibling pairs and 47 control sibling pairs (for prosody: correlations are based on 95 ASD proband-sibling pairs and 25 control sibling pairs)

Significant correlations presented in **bold after correction for multiple testing (FDR).**

higher correlations in control sibling pairs, than in proband-sibling pairs (inhibition). No sibling correlations were found for the local processing task.

Co-segregation of SC, EF and local processing style (weak CC)

In order to examine whether the domains co-segregate in ASD families, sibling cross-correlation (corrected for the age and IQ difference between probands and sibling) were calculated. All social cognition tasks were significantly cross-related between siblings within the SC domain, and a few significant positive sibling cross-correlations were also found between SC tasks (mostly face recognition and facial emotion recognition) and some EF tasks, indicating that poorer SC skills in probands were related to poorer EF skills in their siblings, and vice versa. These correlations became more pronounced (with medium to large effect sizes) after correction for age and IQ difference between siblings. A few significant sibling cross-correlations of small to medium effect size were found between EF tasks within the EF domain. No sibling cross-correlations were found between local processing on the one hand and SC and EF tasks on the other hand, indicating that poorer SC and/or EF skills in probands were unrelated to processing style in his or her sibling. In five of the measures, higher correlations in control sibling pairs compared to proband-sibling pairs were revealed (Table 4).

To further examine whether SC, EF and local processing style co-segregated within families, we examined whether we could predict the cognitive performance of the siblings, based on the SC and EF component score and the local processing score of their ASD proband/control sibling. Results showed that the performance of probands on SC tasks could significantly predict the performance of their siblings on SC as well as

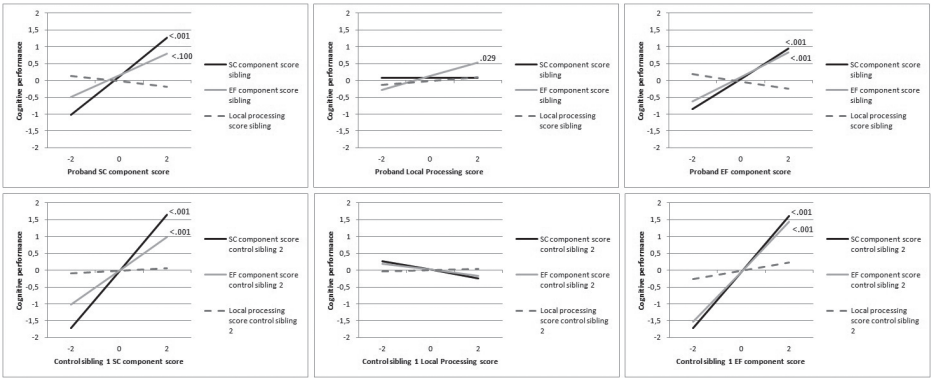

Figure 2 Illustration of the correlations between the cognitive performances of ASD probands/controls and their siblings (z-scores). Siblings' SC, EF and local processing composite scores as a function of the same composite scores in probands/controls.

Note. Higher z-score indicates better performance and a more local (i.e. detailed focused) processing style. The performance of probands grouped on either SC and EF performance could significantly predict the performance of their siblings on both SC and EF composite scores.

EF (all *p*-values < .001). That is, if the proband performed poorly on SC, his or her sibling would perform poorly on SC, and also on EF tasks. Vice versa, probands' EF significantly predicted siblings' EF and SC performances (both *p*-values < .001), see Figure 2. Similar results were found for control sibling pairs. In ASD families only, the local processing score of probands could significantly predict their siblings' EF performance ($p = .029$).

DISCUSSION

The current study set out to examine whether SC (face recognition, facial emotion recognition and emotional prosody), EF (inhibition, cognitive flexibility and verbal working memory) and local processing style (weak CC) segregate independently within ASD. Based on Happé and Frith's (2008) theory, we would have expected independent segregation as reflected by the absence of significant correlations between social cognition, executive function and local processing tasks and no significant cross-correlations between probands and their siblings. This was not what we found. We found that SC and EF tend to co-segregate together. These findings contrast the majority of studies that argue that cognitive deficits tend to decouple and segregate independently in ASD (Happe and Ronald, 2008, Pellicano *et al.*, 2006). Particularly SC and EF (mostly inhibition and cognitive flexibility) appeared strongly interrelated, both within children and within families. This was true for both ASD probands and their siblings and for the control children. All sibling cross-correlations between SC tasks reached significance with correlations of medium size, suggesting that performances in these functions (face and facial emotion recognition and affective prosody) partly originate from the shared familial sources. The same holds true for inhibition, cognitive flexibility and verbal working memory suggesting that these functions have similar familial underpinnings, as has been reported before in ADHD (Rommelse *et al.*, 2008). Furthermore, the majority of sibling cross-correlations between SC and EF were significantly correlated. That is, SC in probands was related to EF in their siblings and vice versa. The sibling (cross) correlations became even more pronounced when variance attributed to differences in age and intellectual ability was controlled for. The interdependence of both domains was further underlined by the finding that poorer performance in siblings on both domains could be predicted by the performance of the proband to which they were familially related. This suggests that especially SC and EF may be fruitful targets in future family studies of the genetic contribution to ASD. Although correlations between SC and EF have been reported before in autistic individuals (Goussé *et al.*, 2009, Ozonoff *et al.*, 1991, Pellicano, 2007, 2010, Pellicano *et al.*, 2006) and typically developing children (Carlson *et al.*, 2004, Carlson and Moses, 2001, Carlson *et al.*, 2002), our study is the first to address the interrelatedness of these two domains in autistic children *and* their (mostly

unaffected) siblings by examining cross-correlations. We build on the studies of Losh and colleagues (2009) and Nydén and colleagues (2011) by including a larger sample of intact biological ASD (and control) sibling pairs and examining family correlations both within *and* between cognitive domains. Our findings demonstrate that the cognitive performance of children with ASD and their first degree relatives (siblings) was not only related within (Nydén *et al.*, 2011), but also between cognitive domains. These findings contrasts Losh and colleagues (2009), who did not report significant associations between children with ASD and their first degree relatives. However, the authors raised the possibility that the lack of power due to the small number of intact families in their study could explain the absence of significant correlations. Another factor that may play a role is that we tested siblings, whereas Losh and colleagues (2009) tested parents of children with ASD. All in all, our results add to knowledge that the two domains are not only interrelated within children, but are also likely influenced by shared familial factors as reflected by significant sibling cross-correlations. Furthermore, most studies examining the relationship between SC and EF have reported findings on the interrelatedness of ToM abilities with various EF functions. Our results expand this relationship between SC and EF to measures of face and (facial and prosodic) emotion recognition as well.

In contrast to the above reported results, local processing style (weak CC) appeared relatively independent from both other domains. With the exception of a small correlation between local processing style and SC (facial emotion recognition) in ASD probands only, no evidence for co-segregation of local processing with the other domains was found. Moreover, local processing performance itself appeared not to be familial, as indicated by a non-significant sibling correlation. These findings suggest that a local processing style might be a relatively independent atypicality in ASD with generally different underlying factors (genetic and environmental) than the other two domains and may be related to etiological factors unique to the affected child not shared by unaffected family members. These findings corroborate with previous findings that favor the current notion that weak CC is dissociable from SC and EF (Happe and Ronald, 2008, Morgan *et al.*, 2003), but contrast the findings of Jarrold and colleagues (2000). Given that we only administered one task to reflect local processing style which was not convincingly associated with ASD in this sample, and the validity of the weak CC construct has been proven to be a complex one (Pellicano *et al.*, 2005, Pellicano *et al.*, 2006); our results need replication before firm conclusions can be drawn on the co-segregation of weak CC, SC and EF.

The finding that the pattern of co-segregation between SC and EF was highly similar within ASD and control families indicates that the reported co-segregation of cognitive functions associated with ASD is not specific to ASD. In other words, it was previously hypothesized that the co-occurrence of cognitive deficits in ASD may be explained by patients being at the extreme end of the ASD continuum and therefore by chance more

likely to be impaired in all three domains, but our data suggests otherwise. Furthermore, the tendency for siblings to display a similar cognitive profile as their affected brother or sister, regardless of their own affected status, puts them at risk for developing impairments in one of multiple domains, even in the absence of (milder) ASD features. This highlights the importance of following siblings over time.

A number of limitations need to be considered. First, although effort was made to include several tasks tapping the domains of SC and EF, we were not able to assess *all* aspects of these domains. For example, theory of mind has not been accessed here. It may be possible that our findings do not generalize across the entire SC spectrum, but only relate to face and emotion recognition. However, given that theory of mind relies greatly on the ability to perceive emotions, it is likely that poor performances on emotion recognition tasks relate to poorer performances on theory of mind tasks (Buitelaar and van der Wees, 1997). Furthermore, several important aspects of EF were not assessed here, such as planning and fluency. These functions, in addition to inhibition, cognitive flexibility and working memory, have been proposed to be key domains within EF and impairments in these domains have been reported in individuals with ASD (Geurts *et al.*, 2004, Hill, 2004a, Hill, 2004b). It would be interesting to examine whether deficits in planning or fluency also co-segregate with other EF functions or SC skills in ASD. This might not necessarily be so given that EF refers to a broad range of related, but distinct high-level cognitive capacities. Therefore, we must be cautious when interpreting the co-segregation between SC and EF as reflective of the entire EF domain. Second, only one task was administered reflecting local processing style. Therefore, caution is warranted when interpreting the results of this task as reflective of weak CC. The validity of the CC construct has been proven to be a complex one (see Pellicano *et al.*, 2006), and future studies would do well by including several tasks designed to favor a detail-focused, local processing style in order to obtain a more comprehensive measure of weak CC. Third, our sample consisted of high-functioning children with ASD which might limit the generalizability of our findings to the broad range of ASD. It might be worthwhile to extend these results to lower-functioning individuals, where the impairments on the various cognitive domains might be more pronounced, perhaps resulting in a different pattern of co-segregation. Previous studies have indeed shown that low- and high functioning individuals with autism demonstrate distinct patterns of EF impairment (Turner, 1997), which could result in a distinct pattern of between-tasks or between-domain correlations. Fourth, error percentage analyses revealed a ceiling effect in the cognitive flexibility task (<10% errors) and the task was only marginally significantly related to ASD in this sample. This suggests that the task was probably too easy due to the predictable nature of the task (the problem solving rule in the flexibility task was known to the subject and constant during the test). However, this effect was not mediated by the presence of ASD (e.g. error percentage was similar for ASD pro-

bands, their (mostly unaffected) siblings and controls and it could not account for the observed overlap between SC and other EF tasks). Last, this study did not include the cognitive performances of parents of autistic individuals, which would have made an interesting contribution to the study. By including parental information, more insight in the parent-offspring (cross) relationships of SC, CC and EF could be gained.

Taken together, compelling evidence was found for co-segregation of SC and EF, but not local processing style, within ASD, indicating that particularly SC and EF may be fruitful domains in future family studies of the genetic contribution to ASD. Given that SC and EF are both strongly related to ASD, using the underlying shared variance of both constructs in genetic research may increase the power for detecting susceptibility genes for ASD (Sham *et al.*, 2000). This approach was recently successfully applied in ADHD research (Frazier-Wood *et al.*, 2012) and is expected to offer great potential for future studies in ASD. Furthermore, our results have shown that performances on SC and EF tasks are highly correlated within probands, suggesting that probands who perform worse on one domain are likely to display deficits in the other domain. Moreover, siblings of autistic individuals (regardless of their affected status) tend to display a similar cognitive profile as their affected brother or sister and are therefore at risk for impairments on SC and EF as well. This finding highlights the importance of following sibling risk groups over time.

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CHAPTER 3

Recognition of facial emotion and affective prosody in children with ASD (+ADHD) and their unaffected siblings

Based on:

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ABSTRACT

Autism is a highly heritable and clinically heterogeneous neuropsychiatric disorder that frequently co-occurs with other psychopathologies, such as attention-deficit/hyperactivity disorder (ADHD). An approach to parse heterogeneity is by forming more homogeneous subgroups of autism spectrum disorder (ASD) patients based on their underlying, heritable cognitive vulnerabilities (endophenotypes). Emotion recognition is a likely endophenotypic candidate for ASD and possibly for ADHD. Therefore, this study aimed to examine whether emotion recognition is a viable endophenotypic candidate for ASD and to assess the impact of comorbid ADHD in this context. A total of 90 children with ASD (43 with and 47 without ADHD), 79 ASD unaffected siblings and 139 controls aged 6-13 years, were included to test recognition of facial emotion and affective prosody. Our results revealed that the recognition of both facial emotion and affective prosody was impaired in children with ASD and aggravated by the presence of ADHD. The latter could only be partly explained by typical ADHD cognitive deficits, such as inhibitory and attentional problems. The performance of unaffected siblings could overall be considered at an intermediate level, performing somewhat worse than the controls and better than the ASD probands. Our findings suggest that emotion recognition might be a viable endophenotype in ASD and a fruitful target in future family studies of the genetic contribution to ASD and comorbid ADHD. Furthermore, our results suggest that children with comorbid ASD and ADHD are at highest risk for emotion recognition problems.

INTRODUCTION

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders, characterized by impairments in interaction, communication and restricted, stereotyped and repetitive behaviour (APA, 2000). Twin studies have demonstrated a high heritability for ASD (> 90%) (Freitag, 2007) and the search for potentially ASD risk genes has become a fast moving area of research. Despite this high heritability, the identification of genes linked to ASD has been a difficult endeavour (Neves *et al.*, 2011). This might be due, among others, to the clinical and etiological heterogeneity of ASD. ASD presents with a wide range of symptoms and symptom severities, associated cognitive deficits and underlying etiological factors (Pelphrey *et al.*, 2011). An approach to parse heterogeneity is to create homogeneous subgroups of ASD patients based on their underlying endophenotypes (Gottesman and Gould, 2003, Wang *et al.*, 2012). Endophenotypes are defined as heritable vulnerability traits that heighten the risk for developing a disorder (Gottesman and Gould, 2003). Endophenotypes offer a simplified approach to dissect complex traits by reducing heterogeneity (Wang *et al.*, 2012) and as such may boost the power for genetic analyses, as well as shed light on the functional outcomes of genes (Almasy and Blangero, 2001, Gottesman and Gould, 2003, Viding and Blakemore, 2007). Homogeneous subgroups of patients based on their endophenotypes may facilitate our understanding of the involved biological processes (Wang *et al.*, 2012). A common approach in endophenotype research is the affected-unaffected sibling design (Almasy and Blangero, 2001, Waldman, 2005). This design is particularly useful under the assumption that psychiatric disorders are caused by a complex interplay between multiple susceptibility genes and environmental factors (i.e. polygenetic or multifactorial heritability model) (Gottesman and Gould, 2003). If a targeted marker is indeed a useful endophenotype, abnormalities should also be present in unaffected siblings, because they are likely to carry susceptibility genes for the disorder (given that they share on average 50% of their genes with their affected brother or sister) and share common environmental influences. This affected-unaffected sibling design has led to many fruitful results in for instance attention-deficit/hyperactivity disorder (ADHD) and schizophrenia cognitive studies, firmly demonstrating subthreshold impairments in unaffected relatives on cognitive functions and brain morphology (Allen *et al.*, 2009, Hoff *et al.*, 2005, Rommelse *et al.*, 2011) and their utility in molecular genetics research (Chen *et al.*, 2012, Doyle *et al.*, 2008, Rommelse *et al.*, 2008). Despite these promising results, a similar strategy has less often been applied in autism research (Rommelse *et al.*, 2011, Tierney *et al.*, 2012).

One of the most promising risk markers that may be present in unaffected relatives of individuals with ASD is impaired *social cognition* (Soorya and Halpern, 2009). Social cognition refers to the interdependence of cognition and social behavior and is substan-

tially heritable (Anokhin *et al.*, 2010). It relies upon a specific network of brain regions, including the medial frontal cortex, temporoparietal junction, superior temporal sulcus and temporal poles (Anokhin *et al.*, 2010). An important aspect of social cognition is *emotion recognition*, which refers to the capacity to perceive and understand affective expressions. Problems in emotion understanding are a core diagnostic feature in ASD (Harms *et al.*, 2010). Research on emotion recognition has traditionally focused on the visual aspect of emotion recognition (the identification of facial expressions) and most studies have shown that children and adolescents with ASD display deficits in their ability to accurately recognize and understand facial expressions of emotions (Charbonneau *et al.*, 2013, Downs and Smith, 2004, Harms *et al.*, 2010, Sinzig *et al.*, 2008, Uljarevic and Hamilton, 2012). Similar impairments have been reported in unaffected first-degree relatives as well (Losh *et al.*, 2009, Neves *et al.*, 2011). Parents and siblings of autistic children showed a remarkable reduction in processing the eye region in faces, a pattern that was previously reported to occur in autism (Adolphs *et al.*, 2008). However, other studies have reported normal abilities in emotion recognition in autistic children (Buitelaar *et al.*, 1999, Castelli, 2005), leaving the status of emotion recognition in ASD uncertain. In a recent meta-analysis, it was reported that there is an emotion recognition difficulty in ASD and that these difficulties might be specifically related to particular emotions or stimuli, rather than being a global emotion recognition deficit that is primary and universal in ASD (Uljarevic and Hamilton, 2012). Much less is known about the auditory aspects of emotion recognition, named affective prosody recognition. Affective prosody plays an important role in the understanding and verbal expression of affect (McCann and Peppe, 2003). Difficulties with using and understanding affective prosody could have implications for recognizing emotions in other persons. Even though impaired prosody is considered a prime characteristic of ASD, relatively little research has been done in this area. Whereas some studies reported impaired affective prosody recognition in ASD (e.g. autistic participants had more difficulty identifying emotional expressions from prosody and were less able to discriminate between vocal emotional expressions) (Charbonneau *et al.*, 2013, Golan *et al.*, 2007, Korpilahti *et al.*, 2007, Lindner and Rosen, 2006, Philip *et al.*, 2010), others did not (Baker *et al.*, 2010, Grossman *et al.*, 2010, Heikkinen *et al.*, 2009, Jones *et al.*, 2011), making it difficult to draw firm conclusions. One study reported on fathers of autistic children demonstrating atypical neural responses to affective prosody (Korpilahti *et al.*, 2007), but more research is needed to examine if affective prosody recognition is impaired in relatives of ASD patients.

Another main cause of heterogeneity in ASD is the co-occurrence with other disorders as psychopathologies, such as ADHD (Baird *et al.*, 2008, de Bruin *et al.*, 2007). Particularly the ADHD characteristic symptoms of increased inattention, hyperactivity and impulsivity appear frequently present in patients with ASD with comorbidity estimates ranging from 30-80% (de Bruin *et al.*, 2007, Leyfer *et al.*, 2006, Rommelse *et al.*, 2010) and

an increased incidence of ADHD was reported in (unaffected) family members of ASD patients and vice versa (Nijmeijer *et al.*, 2009). Although research into social cognition impairments in ADHD is relatively sparse, difficulties in recognizing facial expressions (Corbett and Glidden, 2000, Da Fonseca *et al.*, 2009, Uekermann *et al.*, 2010) and affective prosody (Corbett and Glidden, 2000, Shapiro *et al.*, 1993) have also been described in children with ADHD. Children with ADHD showed mild-to-moderate deficits in the perception of affect (through facial expressions and speech intonation) and were less able to identify emotions using contextual information. Moreover, some studies reported more severe social cognition deficits in the ASD+ADHD group (e.g. slower and less accurate) compared to the ASD only group (Sinzig *et al.*, 2008, Van der Meer *et al.*, 2012). This suggests that we should take comorbid ADHD symptoms into account and assess the impact of these symptoms when studying emotion recognition in ASD.

The present study therefore aimed to examine whether recognition of facial emotion and affective prosody are viable endophenotypic candidates for ASD and to assess the impact of comorbid ADHD symptoms in this context. First, we hypothesized that if emotion recognition would indeed be an endophenotype in ASD, probands would perform worse than controls on both emotion recognition tasks and unaffected siblings would form an intermediate group based on their performance. Second, we hypothesized that probands with both ASD and comorbid ADHD would be more impaired in emotion recognition than children with only ASD. If children with ASD and comorbid ADHD would indeed be more impaired on emotion recognition, it would raise the question of whether children with ASD and comorbid ADHD have more difficulties in emotion recognition per se or perhaps secondary as a consequence of typical ADHD cognitive problems, such as impaired reaction time speed, inhibition and attention. Therefore, we included some general neuropsychological measures as covariates in the analyses, to better understand the mechanisms of comorbid ADHD on emotion recognition.

METHOD

Participants

Families with at least one child with a clinical diagnosis of Autism Spectrum Disorder (Autism, Asperger's Syndrome (AS) or PDD-NOS; diagnosis mostly based on Autism Diagnostic Interview - Revised (ADI-R) and Autism Diagnostic Observation Scale (ADOS) assessment) (APA, 2000) (aged 2 - 20 years) and at least one biological sibling (regardless of age and possible ASD status) were recruited in order to participate in the Biological Origins of Autism (BOA) study at Karakter Child and Adolescent Psychiatry University Center Nijmegen, the Netherlands. The BOA project aims to examine the genetic, bio-

chemical and cognitive origins of ASD and study the overlap between ASD and ADHD on these levels. Control children were recruited from a random population cohort study (School kids Project of Interrelating DNA and Endophenotype Research; SPIDER, 32.4%) and via information leaflets that were send to families living in the same geographical regions as the participating ASD families as part of the BOA project (67.6%). Selected controls were required to have no formal or suspected ASD or ADHD diagnosis.

A subsample of 90 children with ASD (probands), 79 ASD unaffected siblings and 139 controls were included in the current study. Data from these children was collected between December 2008 and July 2012. All selected children were between the ages of 6 and 13 and were of European Caucasian descent. Participants were excluded if they had an IQ ≤ 60, a diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome. The study has been approved by the local medical ethics board and written informed consent was obtained from parents and children over the age of 12 before testing.

Both the children already clinically diagnosed with ASD, their siblings and the control children were screened for the presence of ASD and ADHD symptoms using the parent Social Communication Questionnaire (SCQ) (Berument *et al.*, 1999) and the parent and teacher Conners Rating Scales Revised (CPRS; CTRS) (Conners, 1996) respectively. A raw score of ≥ 10 on the SCQ and T-scores ≥ 63 on the Conners' DSM-IV Inattention, Hyperactivity-Impulsivity or Combined scales were considered as clinical. A lower cutoff was chosen for the parent reported SCQ to avoid false negatives in their undiagnosed offspring (Corsello *et al.*, 2007, Eaves *et al.*, 2006, van Steijn *et al.*, 2012). For all children scoring above cut-off on the SCQ, a formal diagnosis of ASD was made by a certified clinician using the Autism Diagnostic Interview-Revised (ADI-R) (Lord *et al.*, 1994). For all children scoring clinically on at least one of the DSM-IV ADHD scales, a semi-structured, standardized, investigator-based interview that covers the DSM-IV symptoms of ADHD (Parental Account of Childhood Symptoms; PACS) (Taylor *et al.*, 1991) was administered. Thereafter, a standardized algorithm was applied using the scores of the PACS and the teacher version of the CRS-R to construct a formal diagnosis of ADHD (Rommelse *et al.*, 2007). The protocol for screening and measuring ADHD was similar to the protocol used in the International Multicenter ADHD Genetics (IMAGE) study, fully described elsewhere (Brookes *et al.*, 2006). Control children were required to obtain non-clinical (i.e. a raw score on the SCQ of < 10 and T-score < 63 on the Conners' DSM-IV scales) scores in order to be accepted in the current study.

Depending on the child's age, full-scale IQ was estimated by four subtests of the Dutch version of the Wechsler Intelligence Scale for Children (WISC-III): Similarities, Vocabulary, Block design and Picture completion (Wechsler, 2002). For 14.6% (N=45) of control children (SPIDER cohort), the subtest Arithmetic was administered instead of the subtest Vocabulary. These selected WISC-III subtests are known to correlate between

Table 1. Sample characteristics, separately for the facial emotion recognition (FER) and affective prosody recognition (APR) samples.

	1.ASD affected probands with ADHD		2. ASD affected probands without ADHD		3. ASD unaffected siblings		4.Controls		*F*		Contrasts based on *p* values of <.001	
	FER	APR	FER	APR	FER	APR	FER	APR				
	N = 43	N=30	N = 47	N=36	N = 79	N=49	N = 139	N=72	FER	APR	FER	APR
	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)				
Age in years	10.5 (2.0)	11.5 (1.5)	10.7 (2.1)	11.6 (1.2)	9.7 (2.0)	10.9 (1.3)	9.2 (1.9)	10.7 (1.1)	9.1	5.6	1=2>3=4	1=2>3=4
% Male	81.4	83.3	80.6	80.6	43.0	40.8	44.6	45.8	13.2	9.7	1=2>3=4	1=2>3=4
% Right handed	79.1	76.7	80.6	83.3	79.7	77.6	89.9	87.5	ns.	ns.		
Estimated full scale IQ	103.3(13.3)	101.7 (11.9)	103.1 (14.1)	103.4 (14.7)	106.4 (12.5)	106.2 (12.3)	107.4 (11.7)	106.1 (11.3)	ns.	ns.		
Estimated VIQ	100.8 (15.5)	100.4 (15.2)	102.3 (15.9)	102.0 (17.1)	107.6 (15.3)	107.6 (13.8)	108.8 (13.1)	108.2 (12.5)	4.7	3.2	1=2<3=4	1=2<3=4
Estimated PIQ	106.2 (16.6)	103.3 (13.8)	104.2 (17.9)	105.2 (17.9)	105.4 (16.5)	105.2 (16.8)	106.4 (14.7)	104.1 (14.6)	ns.	ns.		
SCQ parents Total	19.7 (7.4)	19.4 (8.6)	19.4 (6.7)	19.8 (6.2)	4.2 (4.8)	4.3 (5.1)	3.4 (2.9)	3.5 (2.8)	221.3	123.2	1=2>3=4	1=2>3=4
Conners' parents												
Oppositional	62.5 (12.7)	60.8 (13.2)	59.4 (14.1)	56.8 (12.2)	53.3 (12.0)	53.0 (12.0)	46.2 (6.2)	46.1 (6.0)	28.1	12.8	1=2>3>4	1=2>4,1>3,2=3
DSM-IV Inattentive	66.7 (8.4)	66.7 (9.0)	59.9 (10.0)	58.2 (9.6)	53.8 (13.2)	52.2 (12.2)	47.9 (9.0)	47.0 (7.1)	42.9	34.2	1>2>3>4	1>2>3>4
DSM-IV Hyp-Imp	71.6 (9.9)	71.2 (10.3)	62.1 (15.9)	59.1 (11.9)	54.0 (12.0)	53.2 (11.6)	49.1 (7.9)	48.1 (6.1)	61.5	44.5	1>2>3>4	1>2>3>4
DSM-IV Combined	70.5 (9.0)	70.4 (9.3)	62.0 (11.5)	59.6 (10.8)	54.1 (12.6)	52.9 (11.9)	48.3 (8.5)	47.4 (6.5)	60.4	47.0	1>2>3>4	1>2>3>4
Fulfilled criteria for comorbid ADHD (%)	100	100	0	0	15.2	10.2	-	-				
Inattentive	37.2	46.7	-	-	6.3	6.1	-	-				
Hyp-Imp	23.3	13.3	-	-	2.6	0	-	-				
Combined	39.5	40.0	-	-	6.3	4.1	-	-				
Medication use during testing (%)	19.1	43.3	34.9	22.2	2.5	4.1	-	-				

Note. ASD = Autism Spectrum Disorders; FER = facial emotion recognition; APR = affective prosody recognition; VIQ = Verbal IQ; PIQ = Performal IQ; SCQ = Social Communication Questionnaire (total score); ADHD = Attention-Deficit/Hyperactivity Disorder; Hyp-Imp = DSM-IV ADHD hyperactive/impulsive subtype; ns = non-significant

.90-.95 with the Full-scale IQ (Groth-Marnat, 1997). Two tasks were administered to test emotion recognition; 1) a visual task assessing the ability to recognize emotional facial expressions and 2) an auditory task to assess the ability to recognize emotions 'in voices' (affective prosody). The affective prosody recognition task was not administered to children younger than 9 years of age. Therefore, affective prosody recognition data was based on 66 ASD affected probands (30 with ADHD and 36 without ADHD), 49 ASD unaffected siblings and 72 controls. See Table 1 for sample characteristics.

Materials

Emotion recognition tasks

Facial emotion recognition

Stimuli consisted of digitized, high quality color photographs of a human face with a distinct affective expression presented on a computer screen (see Figure 1). Each photograph presented a face with either a neutral expression or one of eight different types of emotion (happiness, sadness, anger, fear, disgust, surprise, shame, contempt) and children were asked to judge whether the presented photograph showed the target emotion or not by clicking a mouse button (De Sonneville *et al.*, 2002). For the current study four target emotions were selected: happiness, sadness, anger and fear. If the presented photograph showed the target emotion the child was asked to click the 'yes-button' (right button for right-handed children, and left button for left-handed children). 'No-responses' were given by clicking the 'no-button' (left button for right-handed children and right button for left-handed children). The photograph remained on the screen until the subject pressed the button. Each emotion condition consisted of 40 trails; half of which were the target emotion (requiring a 'yes' response) and half of which were a random selection of other emotions (requiring a 'no' response). Children were required to respond within 200-3,000 ms. After responding the next photograph appeared after a 1,000 ms interval. The sequence of target and nontarget stimuli was

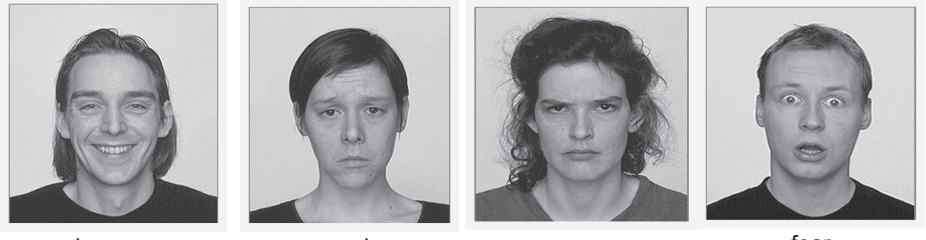

Figure 1 Examples of stimuli in the facial emotion recognition task

randomly assigned. Dependent variables were measures of speed (mean reaction time) and accuracy (percentage of errors) for the four emotions separately.

Affective prosody recognition

Stimulus material, designed by Vingerhoets et al. (2003), was adopted in the Amsterdam Neuropsychological Tests program (De Sonneville, 2005, Vingerhoets *et al.*, 2003) and consisted of spoken sentences with a neutral content, presented through a headphone. Sentences were spoken in a happy, sad, angry or frightened intonation. Children were asked to verbally identify the emotion with which the sentence was spoken. A typical sentence was for example: 'Put the plug in the wall socket' (presented in Dutch). Reaction times were recorded using a voice-key response (i.e. as soon as the child identified the expressed emotion in the tone of voice, the child was required to pronounce that particular emotion in a microphone that was connected to the computer). Subjects were required to respond within 300-6,000ms. Thereafter, the examiner registered the content of the response (type of emotion) the child identified by clicking the corresponding answer button. The next trial was presented 1,200ms after the examiner had registered the response. The complete task consisted of 48 trials (12 per emotion - 6 spoken by a man, 6 spoken by a woman), which were presented in randomized order. Missed trials were replaced automatically. Dependent variables were speed (mean reaction time) and accuracy (percentage of errors) for the four emotions separately.

General neuropsychological measures

Baseline Response Speed

This task was used to measure the speed and variability of motor output, comparable to a simple reaction time task (De Sonneville, 1999). When a fixation cross in the centre of a computer screen changed into a white square, children pressed a key as quickly as possible. To prevent anticipation strategies, the time interval between a response and the emergence of the next square varied randomly between 500 and 2500 ms. Response speed (mean reaction time) was used as covariate in the analyses.

Response inhibition

The Response Organisation Objects (ROO) was used to measure response inhibition (De Sonneville, 1999, Van der Meer *et al.*, 2012). The stimulus was a figure of a colored ball that was presented to the left or right of a fixation cross. Two parts were administrated. In Part 1, baseline speed and accuracy were measured. Children were instructed to click the mouse button that corresponded with the orientation of the stimulus, as soon as they noticed a green ball on the left or the right of a fixation cross. In part 2, the ball was colored red, and children had to click the mouse button on the opposite side. Motor

inhibition was calculated as the difference in mean reaction time and percentage of errors between blocks one and two. Both measures were included as covariates in the analyses.

Verbal Attention

The forward condition of the Digit Span task of the WISC-III (Wechsler, 2002) was used to obtain an indication of verbal attention (Van der Meer *et al.*, 2012). Children were instructed to repeat a sequence of verbally presented numbers. The difficulty level increased after each succeeded trial. The total number of correct sequences in identical order was included as covariate in the analyses.

Procedure

The tasks described were part of the broader neuropsychological assessment batteries used in the BOA and SPIDER projects. Children completed the battery in approximately two hours and the order of task administration was counterbalanced. Testing of the BOA cohort took place at Karakter Child and Adolescent Psychiatry University Center in Nijmegen, The Netherlands, and was conducted for all the children of the same family simultaneously. If possible, stimulants were discontinued for at least 24 hours and non-stimulants according to guidelines to allow for sufficient wash-out. Children were motivated with small breaks and received a reward after test administration. Control children were tested in a similar way at Karakter (BOA) or in a quiet room at their school (SPIDER).

Statistical analyses

A Van der Waerden transformation was used to normalize the dependent measures (Norusis, 1992). The normal scores were mirrored, so that the z-scores of all tasks would have the same meaning: lower z-scores indicated a worse performance (i.e. more errors and slower performance). In first instance, we examined the main effects of possible confounders (sex, total IQ, modality (two modalities: visual/auditory) and age (linear and curvilinear)) on performance on the two tasks. This was done within the control group to avoid dependency with the factor group. Furthermore, interactions between group and the confounders were examined to investigate whether effects of possible confounders were comparable across groups. Analyzing the possible confounding effect of modality was done in children ≥ 9 years of age, so that sample size and composition was comparable for the two tasks.

Group differences on facial emotion recognition and affective prosody were examined using linear mixed models with group (probands, unaffected siblings, and controls) as factor, age and total IQ as covariates, and family as random effect to account for within family correlation. Also, the interactions between group and age and group and total IQ

were implemented in the initial model. When the interactions were non-significant, they were dropped from the model. If the main effects of age and IQ were non-significant, they were dropped from the model as well. Group contrasts were calculated within the mixed model using pairwise comparisons with age, IQ and sex as covariates. The analyses were run separately for facial emotion and affective prosody recognition measures, a) with the four emotions as repeated measure, to examine whether an overall deficit in emotion recognition was present and b) separately for the four emotions (happiness, sadness, anger and fear), to examine post-hoc which emotions specifically were impaired. Subsequently, analyses were rerun, but with factor group defined as: ASD probands with ADHD versus ASD probands without ADHD versus controls, and including response inhibition speed and accuracy measures, verbal attention and baseline response speed as covariates, to investigate the effect of comorbid ADHD and typical ADHD cognitive deficits on emotion recognition. A False Discovery Rate (FDR) correction with a q-value setting of 0.05 was applied to control for multiple testing. All analyses were carried out in SPSS version 20.

RESULTS

Testing of the possible confounding effects of sex, IQ, modality and age

The percentage of missing data was random and less than 3% for accuracy and speed measures on the facial emotion recognition task, <4% for accuracy and speed measures for affective prosody recognition (for children aged 9-13 years) and <4% on response inhibition, verbal attention and baseline response speed. Missing data were replaced by means of Expectation Maximization (Tabachnick and Fidell, 2001). Analyses were carried out with and without expectation maximization, which revealed similar results. Results were therefore reported with missing data replaced. For 24 (26.7%) affected children and 2 (2.4%) ASD unaffected siblings, discontinuation of medication was neither possible nor desirable and 12 (15.2 %) ASD unaffected siblings fulfilled criteria for ADHD. Analyses were performed with and without including the data of these children, which revealed overall similar results. Results were therefore reported including these children.

Main effects of sex, IQ, modality and age

We tested for the effects of sex, IQ, modality and age on emotion recognition within the control group to avoid dependency with the factor group. Overall, no significant effects of sex were found for the speed and accuracy measures of facial emotion and affective prosody recognition, except for a significant sex effect on the visual recognition of fear (accuracy: F (1, 132.2) = 4.27, p = .041) and the auditory recognition of anger (speed: F (1, 64.2) = 4.92, p = .030) and fear (speed: F (1, 67.0) = 8.47, p = .005), with boys performing

less accurate or slower than girls. However, these effects became non-significant after FDR correction. For 8/16 analyses, significant effects of IQ were found that survived FDR correction. Significant IQ effects were found for accuracy on the visual recognition of sadness and fear (F (1, 128.9) = 6.31, p = .013 and F (1, 125.0) = 14.16, p <.001), and for speed on happiness and anger (F (1, 123.5) = 7.84, p = .006 and F (1, 131.8) = 9.22, p = .003) and for the auditory recognition of sadness (speed; F (1, 66.2) = 8.29, p = .005), anger (accuracy; F (1, 64.7) = 9.42, p = .013 and speed; F (1, 64.4) = 6.56, p = .003) and fear (F (1, 67.0) = 6.26, p = .015). Significant effects of modality were found for both accuracy (F (1, 69.5) = 229.4, p <.001) and speed measures (F (1, 70.3) = 310.5, p <.001). Age (in linear terms) had a strong main effect on all speed measures of facial emotion and affective prosody recognition (all p-values < .010) indicating that older children performed better than younger children. Furthermore, significant age effects were found for accuracy on the visual recognition of happiness (F (1, 138.9) = 4.94, p = .028), anger and fear (both p-values <.001) and on accuracy on the auditory recognition of anger (F (49.9) = 6.78, p = .012). Overall, no main effects of curvilinear age were present, except for a small significant effect for accuracy on the auditory recognition of anger (F (1, 129.0) = 4.40, p = .004), which did not survive FDR correction.

Interactions between group and possible confounders

Only 1/16 group*sex interactions had a p-value below .05 (speed of auditory recognition of happiness: F (2, 167.9) = 3.23, p = .042), which did not survive FDR correction. Only 2/16 group*IQ interactions had a p-value below .05 [accuracy of auditory recognition of happiness (F (2, 166.0) = 3.59, p = .030) and sadness (F (2, 167.9) = 4.73, p = .010)], which did not survive FDR correction. No significant group*modality analyses were found for accuracy (F (2, 182.1) = 2.24, p = .109) and speed (F (2, 183.8) = .81, p = .446). Only 2/32 group*age (linear or curvilinear) interactions had a p-value below .05 (facial recognition of happiness (linear: F (2, 303.9) = 4.25, p = .015; curvilinear: F (2, 289.5) = 3.87, p = .022)), which did not survive FDR correction.

Based on the results of these analyses, we decided to omit sex from further analyses, since no sex differences in the control group were found and sex had a similar effect on emotion recognition in the groups. Age and IQ and the interactions between age, IQ and group were included as covariates in the initial analyses, because of the strong age and IQ effects that were found for the majority of dependent measures. Facial emotion recognition and prosody were separately analyzed, because of the main effect of modality. Means and standard deviations of the unstandardized scores are presented in Table 2.

Table 2. Means, standard deviations and 95% confidence interval (lower-upper bound) of the untransformed task variables for ASD probands (with and without comorbid ADHD), their siblings and controls

		1.Probands				2.Siblings				3. Controls				Group contrasts based on *p* values <.05
		Mean	*sd*	95% confidence interval		Mean	*sd*	95% confidence interval		Mean	*sd*	95% confidence interval		
				Lower bound	Upper bound			Lower bound	Upper bound			Lower bound	Upper bound	
Facial Emotion Recognition (FER)														
	happiness	6.3	*0.7*	4.9	7.7	6.7	*0.7*	5.4	8.1	4.3	*0.6*	3.2	5.4	1=2>3
	sadness	20.8	*1.2*	18.3	23.2	19.6	*1.3*	17.1	22.1	18.4	*0.9*	16.4	20.3	1=2=3
Errors (%)	anger	17.5	*1.2*	15.2	19.8	15.0	*1.2*	12.6	17.4	14.3	*1.0*	12.3	16.3	1>2=3
	fear	17.4	*1.3*	15.1	20.4	14.7	*1.3*	12.1	17.4	15.2	*1.7*	13.1	17.3	1=2=3
	total	9.1	*0.7*	7.8	10.4	8.9	*0.7*	7.6	10.2	6.7	*0.5*	5.7	7.7	1=2>3
	happiness	914.9	*19.8*	876.0	953.9	895.4	*19.9*	856.2	934.5	847.3	*15.8*	816.2	878.4	1>2, 1>3, 2=3
	sadness	1210.8	*23.1*	1165.4	1256.3	1145.9	*23.6*	1094.5	1192.4	1129.6	*18.6*	1092.9	1166.2	1>2=3
Speed (in ms)	anger	1139.7	*21.4*	1097.6	1181.7	1097.9	*22.1*	1054.4	1141.4	1067.1	*17.9*	1031.8	1102.3	1>3, 1=2, 2=3
	fear	1161.2	*23.3*	1115.3	1207.2	1111.0	*23.5*	1064.7	1157.2	1072.8	*18.6*	1036.2	1109.5	1>3, 1=2, 2=3
	total	1036.6	*17.9*	1001.4	1071.8	1005.2	*18.1*	969.6	1040.8	964.7	*14.5*	936.1	993.3	1>3, 1=2, 2=3
Affective Prosody Recognition (APR)														
	happiness	22.6	*1.9*	18.7	26.4	24.1	*2.1*	20.1	28.2	22.6	*1.8*	19.0	26.2	1=2=3
	sadness	27.4	*2.7*	22.1	32.7	30.3	*2.8*	24.7	35.9	31.4	*2.5*	26.5	36.2	1<3, 1=2, 2=3
Errors (%)	anger	17.7	*1.8*	14.1	21.4	13.4	*1.9*	9.7	17.2	15.2	*1.7*	11.9	18.5	1<2, 1=3, 2=3
	fear	66.6	*2.2*	62.3	70.8	68.6	*2.3*	64.1	73.1	68.9	*2.0*	65.0	72.9	1=2=3
	total	31.7	*1.3*	29.3	34.2	29.6	*1.3*	27.0	32.2	30.0	*1.1*	27.7	32.3	1=2=3
	happiness	3623.9	*61.0*	3503.7	3744.1	3445.6	*64.5*	3318.6	3572.7	3382.9	*56.6*	3271.3	3494.5	1>2>=3
	sadness	4076.6	*93.6*	3892.1	4261.2	3958.1	*96.9*	3767.1	4149.1	3740.9	*84.8*	3573.7	3908.0	1>2=3
Speed (in ms)	anger	3290.3	*55.1*	3181.6	3399.0	3147.5	*57.7*	3033.8	3261.1	3009.1	*50.1*	2910.2	3108.0	1>3, 1=2, 2=3
	fear	3682.1	*89.7*	3505.4	3858.7	3559.9	*95.8*	3371.2	3748.5	3348.2	*81.7*	3187.2	3509.3	1>3, 1=2, 2>3
	total	3374.3	*52.1*	3279.6	3482.0	3214.4	*54.1*	3106.7	3322.2	3105.9	*47.6*	3012.1	3199.6	1>2=3

Note. ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder; 1= probands; 2 = siblings; 3 = controls; FER = facial emotion recognition; APR = affective prosody recognition; speed measures are presented in ms.

Group differences between ASD probands, unaffected siblings and controls

Facial emotion recognition

A significant group effect was found for **accuracy** (F (2, 181.5) = 3.21, p = .043). Pairwise comparisons revealed a significantly worse performance of probands compared to controls (p = .015). Analyses per emotion revealed a significantly worse performance of probands and unaffected siblings compared to controls on the recognition of happiness (p = .034 and p = .007, respectively). No group differences were found for accuracy on the other emotions. Furthermore, significant group effects were found for **speed** (F (2,182.8) = 4.27, p = .015). Analyses per emotion revealed significant differences between probands and controls on happiness (p = .01), sadness (p = .017), anger (p = .024) and fear (p = .006), with probands performing worse than controls. Unaffected siblings did not differ significantly from probands and controls, see Figure 2a.

Affective prosody recognition

A significant group effect was found for **accuracy** (F (2, 164.2) = 3.20, p = .043). Analyses per emotion revealed a significant group difference between probands and controls on the auditory recognition of sadness (p = .023) and between probands and unaffected siblings on the auditory recognition of anger (p = .022). Furthermore, a significant group*IQ interaction effect was found (F (2, 165.8) = 3.32, p = .038). Analyses revealed that IQ had no significant effect on accuracy in controls (F (1, 67.1) = .01, p = .91), but had large effects in ASD probands (F (1, 62.0) = 13.09, p = .001) and siblings (F (1, 68.0) = 273.4, p <.001), with children with higher IQ's performing better than children with lower IQ's. A significant group effect was found for **speed** (F (2, 179.1) = 9.56, p <.001). Analyses per emotion revealed significant differences between probands and controls on the recognition of happiness, sadness, anger and fear (all p-values <.004) and between unaffected siblings and controls on the recognition of fear (p = .01), see Figure 2b.

Group differences between ASD probands with ADHD, ASD probands without ADHD and controls

Facial emotion recognition

Overall, a significant group effect was found for **accuracy** (F (2, 194.8) = 4.35, p = .014). This group effect remained significant after correction for general cognitive measures and medication use. Pairwise comparisons revealed a significant difference between ASD probands with ADHD and controls (p = .004), but no significant difference between ASD probands with and without ADHD. Analyses per emotion revealed that ASD probands with ADHD performed worse on the recognition of happiness compared to controls (p = .012), but this effect became non-significant after controlling for medica-

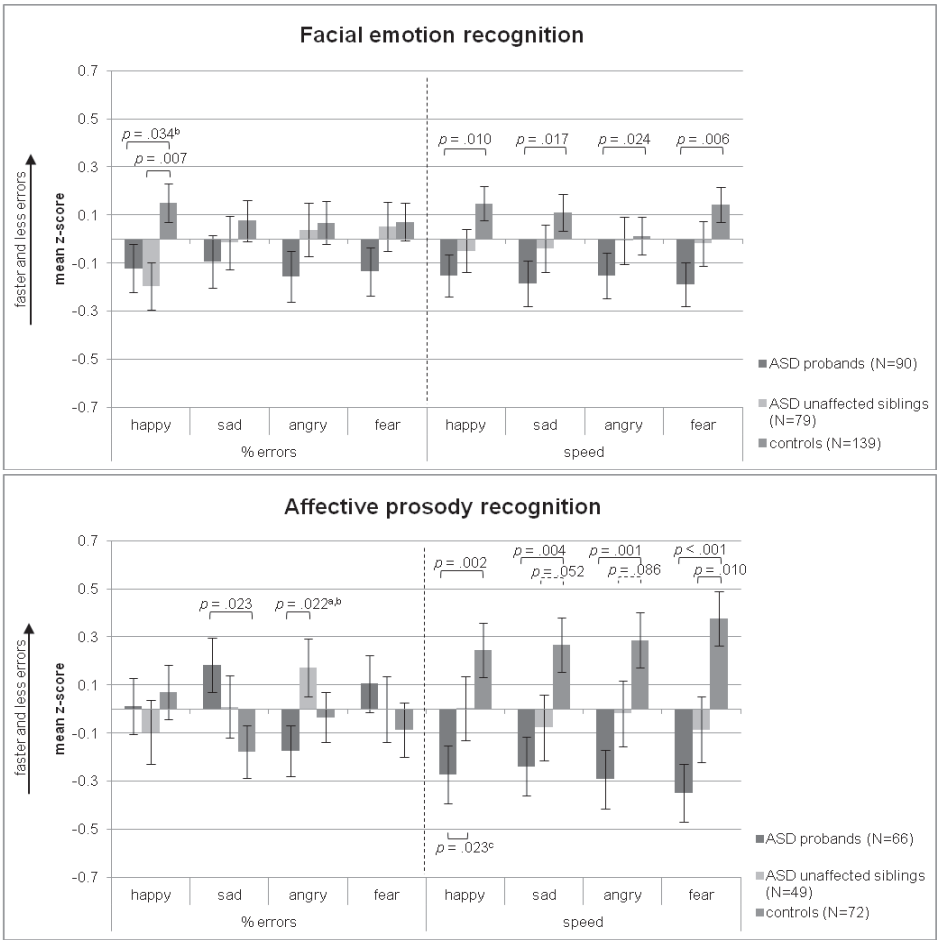

Figure 2a and 2b. Group differences between ASD probands, ASD unaffected siblings and controls on accuracy (% errors) and speed measures of facial emotion and affective prosody recognition, separate for the four emotions

Note. ASD = Autism Spectrum Disorders. The means are adjusted for the covariates age and IQ and error bars represent 1 standard error. A higher z-score indicates a faster or more accurate response. Results were based on 308 children (age 6-13 years; facial emotion recognition) and 187 children (age 9-13 years; affective prosody recognition). The dotted lines indicate marginally significant group differences

[a] Effect became non-significant when unaffected siblings with ADHD were excluded from the analyses

[b] Effect became non-significant after correction for medication use

[c] Significant group difference appeared after correction for medication use

tion use. Furthermore, a significant group effect was found for **speed** (F (2, 192.8) = 4.91, p = .008) with ASD probands with (p = .005) and without ADHD (p = .027) performing significantly worse than controls. Analyses per emotion revealed significant differences between ASD probands with ADHD and controls on happiness (p = .003) and fear (p =

.004) and between ASD probands without ADHD and controls on anger (p = .021) and fear (p = .042; did not survive FDR correction). These effects became non-significant after controlling for inhibition speed and baseline response speed, see Figure 3a. For means and standard deviations of the unstandardized scores of ASD probands with ADHD and ASD probands without ADHD, see Supplementary Table 1.

Affective prosody recognition

A significant effect of group (F (2, 134.0) = 3.12, p = .048) and a group*IQ interaction (F (2, 130.2) = 3.13, p = .047) were found for **accuracy,** which remained significant after correction for ADHD cognitive measures and medication use. Group differences were found between ASD probands with ADHD and controls on sadness (p =. 012) and between ASD probands with and without ADHD on happiness (p =.039). Further, analyses revealed that IQ had no significant effect on accuracy in controls (F (1, 67.1) = .01, p = .91), but had large effects in ASD probands with ADHD (F (1, 90.1) = 107.0, p <.001) and without ADHD (F (1, 27.0) = 11.89, p = .002), with probands with higher IQ's performing better than probands with lower IQ's. A significant group effect was found for **speed (**F (2, 121.0) = 9.01, p <.001). Analyses per emotion revealed group differences between ASD probands with ADHD and controls on all four emotions (all p-values <.016) and between ASD probands without ADHD and controls (all p-values <.042). However, the difference between ASD probands without ADHD and controls on anger did not survive FDR correction. The difference between ASD probands with ADHD and controls on happiness and anger became non-significant after controlling for medication use, see Figure 3b.

DISCUSSION

The present study aimed to examine whether recognition of facial emotion and affective prosody are viable endophenotypic candidates for ASD and to assess the impact of comorbid ADHD in this context. Studying these issues is of vital importance to enhance our understanding of the genetic underpinnings of ASD and comorbid ADHD, and the problems children with autism face in every day social life. The results for the ASD probands are consistent with previous research findings that the recognition of facial expressions and affective prosody are affected in children with ASD (Charbonneau *et al.*, 2013, Golan *et al.*, 2007, Harms *et al.*, 2010, Korpilahti *et al.*, 2007, Lindner and Rosen, 2006, Philip *et al.*, 2010, Sinzig *et al.*, 2008, Uljarevic and Hamilton, 2012). Children with ASD were overall slower and less accurate in identifying the emotional state of others compared to controls. The impairments were present in all four emotions, and were most pronounced for speed measures. This indicates that children with ASD are slower in identifying emotions, which might make it difficult for them to quickly adapt their

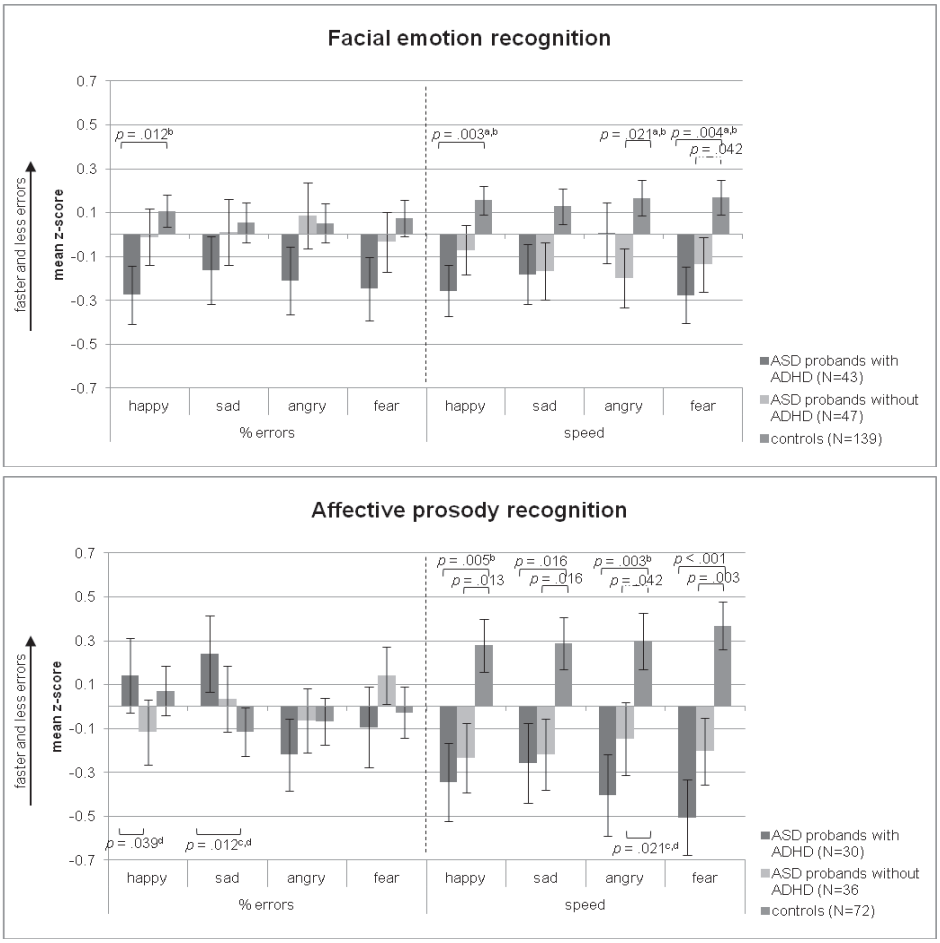

Figure 3a and 3b. Group differences between ASD probands with and without ADHD and controls on accuracy (% errors) and speed measures of facial emotion and affective prosody recognition, separate for the four emotions

Note. ASD = Autism Spectrum Disorders. The means are adjusted for the covariates age and IQ and error bars represent 1 standard error. A higher z-score indicates a faster or more accurate response. Results were based on 229 children (age 6-13 years; facial emotion recognition) and 138 children (age 9-13 years; affective prosody recognition). The dotted lines indicate group difference that did not survive FDR correction.

[a] Effect became non-significant after correction for ADHD cognitive measures.

[b] Effect became non-significant after correction for medication use

[c] Significant group difference appeared after correction for ADHD cognitive measures

[d] Significant group difference appeared after correction for medication use

behavior adequately in social settings, for example responding appropriately to highly emotional charged conversations.

Furthermore, some support was found for emotion recognition being a cognitive endophenotype of ASD. That is, the performance of unaffected siblings could be considered at an intermediate level, performing somewhat worse than the controls and better than the ASD probands regarding the speed measures. This was best visible in the domain of auditory recognition of sadness, anger and fear. This finding confirms previous findings on emotion recognition deficits in relatives of autistic individuals (Neves *et al.*, 2011) and is in line with previous studies employing this affected-unaffected sibling design in for instance ADHD or schizophrenia cognitive studies (Hoff *et al.*, 2005, Rommelse *et al.*, 2011). The findings were less convincing with respect to facial emotion recognition and error measures. Despite a linear trend in group contrast, the performance of unaffected siblings was more like controls than that of affected siblings. An explanation for this finding might be that facial emotion recognition deficits, more so than problems in affective prosody recognition, are part of the defining features of ASD, rather than being an endophenotypic trait that can be seen in unaffected relatives. An alternative explanation however, might be that the task demands differed between the visual and auditory emotion recognition tasks. A difference in difficulty level between visual and auditory tasks has been reported previously (Aylward *et al.*, 2002) and was supported by our findings that overall, the participants performed more poorly on the auditory measures compared to the visual measures. In addition, Spencer et al. demonstrated that unaffected siblings of autistic adolescents demonstrated an atypical implicit response to facial emotional expressions and Kaiser et al. found neural atypicalities in unaffected siblings of children with ASD during a social biological motion task (Kaiser *et al.*, 2010, Spencer *et al.*, 2011). Given that neuroimaging measures may be more sensitive than neuropsychological tests in detecting subtle functional deficits or compensatory activity, it is premature to conclude that visual emotion recognition is normal in unaffected relatives of ASD probands. Overall, our results suggest subtle emotion recognition impairments to be present in unaffected relatives. This may suggest that deficits in emotion recognition are not only associated with the disorder, but may be related to shared familial sources, which may make it a useful tool in future studies aimed at unraveling the genetic underpinnings of ASD. Even though unaffected siblings do not portray obvious ASD symptoms, they appear to share at least some of the underlying neuropsychological difficulties characteristic of ASD, which may be easily overlooked. This highlights the importance of following siblings over time, since they are at risk for impairments in social functioning.

Overall, some support was found for the hypothesis that ASD+ADHD probands would be more impaired than ASD only probands on emotion recognition. The findings suggested that the presence of ADHD might add up to a more severe deficit in emotion recognition than ASD alone. This raised the question of whether children with ASD and comorbid ADHD have more difficulties in emotion recognition per se or perhaps

secondary as a consequence of typical ADHD cognitive problems, such as impaired reaction time speed, inhibition and attention. After controlling for these cognitive measures, group differences remained significant for affective prosody recognition, but became non-significant for facial emotion recognition. This might imply that poorer facial emotion recognition skills in ASD children with ADHD (compared to ASD children without ADHD) might be a result of additive effects of ADHD neurocognitive symptoms on emotion recognition problems. In contrast, poorer performance on the auditory recognition of emotions is more likely reflective of poorer affective prosody recognition skills amongst this comorbid group compared to children with ASD only. Somewhat similar results that poorer performance in facial affect recognition in children with ASD and comorbid ADHD was mostly caused by ADHD inattentive symptoms were reported previously (Sinzig *et al.*, 2008). However, an alternative explanation for the findings may be the different nature and complexity of the tasks: for facial emotion recognition, reaction times were all calculated by clicking a mouse button, whereas a voice-key response (verbally identifying the target emotion, no motor action required) was used in the affective prosody task. This might explain why baseline response speed and inhibition (both manual reaction time tasks) were related to the recognition of facial emotion, but not affective prosody. Another possibility is that the different nature of the tasks (voice-key vs. clicking a button) in itself might lead to different performances in ASD children with and without ADHD, not just the difference in stimuli (facial vs. auditory). Nevertheless, even though some emotion recognition problems may be secondary to core ADHD cognitive problems, our results do suggest that children with comorbid ASD and ADHD are at highest risk for emotion recognition problems and clinicians should therefore pay special attention to these children suffering from symptoms of both spectra.

A number of limitations need to be considered. First, no children with ADHD only and their unaffected siblings were present in this study, limiting our ability to draw conclusions about the endophenotypic properties of emotion recognition in ADHD. We can therefore not conclude that emotion recognition deficits are common deficits across ASD and ADHD. Second, sample sizes were relatively small with regard to the ASD probands with and without ADHD, which might have resulted in undetected effects due to a lack of power. However, even though sample sizes were relatively small, group effects were significant, underlining the presence of group differences between ASD probands with and without ADHD. Third, when comparing performances on the facial emotion recognition and the affective prosody recognition tasks, it should be noted that a) the number of children who have participated in both tasks is not identical, b) the children involved in the affective prosody recognition task are older to the ones who did the facial emotion recognition task, and c) the level of complexity of the two tasks is different (i.e. one task requires a verbal answer while the other requires pressing a button and one involves target detection among distracters, while the other is a labeling

task). Whilst we corrected for the difference in sample composition by analyzing the effect of modality in children > 9 years only (i.e. in the affective prosody sample), we were unable to correct for the difference in task complexity and therefore we cannot firmly conclude that recognition of affective prosody is more impaired than recognition of facial emotional expressions based on the current data. Future research should consider this issue by using facial emotion and affective prosody recognition tasks with comparable experimental designs and difficulty levels. Fourth, we did not report on the treatments and cares that have been offered to the ASD probands. The recognition of emotions is currently at the heart of training and this could have impacted the current data. Several studies have been published that report improvements in social skills (e.g. recognition of emotional expressions) in children with ASD after social skills training (Hopkins *et al.*, 2011, Reichow *et al.*, 2012). This would suggest that emotion recognition deficits might be state-dependent (and can be improved or even resolved after social skills training), and this would imply that emotion recognition deficits might be a weak endophenotypic candidate. However, we feel that our findings reflect shared areas of dysfunction in children with ASD and their unaffected siblings, suggesting that emotion recognition deficits might be, at least to some extent, trait-related. Although we have not specifically asked and therefore cannot report on whether or not the ASD probands received social skills training in their past, it is highly unlikely that their unaffected, undiagnosed siblings have received social skills training. Finding similar deficits in both ASD affected and ASD unaffected siblings supports the hypothesis that emotion recognition might be a viable endophenotypic candidate for ASD. Fifth, we did not assess the presence of Oppositional Defiant Disorder (ODD) or Conduct Disorder (CD) in this sample, even though oppositional behavior is often observed in children with ASD and/or ADHD (Gadow *et al.*, 2008, Guttmann-Steinmetz *et al.*, 2009) and all of these disorders are associated with impairments in social cognition (Cadesky *et al.*, 2000, Downs and Smith, 2004). Future studies should examine ASD, ADHD and ODD/CD in tandem to gain a better understanding of social cognition deficits in children with psychopathology. Last, 15.2% (facial emotion recognition sample)/10.2% (affective prosody recognition sample) of the ASD unaffected siblings presented clinical ADHD symptoms, which could have obscured our findings with regard to the intermediate performance of siblings. However, analyses were run with and without these siblings and revealed similar results.

In conclusion, this study has shown that the recognition of both facial emotion and affective prosody was impaired in children with ASD and aggravated by the presence of ADHD. The latter could only be partly explained by typical ADHD cognitive deficits, such as inhibitory and attentional problems. Some evidence was found for the viability of emotion recognition as endophenotype in ASD. Particularly speed measures of emotion recognition may be of interest in future family studies of the genetic contribution to ASD and comorbid ADHD.

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Supplementary Table 1. Means, standard deviations and 95% confidence interval (lower-upper bound) of the untransformed task variables for ASD probands with and without comorbid ADHD

		1.ASD probands with ADHD				2.ASD probands without ADHD				Group contrasts between ASD probands with and without ADHD compared to controls, based on *p* values <.05
				95% confidence interval				95% confidence interval		
		Mean	*sd*	Lower bound	Upper bound	Mean	*sd*	Lower bound	Upper bound	
Facial Emotion Recognition (FER)										
	happiness	7.8	*.9*	6.2	9.5	4.9	*.8*	3.3	6.6	1>2=c
	sadness	21.2	*1.8*	17.7	24.8	20.2	*1.7*	16.8	23.6	1=2=c
Errors (%)	anger	19.6	*1.6*	16.4	22.9	15.3	*1.6*	12.1	18.4	1>2=c
	fear	19.2	*1.8*	15.6	22.9	16.2	*1.8*	12.7	19.7	1=2=c
	total	10.1	*.8*	8.4	11.7	6.8	*.8*	5.2	8.4	1>2=c
	happiness	934.7	*25.5*	884.4	984.9	899.3	*25.1*	849.8	948.9	1=2, 1>c, 2=c
	sadness	1214.6	*33.4*	1148.7	1280.5	1200.9	*31.9*	1137.9	1263.8	1=2>c
Speed (in ms)	anger	1107.8	*31.2*	1046.2	1169.3	1146.1	*30.6*	1085.9	1206.3	1=2, 1=c, 2>c
	fear	1191.7	*32.8*	1127.2	1256.3	1147.1	*31.8*	1084.4	1209.8	1=2>c
	total	1020.5	*24.2*	972.7	1068.2	1008.4	*23.6*	961.8	1055.0	1=2>c
Affective Prosody Recognition (APR)										
	happiness	22.9	*2.7*	17.6	28.3	22.5	*2.6*	17.4	27.5	1=2=c
	sadness	29.5	*3.7*	22.3	36.8	28.9	*3.5*	21.4	35.7	1=2=c
Errors (%)	anger	18.2	*2.7*	12.9	23.5	14.9	*2.6*	9.8	20.0	1=2=c
	fear	69.6	*3.1*	63.5	75.7	66.0	*2.9*	60.2	71.8	1=2=c
	total	32.2	*1.8*	28.5	35.8	30.6	*1.8*	27.1	34.1	1=2=c
	happiness	3653.7	*85.6*	3484.8	3822.7	3593.6	*81.6*	3432.5	3754.7	1=2>c
	sadness	4137.6	*133.2*	3871.7	4400.6	4022.3	*127.1*	3771.3	4273.4	1=2>c
Speed (in ms)	anger	3335.2	*80.4*	3173.4	3493.8	3242.9	*76.5*	3091.7	3394.0	1=2>c
	fear	3895.1	*126.9*	3644.6	4145.7	3621.8	*117.1*	3390.2	3853.4	1=2>c
	total	3469.2	*75.9*	3319.4	3618.9	3381.7	*72.5*	3238.6	3524.9	1=2>c

Note. ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder; 1= ASD probands with ADHD; 2 = ASD probands without ADHD; FER = facial emotion recognition; APR = affective prosody recognition; speed measures are presented in ms; c = controls.

CHAPTER 4

Simplex and multiplex stratification in ASD and ADHD families: a promising approach for identifying overlapping and unique underpinnings of ASD and ADHD?

Based on:

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ABSTRACT

Autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder (ADHD) are highly heterogeneous neuropsychiatric disorders that frequently co-occur. This study examined whether stratification into single-incidence (SPX) and multi-incidence (MPX) is helpful in a) parsing heterogeneity and b) detecting overlapping and unique underpinnings of the disorders. ASD and ADHD traits were measured in 56 ASD/31 ADHD SPX, 59 ASD/171 ADHD MPX, and 203 control families. In ASD but not in ADHD, behavioral traits were less elevated in SPX compared to MPX unaffected relatives, suggesting that SPX-MPX stratification may thus help parse ASD, but not ADHD heterogeneity. Particularly unaffected relatives from MPX ASD and ADHD families displayed elevated trait levels of both disorders, indicating shared (multifactorial) underpinnings underlying ASD and ADHD in these families. Cross-disorder traits were highest in MPX ASD unaffected siblings.

INTRODUCTION

Autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder (ADHD) are both severely impairing neurodevelopmental disorders. ASD is characterized by impairments in interaction, communication and restricted, stereotyped and repetitive behaviour. ADHD is characterized by symptoms of hyperactivity, impulsivity and/or inattention (APA, 2013). Both ADHD and ASD are disorders with a strong heritable component that frequently co-occur within patients and families due to overlapping etiological factors (Rommelse *et al.*, 2010, Rommelse *et al.*, 2011, Ronald *et al.*, 2008). Common to both disorders is the large within-disorder heterogeneity, both in symptom presentation, developmental course and underlying etiological mechanisms (Jones and Klin, 2009, Wahlstedt *et al.*, 2009). This strongly hinders optimal diagnosis and treatment. Finding methods that may aid in detecting more homogeneous subgroups of patients are therefore needed.

One such method currently applied in the field of ASD research is the stratification according to family history for ASD (Sullivan *et al.*, 2012). In this so-called SPX-MPX design, single-incidence or simplex families (SPX) are defined as nuclear families with only one affected individual and at least one unaffected male sibling, and multi-incidence or multiplex families (MPX) as families with two or more affected individuals in the family. The hypothesis is that the constellation of etiological factors differs between SPX versus MPX families. Research on genetic factors in ASD shows that symptoms of affected individuals from SPX families more often have a sporadic cause, such as *de novo* mutations, that is unshared with other family members (Sebat *et al.*, 2007). In contrast, polygenic or multifactorial modes of inheritance (i.e. multiple (genetic) risk factors shared between family members) appear to play a more important role in MPX families (Freitag, 2007), with studies showing that members of MPX families more often exhibit ASD traits compared to members of SPX families (Bernier *et al.*, 2012, Losh *et al.*, 2008, Schwichtenberg *et al.*, 2010, Virkud *et al.*, 2009). Thus, stratification according to family history seems sensitive in discriminating between (genetic) risk factors uniquely present in the proband (mostly present in SPX families) versus risk factors shared by multiple family members (in MPX families) (Sullivan *et al.*, 2012).

In ADHD research, this approach has rarely been used, probably since the majority of studies suggest that ADHD is caused by small disease-increasing effects of multiple genes in interaction with environmental factors (Banaschewski *et al.*, 2010). The evidence for this model is strong and also supported by numerous studies documenting an increased incidence of ADHD symptoms and ADHD-related cognitive vulnerabilities in family members of ADHD probands (Rommelse, 2008, Rommelse *et al.*, 2011). However, several recent reports do indicate that rare genetic mutations or non-shared environmental factors (such as low birth weight and medical conditions) with a large effect may

relate to ADHD as well (Ben Amor *et al.*, 2005, Elia *et al.*, 2012, Lionel *et al.*, 2011, Williams *et al.*, 2012, Williams *et al.*, 2010). These findings suggest that applying the SPX-MPX stratification in ADHD families may also be worthwhile.

Applying the SPX-MPX stratification in ASD and ADHD families might be a promising approach to form more homogenous groups of patients, thereby enhancing chances to uncover the etiologies of both disorders. This is highly relevant for research of genetic and neurobiological causes of ASD and ADHD, which may be otherwise confounded by the mixing of causally-distinct subgroups within study samples (Virkud *et al.*, 2009). SPX-MPX stratification adds to current genetic designs in that it differentiates between monocausal (such as rare genetic variants or de novo mutations with large penetrance unique to the affected individual; expected in SPX families) and multifactorial genetic factors (shared between siblings; expected in MPX families). Traditional twin and adoption designs do not differentiate between these different causal (genetic) factors which might result in overestimation of heritability (Trzaskowski *et al.*, 2013). Attempting to differentiate between risk factors that are unique to the affected individuals and risk factors shared between affected and unaffected relatives may increase our knowledge of the mechanisms that play a key role in ASD and ADHD. A related approach to ours that also focuses on differentiating between shared and non-shared risk factors is examining monozygotic (MZ) twins discordant for the disorder (Wong *et al.*, 2014, Zwijnenburg *et al.*, 2010). Although MZ twins are generally considered 'genetically identical, evidence for genetic and epigenetic differences within MZ twin pairs has accumulated (Zwijnenburg *et al.*, 2010). Examining the differences between discordant monozygotic twins can contribute to our understanding of the pathogenesis of the disorder, aid in finding new shared and unique risk factors, and guide us to why one twin may manifest symptoms differently than the other (Wong *et al.*, 2014, Zwijnenburg *et al.*, 2010).

Of great interest is further whether SPX-MPX stratification may also be successful in the identification of common versus unique risk factors for ASD *and* ADHD. To our knowledge, no such attempt has been made thus far. Several reports have been published on the overlap between ASD and ADHD (Rommelse *et al.*, 2011), and studies have indicated that a large portion of this covariance might be explained by shared additive genetic factors (Ronald *et al.*, 2008). If these factors are polygenic in nature, then we would expect to find a heightened prevalence of comorbid ASD symptoms in ADHD MPX, but not SPX families (and vice versa higher prevalence of ADHD in ASD MPX but not SPX families). Such knowledge could inform the search for shared and unique genetic risk factors for both disorders. Consideration of (comorbid) ASD and ADHD traits in family members also has important clinical implications in terms of treatment planning. For example, awareness of the impact of family history on the presence of ASD and/or ADHD risk factors in unaffected relatives is crucial for the development of a treatment plan and for genetic counseling.

The aim of this study was twofold. First, we examined whether the SPX-MPX stratification method may be a useful approach to create (etiologically) more homogeneous subgroups of patients based on symptom presentation of unaffected relatives. We hypothesized that (a) unaffected relatives from MPX but not SPX ASD families will have elevated levels of ASD traits compared to controls and (b) unaffected relatives in MPX but not SPX ADHD families will have elevated levels of ADHD traits compared to controls. Second, we examined whether SPX-MPX stratification is a useful tool to detect shared etiological underpinnings of ASD and ADHD. We expected unaffected relatives in MPX more than SPX ADHD families will have elevated levels of ASD traits, and vice versa higher levels of ADHD traits in MPX than SPX ASD unaffected relatives.

METHOD

Participants

ASD and ADHD families were recruited as part of two family-genetic studies: the Biological Origins of Autism (BOA) study and the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (as described previously in van Steijn *et al.*, 2012). Inclusion criteria for all participants were at least two biological siblings (in case families: at least one child with a clinical diagnosis of ASD or ADHD) and one biological parent willing to participate, offspring age between 4 and 20 years, European Caucasian descent, an IQ ≥ 70, and no diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome.

Procedure

Parents were invited to fill out several questionnaires concerning their own and their children's behavior. Additional data collected included blood samples of all family members for DNA-isolation and neuropsychological assessment of the children. The study was approved by the local medical ethics board and parents and children (12 years and older) signed for informed consent.

Screening and measures

Children

The exact screening procedures and measures for ASD and ADHD phenotyping in children have been described in previous publications which can be consulted for greater detail (van Steijn *et al.*, 2012). The parent and teacher Social Communication Questionnaire (SCQ) (Rutter *et al.*, 2003), the Child Social Behavior Questionnaire (CSBQ)(Hartman *et al.*, 2006), and Conners Rating Scales Revised (CPRS; CTRS) (Conners, 1996) were used

to identify children with ASD and/or ADHD symptoms. These questionnaires are validated instruments to measure ASD and ADHD traits (Charman *et al.*, 2007, Conners *et al.*, 1998b, Rutter *et al.*, 2003). All children scoring above cut-off on any of the questionnaires underwent full diagnostic ASD and ADHD assessment, including the Autism Diagnostic Interview-Revised (ADI-R) (Le Couteur *et al.*, 2003) and Parental Account of Childhood Symptoms ADHD subversion (PACS) (Taylor *et al.*, 1991).

Parents

In the ASD cohort, case and control parents were screened for ASD using the Autism Spectrum Quotient (AQ) (Baron-Cohen *et al.*, 2001) and the Adult Social Behavior Questionnaire (ASBQ) (Horwitz *et al.*, 2005). The ASBQ is the adult version of the CSBQ and, although still under development, shows promising first results in terms of reliability and validity for the ASBQ (Horwitz et al. submitted for publication). In the ADHD cohort, case and control parents were screened for ADHD using the self-report questionnaire for ADHD (Kooij *et al.*, 2005), the self and spouse Conners Adult Rating Scales Self Report (CAARS:S-L) (Conners *et al.*, 1998a, 1999), and the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL; administered in follow-up study [NeuroIMAGE]) (Kaufman *et al.*, 1997). Parents scoring above cut-off (Hoekstra *et al.*, 2008, Kooij *et al.*, 2005) on any of the ASD/ADHD questionnaires, or on the semi-structured ADHD interview, were considered a suspected case.

Control children and their parents were required to obtain non-clinical scores in order to be accepted in the study.

Family classification

Families were then stratified into SPX and MPX families, see Figure 1 for details. SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ASD or ADHD on the basis of non-clinical scores on the screenings questionnaires and/or administered diagnostic interviews. Families with siblings and/or parents who displayed (sub) threshold ASD and/or ADHD symptoms, in addition to the proband, were categorized as multiplex (MPX). Families were excluded if a) only one unaffected parent from a presumed SPX family based on number of affected children participated in this study (to minimize the risk of erroneous categorization because of missing parental data) and b) if the affected proband had only female unaffected siblings (to account for higher sibling recurrence risk in male siblings than female siblings). SPX-MPX stratification was made on the basis of the primary clinical diagnosis of the probands. That is, SPX and MPX ASD families originate from an ASD cohort in which the primary clinical diagnosis of the proband is ASD, SPX and MPX ADHD families originate from an ADHD cohort in which the primary clinical diagnosis of the proband is ADHD. ASD+ADHD probands were not considered

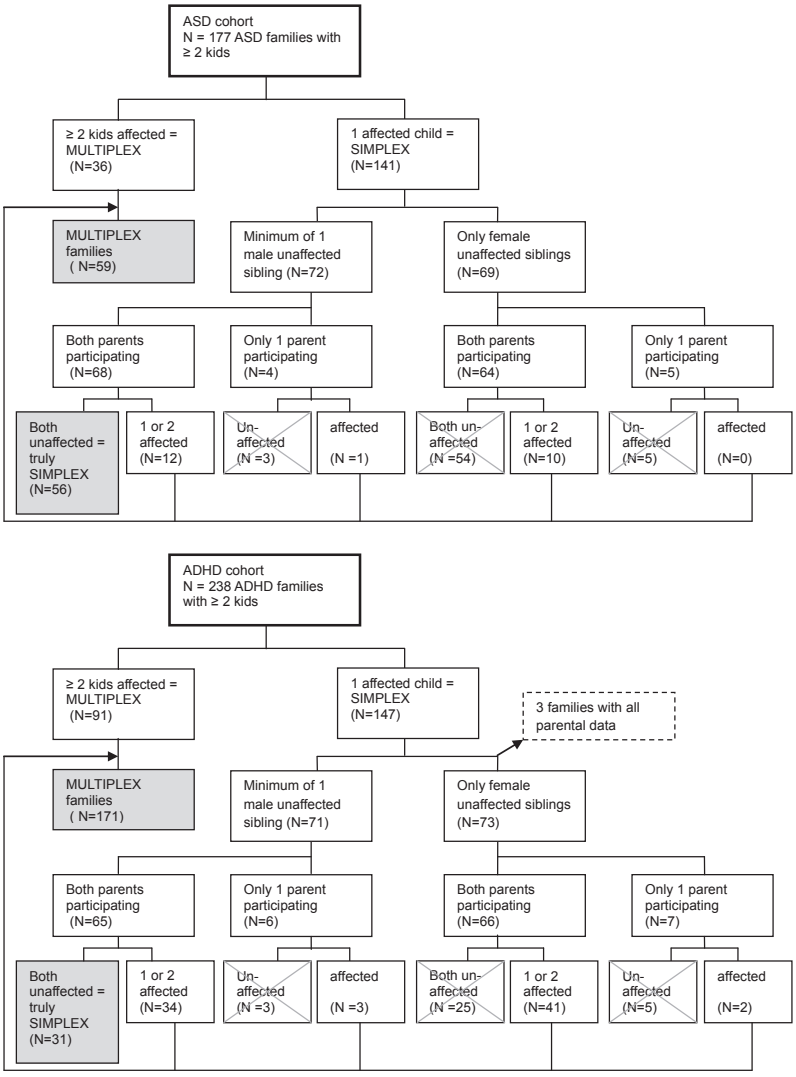

Figure 1 Flowcharts of SPX-MPX stratification in the ASD and ADHD cohorts

Note. A conservative approach was adopted to the inclusion of simplex (SPX) families in this study. All SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ASD or ADHD on the basis of non-clinical scores on the screenings questionnaires and/or administered diagnostic interviews. Families with siblings and parents who displayed (sub) threshold ASD and/or ADHD symptoms, in addition to the affected child, were categorized as multiplex (MPX). Families were excluded (ASD: N = 62, 35.0% of ASD cohort; ADHD: N=33, 13.9% of ADHD cohort) if a) only one unaffected parent from a presumed SPX family based on number of affected children participated in this study (to minimize the risk of erroneous categorization because of missing parental data) (ASD: N=3, 1.7%; ADHD: N=3, 1.3%) and b) if the affected proband had only female unaffected siblings (to account for higher sibling recurrence risk in male siblings than female siblings) (ASD: N=54, 30.5%; ADHD: N=25, 10.5%) or both a and b (ASD: N = 5, 2.8%; ADHD: N=5, 2.1%). In grey, the final number of selected SPX and MPX families that fulfilled inclusion criteria.

a separate category within the SPX-MPX stratification, because of the main reason that this would interfere with our aim to examine quantitatively the cross-disorder relationships between both symptom dimensions. By treating ASD+ADHD families as a separate category –families with a likely high cross-disorder loading- the quantitative cross-disorder loading would be artificially reduced. A total of 56 ASD and 31 ADHD SPX nuclear families, 59 ASD and 171 ADHD MPX nuclear families, and 203 control nuclear families were included in the current sample, see Table 1 for sample characteristics.

Dependent measures

Dependent measures were the CSBQ (or ASBQ) 'ASD Composite score' (i.e. aggregate score of the four subscales: reduced contact and social interests, difficulties in understanding social information, stereotyped behaviour and fear of and resistance to changes), the SCQ Total score (children), and the parent and teacher (children) and self-report (parents) Conners' DSM-IV Combined Subscale scores of the ADHD questionnaires. We choose to use the ASD composite score (based on four CSBQ subscales) instead of the summed score of all six CSBQ subscales, because the subscales 'orientation' and 'tuned' are not specific for ASD (i.e. similar behaviors are scored in ADHD) and it was our aim to specifically focus on the most differentiating ASD core symptoms (Hartman *et al*., 2012).

Data analyses

Most of the dependent variables were not normally distributed, therefore, a van der Waerden transformation was used to normalize the dependent measures (Norusis, 1992). This facilitated the comparison between variables since variables were all depicted on the same scale (i.e. higher z-scores indicated more ASD or ADHD symptoms). For child data, the percentage of missing data was < 5% for each dependent measure. The SCQ was not administered in control children (N = 275) of the IMAGE cohort. The CSBQ was not administered to 23.0% (N = 99) children from the BOA cohort since it was added at a later stage to the protocol. For parental data, the percentage of missing data was 5.2% for self-reported ADHD symptoms. ADHD symptoms were not assessed in control parents of the IMAGE cohort (N=282). For self-reported ASD symptoms (ASBQ), the percentage of missing data was 37.0% for ASD families, 28.5% for ADHD families, 26.2% for control families. Variables with less than 5% missing data were subjected to expectation maximization (EM) algorithm to replace the missing data (Tabachnick and Fidell, 2001). All analyses were carried out with and without expectation maximization and Van der Waerden transformation, and revealed similar results. Results were therefore reported for transformed measures with missing data replaced. Missing data was not imputed for variables with > 5% missing values.

The analyses were run separately for child and parental data. For children, we conducted linear mixed models with diagnosis (probands versus unaffected siblings), type

Table 1. Sample Characteristics

	c. Control families[a] (N=203)	ASD cohort				ADHD cohort				Group contrasts ASD versus controls based on $p < .05$	Group contrasts ADHD versus controls based on $p < .05$
		SPX families (N = 56)		MPX families (N= 59)		SPX families (N = 31)		MPX families (N=171)			
		1. ASD probands	2. ASD Unaffected siblings	3. ASD probands	4. ASD Unaffected siblings	5. ADHD probands	6. ADHD Unaffected siblings	7. ADHD probands	8. ADHD Unaffected siblings		
	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)		
Child data											
Number of children	N = 408	N = 56	N = 81	N = 96	N = 55	N = 31	N = 47	N = 270	N = 128		
Age	11.2 (3.5)	12.0 (3.7)	12.1 (3.8)	11.3 (3.6)	10.9 (4.2)	11.8 (2.4)	10.9 (3.4)	11.4 (2.7)	11.4 (3.6)	1=2=3=4=c	5=6=7=8=c
Sex (% males)	41.9	85.7	74.1	72.9	45.5	87.1	74.5	73.6	39.1	1=2=3>4=c	5=6=7>8=c
Diagnose (%)											
ASD	0	100	0	100	0	25.8	2.1	14.1	1.6		
ADHD	0	35.7	12.3	43.8	18.1	100	0	100	0		
Parental data		-[b]	10. ASD unaffected parents	11. ASD affected parents	12. ASD unaffected parents	-[c]	13. ADHD unaffected parents	14. ADHD affected parents	15. ADHD unaffected parents		
Number of parents	N = 404		N = 112	N = 42	N = 76		N = 62	N = 150	N = 178		
Age	43.6 (5.2)		44.6 (5.2)	43.4 (6.2)	44.0 (5.9)		41.0 (4.6)	42.4 (5.7)	41.6 (4.4)	10=11=12=c	14=c>13=15
Sex (% males)	50.0		50.0	71.4	38.2		50.0	60.7	37.6	11>10=12=c	14>13=15=c
Diagnose (%)[c]											
ASD	0		0	100	0		-	-	-		
ADHD	0		-	-	-		0	100	0		

Note ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder: SPX = simplex family; MPX = multiplex family; c = controls

[a]control families were combined from both datasets

[b]by definition, no affected parents are present in ASD and ADHD SPX families

[c]no formal diagnoses were made for parents. Details of the diagnostic procedure are described in the method section

of disorder (ASD [BOA cohort] versus ADHD [IMAGE cohort]) and type of family (SPX versus MPX) as fixed factors, age and sex as covariates and family number as random effect to account for within family correlation. The two-way interactions and three-way interaction between diagnosis, type of disorder and type of family were implemented in the initial model and dropped from the model when non-significant. The control group was used as reference value. Dependent variables were the four parent- and teacher-reported ASD and ADHD scales. Separate analyses for subscales were run.

For parents, we conducted linear mixed models with type of disorder of the child (ASD versus ADHD) and type of family (SPX versus MPX) as fixed factors and family as random effect. Affected (MPX) parents were only used as reference but further omitted from the analyses, because by definition no affected SPX parents were available for comparison. Dependent measures were the two parental spouse-, and self-report ASD and ADHD scales. Pairwise comparisons between unaffected relatives from SPX ASD, SPX ADHD, MPX ASD and MPX ADHD families (separately for siblings and parents) were calculated to test whether the unaffected relatives differed with regard to symptom presentation severity. Correction for multiple comparisons was applied using the False Discovery Rate (FDR) controlling procedure with a q-value setting of 0.05 (Benjamini, 2010). Only the effects that remained significant after FDR correction were reported in the tables and figures. FDR-adjusted *p*-values (*q*-values) were reported for effects that became non-significant after FDR correction. All analyses were carried out in SPSS version 20.

RESULTS

Regarding child data, none of the three-way interactions between diagnosis * type of disorder * type of family were significant (*p*-values >.65) and were therefore dropped from the models. Further, 9/12 two-way interactions between diagnosis*type of disorder, diagnose*type of family and type of disorder*type of family for the four dependent measures had a corrected *p*-value below .05 and therefore were retained in the model. The multitude of significant interactions in child data involving the factor type of family indicated that the stratification into SPX and MPX moderated the effects of diagnosis in both cohorts. Therefore, post-hoc analyses were performed testing the effect of diagnosis separately for ASD/ADHD SPX and ASD/ADHD MPX families. In parental data, a marginally significant two-way interaction between type of disorder*type of family was found for the ADHD (F (1, 236.5) = 3.66, p = .057). No type of disorder*type of family interaction was found for the ASD dependent measure (F (1, 171.3) = 0.66, p = .42). Here, analyses were performed to examine the effect of type of family separately for ASD and ADHD families.

Multiplex families

For ASD families, a significant main effect of **diagnosis** was found for both parent-reported ASD measures (SCQ: F (2, 160.4) = 213.5, $p < .001$ and CSBQ: F (2, 297.7) = 199.42, $p < .001$) and for parent- and teacher-reported ADHD measures (CPRS: F (2, 351.3) = 117.81, $p < .001$ and CTRS: F (2, 452.4) = 24.45, $p < .001$). ASD probands demonstrated significantly more ASD and ADHD symptoms than controls (p-values <.001) and ASD unaffected siblings formed an intermediate group, demonstrating less ASD/ADHD symptoms than their affected brothers and sisters (p-values <.001), but significantly more symptoms than controls (p-values < .001), see Figure 2. For untransformed means and standard deviations, see Table 2.

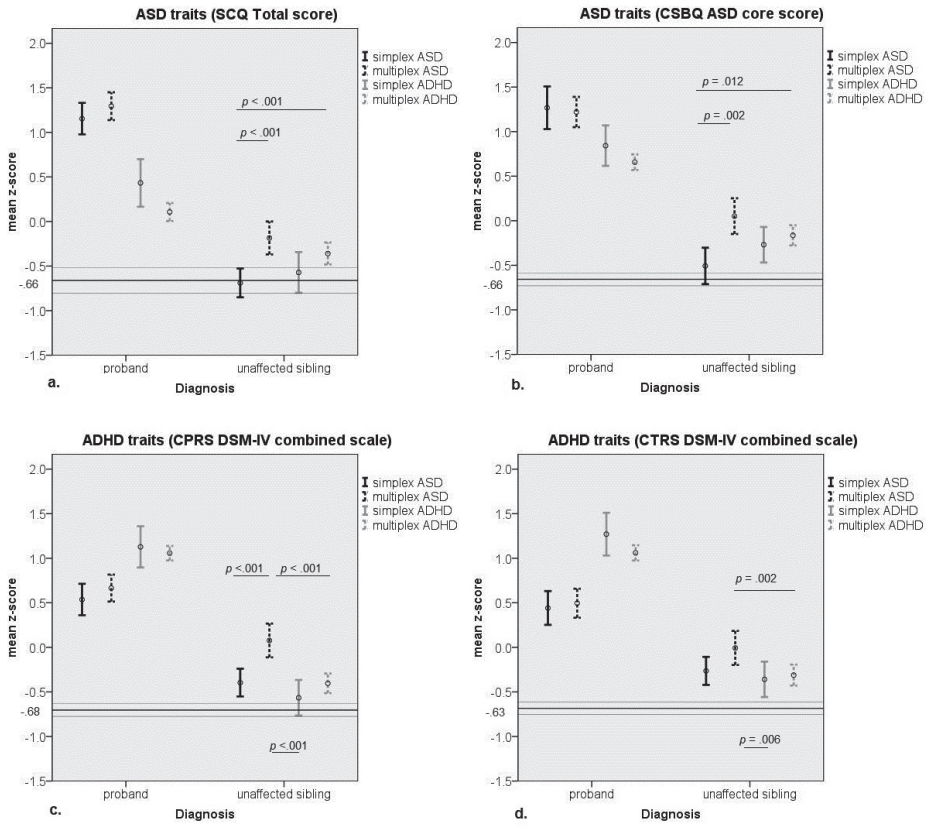

Figure 2 ASD and ADHD traits in SPX and MPX probands and unaffected siblings from ASD and ADHD families

Note. The interpolation lines represent the mean and 95% confidence interval (CI) of the standard errors of controls. The *error bars* represent the 95% CI of the standard errors of cases. Higher z-scores indicate more symptoms. Figures a and b display ASD traits; figures c and d display ADHD traits.

Table 2. Means and standard deviations of the untransformed dependent variables for the ASD and ADHD affected and unaffected children/parents from SPX and MPX families, and for controls

	Controls (c)	ASD cohort				ADHD cohort				Group contrasts ASD versus controls based on FDR-adjusted *p*-values < .05	Group contrasts ADHD versus controls based on FDR-adjusted *p*-values < .05
		SPX families		MPX families		SPX families		MPX families			
		1. Affected	2. Unaffected	3. Affected	4. Unaffected	5. Affected	6. Unaffected	7. Affected	8. Unaffected		
	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)		
Child data											
SCQ Total	3.01 (2.59)	17.84 (6.46)	3.15 (3.24)	19.58 (6.65)	5.75 (5.84)	10.68 (7.29)	4.00 (3.58)	8.33 (5.80)	4.64 (3.67)	1=3>4>2=c	5=7>6=8/6=c,8>c
CSBQ ASD core	3.21 (3.81)	26.26 (10.95)	5.53 (6.07)	27.68 (8.76)	10.35 (9.68)	21.87 (11.53)	7.34 (6.87)	18.47 (10.20)	7.57 (7.22)	1=3>4>2=c	5=7>6=8>c
CPRS	46.59 (5.20)	64.11 (10.19)	50.27 (9.95)	67.34 (13.06)	57.87 (14.36)	76.06 (7.96)	48.06 (7.30)	74.73 (10.24)	50.54 (9.20)	1=3>4>2>c	5=7>6=8/6=c, 8>c
CTRS	46.74 (5.56)	57.32 (9.63)	49.76 (8.26)	58.56 (12.54)	53.33 (9.69)	69.71 (8.02)	48.28 (6.33)	67.69 (10.73)	50.11 (7.75)	1=3>2=4>c	5=7>6=8>c
Parental data											
ASBQ ASD core[a]	3.69 (4.76)	-[b]	5.31 (5.53)	19.62 (9.20)	7.54 (7.14)	-[b]	5.09 (5.95)	9.41 (8.22)	5.64 (5.41)	3>2=4>c	7>8>c/6=c, 6=8
ADHD DSM Combined	7.56 (5.34)	-[b]	8.78 (6.67)	16.43 (9.73)	10.48 (8.63)	-[b]	10.96 (5.63)	22.28 (8.44)	9.90 (5.34)	3>4>c, 2=4, 2=c	7>6=8>c

ASD = Autism Spectrum Disorders; ADHD = Attention-Deficit/Hyperactivity Disorder; SPX = simplex; MPX = multiplex; SCQ = Social Communication Questionnaire; CSBQ = Child Social Behavior Questionnaire; CPRS = Conners Parent Rating Scales; CTRS = Conners Teacher Rating Scales; ASBQ = Adult Social Behavior Questionnaires; c = controls

[a]the ASD Core score is the combined total score of CSBQ/ASBQ subscales: tendency to withdraw, not understanding, stereotypic behavior and fear of changes

[b]by definition, no affected parents are present in SPX families

Reported contrasts were significant after correction for multiple testing (FDR).

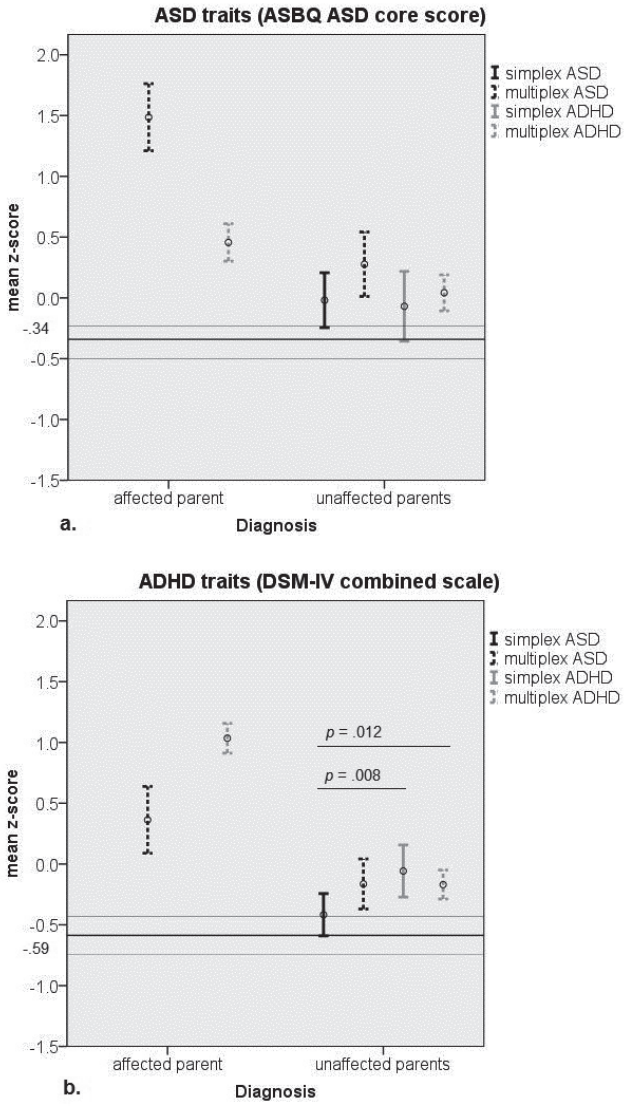

Figure 3 ASD and ADHD traits in SPX and MPX affected and unaffected parents from ASD and ADHD families

Note. The interpolation lines represent the mean and 95% confidence interval (CI) of the standard errors of controls. The *error bars* represent the 95% CI of the standard errors of cases. Higher z-scores indicate more symptoms. Figure a displays ASD traits; figure b displays ADHD traits. No affected parents are present in SPX ASD and ADHD families.

For ADHD families a similar pattern was found. A significant main effect of **diagnosis** was found for both ADHD measures (CPRS: F (2, 748.9) = 55.75, $p < .001$ and CTRS: F (2, 750.6) = 60.86, $p < .001$) and both ASD measures (SCQ: F (2, 304.4) = 46.44, $p < .001$ and CSBQ: F (2, 515.9) = 259.70, $p < .001$). Again, ADHD probands showed significantly elevated levels of ADHD and ASD symptoms compared to controls (p-values < .001) and ADHD unaffected siblings formed an intermediate group differing from affected siblings (p-values < .001) and controls (p-values < .001). Subscale analyses for SCQ, CSBQ and CRS (parent- and teacher reported) revealed similar results, see Supplementary Table 1.

Unaffected parents from MPX ASD families and MPX ADHD families demonstrated significantly more ASD (both p-values < .001) and ADHD symptoms ($p = .002$ and $p < .001$, respectively) compared to control parents, see Figure 3.

Simplex families

For ASD families, a significant main effect of **diagnosis** was found for both ASD measures (SCQ: F (2, 138.7) = 215.6, $p < .001$ and CSBQ: F (2, 344.9) = 121.39, $p < .001$) and both ADHD measures (CPRS: F (2, 325.6) = 68.97, $p < .001$ and CTRS: F (2, 525.4) = 13.25, $p < .001$). Pairwise comparisons revealed that ASD probands showed elevated levels of ASD and ADHD symptoms compared to controls (p-values < .001). ASD unaffected siblings showed no significantly elevated levels of ASD symptoms according to the SCQ ($p = .91$) and CSBQ ($p = .18$), but intermediate levels on CPRS and CTRS were found (p-values < .001). Compared to MPX ASD unaffected siblings, the SPX ASD unaffected siblings showed significantly less ASD and ADHD symptoms (SCQ; $p = .001$; CSBQ: $p = .006$; CPRS: $p = .001$, and CTRS: $p = .066$). SPX and MPX ASD probands did not differ from each other (p-values > .10); see Figure 2 and Table 2.

In ADHD families, largely comparable results were found. A significant main effect of **diagnosis** was found for all measures (SCQ: F (2, 199.4) = 9.99, $p < .001$; CSBQ: F (2, 257.9) = 77.97, $p < .001$; CPRS: F (2, 287.4) = 115.90, $p < .001$, and CTRS: F (2, 410.7) = 113.62, $p < .001$). ADHD probands demonstrated elevated levels of ADHD and ASD symptoms compared to controls (p-values < .001). ADHD unaffected siblings either a) did not differ from controls (SCQ; $p = .52$, CPRS: $p = .37$) or b) demonstrated significantly elevated levels of ADHD and ASD symptoms (CSBQ and CTRS: both p-values < .01) compared to controls. SPX and MPX ADHD unaffected siblings did not differ significantly from each other on all measures (all p-values > .10). Except for a trend-level effect on CSBQ ($p = .062$), SPX and MPX ADHD probands could not be dissociated from each other (other p-values > .19). Comparisons between ASD and ADHD unaffected siblings from the two types of families revealed that SPX ASD unaffected siblings showed significantly less ASD traits than MPX ADHD unaffected siblings ($p = .012$), and MPX ASD unaffected siblings showed more ASD *and* ADHD traits than (SPX and MPX) ADHD unaffected siblings

(SCQ: p= . 033; CPRS and CTRS: $p < .006$), although the first effect did not survive FDR correction (*q-value* = .075).

Unaffected parents from SPX ASD families demonstrated significantly more ASD symptoms ($p = .012$), but not ADHD symptoms ($p = .165$) compared to control parents. Vice versa, unaffected parents from SPX ADHD families demonstrated more ADHD ($p < .001$), but not ASD ($p = .082$) symptoms, see Figure 3. Comparisons between cohorts revealed that unaffected parents from (SPX and MPX) ADHD families demonstrated significantly more ADHD symptoms than unaffected parents from SPX ASD families ($p = .008$ and $p = .012$, respectively).

DISCUSSION

This is the first study to examine whether the SPX-MPX stratification method is a promising approach for creating (etiologically) more homogeneous subgroups of ASD and ADHD patients based on the symptom presentation of unaffected relatives. This approach builds on the idea that polygenic and multifactorial causes of disease will increase symptom levels in most or all members of the family, whereas sporadic genetic and non-genetic causes will be strictly personal to the patient. In addition, we wished to test the SPX-MPX stratification method as a means to detect overlapping and unique underpinnings of both ASD and ADHD. Results indicate that the symptom presentation of SPX and MPX (ASD and ADHD) probands was highly similar, showing equally elevated levels of both ASD and ADHD symptoms compared to controls. Unaffected relatives (siblings and parents) from MPX ASD and ADHD families displayed a similar (yet milder) profile as the affected child, with elevated symptom levels of both ASD and ADHD, consistent with what would be expected based on existing literature. Novel findings are that in ASD families, behavioral traits were indeed found less frequently in unaffected relatives of SPX families compared to unaffected relatives of MPX families. In ADHD families, SPX and MPX unaffected relatives could not be clearly dissociated based on symptom presentation. Importantly, higher levels of ADHD symptoms in ASD unaffected family members were found compared to ASD symptoms in ADHD unaffected family members.

These behaviorally based findings suggest that causes of ASD might differ between SPX and MPX families. Undiagnosed first-degree relatives of MPX ASD families might be more prone to develop ASD symptoms, as previously reported (Gerdts *et al.*, 2013, Virkud *et al.*, 2009). However, the finding that unaffected parents from both SPX and MPX displayed equally heightened levels of ASD traits contradicted previous reports (Gerdts *et al.*, 2013, Losh *et al.*, 2008). Also, SPX unaffected siblings were not entirely 'normal' either, displaying somewhat elevated levels of ASD traits compared to controls. This suggests that the SPX-MPX stratification detects some quantitative differences,

with SPX ASD families being less densely affected and possibly having a smaller genetic loading for ASD than MPX families, yet that there is no simple dichotomy, with only the proband showing behavioral symptoms in SPX families. Thus, whilst factors uniquely present in the affected child (such as *de novo* mutations) might underlie ASD in some of the SPX cases, multifactorial risk factors might still underlie the disorder in others. This is supported by genetic findings that de novo variations most likely play a role in the development of simplex ASD, but do not fully explain genetic etiology (Krumm *et al.*, 2013).

The validity of SPX-MPX stratification in ADHD families was not confirmed. SPX and MPX unaffected relatives (siblings and parents) both showed equally elevated levels of ADHD traits compared to controls. This suggests that in most ADHD cases, multifactorial risk factors underlie the disorder and supports the prevailing model that ADHD is caused by small disease-increasing effects of multiple genetic and environmental risk factors (Banaschewski *et al.*, 2010, Franke *et al.*, 2009). It might nonetheless be premature to conclude that SPX-MPX stratification is not a valid approach for creating more homogeneous subgroups of ADHD patients based on behavioral findings alone, taking into consideration (subtle) neurocognitive/functional deficits might improve dissociation of these unaffected relatives (Rommelse *et al.*, 2011). In any case, a small proportion of ADHD families exist in which only one individual has developed ADHD whilst the other family members are also at heightened risk (given elevated levels of ADHD traits in unaffected relatives). These families might be particularly valuable for detecting protective mechanisms decreasing the risk of developing ADHD.

SPX-MPX stratification proved useful in identifying shared etiological underpinnings of ASD and ADHD. Particularly unaffected relatives from MPX ASD and ADHD probands displayed elevated levels of cross-disorder behavioral traits (i.e., elevated levels of ASD in the ADHD cohort and ADHD in the ASD cohort) compared to controls, suggesting that pleiotropic risk factors for ASD and ADHD are likely polygenic and multifactorial in nature. That is, pleiotropic risk factors are likely common genetic influences operating across ASD traits and ADHD behaviors throughout normal variation and at the extreme as was previously suggested by Ronald and colleagues (Ronald *et al.*, 2008) and also discussed in a recent publication by Martin and colleagues (Martin *et al.*, 2014). The presence of ASD traits in ADHD probands and vice versa has been extensively documented before (Lichtenstein *et al.*, 2010, Rommelse *et al.*, 2011, Ronald *et al.*, 2008). Previous studies on cross-disorder traits in unaffected relatives of ASD or ADHD probands are relatively scarce, but report similar findings (e.g. ADHD unaffected siblings had higher levels of ASD compared to the population) (Mulligan *et al.*, 2009, Nijmeijer *et al.*, 2009). Importantly, it was found that the type of diagnosis (ASD or ADHD) of the proband was informative for unaffected relatives' ADHD outcomes. That is, significantly higher levels of ADHD traits were found in unaffected siblings of ASD MPX families compared

to ADHD (SPX and MPX) families, which was not the case for ASD traits. This is in line with findings by Lichtenstein and colleagues (2010) who reported that among co-twins of children with ASD, the probability of having ADHD was 44.4% for monozygotic- and 15.4% for dizygotic twins, yet the probability of co-twins of ADHD children of having ASD was 22.5% and 7.0%, respectively (Lichtenstein *et al.*, 2010). In other words, the risk for ADHD in siblings of ASD probands is higher, than vice versa the risk for ASD in siblings of ADHD probands. This suggests that ADHD might be seen as a milder, less severe subtype within the ASD spectrum as has been stated in the gradient overarching disorder hypothesis (Van der Meer *et al.*, 2012) or that risk factors underlying ASD may overlap to a larger degree with risk factors underlying ADHD than vice versa (Rommelse *et al.*, 2010, van Steijn *et al.*, 2012). However, since this pattern of results was not found in parents, further research is needed to confirm these hypotheses.

A number of strengths and limitations of this study should be considered when weighing the results. Strengths were the relatively large sample size, the use of well-established measures and the differentiation between different heritable forms (i.e. monocausal [most likely present in SPX families] and multifactorial genetic risk factors [most likely present in MPX families]) of ASD and ADHD. Another strength was the inclusion of parental affected status into the SPX-MPX classification. Most studies base the SPX-MPX classification solely on the number of affected children in the family (Gerdts and Bernier, 2011, Gerdts *et al.*, 2013, Virkud *et al.*, 2009), but given the high heritability of both disorders, it is highly relevant to include parental affected status as well. That said, it should be noted that no formal diagnosis was made in parents. Instead, we based the affected status of parents on self- and spouse report questionnaires (particularly for parents of ASD families), which may have overstepped the clinical boundaries of these instruments. Self reported symptoms may be less valid in high scoring individuals due to limited self awareness (Mazefsky *et al.*, 2011), which may results in an underestimation of behavioral symptoms. However, we believe that by using multiple screening questionnaires and selecting low cut-off scores in order to avoid false negatives, all parents with threshold ASD or ADHD traits (i.e. suspected cases) were successfully identified and accounted for in the SPX-MPX classification. A second limitation is that SPX-MPX classification is not a straightforward one. Correct classification requires confident knowledge of family history and fecundity is a major confounder (Sullivan *et al.*, 2012). However, we are confident that by including parental data from both parents and accounting for higher sibling recurrence risk in male compared to female siblings, we acquired the most optimal SPX-MPX classification in our sample. Third, only a small proportion of ADHD families could be classified SPX (about 1/8 families), compared to the large number of ADHD families that were considered MPX (about 3/4 families), which might have resulted in undetected effects due to lack of power. It follows that our study need replication. Fourth, only 'intact' families were included in this study (i.e. families with

available data of both parents). Possibly, families where one or both biological parents are absent or unwilling to participate in research may have children more severely affected. This could have implications for the representativeness of the findings for ASD and ADHD families in general.

All in all, our study suggests that the SPX-MPX stratification detects quantitative rather than qualitative differences between ASD families and in its current form does not appear to be helpful in stratifying ADHD families. Nevertheless, unaffected relatives from MPX families are at highest risk of displaying subthreshold ASD and/or ADHD symptoms, making these families of particular interest for genetic counseling and therapeutic interventions. Some evidence was found suggesting risk factors underlying ASD may overlap to a larger degree with risk factors underlying ADHD than vice versa, but further research herein is warranted.

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Supplementary Table 1. Means, and standard deviations (sd's) of the normalized dependent variables for the ASD and ADHD simplex and multiplex probands, unaffected siblings, and parents compared to controls

			ASD cohort				ADHD cohort				Group contrasts ASD versus controls based on *p*-values < .05	Group contrasts ADHD versus controls based on *p*-values < .05
			Simplex families		Multiplex families		Simplex families		Multiplex families			
		Controls (c)	1.Affected probands	2.Unaffected siblings	3.Affected probands	4. Unaffected siblings	5.Affected probands	6.Unaffected siblings	7.Affected probands	8.Unaffected siblings		
		M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)		
Child data												
SCQ	Total Score	-.66 (.07)	1.15 (.09)	-.69 (.08)	1.29 (.08)	-.18 (.09)	.43 (.14)	-.57 (.12)	.11 (.05)	-.36 (.06)	1=3>4>2=c	5=7>6=8/ 6=c, 8>c
	Social interaction	-.64 (.07)	1.02 (.09)	-.57 (.08)	1.23 (.07)	-.09 (.09)	.37 (.13)	-.45 (.12)	.06 (.05)	-.31 (.07)	1=3>4>2=c	5=7>6=8/ 6=c, 8>c
	Communication	-.45 (.08)	.91 (.10)	-.63 (.09)	1.00 (.09)	-.18 (.11)	.29 (.14)	-.34 (.13)	.16 (.06)	-.23 (.07)	1=3>4>2=c	5=7>6=8/ 6=c, 8>c
	Stereotypic behavior	-.32 (.07)	.96 (.09)	-.35 (.08)	1.07 (.07)	-.19 (.09)	.13 (.12)	-.53 (.11)	.10 (.05)	-.38 (.06)	1=3>2=4=c	5=7>6=8=c
CSBQ	ASD core score[a]	-.66 (.04)	1.27 (.12)	-.51 (.10)	1.22 (.09)	.05 (.10)	.84 (.12)	-.27 (.10)	.66 (.05)	-.16 (.06)	1=3>4>2=c	5=7>6=8>c
	Tendency to withdraw	-.48 (.03)	1.16 (.10)	-.25 (.09)	1.30 (.08)	.08 (.10)	.61 (.13)	-.12 (.11)	.39 (.05)	-.16 (.06)	1=3>4>2>c	5=7>6=8>c
	Not understanding	-.62 (.04)	1.02 (.10)	-.36 (.10)	1.06 (.09)	.19 (.10)	.93 (.12)	-.22 (.10)	.69 (.04)	-.16 (.06)	1=3>4>2>c	5=7>6=8>c
	Stereotypic behavior	-.43 (.03)	.74 (.09)	-.33 (.08)	.73 (.07)	-.19 (.09)	.92 (.12)	-.19 (.11)	.67 (.05)	-.27 (.06)	1=3>2=4, 2=c, 4>c	5=7>6=8>c
	Fear of changes	-.35 (.03)	.66 (.09)	-.23 (.08)	.96 (.08)	.09 (.09)	.64 (.12)	-.21 (.10	.49 (.04)	-.12 (.06)	3>1>4>2=c	5=7>6=8/ 6=c, 8>c
CPRS	DSM-IV Combined	-.68 (.03)	.38 (.08)	-.38 (.07)	.56 (.07)	.07 (.09)	1.02 (.11)	-.58 (.10)	.98 (.04)	-.44 (.06)	1=3>4>2>c	5=7>6=8/ 6=c, 8>c
	Inattentive	-.63 (.03)	. 38 (.09)	-.38 (.07)	.53 (.07)	.09 (.09)	.94 (.11)	-.57 (.09)	.93 (.04)	-.43 (.05)	1=3>4>2>c	5=7>6=8/ 6=c, 8>c
	Hyp/imp	-.62 (.04)	.45 (.08)	-.32 (.07)	.57 (.07)	.03 (.09)	1.07 (.11)	-.53 (.09)	.95 (.04)	-.42 (.06)	1=3>4>2>c	5=7>6=8/ 6=c, 8>c
CTRS	DSM-IV Combined	-.63 (.03)	.33 (.09)	-.27 (.07)	.43 (.08)	-.06 (.09)	1.13 (.11)	-.38 (.09)	.95 (.04)	-.38 (.06)	1=3>2=4>c	5=7>6=8>c
	Inattentive	-.61 (.03)	.32 (.09)	-.17 (.08)	.36 (.07)	-.04 (.09)	1.22 (.11)	-.31 (.10)	.95 (.04)	-.39 (.06)	1=3>2=4>c	5=7>6=8/ 6=c, 8>c
	Hyp/imp	-.53 (.03)	.26 (.09)	-.31 (.08)	.35 (.07)	-.09 (.09)	.99 (.12)	-.38 (.10)	.92 (.04)	-.36 (.06)	1=3>2=4>c	5=7>6=8/ 6=c, 8>c

Supplementary Table 1. Means, and standard deviations (sd's) of the normalized dependent variables for the ASD and ADHD simplex and multiplex probands, unaffected siblings, and parents compared to controls (continued)

			ASD cohort				ADHD cohort				Group contrasts ASD versus controls based on *p*-values < .05	Group contrasts ADHD versus controls based on *p*-values < .05
			Simplex families		Multiplex families		Simplex families		Multiplex families			
		Controls (c)	1.Affected probands	2.Unaffected siblings	3.Affected probands	4. Unaffected siblings	5.Affected probands	6.Unaffected siblings	7.Affected probands	8.Unaffected siblings		
		M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)			
Parental data												
	ASD Core score	-.34 (.06)	-[b]	-.02 (.12)	1.49 (.14)	.28 (.14)	-[b]	-.07 (.15)	.46 (.08)	.04 (.08)	3>2=4>c	7>8>c,6=c, 6=8
	Tendency to withdraw	-.24 (.05)	-	.12 (.10)	1.35 (.13)	.31 (.12)	-	-.04 (.13)	.26 (.07)	.09 (.07)	3>2=4>c	7>8>c,6=c, 6=8
ASBQ	Not understanding	-.22 (.05)	-	-.12 (.10)	1.23 (.14)	.25 (.13)	-	-.09 (.13)	.50 (.08)	.02 (.07)	3=4>2=c	7>6=8/ 6=c, 8>c
	Stereotypic behavior	-.08 (.04)	-	-.18 (.09)	.77 (.12)	.16 (.11)	-	.04 (.12)	.41 (.07)	.03 (.07)	3>4>2=c	7>6=8=c
	Fear of changes	-.14 (.04)	-	.02 (.08)	1.14 (.11)	-.01 (.10)	-	.01 (.11)	.35 (.07)	.07 (.06)	3>2=4=c	7>8>c,6=c, 6=8
	DSM-IV Combined	-.59 (.08)	-	-.42 (.09)	.36 (.14)	-.16 (.11)	-	-.06 (.11)	1.04 (.06)	-.17 (.06)	3>4>c, 2=4, 2=c	7>6=8>c
ADHD	Inattentive	-.53 (.08)	-	-.39 (.09)	.49 (.14)	-.21 (.10)	-	-.09 (.10)	.95 (.06)	-.14 (.06)	3>4>c, 2=4, 2=c	7>6=8>c
	Hyp/imp	-.48 (.08)	-	-.37 (.08)	.20 (.14)	-.15 (.10)	-	-.03 (.10)	.98 (.07)	-.21 (.06)	3>4>c, 2=4, 2=c	7>6=8>c

ASD = Autism Spectrum Disorders; ADHD = Attention-Deficit/Hyperactivity Disorder; SCQ = Social Communication Questionnaire; CSBQ = Child Social Behavior Questionnaire; CPRS = Conners Parent Rating Scales; CTRS = Conners Teacher Rating Scales; Hyp/imp = Hyperactive/Impulsive; ASBQ = Adult Social Behavior Questionnaires; c = controls

[a]the ASD Core score is the combined total score of CSBQ/ASBQ subscales: tendency to withdraw, not understanding, stereotypic behavior and fear of changes

[b]by definition, no affected parents are present in SPX families

CHAPTER 5

Cognitive impairments are different in single-incidence and multi-incidence ADHD families

Based on:

Oerlemans, A.M., Hartman, C.A., de Bruijn, Y.G.E., Franke, B., Buitelaar, J.K. & Rommelse, N.N.J. (2014) Cognitive impairments are different in single-incidence and multi-incidence ADHD families.

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ABSTRACT

Background: We may improve our understanding of the role of common versus unique risk factors in attention-deficit/hyperactivity disorder (ADHD) by examining ADHD-related cognitive deficits in single- (SPX), and multi-incidence (MPX) families. Given that individuals from MPX families are likely to share genetic vulnerability for the disorder, whereas SPX ADHD may be the result of sporadic (non-)genetic causes unique to the patient, we hypothesized that cognitive impairments may be different in SPX and MPX ADHD as indicated by (a) the presence of cognitive deficits in MPX, but not SPX unaffected siblings and (b) dissimilar cognitive profiles in SPX and MPX ADHD patients.
Methods: Tasks measuring total IQ, verbal attention, executive functioning, motor functioning, and time estimation were administered to 31 SPX/264 MPX ADHD probands, 47 SPX/123 MPX unaffected siblings, and 263 controls, aged 6-19 years.
Results: SPX unaffected siblings were unimpaired compared to controls, except for verbal working memory, whereas MPX unaffected siblings showed impairments on most cognitive domains. The cognitive profiles of SPX and MPX probands were highly similar, except that verbal attention, response inhibition and motor control deficits were more pronounced in MPX probands, and -compared to their unaffected siblings- impairments in IQ, visual working memory and timing abilities were more pronounced in SPX cases.
Conclusions: Our results support the hypothesis that a partly different cognitive architecture may underlie SPX and MPX forms of ADHD, which becomes evident when contrasting cognitive performances *within* families. Cognitive factors underlying MPX forms of ADHD are familial, whereas non-familial in SPX ADHD. SPX-MPX stratification may be a step forward in unravelling diverse causal pathways.

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a severely impairing neurodevelopmental disorder, characterized by symptoms of hyperactivity, impulsivity and/or inattention (Diagnostic and Statistical Manual of Mental Disorders; DSM-5) (APA, 2013). ADHD is a highly heritable disorder, with heritability estimates ranging to 76% (Faraone *et al.*, 2005, Thapar *et al.*, 2013). Common to ADHD is the large within-disorder heterogeneity, in symptom presentation, developmental course and underlying etiological mechanisms (Wahlstedt *et al.*, 2009). The prevailing etiological model suggests that ADHD is caused by small disease-increasing effects of multiple common genetic and environmental risk factors (Franke *et al.*, 2009, Thapar *et al.*, 2013). However several recent studies report that rare genetic mutations or non-shared environmental factors (such as low birth weight and medical conditions) with a large effect may relate to ADHD aetiology as well (Ben Amor *et al.*, 2005, Williams *et al.*, 2010). This suggests that while in many cases multifactorial factors, possibly shared with (unaffected) relatives, might underlie ADHD, factors uniquely present in affected individuals, such as *de novo* mutations, might underlie the disorder in at least some cases.

More insight into the role of shared versus unique genetic factors for ADHD might be obtained by examining the presence of ADHD-related cognitive deficits in unaffected siblings of ADHD probands in the search for cognitive endophenotypes of ADHD. Endophenotypes are defined as heritable vulnerability traits that heighten the risk for developing a disorder (Gottesman and Gould, 2003). Endophenotypes offer a simplified approach to dissect complex traits by reducing heterogeneity and as such may boost the power for genetic analyses, as well as shed light on the functional outcomes of genes (Gottesman and Gould, 2003). Cognitive deficits that are present in unaffected siblings and thus shared between affected and unaffected relatives are assumed to provide an index of the multifactorial liability to ADHD (Waldman, 2005). Conversely, cognitive deficits that are not shared between affected and unaffected siblings may have a unique effect on the development of the disorder. This affected-unaffected siblings design has been frequently applied in ADHD research and has led to many studies documenting an increased incidence of behavioral symptoms, comorbid symptomatology, and ADHD-related cognitive deficits in unaffected family members of ADHD probands (for an extensive review see Rommelse *et al.*, 2011).

We may improve our understanding of the role of common versus unique genetic risk factors in ADHD by examining ADHD related cognitive deficits in single-, and multi-incidence ADHD families. We hypothesized that ADHD-related cognitive deficits are only present in unaffected family members from multi-incidence (here referred to as multiplex; MPX), but not single-incidence (here referred to as simplex: SPX) ADHD families. SPX families are defined as nuclear families with only one affected individual and at

least one unaffected male sibling. MPX families consist of at least two (or more) affected individuals in the family (Sullivan *et al.*, 2012). The assumption is that individuals from SPX families are more likely than individuals from MPX families to develop ADHD as a result of sporadic genetic and/or non-genetic causes strictly unique to the patient. Then unaffected relatives in SPX families would show less or even no behavioral or cognitive deficits compared controls and would deviate more from the cognitive profile of their affected brother or sister. In contrast, unaffected relatives in MPX families would show cognitive deficits, compared to controls, and about as similar as the probands. In other words, the within-family contrast between probands and unaffected siblings regarding cognitive or behavioral aspects of the disorder is larger in SPX compared to MPX families. Unaffected siblings can be viewed as an ideal reference group, indexing the 'full potential' of children with ADHD had they not developed the disorder (while correcting for shared environmental influences). Higher within-family contrasts might thus be indicative of more severely impaired cognitive abilities in the affected children from those families. This model of different etiologies in SPX and MPX families has been developed and confirmed in research in Autism Spectrum Disorders (ASD) (Gerdts *et al.*, 2013, Sebat *et al.*, 2007). For example, a more than threefold rate of de novo mutations were identified in ASD SPX families (~7-10%), compared to ASD MPX families (~2-3%) or control families (~1%) (Sebat *et al.*, 2007). In contrast, members of MPX families more often exhibit ASD traits compared to members of SPX families, indicative of a more pronounced role of shared genetic predispositions (Gerdts *et al.*, 2013). The association between de novo mutations and ADHD has received little research attention, unlike ASD (D'Onofrio *et al.*, 2014). Recent studies that point towards a role for rare genetic variants such as de novo mutations in ADHD highlight the need for future studies exploring this issue (Ben Amor *et al.*, 2005, D'Onofrio *et al.*, 2014, Williams *et al.*, 2010).

The present study extends the findings by Rommelse et al. (Rommelse *et al.*, 2008a, Rommelse *et al.*, 2007a, Rommelse *et al.*, 2008b, Rommelse *et al.*, 2007b, Rommelse *et al.*, 2008c, Rommelse *et al.*, 2007c, Rommelse *et al.*, 2008d) by testing whether ADHD-related cognitive deficits are only present in unaffected siblings from MPX ADHD families. If correct, then the use of cognitive endophenotypes in the search for ADHD risk genes might be of particular use for MPX, but not SPX ADHD. Further, we aimed to examine whether cognitive impairments may be different in SPX and MPX ADHD as indicated by dissimilar cognitive profiles in SPX and MPX ADHD patients. So far, no studies have been undertaken that differentiate between single- and multi-incidence ADHD, but it is plausible that different heritable forms of ADHD might result in dissimilar cognitive disabilities.

METHODS

Participants

ADHD families were recruited as part of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (as previously described in Rommelse *et al.*, 2008a). Inclusion criteria for all participants were at least two biological siblings (in case families: at least one child with a clinical diagnosis of ADHD) and one biological parent willing to participate, offspring age between 4 and 20 years, European Caucasian descent, an IQ ≥ 70, and no diagnosis of autism, epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome. All children and parents were carefully phenotyped for ADHD using validated and standardized questionnaires and diagnostic interviews. Families were stratified into SPX and MPX based on the number of affected individuals. SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ADHD; MPX families were required to have two or more affected individuals. A total of 31 ADHD SPX nuclear families (including 31 probands and 47 unaffected siblings), 171 ADHD MPX nuclear families (including 264 probands and 123 unaffected siblings), and 142 control nuclear families (263 children) were included in the current study, see Table 1 for sample characteristics and Oerlemans *et al.*, 2014 (chapter 4) for a full description of phenotyping and family classification.

Table 1. Sample characteristics

	Controls (c)		ADHD probands				Unaffected siblings				Group contrasts ADHD vs. controls
			1. SPX		2. MPX		3. SPX		4. MPX		
	M	sd	M	sd	M	sd	M	sd	M	sd	
Number of kids	N = 263		N = 31		N = 264		N = 47		N = 123		
Age	11.7	3.2	11.8	2.5	11.5	2.6	10.9	3.4	11.6	3.5	ns
Sex (% males)	41.1		87.1		73.6		74.5		38.2		1=2=3>4=c
CPRS DSM-IV Combined Scale	46.5	4.5	76.1	8.0	74.8	10.3	48.1	7.3	50.6	9.5	1=2>3=c, 3=4, 4>c
CTRS DSM-IV Combined scale	46.4	4.5	69.7	8.0	67.7	10.8	48.3	6.3	50.1	7.9	1=2>3=c, 3=4, 4>c

Note. ADHD = attention-deficit/hyperactivity disorder; SPX = simplex; MPX = multiplex, CPRS = Conners Parent Rating Scale; CTRS = Conners teacher rating scale; c = controls; 1 = SPX probands; 2 = MPX probands; 3 = SPX unaffected siblings; 4 = MPX unaffected siblings; ns = non significant

Measures

Cognitive functioning was examined across a range of domains. Full scale IQ was prorated by four subtests of the Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale: Similarities, Vocabulary, Block Design and Picture Completion

(Wechsler, 2000, 2002). The forward condition of Digit Span was used to obtain an indication of verbal attention. Four executive function tasks were included: response inhibition, visual and verbal working memory, and set shifting. Response inhibition was measured with the commonly used Go-NoGo paradigm where participants were instructed to withhold a response when the NoGo target was depicted. Visual and verbal working were measured by instructing the participants to correctly reproduce sequences of figures (visual) or digits (verbal) that increased in difficulty after each succeeded trial. Set shifting was measured by administering a task that required a mixture of compatible and incompatible responses, hypothesized to require a higher level of cognitive flexibility. Motor functioning was measured using a simple reaction time task and a motor control task. Last, a timing measure was included to measure the variability of motor timing. Table 2 provides an overview of the neurocognitive tasks used. For full task descriptions, see Appendix 1 or elsewhere (Rommelse *et al.*, 2008a).

Table 2. Description of the neurocognitive tasks

Task	Measurement potential	Dependent variables
Intelligence		
Vocabulary, Similarities, Block Design, Picture Completion[a]	intelligence	Total IQ (TIQ)
Attention		
Digit Span[a]	Verbal attention	maximum span forward
Executive functions		
Stop Task	inhibition	stop signal reaction time (SSRT)
Digit Span[a]	verbal working memory	maximum span backwards
Visuospatial Sequencing	visuospatial working memory	percentage correct identified targets in correct order (part forward)
Shifting Attentional Set Visual	set shifting	percentage errors
Motor functions		
Baseline Speed	baseline variability	variability of reaction time (SD in ms).
Tracking	motor control without continuous adaptation	stability (SD of distances in mm). (non-preferred hand)
Timing		
Motor Timing	timing estimation	variability in reaction time (SD)

Note. For task details, see Appendix 1 or elsewhere (Rommelse et al., 2008a).
[a] based on Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale

Procedure

Neurocognitive assessment of the children with ADHD and their siblings took place at the VU University Amsterdam or at the Radboud university medical center in Nijmegen, the Netherlands and is described in more detail elsewhere (Rommelse *et al.*, 2008c).

To avoid possible inter-rater or location effects, cognitive performance was measured using standardized computerized tasks with fixed settings and computer-calculated outcome measures (e.g. error percentages or mean reaction times) across the two sites. In addition, all examiners were thoroughly trained using a standardized training protocol and were regularly supervised and observed during task administration to monitor standardized assessment across sites and examiners. Stimulants were discontinued for at least 24 hours before testing and non-stimulants according to their plasma half life to allow for sufficient wash-out. Children were motivated with small breaks and received a gift at the end of the session. Additional data collected included blood or saliva samples and behavioral data of all family members. The study was approved by the local medical ethics board. After the study procedures had been fully explained, parents and children (12 years and older) signed for informed consent. Children younger than 12 years of age were asked to give their assent for participation.

DATA-ANALYSES

The percentage of missing data was <5% for all dependent measures, except for stop signal reaction time (SSRT). Here, 8.4% of the data were missing. Missings were imputed by means of Expectation Maximization (Tabachnick and Fidell, 2001). Analyses were carried out with and without expectation maximization, which revealed similar results and led to the same conclusions. Results were therefore reported with missing data replaced. To account for the influence of age and sex on neurocognitive performance, we regressed scores for each measure on age and sex and used the unstandardized residuals as dependent variables. Most of the unstandardized residuals were not normally distributed, therefore, a van der Waerden transformation was applied to normalize the dependent measures (Norusis, 1992). This also facilitated the comparison between variables since variables were all depicted on the same scale. A number of dependent variables were mirrored so that the z-scores of all measures had the same meaning: lower z-scores indicated poorer performance (e.g. more errors or more variable responses).

Linear mixed models (LMM) were used to account for the dependency in the data due to inclusion of siblings and probands by estimating a random intercept. Dependent variables were the neurocognitive measures and group was the independent variable. We contrasted specific groups of interest to answer our research questions. LMM analyses were run with group defined as (a) probands versus unaffected siblings versus controls, separately for SPX and MPX families, to examine whether cognitive deficits were present in (SPX and MPX) probands and MPX, but not SPX, unaffected siblings, (b) SPX versus MPX unaffected siblings to examine whether cognitive performance of first-degree relatives was poorer in MPX compared to SPX families, and (c) MPX versus SPX probands

to examine whether potentially different heritable forms of ADHD would result in (dis) similar cognitive profiles in ADHD patients. Furthermore, within family discrepancy scores (estimated mean of proband minus mean of unaffected sibling) in SPX versus MPX families were compared to examine whether within family contrast was higher in SPX than MPX families. A False Discovery Rate (FDR) correction with a q-value setting of 0.05 was applied to control for multiple testing (Benjamini, 2010). Given the unequal sample size for MPX and SPX families, emphasis was given to effect sizes next to the p-values. Effect sizes (Cohen's d) were calculated to define small (d = .20), medium (d = .50), and large effects (d = .80) (Cohen, 1988). All analyses were carried out in SPSS version 20.

RESULTS

Cognitive measures sensitive for SPX-MPX stratification

Endophenotypes in MPX but not SPX ADHD families

Testing our first hypothesis, we found indeed that SPX unaffected siblings were unimpaired compared to controls on all cognitive domains (all p-values >.17, effect sizes in terms of Cohen's d ranging from .00-.22) except for verbal working memory (p=.029, d=.32), whereas MPX unaffected siblings performed poorer than controls on all cognitive domains (p-values <.033, d-values =.21-.49), except for stability of motor control (p=.151, d=.15). Moreover, comparisons between SPX and MPX unaffected siblings revealed a significantly better performance of SPX unaffected siblings in three domains, namely visual working memory (p=.024, d=.40), inhibition (p=.005, d=.51), and time estimation: p=.005, d=.49). Within-family discrepancy (proband-unaffected sibling contrast) was larger for SPX probands than for MPX probands for visual working memory (t=2.65, p=.012). SPX probands differed significantly from their unaffected siblings on TIQ, visual working memory, and variability of time estimation (p-values <.009, d-values =.53-.74), whereas MPX probands differed from their unaffected siblings only on TIQ (p<.001, d=.29), see Figure 1 and Table 3.

Cognitive deficits in MPX versus SPX ADHD probands

Testing our second hypothesis, we found that the cognitive profiles of SPX and MPX probands were highly similar. Both probands from SPX and from MPX families performed significantly worse than controls on estimated TIQ, verbal and visual working memory, and variability of time estimation (SPX; p-values <.006, d-values =.52-.71; MPX: p-values <.001, d-values =.43-.64) and could not be dissociated from each other (p-values >.20, d-values <.22).

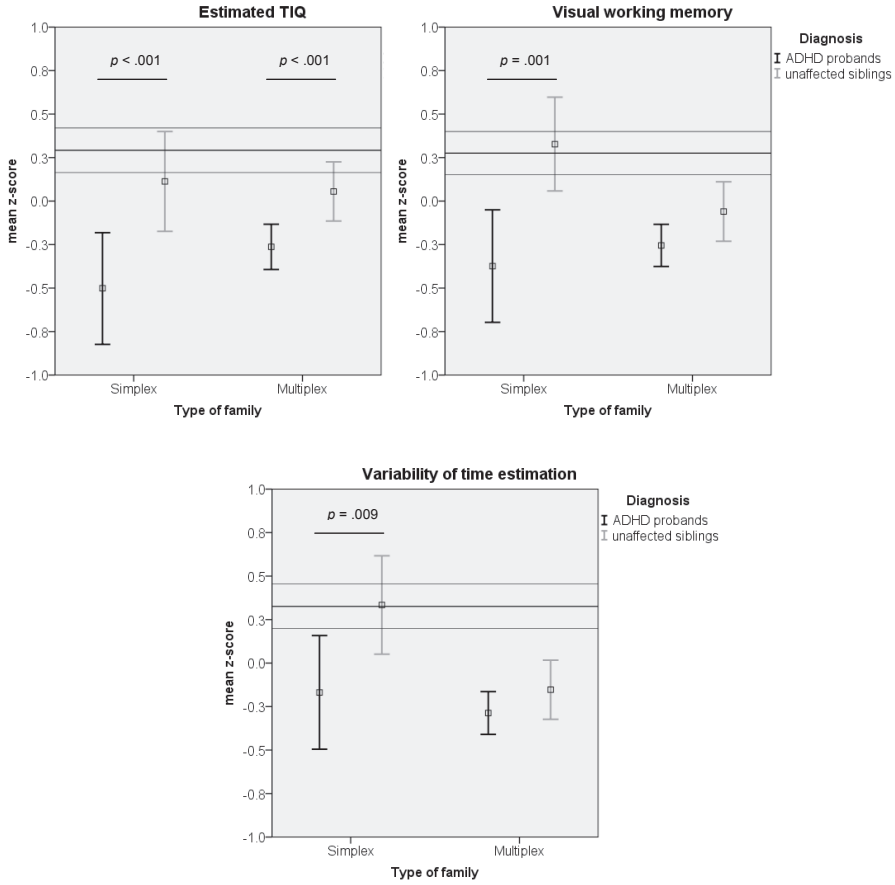

Figure 1 Cognitive deficits in MPX, but not SPX unaffected siblings from ADHD families.
Note. The interpolation lines represent the mean z-score and the 95% CI of normal controls. The *error bars* represent the 95% confidence interval (CI). Lower z-scores indicate worse performance. Significant group differences that survived FDR correction between case groups and controls, are depicted using asterisks (*** $p < .001$, ** $p < .01$). SPX and MPX ADHD probands performed significantly worse than controls and could not be dissociated from each other on TIQ, visual memory and variability of time estimation. MPX, but not SPX unaffected siblings showed similar cognitive impairments on these domains.

Impairments in verbal attention, response inhibition, set shifting, and stability of motor control appeared to be most pronounced in MPX ADHD probands. Relative to normal controls, MPX probands showed significant impairments (p-values <.029, d-values =.21-.45), whereas SPX probands showed no problems on inhibition (p=.341, d=.18), set shifting (p=.218, d=.25), or motor control problems (p=.445, d=.13). The significant difference between SPX probands and controls on verbal attention (p=.043, d=.38) did not survive FDR correction (q-value =.132). However, SPX and MPX probands could not

Table 3. Means and standard errors of the transformed task variables for SPX and MPX probands, their unaffected siblings and normal controls

	Controls (c)		ADHD probands		unaffected siblings		Group contrasts		Within family contrasts		Comparisons between SPX and MPX family members			
											probands		unaffected siblings	
	M (se)	Family type	M	se	M	se	*p-values**	*d-values**	*t*	*p*	*p*	*d*	*p*	*d*
Endophenotypes in MPX but not SPX ADHD families														
TIQ	.29 (.07)	SPX	-.50	.17	.11	.15	**<.001**/.264/**<.001**	.71/.16/.62	1.421	.164	.212	.22	.496	.05
		MPX	-.26	.07	.06	.09	**<.001/.033/<.001**	.49/.21/.29						
Visual WM	.28 (.06)	SPX	-.37	.17	.33	.14	**<.001**/.726/**.001**	.67/.05/.74	**2.652**	**.012**	.502	.11	**.024**	**.40**
		MPX	-.26	.06	-.06	.09	**<.001/.002**/.054	.56/.35/.20						
Variability of time estimation	.33 (.06)	SPX	-.17	.17	.33	.14	**.006**/.960/**.009**	.52/.00/.53	1.909	.065	.516	.12	**.005**	**.49**
		MPX	-.29	.06	-.15	.09	**<.001/<.001**/.168	.64/.49/.14						
Cognitive deficits in MPX but not SPX ADHD probands														
Verbal attention	.23 (.06)	SPX	-.14	.17	.04	.14	.043/.239/.378	.38/.20/.19	.463	.677	.880	.03	.463	.12
		MPX	-.17	.06	-.08	.09	**<.001/.006**/.352	.41/.32/.10						
Inhibition SSRT	.24 (.06)	SPX	.07	.17	.29	.15	.341/.792/.273	.18/.05/.22	.834	.322	.157	.28	**.005**	**.51**
		MPX	-.20	.06	-.22	.09	**<.001/<.001**/.856	.45/.47/.02						
Set shifting % errors	.13 (.06)	SPX	-.11	.18	.06	.15	.218/.690/.444	.25/.07/.17	.651	.518	.853	.04	.248	.17
		MPX	-.07	.06	-.11	.09	**.029/.032**/.708	.21/.25/.04						
Stability of motor control	.22 (.07)	SPX	.07	.18	-.02	.16	.445/.167/.666	.13/.22/.09	.833	.411	.159	.27	.777	.07
		MPX	-.19	.06	-.09	.09	**<.001/.005**/.340	.39/.28/.10						
Measures not sensitive to SPX-MPX stratification														
Verbal WM	.26 (.07)	SPX	-.35	.18	-.10	.15	**.001**/.029/.237	.55/.32/.25	.673	.654	.363	.16	.606	.07
		MPX	-.19	.06	-.03	.09	**<.001/.006**/.138	.43/.27/.16						
Baseline variability	.08 (.07)	SPX	-.01	.18	.-.10	.15	.667/.878/.632	.08/.16/.09	.400	.691	.764	.06	.340	.02
		MPX	-.07	.06	-.08	.09	.108/.151/.898	.14/.15/.01						

Note. ADHD = attention-deficit/hyperactivity disorder, SPX = simplex, MPX = multiplex, M = mean, se = standard error, WM = working memory. SSRT = stop signal reaction time. Significant group contrasts after FDR correction, are presented in bold.

*p-values and *d*-values are presented in the following order: probands vs. controls / siblings vs. controls / probands vs. siblings

be dissociated from each other (*p*-values >.15, *d*-values <.28) nor from their unaffected siblings (*p*-values >.27, *d*-values <.22) on these domains and within-family discrepancy did not differ between SPX and MPX families (*t*-values <.85, *p*-values >.30), see Figure 2.

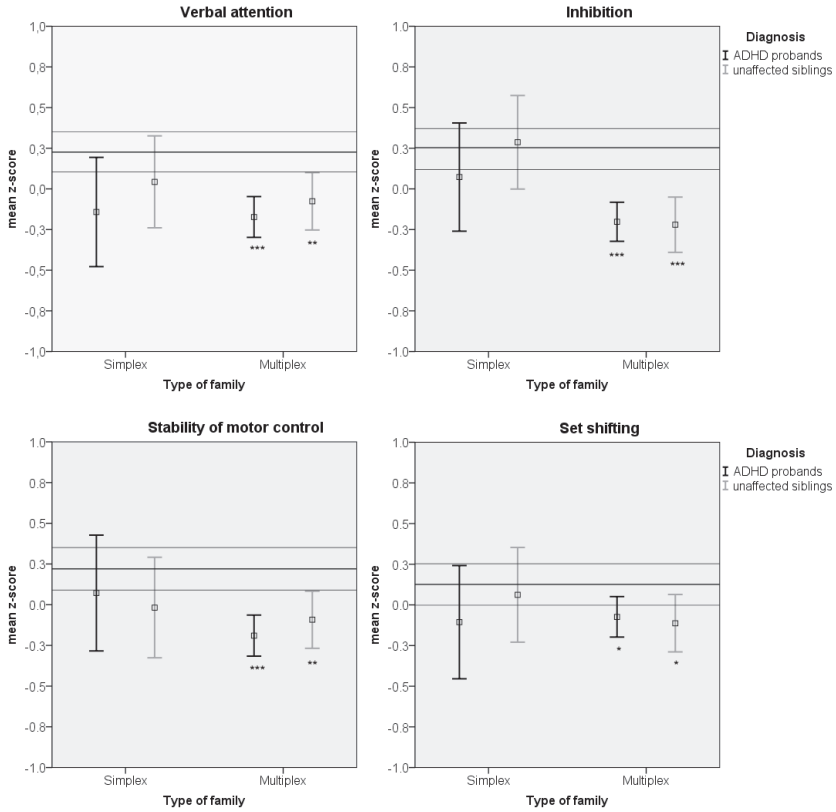

Figure 2 Cognitive domains most pronounced in MPX ADHD families
Note. The interpolation lines represent the mean z-score and the 95% CI of normal controls. The *error bars* represent the 95% confidence interval (CI). Lower z-scores indicate worse performance. Significant group differences that survived FDR correction between case groups and controls, are depicted using asterisks (*** $p < .001$, ** $p < .01$, *$p < .05$). Probands and unaffected sibling from MPX, but not SPX ADHD families were impaired on verbal attention, inhibition, set shifting and motor control.

Measures not sensitive to SPX-MPX stratification

A few domains were insensitive to SPX-MPX stratification. First, for verbal working memory, a performance intermediate between cases and controls was found in MPX *and* SPX unaffected siblings. Both SPX and MPX unaffected siblings performed significantly worse than controls (*p*=.029, *d*=.32 and *p*=.006, *d*=.27, respectively), but similar to their affected brothers/sisters (*p*-values >.13, *d*-values <.25). However, the difference between SPX

unaffected siblings and controls became non-significant after FDR correction (*q*-value =.077). Second, probands and unaffected siblings from both SPX and MPX families were equally unimpaired on baseline variability (*p*-values >.10, *d*-values <.16), see Table 3.

DISCUSSION

In the current study, we aimed to examine whether the cognitive architecture underlying SPX and MPX ADHD families is different and useful for parsing the etiological heterogeneity of ADHD. Based on the assumption that individuals from SPX families are more likely than individuals from MPX families to develop ADHD as a result of sporadic genetic and/or non-genetic causes strictly personal to the patient, we hypothesized that shared cognitive deficits between affected and unaffected siblings are present in MPX, but not SPX families. Further, we hypothesized that potentially different heritable forms of ADHD might result in dissimilar cognitive profiles in SPX and MPX ADHD probands. Consistent with our hypothesis, SPX unaffected siblings were unimpaired compared to controls, except for verbal working memory, whereas MPX unaffected siblings showed an intermediate performance between cases and controls on most domains. Furthermore, the cognitive profiles of SPX and MPX probands were highly similar, except that (a) impairments in response inhibition and stability of motor control were more pronounced in MPX probands than in SPX probands, and (b) when compared to their unaffected siblings, impairments in TIQ, visual working memory and timing abilities were more pronounced in SPX cases compared to MPX cases.

Results largely confirmed the hypothesized dissociation between SPX and MPX families based on cognitive performance of probands and their unaffected siblings. Indeed, unaffected siblings from MPX families demonstrated a similar (but milder) cognitive vulnerability profile as probands from those families, whereas unaffected siblings from SPX families were indistinguishable from controls on all measures but verbal working memory. The former finding replicates previous analyses in this sample (Rommelse *et al.*, 2008a, Rommelse *et al.*, 2007a, Rommelse *et al.*, 2008b, Rommelse *et al.*, 2007b, Rommelse *et al.*, 2008c, Rommelse *et al.*, 2007c, Rommelse *et al.*, 2008d) as well as many previous studies without stratification according to family history. The latter finding is novel and indeed suggests that in a percentage of ADHD cases (15.3% in our sample) different modes of inheritance may underlie the disorder in the proband that are mostly not shared with the unaffected family members. These SPX probands further seem to be relatively more strongly impaired in TIQ, visual working memory and timing abilities. When using unaffected siblings as an ideal reference group (viewed as indexing the 'full potential' of children with ADHD had they not developed the disorder), an 'SPX subtype' of ADHD may relate to factors that particularly decrease overall intelligence, visual work-

ing memory and time estimation. (Rare) genetic variations in genes associated with IQ/ intellectual (dis)ability and working memory (e.g. *COMT*) (Boonstra *et al.*, 2008, Green *et al.*, 2013), or environmental factors that have a detrimental effect on the development of the brain (e.g. prematurity, low birth weight and fetal distress) (Bilder *et al.*, 2013), might thus play particularly important roles in the development of SPX ADHD. This suggests that sporadic ADHD might be more prevalent among children with lowered (but still normal) intelligence levels. In contrast, the classical response inhibition difficulties as well as verbal attention and motor coordination problems were less outspoken in SPX versus MPX ADHD. This may suggest that factors related to these traits (e.g. genetic polymorphisms in *DAT1* are associated with response inhibition (Boonstra *et al.*, 2008)) are less involved in SPX forms of ADHD. The dopamine-modulated basal ganglia neurocircuits are proposed to underpin inhibitory control and also play an important role in motor control (Fliers *et al.*, 2009, Sonuga-Barke, 2005). Moreover, dopamine plays an important role in attention and auditory processing (Bailey, 2012). Abnormalities in structure and function of these circuits caused by genetic variation might thus be hypothesized to be less often observed in SPX ADHD. It is challenging to explain why SPX unaffected siblings were impaired in verbal working memory and not other cognitive domains. A possible explanation might be that auditory (or verbal) tasks are generally more difficult than visual tasks, because auditory measures are more closely related to the attentiveness required for daily life than visual measures (Park *et al.*, 2011). Given that (a) SPX unaffected siblings displayed somewhat elevated levels of ADHD traits compared to controls (see sample characteristics) and (b) inattention is a core characteristic of ADHD, this might explain why SPX unaffected siblings did show some problems in this particular area. However, since SPX unaffected siblings were unimpaired on verbal attention, this suggests that the verbal working memory deficit is not fully explained by attention problems. Working memory problems in SPX unaffected siblings did not extent to the visuo-spatial domain. Possibly, verbal working memory is most sensitive to (mild) susceptibility for ADHD. Additional research is however needed to further investigate this issue. In any case, these preliminary findings suggest that the rarer SPX forms of ADHD may have partially different cognitive underpinnings compared to MPX forms of ADHD and a different pattern of familial-determined cognitive vulnerabilities, with minimal cognitive vulnerabilities in unaffected siblings.

In contrast to the situation in SPX families, when selectively analyzing cognitive traits in family members from MPX families, virtually for all cognitive domains a strong endophenotypic group pattern was found: non-affected siblings originating from families in which at least two members had ADHD, showed substantial cognitive vulnerabilities, similar to their affected sibling. These findings suggest that in families with shared risk factors for ADHD, using cognitive traits to detect these underlying causal factors may be a powerful approach. Particularly impairments in inhibition, motor control, visual working memory and

time estimation seem sensitive to such effects and promising areas for further research in this context. These neurocognitive functions may be useful in creating more homogeneous subgroups of patients with (MPX) ADHD. This reduces heterogeneity and may facilitate our understanding of the involved biological processes, boost power for genetic analyses, as well as shed light on the functional outcomes of genes (Gottesman and Gould, 2003).

The direct comparison between SPX and MPX probands revealed very similar cognitive problems. These findings suggest a phenomenon referred to in developmental psychopathology as equifinality (Cicchetti and Rogosch, 1996), that is, even though partly different developmental pathways might underlie SPX and MPX ADHD, these result in quite similar cognitive deficits and also in similar severity of ADHD symptoms. By examining cognitive functions or behavioral symptoms alone, these different underlying etiological factors cannot be identified. Instead, causal effects might be muted by the presence of multiple distinct subgroups of ADHD patients with different etiologies (Nigg *et al.*, 2005). Although the reality of equifinality is well-recognized, few solutions have been provided to tease etiological heterogeneity apart. Stratification into SPX and MPX seems therefore highly relevant in defining relevant subgroups in order to facilitate research that aims to unravel these multiple pathways leading to the same cognitive impairments and ADHD symptomatology

Our findings further highlight the fact that there is clearly no 1:1 relationship between cognitive problems and behavioral problems (de Zeeuw *et al.*, 2008): unaffected siblings from SPX and MPX families did not differ from each other regarding (the absence of) ADHD symptoms, yet substantial cognitive vulnerabilities were only present in MPX unaffected siblings. This corroborates with the conclusions from a systematic review on cognitive (dis)similarities in ADHD persisters and remitters; both were equally impaired at follow-up on almost all domains assessed (van Lieshout *et al.*, 2013). It suggests that cognitive vulnerabilities and behavioral problems are to some extent disentangled during the course of development. It has been hypothesized that neurocognitive deficits in ADHD are epiphenomena instead of core causal factors, that are related to the same etiological factors but do not mediate between genes and behavior (Kebir and Joober, 2011, Kendler and Neale, 2010, Rommelse *et al.*, 2011). This could explain the highly similar cognitive profiles of MPX affected and unaffected siblings (who are likely to share etiological risk factors for ADHD), and the highly deviant cognitive profiles of SPX affected and unaffected siblings (where causal risk factors for ADHD appear strictly personal to the patient). More longitudinal studies are definitely needed in this fascinating area of research.

A number of limitations of this study need to be considered. First, only a small proportion of families could be classified SPX (13.5% in our sample). Therefore, we calculated effect sizes to accompany statistical testing. We nonetheless restricted interpretation to significant findings; it follows that our study need replication in larger samples. Further, boys were overrepresented in both proband groups and in SPX unaffected siblings, but were under-

represented in MPX unaffected siblings and controls. This was due to the fact that a) in childhood, ADHD is more frequently diagnosed in males and b) the presence of male unaffected siblings was only required for SPX, but not MPX families. However, we do not believe that this has affected the results, since the effect of sex was controlled for in this study.

In all, our results support the hypothesis that a partly different cognitive architecture may underlie SPX and MPX forms of ADHD, which becomes evident when contrasting cognitive performances *within* families. When using performance of unaffected siblings as a reference, TIQ, visual working memory and time estimation are particularly impaired in SPX ADHD, suggesting sporadic (non-)genetic causes acting predominantly on these domains. Response inhibition and motor control seem relatively unimpaired in SPX forms of ADHD. In contrast, familial (MPX) ADHD is related to a wide range of cognitive vulnerabilities, translated to comparable (but milder) impairments in non-affected siblings. These findings suggest that different causal pathways may lead up to –on the surface- comparable cognitive deficits and ADHD symptoms in children with ADHD, and that SPX-MPX stratification may be a step forward in unravelling these various causal pathways. Clinically, subgroups of ADHD patients may have distinct prognoses and benefit most from different treatment strategies (Nigg *et al.*, 2005), which indicates that awareness of the impact of family history on the presence of ADHD traits and cognitive impairments in probands and their unaffected siblings is relevant for the development of treatment plans and for genetic counseling.

Key bullet points

- The etiology of ADHD is heterogeneous; small disease-increasing effects of multiple common genetic variants likely play a role in multi-incidence (MPX) families, while rare genetic variants (e.g. de novo mutations) likely play a role in single-incidence (SPX) families.
- We may improve our understanding of etiological heterogeneity of ADHD by studying cognitive deficits in SPX versus MPX ADHD.
- A partly different cognitive architecture appears to underlie SPX and MPX ADHD. MPX ADHD is related to a wide range of cognitive vulnerabilities, translated to comparable (but milder) impairments in unaffected siblings. SPX ADHD is related to TIQ, visual working memory and time estimation impaired in probands but not in siblings.
- Clinically, SPX and MPX ADHD patients may have distinct prognoses and benefit from different treatment strategies.

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APPENDIX 1. FULL DESCRIPTION OF NEUROPSYCHOLOGICAL MEASURES

Measures

Four of the tasks described below were selected from the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville, 1999b). The ANT is a computer-aided assessment battery that allows for the systematic evaluation of information processing capacities. Test-retest reliability and validity of the ANT-tasks are satisfactory (De Sonneville, 2005). Each computer task contained an instruction trial where the examiner provided a typical item of the task, and a separate practice session. If necessary, the instruction was repeated. All subjects were able to perform the training items before testing. Furthermore, several subtests from the Wechsler Intelligences Scales for Children (WISC-III) or the Wechsler Adult Intelligence Scale (WAIS-III) were selected (Wechsler, 2000, 2002). These subtests were administered following manual guidelines.

Intelligence

Full scale IQ was prorated by four subtests of the WICS-III or WAIS-III: Similarities, Vocabulary, Block Design and Picture Completion (Wechsler, 2000, 2002). Verbal IQ (VIQ) and Performal IQ (PIQ) were each prorated by two subtests (i.e. Similarities and Vocabulary for VIQ and Block Design and Picture Completion for PIQ). These selected WISC-III subtests are known to correlate between .90-.95 with the Full-scale IQ (Groth-Marnat, 1997).

Executive function

Executive function (EF) was measured using tests that tap into three major aspects of EF, namely inhibition, cognitive flexibility and working memory. All measures were standardized and some scores were mirrored so that low z-scores indicated poorer performance). Inhibition was measured using the Stop task (Logan, 1994, Logan *et al.*, 1984). Children were presented with two types of trials: go-trails and stop-trails. Children were asked to press a mouse key as quickly and accurately as possible when the go-stimulus was presented, but withhold their response to the stop-trial. Dependent variable was the Stop signal reaction time (SSRT). Cognitive flexibility was measured using the Shifting Attentional Set (De Sonneville, 1999a). A horizontal bar with ten grey squares was presented permanently at the centre of the screen. The stimulus was a colored square that moved across the bar in a random direction (either one square to the left or to the right). Three parts were administrated: in part 1, the stimulus was colored green and *compatible responses* were required (i.e. children were instructed to click the mouse key that corresponded to the direction in which the stimulus moved). In part 2, the stimulus was colored red and *incompatible responses* were required (i.e. children were instructed to click the response mouse button opposite to the direction of the moving stimulus). In part 3, the color of the stimulus shifted randomly between green and

red and *both compatible and incompatible responses* were required. Cognitive flexibility was operationalized as the difference in percentage of errors (accuracy) between part 1 and the compatible trials of part 3 (Oerlemans *et al.*, 2013). Working memory (WM) was measured using two tasks: one spatial and one verbal task. Spatial WM was tested using the Visuo-Spatial Sequencing (VSS) task. Stimuli consisted of nine figures presented symmetrically in a 3 by 3 square. On each trail, a sequence of figures was pointed at by a computer-driven hand. Children were then instructed to reproduce the sequence in forward order. The difficulty level increased after each succeeded trial. Dependent measure was the total percentage of correctly identified targets in the correct order. Verbal WM was measured using the maximum span of the backward condition of the Digit Span subtest of the WISC-III/WAIS-III. Children were instructed to repeat a sequence of digits in backward order. One digit was added to the sequence if the child reproduced the sequence successfully (Wechsler, 2002).

Motor functioning

Motor functioning was measured using a simple reaction time task and a motor control task. The Baseline Speed (BS) task was used to measure the variability of motor output (De Sonneville, 1999a). When a fixation cross in the center of a computer screen changed into a white square, children were asked to press a mouse key as quickly as possible. To prevent anticipation strategies, the time interval between a response and the next emergence of the square varied randomly. Dependent measure was the standard deviation of reaction time in ms. Tracking (TR) was used to test motor control (De Sonneville, 1999a). The task was completed with the non-preferred hand; hand preference was ascertained by asking children with which hand he/she preferred to draw or write. Children were instructed to trace an invisible midline (radius 8 cm) between an outer (radius 8.5 cm) and inner (radius 7.5cm) circle with a mouse cursor, clockwise with the right hand and counter clockwise with the left hand. Dependent variable was the standard deviation of the distances in mm (stability).

Timing

The Motor Timing task was designed to measure the variability of motor timing (Le Couteur *et al.*, 2003). Children were asked to press a mouse button when they thought a 1-second time interval had elapsed. The start of the interval was announced by a tone. After the response, visual feedback concerning the accuracy of the response was presented on the screen. A response was corrected if it fell between the lower and upper boundary set by a dynamic tracking algorithm. The dependent measure was the standard deviation of reaction times in ms.

CHAPTER 6

Does the cognitive architecture of simplex and multiplex ASD families differ?

Based on:

Oerlemans, A.M., Hartman, C.A., Franke, B., Buitelaar, J.K. & Rommelse, N.N.J. Does the cognitive architecture of simplex and multiplex ASD families differ?

(submitted for publication)

ABSTRACT

Background: The heterogeneity of autism spectrum disorders (ASD) hinders research into etiology and effective treatment. An approach to parse etiologic heterogeneity is to form more homogeneous subgroups of patients based on their familial occurrence of the disorder (stratification into so-called multiplex [MPX] versus simplex [SPX] families). The aim of this study was to test the hypothesis that SPX and MPX ASD families differ in their cognitive architecture.

Methods: Tasks measuring intelligence, social cognition, and executive functioning were administered to 54 SPX and 91 MPX ASD probands, 77 SPX and 46 MPX unaffected siblings, and 124 controls aged 6-20 years.

Results: SPX and MPX ASD cases did not differ in cognitive performance; both showed similar impairments in verbal and performal IQ, face and affective prosody recognition, and verbal working memory. Unaffected siblings (regardless SPX or MPX) performed normal on most cognitive tasks, except for impaired affective prosody recognition in SPX and MPX siblings and lower IQ scores in MPX (but not SPX) siblings. Significant within-family contrasts were found in SPX (but not MPX) ASD families.

Conclusions: These results suggest quantitative rather than qualitative differences between SPX and MPX forms of ASD: SPX affected children were more dissimilar from their non-affected sibling compared to MPX affected children from their non-affected sibling. Further, cognitive deficits associated with ASD may have a stronger determining effect on the disorder compared to related disorders such as attention-deficit/hyperactivity disorder (ADHD). Recognition of affective prosody may be particularly sensitive towards familial risk factors for ASD.

INTRODUCTION

Autism spectrum disorder (ASD) is a group of highly heritable and severely impairing neurodevelopmental disorders, characterized by impairments in interaction, communication and restricted, stereotyped and repetitive behaviour (APA, 2013). ASD is the most heritable of all complex neuropsychiatric conditions, with heritability estimates ranging up to 90% (Lichtenstein *et al.*, 2010). ASD is marked by substantial heterogeneity in symptom presentation, developmental course and etiologic mechanisms (Jones and Klin, 2009). The genetics of ASD is complex with involvement of both rare and common genetic variants. Rare genetic variants predisposing to ASD are currently thought to account for 10-20% of all ASD cases (Betancur, 2011). They include rare mutations in genes which lead to monogenic disorders that are frequently associated with ASD, such as fragile X syndrome and tuberous sclerosis, as well as mutations and copy number variations (CNVs, these constitute deletions or duplications of larger fragments of DNA often involving several genes) that may contribute to (mono- and) oligogenic forms of ASD (Berg and Geschwind, 2012, Betancur, 2011, Devlin and Scherer, 2012, Persico and Napolioni, 2013). Common variants, e.g. single nucleotide polymorphism (SNPs), implicated in the etiology of ASD, on the other hand, are assumed to each contribute a (very) small increase in disease risk (Wang *et al.*, 2009). As ASD strongly reduces reproductive fitness, it has been argued that part of the genetic contribution to ASD is due to *de novo* mutations (D'Onofrio *et al.*, 2014, Neale *et al.*, 2012). In addition to the strong genetic background, environmental influences, gene x environment interaction, epigenetic factors, and pre-/perinatal complications also play an important role in susceptibility to ASD (Dietert *et al.*, 2011, Gardener *et al.*, 2009, 2011, Kinney *et al.*, 2010, Wong *et al.*, 2014). Multiple causal pathways may thus underlie the same clinical profiles, and, at the same time, the complex etiology may result in highly heterogeneous clinical profiles.

The heterogeneous character of ASD strongly hinders research into etiology and effective treatment. An approach to parse etiologic heterogeneity is to form more homogeneous subgroups of patients based on the familial occurrence of the disorder. Several studies have reported on the genetic differences between families with only one individual with ASD (the so-called single-incidence or simplex [SPX] families) compared to families with two or more affected individuals (multiple-incidence or multiplex [MPX] families). These studies reported a more than threefold rate of de novo mutations in SPX families (~7-10%), compared to MPX families (~2-3%) or control families (~1%) (Marshall *et al.*, 2008, Sebat *et al.*, 2007). In MPX families, shared genetic predispositions based on a multifactorial etiology of common genes appear to play a more important role (Freitag, 2007), with members of MPX families more often exhibiting ASD traits compared to members of SPX families (Gerdts *et al.*, 2013, Virkud *et al.*, 2009). We recently replicated the latter finding in the sample described in the current study (Oerlemans *et al.*, 2014a).

These findings suggest that individuals from SPX families are more likely than individuals from MPX families to develop ASD as a result of sporadic genetic and/or non-genetic causes strictly personal to the patient.

Assuming that SPX-MPX stratification identifies forms of ASD with a different genetic architecture, we aimed to study whether cognitive deficits differ between SPX and MPX families, in probands and/or in unaffected siblings. Cognitive studies in individuals with ASD have found deficits in intelligence (typically strengths in performal IQ over verbal IQ), social cognition (SC), executive functions (EF) and central coherence (CC) (Black *et al.*, 2009, Happe and Ronald, 2008, Joseph *et al.*, 2002). Direct comparisons of cognitive deficits between individuals with SPX and MPX ASD are mostly lacking thus far, and the vast majority of cognitive studies have failed to clearly specify or adjust for simplex or multiplex ascertainment process. So far, studies in SPX ASD-only samples report a higher frequency of performal > verbal IQ discrepancy in cases compared to controls (Ankenman *et al.*, 2014), and an altered cortical shape in brain regions that have been implicated in communication, higher order social processes (e.g. empathy and theory of mind), spatial attention, visual processing and face recognition (Dierker *et al.*, 2013). Studies in MPX ASD-only samples report deficits in EF components such as planning and set-shifting, theory of mind, and fluid and crystallized intelligence (Nydén *et al.*, 2011). To our knowledge, only one study has examined the association of SPX versus MPX status with cognitive functioning. Verbal and non-verbal IQ and head circumference [HC; associated with impaired brain connectivity and higher order abilities (Courchesne and Pierce, 2005)] were compared between children and adolescents with autism from SPX and MPX families. The authors reported that enlarged HC was related to social deficits in SPX, but not MPX individuals, and that individuals with the lowest nonverbal IQ scores were mostly classified SPX, whereas individuals with a higher than average nonverbal IQ were mostly MPX (Davis *et al.*, 2013). These findings suggest that both SPX and MPX forms of ASD are associated with a wide range of similar disabilities in higher order cognitive processes, but that some cognitive factors may be uniquely related to either SPX or MPX ASD (e.g. lower IQ scores were reported for SPX ASD), and more research is needed to clarify this issue.

Studies reporting on the presence of ASD-related cognitive deficits in first-degree relatives are sparse and report inconsistent findings (Gokcen *et al.*, 2009, Hilton *et al.*, 2012, Oerlemans *et al.*, 2014b, Wong *et al.*, 2006). A possible explanation for these discrepant findings might be that these studies did not differentiate between etiologically different (inherited versus non-inherited) forms of ASD and thus might have investigated relatives with and without familial loading as a mixed group. A recent study using SPX-MPX stratification to examine executive function of the parents of patients with familial versus non-familial (sporadic) schizophrenia confirmed this idea and reported that executive functions were only impaired in parents with a family history of schizophrenia (Erol *et*

al., 2012). Of interest to us is whether similar patterns can also be found in familial (MPX) versus sporadic (SPX) ASD.

To test whether the cognitive architecture underlying SPX and MPX autism families is different and useful for parsing the etiological heterogeneity of ASD, the cognitive performance of ASD probands and unaffected siblings from SPX and MPX families was compared with each other and with healthy controls. We selected cognitive tasks that assess various cognitive domains previously implicated in ASD (Eapen *et al.*, 2013, Gokcen *et al.*, 2009), or have been described as promising cognitive endophenotypes for ASD in previous literature (Oerlemans *et al.*, 2013, Oerlemans *et al.*, 2014b, Rommelse *et al.*, 2011). We hypothesized that potentially different forms of ASD might result in dissimilar cognitive profiles in SPX and MPX ASD probands, a finding with implications for treatment. Further, we hypothesized that the within family contrast between probands and unaffected siblings regarding cognitive aspects of the disorder was larger in SPX compared to MPX families as indicated by (mild) cognitive deficits (similar to their affected brother/sister) compared to controls in unaffected siblings from MPX, but not SPX families, a finding highly relevant to the identification of cognitive endophenotypes for genetic research.

METHOD

Participants

ASD families were recruited as part of the large family-genetic Biological Origins of Autism (BOA) study, (as described previously in Van Steijn *et al.*, 2012). Inclusion criteria for all participants were at least two biological siblings (in case families: at least one child with a clinical diagnosis of ASD) and one biological parent willing to participate, offspring age between 4 and 20 years, European Caucasian descent, an IQ ≥ 70, and no diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome. All children and parents were carefully phenotyped for ASD using validated and standardized questionnaires and a diagnostic interview. Families were then stratified into SPX and MPX based on the number of affected individuals. SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ASD; MPX families were required to have two or more affected individuals. A total of 54 ASD SPX families (including 54 probands and 77 unaffected siblings), 59 ASD MPX families (including 91 probands and 46 unaffected siblings) and 124 control children were included in the current sample, see Table 1 for sample characteristics and Oerlemans *et al.*, 2014a (chapter 4) for a full description of phenotyping and family classification.

Table 1. Sample characteristics

	Controls (c)	ASD probands		Unaffected siblings		Group contrasts ASD vs. controls
		1. SPX	2. MPX	3. SPX	4. MPX	
	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	
Number of children[a]	124	54	91	77	46	
Mean number of children per family	2.3	2.7		2.8		SPX = MPX > controls
Age	10.9 (3.6)	12.3 (3.5)	11.6 (3.4)	12.4 (3.6)	12.0 (3.7)	1=2=3=c, 4>c
Sex (% males)	41.9	85.2	71.4	72.7	41.3	1=2=3>4=c
SCQ Total Score	3.0 (2.6)	17.9 (6.6)	19.6 (6.5)	3.2 (3.3)	6.2 (6.3)	1=2>4>3=c
CSBQ ASD core[b]	2.6 (3.8)	26.2 (11.4)	27.5 (8.6)	5.4 (6.2)	11.5 (10.1)	1=2>4>3=c

Note. ASD = Autism spectrum disorders; SPX = simplex; MPX = multiplex; SCQ = social communication questionnaire; CSBQ = child social behavior questionnaire; c = controls; 1 = SPX probands; 2 = MPX probands; 3 = SPX unaffected siblings; 4 = MPX unaffected siblings

[a]affective prosody was not administered to children younger than 9 years of age and therefore based on 42 SPX probands, 70 MPX probands, 62 SPX unaffected siblings, 34 MPX unaffected siblings and 79 controls.

[b]ASD core is an aggregate score of the CSBQ subscales reduced contact and social interests, difficulties in understanding social information, stereotyped behaviour and fear of and resistance to changes.

Measures

Table 2 provides an overview of the cognitive tasks used. For full task descriptions, see Appendix 1 or elsewhere (Oerlemans *et al.*, 2013).

Table 2. Description of the neuropsychological tasks.

Task[a]	Measurement potential	Dependent variables
Intelligence		
Vocabulary, Similarities, Block Design, Picture Completion	estimated IQ	VIQ and PIQ
Social cognition		
Face Recognition	face recognition	mean reaction time (ms)
Identification of Facial Emotions	identification of facial emotional expressions	mean reaction time (ms)
Prosody	affective prosody	mean reaction time (ms)
Executive function		
GoNoGo	inhibition	percentage false alarms – percentage misses
Digit Span	verbal working memory	max span backwards
Spatial Temporal Span	visuospatial working memory	percentage correct identified targets in correct order (part backward)
Response Organization Objects	cognitive flexibility	percentage errors

Note. WISC/WAIS-III = Wechsler Intelligence Scale for Children or Wechsler Adult Intelligence Scale-III

[a] Details on each of the paradigms are provided elsewhere (Oerlemans *et al.*, 2013a)

Procedure

Cognitive assessment of participants took place at Karakter Child and Adolescent Psychiatry University Centre Nijmegen and is described in more detail elsewhere (Oerlemans *et al.*, 2013). If possible, stimulants were discontinued for at least 24 h before testing and non-stimulants according to guidelines to allow for sufficient wash-out. Children were motivated with small breaks and received a gift at the end of the session. Additional data collected included blood or saliva samples and behavioral data of all family members. The study was approved by the local medical ethics board and parents and children (12 years and older) signed for informed consent. Children younger than 12 years of age were asked to give their assent for participation.

Data Analyses

Unlike the other tasks, the affective prosody recognition task was not administered to children younger than 9 years of age. The affective prosody recognition data was based on 42 SPX probands, 70 MPX probands, 62 SPX unaffected siblings, 34 MPX unaffected siblings and 79 controls. The percentage of missing data was < 5% for the majority of dependent measures. Exceptions were missing values of 9.4% for inhibition and 9.9% for variability of time estimation. Missings were replaced by means of Expectation Maximization(Tabachnick and Fidell, 2001). Analyses were carried out with and without expectation maximization, which revealed similar results and conclusions. Results were therefore reported with missing data replaced. To account for the influence of age and sex on neuropsychological performance, we regressed scores for each measure on age and sex and used the unstandardized residuals as dependent variables. Most of the unstandardized residuals were not normally distributed, therefore a van der Waerden transformation was used to normalize the dependent measures (Norusis, 1992). This facilitated the comparison between variables since variables were all depicted on the same scale. Several of the dependent variables were mirrored so that the z-scores of all measures had the same meaning: lower z-scores indicated poorer performance (e.g. more errors, slower and more variable responses).

Linear mixed models (LMM) were used to account for the dependency in the data due to inclusion of siblings and probands by estimating a random intercept. Dependent variables were the cognitive measures and group was the independent variable. We contrasted specific groups of interest to answer our research questions. LMM analyses were run with group defined as (a) probands versus unaffected siblings versus controls, separately for SPX and MPX families, to examine whether cognitive deficits were present in (SPX and MPX) probands and MPX, but not SPX, unaffected siblings, (b) MPX versus SPX probands to examine whether potentially different heritable forms of ASD would result in (dis)similar cognitive profiles in ASD patients, and (c) SPX versus MPX unaffected siblings to examine whether cognitive performance of first-degree relatives

was poorer in MPX compared to SPX families. Furthermore, within family discrepancy scores (estimated mean of proband minus mean of unaffected sibling) in SPX versus MPX families were compared to examine whether within family contrast was higher in SPX than MPX families. A False Discovery Rate (FDR) correction with a q-value setting of 0.05 was applied to control for multiple testing (Benjamini, 2010). Effect sizes (Cohen's d) were calculated to define small (d = .20), medium (d = .50), and large effects (d = .80) (Cohen, 1988). All analyses were carried out in SPSS version 20.

RESULTS

Cognitive measures sensitive to SPX-MPX stratification

Cognitive deficits are more pronounced in SPX than MPX ASD probands

Testing our first hypothesis, we found that the cognitive profiles of SPX and MPX probands were very similar. Both SPX and MPX probands had significantly lower VIQ (SPX: $p < .001$, effect size in terms of Cohen's d = .69; MPX: $p < .001$, d = .68) and PIQ (SPX: p = .008, d = .42; MPX: p = .045, d = .28), and poorer face recognition (SPX: $p < .001$, d = .65; MPX: p = .004, d = .40), affective prosody recognition (SPX: $p < .001$, d = .92; MPX: $p <$ .001, d = .70), and verbal working memory (SPX: p = .003, d = .46; MPX: p = .031, d = .31) than controls. However, the effects on PIQ and verbal working memory in MPX (but not SPX) probands became non-significant after FDR correction (q-values > .10). Further, SPX (but not MPX) probands differed significantly from controls in the identification of facial emotions (SPX: p = .010, d = .40; MPX: p = .097, d = .19), suggesting that SPX forms of ASD makes patients more prone to deficits in these domains, see Figure 1 and Table 3.

Comparing siblings within families revealed that affected and unaffected siblings from MPX families resembled each other more closely in cognitive functioning than affected-unaffected siblings from SPX families. In SPX families, within-family discrepancy (proband-unaffected sibling contrast) was larger for SPX than for MPX families for VIQ (t = 2.56, p = .012) and identification of facial emotions (t = 2.38, p = .019). SPX probands differed significantly from their unaffected siblings on both measures (VIQ: $p < .001$, d = .59; facial emotions: p = .002, d = .50), whereas MPX affected and unaffected siblings did not differ significantly from each other (p-values > .12, all d values = .03-.29). This might suggest that impairments in these cognitive domains are more pronounced in SPX than MPX cases. Significant differences between SPX affected and unaffected siblings were also found for PIQ (p = .003, d = .42), face recognition (p = .004, d = .52) and verbal working memory (p = .039, d = .36), although the latter effect became non-significant after FDR correction (corrected p = .07). For visual working memory, significant affected-unaffected sibling contrasts were found for both SPX (p = .020, d = .39) and MPX (p =

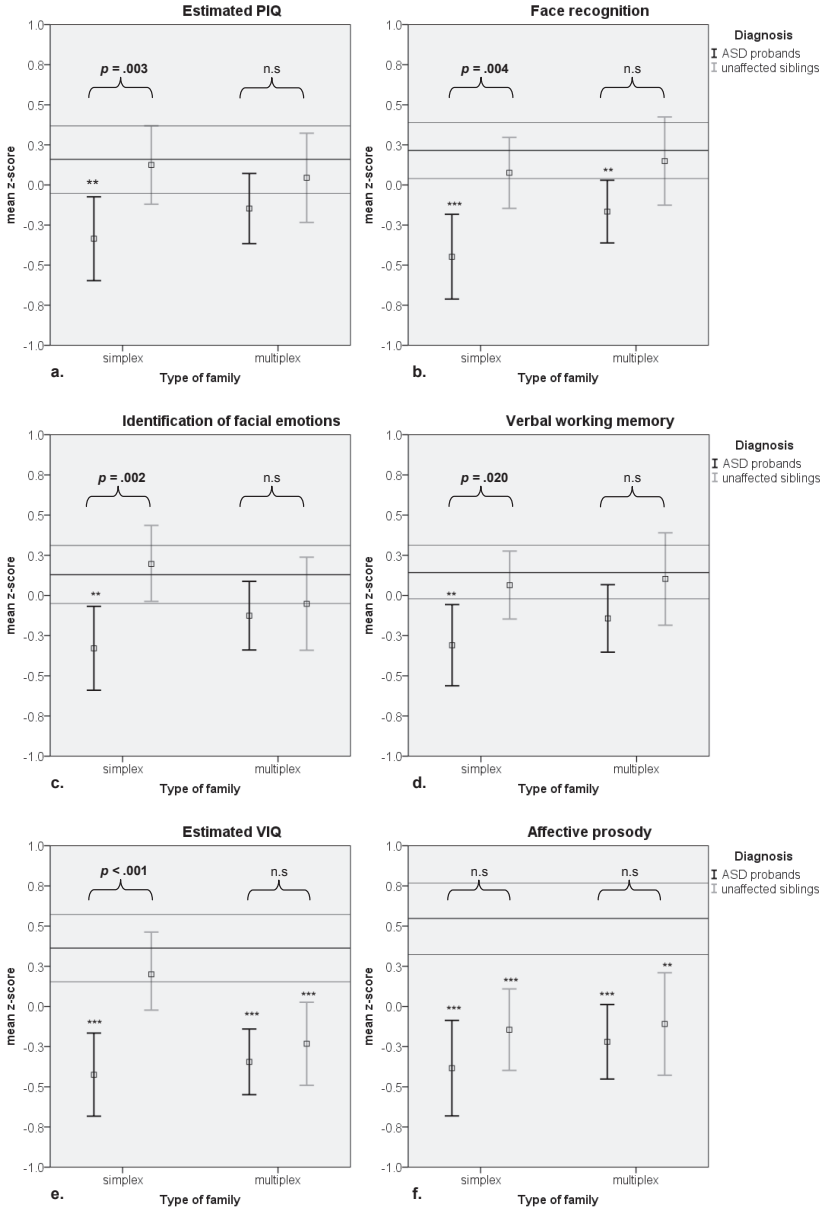

Figure 1 Comparing unaffected siblings from, and within-family contrasts in SPX and MPX ASD families
Note. ASD = autism spectrum disorder; n.s. = non significant. The interpolation lines represent the mean z-score and the 95% CI of normal controls. The *error bars* represent the 95% confidence interval (CI). Lower z-scores indicate worse performance. Significant group differences (case groups versus controls) that survived FDR correction are depicted using asterisks (*** $p < .001$, ** $p < .01$). Within-family contrasts are depicted using squiggly brackets. Within-family contrasts were higher in SPX compared to MPX families for IQ, emotion recognition and visual working memory, suggesting that affected and unaffected siblings from MPX families resembled each other more closely in cognitive functioning than affected-unaffected siblings from SPX fami-

lies. Unaffected siblings from both SPX and MPX families were unimpaired on these cognitive domains (a-e). In line with our expectations, we found that MPX unaffected siblings had a significantly lower VIQ (similar to their affected brother/sister) compared to controls, whereas SPX unaffected siblings were unimpaired in this domain. In addition, within-family contrast was highest in SPX ASD families, but non-significant in MPX ASD families for VIQ (e). An unexpected finding was that SPX (like MPX) unaffected siblings differed significantly from controls (but not from their affected brother/sister) on affective prosody (f).

.043, d = .33) families, but, the effect in MPX families did not survive FDR correction (corrected p = .15). These findings support the hypothesis that MPX, but not so much SPX, unaffected siblings share some of the ASD-related cognitive deficits.

Comparing unaffected siblings from SPX and MPX ASD families

In agreement with our second hypothesis, we found that unaffected siblings from MPX families had a significantly lower VIQ (similar to their affected brother/sister) compared to controls (siblings vs. controls: $p < .001$, d = .57; siblings vs. probands: p = .409, d = .12), whereas SPX unaffected siblings were unimpaired in this domain (p = .392, d = .13). SPX and MPX unaffected siblings also differed significantly from each other on this measure (p = .011, d = .47). Opposing our hypothesis, both SPX and MPX unaffected siblings scored significantly worse than controls, but similar to their affected brother or sister on affective prosody (SPX: $p < .001$, d = .65; MPX: p = .002, d = .65), see Figure 1. The unaffected siblings from both SPX and MPX families displayed a normal performance on all other cognitive measures (SPX: all p-values > .27, all d-values < .16; MPX: all p-values > .25, all d-values < .20).

Measures not sensitive to SPX-MPX stratification

As describe above, both MPX and SPX unaffected siblings differed significantly from controls (but not from their affected brother/sister) on affective prosody. Further, SPX and MPX probands and unaffected siblings were unimpaired on visual working memory (p-values > .17, all d values < .21), inhibition (p-values > .07, all d values < .31), and set shifting (p-values > .09, all d values < .20), see Table 3.

DISCUSSION

The main goal of the current study was to examine whether the cognitive architecture underlying SPX and MPX autism families is different and useful for parsing etiological heterogeneity of ASD. This model of different etiologies in SPX and MPX families is based on evidence from behaviorally-based and genetic research (Freitag, 2007, Gerdts *et al.*, 2013, Marshall *et al.*, 2008, Sebat *et al.*, 2007, Virkud *et al.*, 2009). We hypothesized that (a) the different forms of ASD might result in dissimilar cognitive profiles in SPX and MPX

Table 3. Means and standard errors of the transformed task variables for SPX and MPX probands, their unaffected siblings and normal controls

	Controls (c)		ASD probands	unaffected siblings	Group contrasts		Within family contrasts		Comparisons between SPX and MPX family members			
									probands		unaffected siblings	
	M (se)	Family type	M (se)	M (se)	*p-values**	*d*-values*	*t*	*p*	*p*	*d*	*p*	*d*
VIQ	.36 (.10)	SPX	-.37 (.13)	.22 (.12)	**<.001**/.392/**<.001**	.69/.13/.59	**2.56**	**.012**	.930	.02	**.011**	**.47**
		MPX	-.35 (.10)	-.24 (.13)	**<.001/<.001**/.409	.68/.57/.12						
PIQ	.15 (.10)	SPX	-.30 (.13)	.12 (.12)	**.008**/.841/**.003**	.42/.03/.42	1.02	.311	.418	.15	.848	.05
		MPX	-.15 (.11)	.07 (.14)	.045/.620/.125	.28/.08/.22						
Face recognition	.22 (.09)	SPX	-.42 (.13)	.08 (.11)	**<.001**/.315/**.004**	.65/.14/.52	1.29	.203	.149	.26	.893	.02
		MPX	-.17 (.10)	.10 (.14)	**.004**/.471/.128	.40/.12/.29						
Identification of facial emotions	.12 (.10)	SPX	-.30 (.13)	.20 (.12)	**.010**/.622/**.002**	.40/.07/.50	**2.38**	**.019**	.305	.14	.102	.27
		MPX	-.12 (.15)	-.08 (.15)	.097/.254/.806	.19/.18/.03						
Affective prosody	.51 (.11)	SPX	-.38 (.15)	-.13 (.13)	**<.001/<.001**/.180	.92/.65/.25	.88	.379	.282	.20	.812	.02
		MPX	-.18 (.12)	-.11 (.16)	**<.001/.002**/.706	.70/.65/.07						
Inhibition	.12 (.09)	SPX	-.18 (.13)	.05 (.12)	.066/.655/.160	.31/.07/.23	.53	.596	.623	.10	.941	.01
		MPX	-.09 (.10)	.04 (.14)	.137/.639/.431	.21/.08.14						
Verbal WM	.16 (.09)	SPX	-.29 (.13)	.05 (.11)	**.003**/.428/.039	.46/.11/.36	.48	.624	.431	.13	.592	.04
		MPX	-.16 (.11)	.09 (.15)	.031/.711/.143	.31/.07/.24						
Visual WM	.00 (.09)	SPX	-.21 (.13)	.16 (.11)	.171/.274/**.020**	.21/.16/.39	.16	.873	.727	.07	.655	.04
		MPX	-.14 (.11)	.20 (.15)	.341/.267/.043	.14/.20/.33						
Set shifting % errors	.09 (.09)	SPX	-.09 (.13)	.08 (.11)	.244/.907/.314	.18/.01/.18	.21	.832	.736	.06	.958	.02
		MPX	-.15 (.11)	.06 (.15)	.088/.854/.260	.24/.03/.20						

Note. ASD = autism spectrum disorders, SPX = simplex, MPX = multiplex, M = mean, se = standard error, WM = working memory. Significant group contrasts that survived FDR correction are presented in bold.

*p-values and effect sizes in terms of Cohen's d (*d*-values) are presented in the following order: probands versus controls/siblings versus controls/probands versus siblings

ASD probands, and (b) unaffected siblings from MPX but not SPX would display (mild) cognitive deficits compared to controls. Our results showed that directly comparing SPX and MPX ASD cases, no cognitive differences were detected and both were associated with impairments in VIQ, PIQ, face recognition, affective prosody recognition, and verbal working memory compared to healthy controls. However, when compared to their unaffected siblings, impairments in identification of facial emotions, VIQ, PIQ, and verbal working memory were more pronounced in SPX cases compared to MPX cases. Unaffected siblings from MPX families had a significantly lower VIQ (similar to their affected brother/sister) compared to controls, whereas SPX unaffected siblings were unimpaired in this domain. Both MPX and SPX unaffected siblings differed significantly from controls on affective prosody and were unimpaired on the other cognitive domains. ASD probands and unaffected siblings from MPX families resembled each other more closely in cognitive functioning than affected-unaffected siblings from SPX families.

Results support the hypothesis that a partly different cognitive architecture may underlie SPX and MPX forms of ASD, which only becomes evident when contrasting cognitive performances within families. That is, the direct comparison between autistic children from SPX and MPX families revealed very similar cognitive problems, but when using unaffected siblings as an ideal reference group (viewed as indexing the 'full potential' of children with ASD had they not developed the disorder and correcting for shared environmental factors), SPX probands seem to be relatively more strongly impaired in intelligence, verbal working memory and emotion recognition than MPX probands, which is not explained by a more severe ASD phenotype in SPX probands (i.e., in our sample, SPX and MPX ASD probands demonstrate equally severe ASD traits, see sample characteristics). This could indicate that partly different developmental pathways may result in a similar phenotype and similar cognitive deficits, a phenomenon that has been referred to in developmental psychopathology as *equifinality* (Cicchetti and Rogosch, 1996). ASD has often been associated with lower full scale IQ or intellectual disability (ID) (Charman *et al.*, 2011). One model that has been proposed for the overlap between ID and ASD suggests that rare, highly penetrant mutations set the stage for abnormal developmental trajectories including ASD, developmental delay and mental retardation (Eapen, 2011). Assuming that SPX ASD is more likely than MPX ASD to develop as a result of such rare (sporadic) genetic causes, our finding and the finding of Davis et al. that ASD children with low(ered) intelligence levels more often had SPX than MPX forms of ASD corroborate this theory (Davis *et al.*, 2013).

SPX unaffected siblings were largely unimpaired on cognitive measures compared to controls, except for affective prosody, whereas MPX unaffected siblings were impaired on both affective prosody and VIQ. Several implications may result from this finding. First of all, it suggests that affective prosody is the most sensitive cognitive marker for detecting familial risk for ASD. This finding is in line with previous analyses using the

same cognitive task in a younger subsample of this cohort (Oerlemans *et al.*, 2014b). The perception of emotional expressions via affective prosody is highly relevant for the development of Theory of Mind (ToM), which refers to the ability to understand other people's thoughts, beliefs, and other internal states (Korkmaz, 2011, Korpilahti *et al.*, 2007). Many believe that social cognition deficits are central to explaining the difficulties experienced by people with ASD (Baron-Cohen, 1995, Korkmaz, 2011). Our finding that unaffected siblings (regardless SPX/MPX status) were impaired on affective prosody, but not on other cognitive domains, might suggest that impaired social cognition is the primary cognitive deficit in ASD, resulting from shared (genetic and/or environmental) risk factors that disrupt the ability to process emotional cues in individuals with autism and (to some extent) their unaffected first-degree relatives. Subsequently, impaired social cognition might lead to other cognitive problems (such as poor EF or verbal working memory), most pronounced or uniquely present in affected individuals. Second, it suggests that the unaffected siblings from SPX families are not completely clean from cognitive deficits. The finding is consistent with findings that although *de novo* genetic variations most likely play a role in the development of simplex ASD, they do not fully explain genetic etiology (Krumm *et al.*, 2013). In other words, also in SPX ASD families some risks may be shared between family members (Klei *et al.*, 2012), and the distinction between MPX and SPX ASD may rather be quantitative and not qualitative. Third, only a few comparisons between MPX unaffected siblings and controls reached significance. This finding clearly contrasts with studies in ADHD that firmly demonstrate significant impairments on cognitive functions and brain morphology in first-degree unaffected relatives who are at risk of the disorder (Allen *et al.*, 2009, Rommelse *et al.*, 2011). This does not seem to be due to a simple lack of power: visual inspection of the data indicate no or only very minor cognitive impairments on several domains that are impaired in the MPX probands (face recognition, PIQ, verbal working memory). This suggests that –in contrast to ADHD - cognitive factors in ASD may have a stronger determining effect on the development of the final phenotype. Or, alternatively, the reversed effect from phenotype to aberrant cognition is stronger in ASD than it is in ADHD: the presence of the core social problems characteristic of ASD might seriously hinder cognitive development in other domains (e.g. poorer EF skills might be a consequence of early atypical input from another cognitive system such as theory of mind, as discussed by (Pellicano, 2012)), which is less true for the core (often intermittent and receptive towards treatment) symptoms of ADHD. In any case, our findings suggest that cognitive deficits associated with ASD may have a stronger determining effect on the disorder compared to related disorders such as ADHD. An important exception is affective prosody, suggesting this domain may be sensitive towards familial risk factors for ASD.

Some limitations to this study need to be acknowledged when interpreting the results. First, sample sizes were moderate; it follows that our study needs replication in larger

samples to fully uncover effects. Second, boys were overrepresented in both proband groups and in SPX unaffected siblings, but were underrepresented in MPX unaffected siblings and controls. This was likely due to the fact that a) ASD is more frequently diagnosed in males and b) because the presence of male unaffected siblings was only required for SPX, but not MPX families. However, we do not believe that this has affected the results, since the effect of sex was controlled for in this study. Third, although effort was made to include several tasks tapping the domains of SC and EF, we were not able to assess all aspects of these cognitive domains. For example, fluency, planning and theory of mind were not assessed here. We cannot rule out the possibility that the cognitive functions not studied here are sensitive to familial effects. All in all, results suggest quantitative differences between SPX and MPX forms of ASD, which becomes evident when contrasting cognitive performances *within* families. These findings may help parse etiological heterogeneity of ASD by stratifying ASD families into families with stronger versus weaker familial aggregation of ASD-related neurocognitive deficits.

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APPENDIX 1. FULL DESCRIPTION OF NEUROPSYCHOLOGICAL MEASURES

Measures

Six of the tasks described below were selected from the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville, 1999). The ANT is a computer-aided assessment battery that allows for the systematic evaluation of information processing capacities. Test-retest reliability and validity of the ANT-tasks are satisfactory (De Sonneville, 2005). Each computer task contained an instruction trial where the examiner provided a typical item of the task, and a separate practice session. If necessary, the instruction was repeated. All subjects were able to perform the training items before testing. Furthermore, several subtests from the Wechsler Intelligences Scales for Children (WISC-III) or the Wechsler Adult Intelligence Scale (WAIS-III) were selected (Wechsler, 2000, 2002). These subtests were administered following manual guidelines.

Intelligence

Verbal and Performal IQ were prorated by four subtests of the Wechsler Intelligences Scales for Children (WISC-III) or the Wechsler Adult Intelligence Scale (WAIS-III): Similarities and Vocabulary for VIQ and Block Design and Picture Completion for PIQ (Wechsler, 2000, 2002). These selected WISC-III subtests are known to correlate between .90-.95 with the Full-scale IQ (Groth-Marnat, 1997).

Social cognition

Social cognition (SC) was measured using three tasks from the ANT. The Face Recognition (FR) task was used to measure the capacity to process social (facial) stimuli (De Sonneville, 1999). Stimuli consisted of color photographs of a human face with a neutral expression, were presented on a computer screen. Children were then asked to identify a target face in a display set that consisted of four faces. If the target face was present in the display set, the subject was asked to click the 'yes-button' (right computer mouse button for right-handed subjects, and left computer mouse button for left-handed subjects), if the display set did not contain the target face the subject was asked to press the 'no-button' (left computer mouse button for right-handed subjects, right computer mouse button for left-handed subjects). The Identification of Facial Emotions (IFE) task was used to measure the capacity to understand facial emotional expressions (De Sonneville, 1999). Stimuli consisted of photographs of a human face, presented on a computer screen, with each photograph presenting a face with either a neutral or emotional (happy, sad, angry, fear, disgust, surprise, shame, contempt) expression. Children were asked to judge whether the presented photograph showed the target emotion or not by clicking a mouse button (responses were to be given as described above). The Prosody (PR) task was administered to test the ability to recognize 'emotions

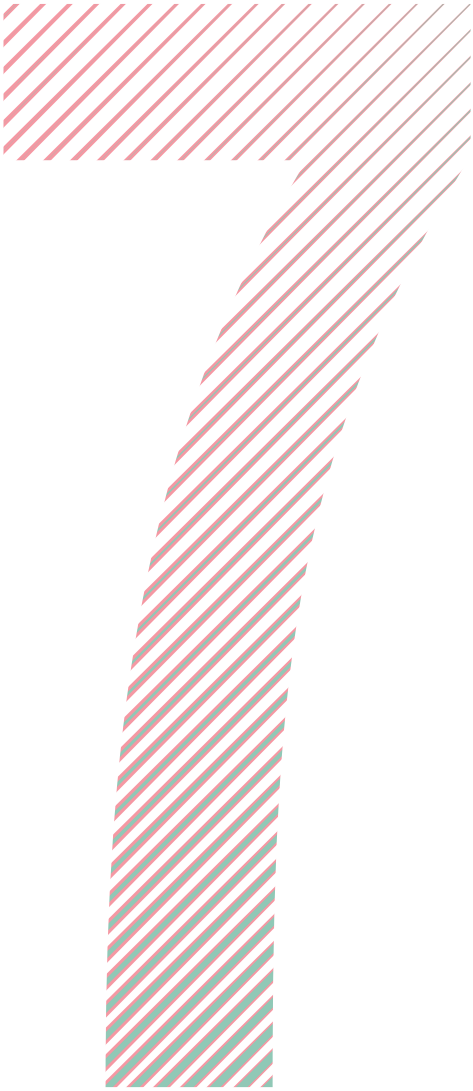
in voices' (De Sonneville, 1999). Stimuli consisted of spoken sentences with a neutral content, presented through a headphone. Sentences were spoken in a happy, sad, angry or frightened manner, with each emotion represented by twelve sentences, in random order. The children were asked to verbally identify the emotion in the voice. Response time was recorded using a headphone that acted like a voice-key response. Dependent variable in all three tasks was mean reaction time (in ms).

Executive function

Executive function (EF) was measured using tests that tap into three major aspects of EF, namely inhibition, cognitive flexibility and working memory. All measures were standardized and some scores were mirrored so that low z-scores indicated poorer performance). Inhibition was measured using the GoNoGo (GNG) task (De Sonneville, 1999). Children were presented with two types of trials: go-trails and no-go-trails. Children were asked to press a mouse key as quickly and accurately as possible when the go-stimulus was presented, but withhold their response to the stop-trial. Dependent variable was accuracy (% false alarms - % misses) (Oerlemans *et al.*, 2013). Cognitive flexibility was measured using the Response Organization Objects (ROO) (De Sonneville, 1999). The stimulus was a colored circle that was presented to the left or right of a fixation cross. Three parts were administrated: in part 1, the stimulus was colored green and *compatible responses* were required (i.e. children were instructed to click the mouse key that corresponded to the direction in which the stimulus moved). In part 2, the stimulus was colored red and *incompatible responses* were required (i.e. children were instructed to click the response mouse button opposite to the direction of the moving stimulus). In part 3, the color of the stimulus shifted randomly between green and red and *both compatible and incompatible responses* were required. Cognitive flexibility was operationalized as the differences in mean number of errors (accuracy) between part 1 and the compatible trials of part 3 (Oerlemans *et al.*, 2013). Working memory (WM) was measured using two tasks: one spatial and one verbal task. Spatial WM was tested using the Spatial Temporal Span (STS) task. Stimuli consisted of nine figures presented symmetrically in a 3 by 3 square. On each trail, a sequence of figures was pointed at by a computer-driven hand. Children were then instructed to reproduce the sequence in backward order. The difficulty level increased after each succeeded trial. Dependent measure was the total percentage of correctly identified targets in the correct order. Verbal WM was measured using the maximum span of the backward condition of the Digit Span subtest of the WISC-III/WAIS-III (Wechsler, 2002).

CHAPTER 7

Identifying unique versus shared pre- and perinatal risk factors for ASD and ADHD using a simplex-multiplex stratification



Based on:

Oerlemans, A.M., Burmanje, M.J., Franke, B., Buitelaar, J.K., Hartman, C.A.* & Rommelse, N.N.J.* Identifying unique versus shared pre-, and perinatal risk factors for ASD and ADHD using a simplex-multiplex stratification.

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(submitted for publication)

ABSTRACT

Background: Autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) frequently co-occur. Besides shared genetic factors, pre- and perinatal risk factors (PPFs) may determine if ASD, ADHD, or the combination of both disorders becomes manifest. This study aimed to test shared and unique involvement of PPFs for ASD and ADHD, using an approach that stratifies the sample into affected/unaffected offspring and single-incidence (SPX) versus multi-incidence (MPX) families.

Methods: Pre- perinatal data based on retrospective parent-report were collected in 288 children from 31 SPX and 59 MPX ASD families, 476 children from 31 SPX and 171 MPX ADHD families, and 408 control children.

Results: Except for large family size and more firstborns amongst affected offspring, no shared PFFs were identified for ASD and ADHD. PPFs specifically related to ASD (maternal infections and suboptimal condition at birth) were more often reported in affected than unaffected siblings. PPFs associated with ADHD (low parental age, maternal diseases, smoking and stress) were shared between affected and unaffected siblings. Firstborn-ship was more frequent in SPX than MPX ASD probands.

Conclusions: Our results suggest that the co-morbidity of ASD and ADHD is not likely explained by shared PPFs. Instead, PPFs might play a crucial role in the developmental pathways leading up to either disorder. PPFs in ADHD appear to index an increased shared risk, whereas in ASD PPFs possibly have a more determining role in the disorder. SPX-MPX stratification detected possible etiological differences in ASD families, but provided no deeper insight in the role of PPFs in ADHD.

INTRODUCTION

Autism spectrum disorder (ASD) and attention deficit/hyperactivity disorder (ADHD) are both highly heritable, impairing neurodevelopmental disorders that manifest early in development and frequently co-occur (Lichtenstein *et al.*, 2010). ASD is characterized by impairments in social interaction, deficits in verbal and non-verbal communication and by restricted or repetitive patterns of behavior and interests. ADHD is characterized by symptoms of hyperactivity, impulsivity and/or inattention (American Psychiatric APA, 2013). High co-morbidity might be explained by shared genetic factors, as indicated by twin studies (Lichtenstein *et al.*, 2010, Ronald *et al.*, 2008). However, genetic effects do not account for all phenotypic covariation (Ronald *et al.*, 2008), implying that the high co-morbidity rates of ASD and ADHD might also be explained by other factors, such as shared pre- and perinatal risk factors (PPFs) (Rutter and Silberg, 2002).

PPFs have proven important in the etiology of both ASD and ADHD. However, since ASD or ADHD have been studied mostly in isolation, we have little knowledge about whether PPFs are shared between the disorders. In meta-analyses of ASD, advanced parental age at birth, maternal prenatal medication use, gestational bleeding, diabetes, being firstborn, fetal distress, birth injury or trauma, low 5-minute APGAR score and low birth weight (< 5.5 pounds or 2,500 gram) were more frequently observed in ASD than in controls (Gardener *et al.*, 2009, 2011). Maternal infections, maternal stress, suboptimal condition of the child at birth, prematurity, and smoking during pregnancy were also found related to ASD (Visser *et al.*, 2013). Additionally, a recent study reported that a birth weight more than two standard deviations above average for gestational age also increases the risk of developing ASD (Abel *et al.*, 2013). Research on ADHD indicates that prenatal exposure to nicotine, alcohol, drugs or toxins, and maternal stress, low birth weight, low maternal age and poor maternal diet are associated with an increased likelihood of developing ADHD (Langley *et al.*, 2005, Mick *et al.*, 2002, Mill and Petronis, 2008, Thapar *et al.*, 2013, Throckmorton-Belzer *et al.*, 2009). Furthermore, an association has been reported between neonatal complications and the severity of ADHD symptoms (Ben Amor *et al.*, 2005). Only two studies so far investigated which early childhood indicators (amongst which, PPFs) might be shared between ASD and ADHD, by examining these risk factors in the general population (Jaspers *et al.*, 2013, St Pourcain *et al.*, 2011). St Pourcain et al. found that maternal smoking might be common to ASD- and ADHD-like symptoms (St Pourcain *et al.*, 2011). Jaspers and colleagues reported that male gender and low educational level of the mother were overlapping indicators, yet PPFs such as maternal smoking and low birth weight were specific for ADHD and ASD traits, respectively (Jaspers *et al.*, 2013). All considered, these findings on population-based samples suggest limited overlap of PPFs in ASD and ADHD, except perhaps for low birth weight and maternal smoking. The role of shared PPFs might, however, be different in

clinical samples, given greater severity of clinical symptoms among referred cases. In the current study that combines two clinical ASD and ADHD cohorts, we aimed to explore this issue further.

Which role PPFs play in a disorder can be understood by looking at the prevalence of these risk factors in unaffected siblings of patients. The advantage of an affected-unaffected sibling design is that it controls for familial (genetic background and shared environmental) risk factors (Ben Amor *et al.*, 2005). When certain PPFs are present in both affected and unaffected siblings, these factors are probably related to an overall increased risk of developing the disorder (trait factors), without a unique, determining contribution to the disorder. Vice versa, PPFs (predominantly) found in affected offspring but not in unaffected offspring may have a more penetrant, possibly uniquely determining effect on the development of the disorder (state factors). Research that compared affected and unaffected ASD siblings showed that medication use during pregnancy, being firstborn, higher non-optimality scores, low birth weight and low APGAR scores are all relatively more prevalent in autistic children compared to their unaffected siblings (Deykin and MacMahon, 1980), suggesting that these factors may explain why the probands did develop ASD, yet their siblings did not. In ADHD families, affected children appear to have significantly higher rates of perinatal complications such as low birth weight and medical conditions when compared to unaffected siblings, but intriguingly, smoking and alcohol consumption during pregnancy does not appear to differ among siblings (Ben Amor *et al.*, 2005). Recent studies using genetically informative designs suggest the latter factors to be a proxy of ADHD risk genes passed from mother to offspring, rather than predominantly environmental toxic factors (D'Onofrio *et al.*, 2013, Thapar *et al.*, 2013). The above described research indicates that there might indeed be specific PPFs that are present in affected individuals, but also PPFs that are shared by affected and non-affected (ADHD) siblings. This is the first study to examine which PPFs are related to ASD and/or ADHD and to what extend these factors are uniquely present in affected offspring or shared between affected and unaffected siblings.

A second and more in depth approach that can improve our understanding of the role of PPFs in ASD and ADHD is to stratify families into simplex (SPX) or multiplex (MPX) affected ones. Families with only one affected individual are referred to as SPX and families in which two or more individuals are affected, are defined as MPX. This stratification allows us to differentiate between common risk factors present in multiple family members (which will be more frequent in MPX families) versus non-shared, unique risk factors, only present in affected persons (more frequent in SPX families). This approach has been proven to be helpful in research on genetic factors in ASD. Individuals with ASD from MPX families generally carry more common, shared genetic factors for the disorder (Freitag, 2007), while the symptoms of ASD-affected individuals from SPX families are more likely to have a unique cause for their disorder, such as de novo mutations or rare

copy number variations that are unshared with other family members (Marshall *et al.*, 2008, Sebat *et al.*, 2007). Consistent with this, individuals from MPX families show higher levels of ASD traits than individuals from SPX families (Constantino *et al.*, 2010, Virkud *et al.*, 2009). With regard to the PPFs, MPX and SPX stratification may add to insights which PPFs reflect mostly genetically driven rather than incidental environmental risk factors.

In sum, this is the first study that tests sharing and uniqueness of the involvement of PPFs for ASD and ADHD using an approach that stratifies the sample into affected versus unaffected offspring and SPX versus MPX-affected families. We tested whether (1) PPFs only present in affected –but not unaffected- offspring may have a unique, highly penetrant contribution to the disorder instead of increasing the overall liability for the disorder only slightly, and (2) offspring from MPX families shares a larger proportion of PPFs than that of SPX families indicating that PPFs in MPX families most likely reflect genetically driven risk factors, whereas PPFs in SPX families might reflect (purely environmental) risk factors.

METHOD

Participants

ASD and ADHD families were recruited as part of two large family-genetic studies: the Biological Origins of Autism (BOA) study and the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (as described previously in van Steijn *et al.*, 2012). Inclusion criteria for all participants were at least two biological siblings (in case families: at least one child with a clinical diagnosis of ASD or ADHD) and one biological parent willing to participate, offspring age between 4 and 20 years, European Caucasian descent, and an IQ ≥ 70. Children with a diagnosis of epilepsy, brain disorders or known genetic disorders, such as Down-syndrome or Fragile-X-syndrome were excluded from participation in order to reduce etiological heterogeneity and providing ASD and ADHD samples with considerable clinical homogeneity. All children and parents were carefully phenotyped for ASD and ADHD using validated and standardized questionnaires and diagnostic interviews. Families were stratified into SPX and MPX based on the number of affected individuals. SPX families were required to have a single-affected proband, a minimum of one male sibling and all siblings and parents of the proband unaffected by ASD or ADHD; MPX families were required to have two or more affected individuals. A total of 288 children from ASD families (including: 56 SPX probands, 96 MPX probands, 81 SPX unaffected siblings, and 55 MPX unaffected siblings), 476 children from ADHD families (including: 31 SPX probands, 270 MPX probands, 47 SPX unaffected siblings, and 128 MPX unaffected siblings), and 408 control children were included in this study, see Table 1 for sample characteristics and Oerlemans *et al.*, 2014 (chapter 4) for a full description of phenotyping and family classification.

Table 1. Sample Characteristics

	Control families (c)[a] (N=203)	ASD cohort				ADHD cohort				Group contrasts ASD vs. controls	Group contrasts ADHD vs. controls
		SPX families (N = 56)		MPX families (N= 59)		SPX families (N = 31)		MPX families (N=171)			
		1. ASD probands	2. ASD Unaffected siblings	3. ASD probands	4. ASD Unaffected siblings	5. ADHD probands	6. ADHD Unaffected siblings	7. ADHD probands	8. ADHD Unaffected siblings		
	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)	M (sd)		
Child data											
Number of kids	N = 408	N = 56	N = 81	N = 96	N = 55	N = 31	N = 47	N = 270	N = 128		
Mean family size[b]	2.3	2.7		2.8		2.6		2.5		SPX = MPX > controls	SPX = MPX > controls
Age	11.2 (3.5)	12.0 (3.7)	12.1 (3.8)	11.3 (3.6)	10.9 (4.2)	11.8 (2.4)	10.9 (3.4)	11.4 (2.7)	11.4 (3.6)	1=2=3=4=c	5=6=7=8=c
Sex (% males)	41.9	85.7	74.1	72.9	45.5	87.1	74.5	73.6	39.1	1=2=3>4=c	5=6=7>8=c
Diagnose (%)											
ASD	0	100	0	100	0	25.8	2.1	14.1	1.6		
ADHD	0	35.7	12.3	43.8	18.1	100	0	100	0		

Note ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder: SPX = simplex family; MPX = multiplex family; c = controls

[a]control families were combined from both datasets

[b]mean number of children per family.

Measure

Pre- and perinatal information was retrospectively collected from the parents using standardized questionnaires, derived from the Prechtl optimality scales (Gillberg and Gillberg, 1983). The items were grouped into PPFs based on related content following the division successfully used in other studies (Schrieken *et al.*, 2013, Visser *et al.*, 2013), see Table 2. For each factor, a dichotomous variable was created, coding '1' if the risk factor was present and '0' indicating absence of the risk factor. Some additions were made. First, the factor 'pregnancy after fertility treatment' was added, because children born following assisted reproductive technologies (ARTs) have been found to be at higher risk of autism than the general population (Zhan *et al.*, 2013). Second, given that both low and high birth weight and parental age were found to be associated with adverse outcomes, it was decided to examine the effect of these categories separately (Abel *et al.*, 2013).

Table 2. Pre-and perinatal risk factors

Prenatal	**Perinatal**
1. Parental age at conception - low *(mothers < 25 years, fathers < 30 years)* - high *(mothers ≥ 35 years, father ≥ 40 years)*	7. Labor/parturition *prolonged parturition (≥24 hours), caesarian section, forceps extraction, vacuum extraction, breech presentation*
2. Miscarriages / bleeding *miscarriages in history, gestational bleeding*	8. Prematurity *(< 37 weeks)*
3. Maternal diseases *diabetes, (pre-) eclampsia, high blood pressure, severe nausea*	9. Birth weight - low birth weight *(< 2,500 grams)* - high birth weight *(> 4,500 grams)*
4. Maternal infections *virus, severe infections*	10. Suboptimal condition of child at birth *low APGAR score at 5 min (<8), respiratory distress, faeces in amniotic fluid, umbilical cord around neck, physical injury*
5. Maternal intoxications - *alcohol use during pregnancy* - *tobacco use during pregnancy*	11. Family size/Firstborn[#]
6. Stress during pregnancy *severe tensions, concerns about the child*	12. Pregnancy after fertility treatment

Note. the items underlying the factors are presented in *italic*. For each item, a dichotomous variable was created, coding '1' if the complication was present and '0' if the complication was not present. Then, items were grouped into the pre/perinatal factors based on related content. If at least one complication was present during pregnancy or delivery, '1' was coded on the overlapping factor.

[#] Because siblings were included in this study, we compared mean family size between disorders and examined firstborn-ship in the post hoc analyses only.

Procedure

Questionnaires were filled in separately for each child at home, mostly by the mother of the child. Additional data collection included demographic information, blood samples of all family members and neuropsychological data of the children. The study was approved by the local medical ethics board, and parents and children (12 years and older) signed for informed consent.

Data Analyses

In the BOA (ASD) study, the pre-/perinatal risk factor questionnaire was part of the standard test protocol and administered to all participating families. The percentage of missing data within the ASD cohort was random and < 5% for all risk factors. The full pre-/and perinatal risk factor questionnaire was only administered to about 50% of the participating ADHD families (IMAGE study), since it was added to the protocol at a later stage. A shorter version of the questionnaire (which did not include all pre-/perinatal exposures) was later sent to the remaining families. This resulted in missing data > 50% for the PFFs miscarriages/bleeding, maternal diseases, maternal infections, labor/parturition, stress during pregnancy, suboptimal condition at birth, and pregnancy after fertility treatment, because these PFFs were not assessed in about half of the sample (note that families entered the study randomly and that therefore missingness is random, including an equal proportion of case and control families). Missing data for the other PFFs was 17.1% for prematurity, 16.2% for high and low birth weight, 15.9% for alcohol use and 15.6% for smoking during pregnancy. Missing data was not imputed to prevent spurious associations.

First, to examine which PPFs were associated with ASD and/or ADHD, Wald chi-square values were calculated using generalized estimated equations (GEE) with a binary logistic model, robust estimators, and exchangeable structure for working correlation matrices. To correct for familial dependency within the data set, family number was used a repeated measure. Independent variables were type of disorder (ASD vs. ADHD vs. control) and sex and the two-way interaction type of disorder*sex. The two-way interaction was dropped from the model when non-significant. Sex was added to the model because previous studies reported that pre-/perinatal complications are more prevalent in boys (Lukkari *et al.*, 2012) and because groups differed in percentage males (see Table 1). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Dependent variables were the PPFs described above and separate analyses were run for each of the dependent variables. Because siblings were included in this study and only one child per family can be firstborn, the factor firstborn could not be compared between ASD vs. ADHD vs. controls. Instead, family size was examined between disorders. The factor firstborn was examined in post hoc analyses described below.

Second, for all PPFs significantly associated with either ASD or ADHD or both post hoc analyses were conducted to test a) whether the effect was specific to affected children or shared between affected and unaffected siblings and b) whether the effect was selectively found in either SPX or MPX families. Similar GEE analyses were run with independent variables a) diagnosis (affected vs. unaffected siblings) and sex or b) type of family (SPX vs. MPX families) and sex. For the factor firstborn, mean family size was included as covariate to account for the number of children per family. The post hoc analyses were run separately for ASD and ADHD cohorts. A False Discovery Rate (FDR) correction with a q-value setting of 0.05 was applied to control for multiple testing (Benjamini, 2010). However, because of the small numbers of individuals available for some of the exposures and the hypothesis-generating nature of our study, a distinction was made between significance (i.e. findings that remained significant after multiple testing), nominal significance (i.e. findings that did not remain significant after multiple testing), and trend-level significance (p <.10), in order to not miss out on possible relevant findings that can be tested in future studies. Note, therefore, that trend-level and nominal significant findings should be interpreted with caution, while associations that survived correction for multiple testing have the highest chance of being replicated in future studies. All analyses were carried out in SPSS version 20.

RESULTS

Table 3 presents the χ^2 tests for the PPFs for ASD and ADHD families. Significant main effects of type of disorder (ASD families vs. ADHD families vs. controls) were found for low parental age (χ^2 (2, N=1166) = 35.30, p<.001), tobacco use during pregnancy (χ^2 (2, N=1050) = 11.61, p=.003), stress during pregnancy (χ^2 (2, N=783) = 15.45, p<.001), and family size (χ^2 (2, N=1172) = 38.81, p<.001). Trend-level effects were found for maternal diseases (χ^2 (2, N=783) = 5.73, p=.057) and maternal infections (χ^2 (2, N=779) = 4.64, p=.098). Comparisons between ASD and ADHD families revealed that only one of these PPFs was significantly associated with both disorders, namely the factor family size (ASD families vs. controls: χ^2 (1, N=696) = 28.54, p<.001, OR=1.64 [95% CI: 1.37-1.97]; ADHD families vs. controls χ^2 (1, N=884) = 27.98, p<.001, OR=1.41 [95% CI: 1.24-1.61]; ASD vs. ADHD families: p=.084). Case parents had significantly more children than control families and this did not differ between ASD and ADHD. The other identified PPFs were associated with either ASD or ADHD. Nominally significant associations between ASD and the factors maternal infections and suboptimal condition at birth (χ^2 (1, N=539) = 3.83, p=.050, OR=3.97 [95% CI: 1.00-15.82]) and χ^2 (1, N=540) = 3.88, p=.049, OR=1.52 [95% CI: 1.00-2.31], respectively) were found. Children from ASD families were almost 4 times more likely to have suffered from a severe infection during pregnancy and were

Table 3. Comparisons between controls on the one hand and individuals from ASD and ADHD families, stratified into affected vs. unaffected siblings and SPX vs. MPX families on the other hand

	Controls		ASD families			ADHD families			Contrasts based on *p*-values < .05*				
									Identification of PPFs associated with ASD and/or ADHD	Further stratification in significantly associated PFFs			
										Affected vs. unaffected siblings		SPX vs. MPX families	
			Probands	Unaffected sibling	total	Probands	Unaffected sibling	total					
	% (n/N)		% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)		ASD	ADHD	ASD	ADHD
1. Parental age at conception													
Low (♀<25y; ♂<30y)	28.5 (115/403)	SPX	21.4 (12/55)	19.8 (16/81)	20.6 (28/136)	51.6 (16/31)	46.8 (22/47)	48.7 (38/78)	**ADHD>ASD=c**	A=U	A=U	S=M	S=M
		MPX	30.2 (29/96)	27.3 (15/55)	29.1 (44/151)	45.2 (122/269)	50.8 (65/128)	47.0 (187/397)					
		total	27.2 (41/151)	22.8 (31/136)	25.0 (72/288)	46.0 (138/300)	49.7 (87/175)	47.4 (225/475)					
High (♀≥35y; ♂≥40y)	17.6 (71/403)	SPX	16.1 (9/56)	14.8 (12/81)	15.3 (21/137)	6.5 (2/31)	6.4 (3/47)	6.4 (5/78)	**ADHD<ASD=c**	A=U	A=U	S=M	S=M
		MPX	19.8 (19/96)	14.5 (8/55)	17.9 (27/151)	10.4 (28/269)	7.8 (10/128)	9.6 (38/397)					
		total	18.4 (28/152)	14.7 (20/136)	16.7 (48/288)	10.0 (30/300)	7.4 (13/175)	9.3 (43/475)					
2. Miscarriages / bleeding	29.6 (75/253)	SPX	28.6 (16/56)	30.0 (24/80)	29.4 (40/136)	20.0 (3/15)	15.8 (3/19)	17.6 (6/34)	ADHD=ASD=c				
		MPX	37.5 (36/96)	30.9 (17/55)	35.1 (53/151)	29.3 (43/147)	30.2 (19/63)	29.5 (62/210)					
		total	34.2 (52/152)	30.4 (41/135)	32.4 (93/287)	28.4 (46/162)	26.8 (22/82)	27.9 (68/244)					
3. Maternal diseases	26.9 (68/253)	SPX	35.7 (20/56)	24.1 (19/79)	28.9 (39/135)	40.0 (6/15)	21.1 (4/19)	29.4 (10/34)	**ADHD>c, ASD=c, ADHD=ASD**	A=U	A=U	S=M	S=M
		MPX	32.3 (31/96)	34.5 (19/55)	33.1 (50/151)	40.1 (59/147)	46.0 (29/63)	41.9 (88/210)					
		total	33.6 (51/152)	28.4 (38/134)	31.1 (89/286)	40.1 (65/162)	40.2 (33/82)	40.2 (98/244)					
4. Maternal infections	1.2 (3/253)	SPX	3.6 (2/56)	2.5 (2/79)	3.0 (4/135)	0.0 (0/15)	0.0 (0/19)	0.0 (0/34)	**ASD>c, ADHD=c, ASD=ADHD**	A=U	A=U	S=M	S=M
		MPX	7.3 (7/96)	1.8 (1/55)	5.3 (8/151)	2.1 (3/144)	1.6 (1/62)	1.9 (4/206)					
		total	5.9 (9/152)	2.2 (3/134)	4.2 (12/286)	1.9 (3/159)	1.2 (1/81)	1.7 (4/240)					

Table 3. Comparisons between controls on the one hand and individuals from ASD and ADHD families, stratified into affected vs. unaffected siblings and SPX vs. MPX families on the other hand (continued)

	Controls		ASD families			ADHD families			Contrasts based on *p*-values < .05*				
									Identification of PPFs associated with ASD and/or ADHD	Further stratification in significantly associated PFFs			
			Probands	Unaffected sibling	total	Probands	Unaffected sibling	total		Affected vs. unaffected siblings		SPX vs. MPX families	
	% (n/N)		% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)		ASD	ADHD	ASD	ADHD
5. Maternal intoxications													
Alcohol	19.7 (75/381)	SPX	12.5 (7/56)	12.7 (10/79)	12.6 (17/135)	4.0 (1/25)	5.3 (2/38)	4.8 (3/63)	ADHD=ASD=c				
		MPX	13.7 (13/95)	9.1 (5/55)	12.0 (18/150)	18.8 (41/218)	17.0 (18/104)	18.3 (59/322)					
		total	13.2 (20/151)	11.2 (15/134)	12.3 (35/285)	17.3 (42/243)	14.1 (20/142)	16.1 (62/385)					
Tobacco	12.9 (49/381)	SPX	10.9 (6/55)	11.4 (9/79)	11.2 (15/134)	15.4 (4/26)	17.9 (7/39)	16.9 (11/65)	**ADHD>ASD=c**	A=U	A=U	S=M	S=M
		MPX	11.8 (11/93)	9.1 (5/55)	10.8 (16/148)	28.0 (61/218)	16.3 (17/104)	24.2 (78/322)					
		total	11.5 (17/148)	10.4 (14/134)	11.0 (31/282)	26.6 (65/244)	16.8 (24/143)	23.0 (89/387)					
6. Stress during pregnancy	13.4 (34/253)	SPX	25.0 (14/56)	10.1 (8/79)	16.3 (22/135)	26.7 (4/15)	10.5 (2/19)	17.6 (6/34)	**ADHD>ASD=c**	**A>U**	A=U	S=M	S=M
		MPX	17.7 (17/96)	12.7 (7/55)	15.9 (24/151)	31.3 (46/147)	25.4 (16/63)	25.8 (62/240)					
		total	20.4 (31/152)	11.2 (15/134)	16.1 (46/286)	30.9 (50/162)	22.0 (18/82)	27.9 (68/244)					
7. Labor/ parturition	22.5 (57/253)	SPX	30.4 (17/56)	21.3 (17/80)	25.0 (34/136)	26.7 (4/15)	36.8 (7/19)	32.4 (11/34)	ADHD=ASD=c				
		MPX	38.5 (37/96)	18.2 (10/55)	31.1 (47/151)	23.3 (34/146)	19.4 (12/62)	22.1 (46/208)					
		total	35.5 (54/152)	20.0 (27/135)	28.2 (81/287)	23.6 (38/161)	23.5 (19/81)	23.6 (57/242)					
8. Prematurity	6.1 (23/374)	SPX	8.9 (5/56)	8.6 (7/81)	8.8 (12/137)	3.8 (1/26)	15.4 (6/39)	10.8 (7/65)	ADHD=ASD=c				
		MPX	9.4 (9/96)	3.6 (2/55)	7.3 (11/151)	5.6 (12/213)	4.8 (5/105)	5.3 (17/318)					
		total	9.2 (14/152)	6.6 (9/136)	8.0 (23/288)	5.4 (13/239)	7.6 (11/144)	6.3 (24/383)					

Table 3. Comparisons between controls on the one hand and individuals from ASD and ADHD families, stratified into affected vs. unaffected siblings and SPX vs. MPX families on the other hand (continued)

	Controls		ASD families			ADHD families			Contrasts based on *p*-values < .05*				
									Identification of PPFs associated with ASD and/or ADHD	Further stratification in significantly associated PFFs			
			Probands	Unaffected sibling	total	Probands	Unaffected sibling	total		Affected vs. unaffected siblings		SPX vs. MPX families	
	% (n/N)		% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)	% (n/N)		ASD	ADHD	ASD	ADHD
9. Birth weight													
Low (>2,500g)	5.8 (22/380)	SPX	1.8 (1/56)	7.7 (6/78)	5.2 (7/134)	0.0 (0/26)	12.8 (5/39)	7.7 (5/65)					
		MPX	4.5 (4/88)	3.9 (2/51)	4.3 (6/139)	2.8 (6/214)	2.9 (3/105)	2.8 (9/319)	ADHD=ASD=c				
		total	3.5 (5/144)	6.2 (8/129)	3.7 (10/273)	2.5 (6/240)	5.6 (8/144)	3.6 (14/384)					
High (<4,500g)	2.1 (8/380)	SPX	1.8 (1/56)	7.7 (6/78)	5.2 (7/134)	3.8 (1/26)	7.7 (3/39)	6.2 (4/65)					
		MPX	9.1 (8/88)	5.9 (3/51)	7.9 (11/139)	2.8 (6/214)	2.9 (3/105)	2.8 (9/319)	ADHD=ASD=c				
		total	6.3 (9/144)	7.0 (9/129)	6.6 (18/273)	2.9 (7/240)	4.2 (6/144)	3.4 (13/384)					
10. Suboptimal condition	21.7 (55/253)	SPX	35.7 (20/56)	18.8 (15/80)	25.7 (35/136)	46.7 (7/15)	21.1 (4/19)	32.4 (11/34)					
		MPX	35.4 (34/96)	30.9 (17/55)	33.8 (51/151)	27.2 (40/147)	15.9 (10/63)	20.8 (50/240)	**ASD>c, ADHD=c, ASD=ADHD**	**A>U**	**A>U**	S=M	S=M
		total	35.5 (54/152)	23.7 (32/135)	30.0 (86/287)	29.0 (47/162)	17.1 (14/82)	25.0 (61/244)					
11. Family size/ Firstborn	49.8 (203/408)	SPX	57.1 (32/56)	27.2 (22/81)	39.4 (54/137)	54.8 (17/31)	29.8 (14/47)	39.7 (31/78)					
		MPX	45.8 (44/96)	21.8 (12/55)	37.1 (56/151)	45.6 (123/270)	37.5 (48/128)	43.0 (171/398)	**ASD=ADHD<c#**	**A>U**	*A>U*	*S>M*	S>M
		total	50.0 (76/152)	25.0 (34/136)	38.2 (110/288)	46.5 (140/301)	35.4 (62/175)	42.4 (202/476)					
12. Fertility treatment	5.2 (13/251)	SPX	7.1 (4/56)	2.5 (2/79)	4.4 (6/135)	13.3 (2/15)	0.0 (0/19)	5.9 (2/34)					
		MPX	7.3 (7/96)	3.9 (2/53)	6.0 (9/149)	8.3 (12/145)	6.3 (4/63)	7.7 (16/208)	ADHD=ASD=c				
		total	7.2 (11/152)	3.0 (4/132)	5.3 (15/284)	8.8 (14/160)	4.9 (4/82)	7.4 (18/242)					

Note. ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder; SPX = simplex; MPX = multiplex; PPFs = pre- and perinatal risk factors; n = wwnumber of children with risk factor present; N = total number of children within group; c = controls; A = affected siblings; U = unaffected siblings; S = individuals from simplex families; M = individuals from multiplex families; ♀ = mothers; ♂ = fathers. Grey bars indicate the risk factors that are significantly associated with ASD and/or ADHD. The factor firstborn (in dark grey) was the only factor associated with both disorders. *Significant contrasts (p <.05) are presented in **bold**. Marginally significant contrasts (p <.10) are presented in *italic*. # contrasts for mean family size

1.5 times more likely to have experienced at least one suboptimal condition at birth than controls. These factors were not significantly associated with ADHD. Further, a trend level effect of high birth weight was found in ASD (χ^2 (1, N=668) =2.74 p=.098; OR=2.38 [95% CI: .85-6.63]). Specifically associated with ADHD were the PPFs low parental age (χ^2 (1, N=878) = 21.20, p<.001, OR=2.26 [95% CI: 1.60-3.21]), tobacco use during pregnancy (χ^2 (1, N=768) = 7.87, p=.005, OR=2.12 [95% CI:1.52-4.48]), and stress during pregnancy (χ^2 (1, N=497) = 12.17, p<.001; OR=2.61 [95% CI: 1.52-4.48]). A nominally significant association was found between ADHD and maternal diseases (χ^2 (1, N=497) = 5.67, p=.017, OR=1.84 [95% CI: 1.11-3.04]). Children from ADHD families were over 2 times more likely to have young parents than control children. Furthermore, case mothers were almost twice as likely to have suffered from at least one disease and were over twice as likely to have smoked or experienced stress during pregnancy as control mothers, see Table 3 and Figure 1. Moreover, the proportion of children from ADHD families exposed to these four risk factors was significantly higher than that of individuals from ASD families

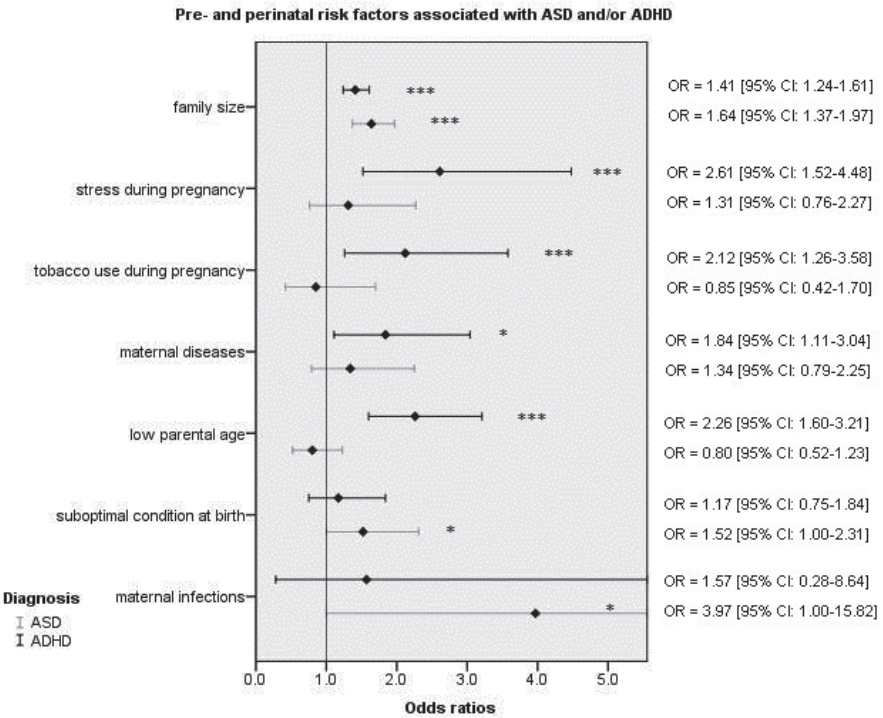

Figure 1 Identified risk factors for individuals from ASD or ADHD families.
Note. ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder. OR = odds ratio. Represented are the odds that a risk factor was present in children from ASD/ADHD families compared to control children (reference line). Significant odd ratios are indicated with an asterisk (*** p < .001, * p < .05)

(*p*-values <.006). A reverse effect was found for high parental age (χ^2 (2, N=1168) = 11.51, *p*=.003), with parents of ADHD cases being less likely to have an advanced parental age than control parents (χ^2 (1, N=878) =10.85 *p*=.001; OR=.45 [95% CI; .28-.72]).

As a next step, we compared affected vs. unaffected siblings for their exposure to PPFs. We found that after controlling for family size, ASD affected children were more likely to be firstborn than their unaffected siblings (χ^2 (1, N=288) = 8.77, *p*=.003, OR=6.48 [95% CI: 1.88-22.33]). A trend-level effect of being firstborn was found for ADHD as well (ADHD: χ^2 (1, N=476) = 3.23, *p*=.072, OR=1.16 [95% CI: .99-1.35]). A suboptimal condition at birth was nominally significantly more frequently reported in ASD-affected compared to ASD-unaffected siblings (χ^2 (1, N=287) = 4.47, *p*=.034, OR=1.70 [95% CI: 1.04-2.79]), whereas ASD-affected and unaffected offspring did not differ from each other with regard to the prevalence of maternal infections (*p* = .114*)* or high birth weight (*p*=.460). Within ADHD

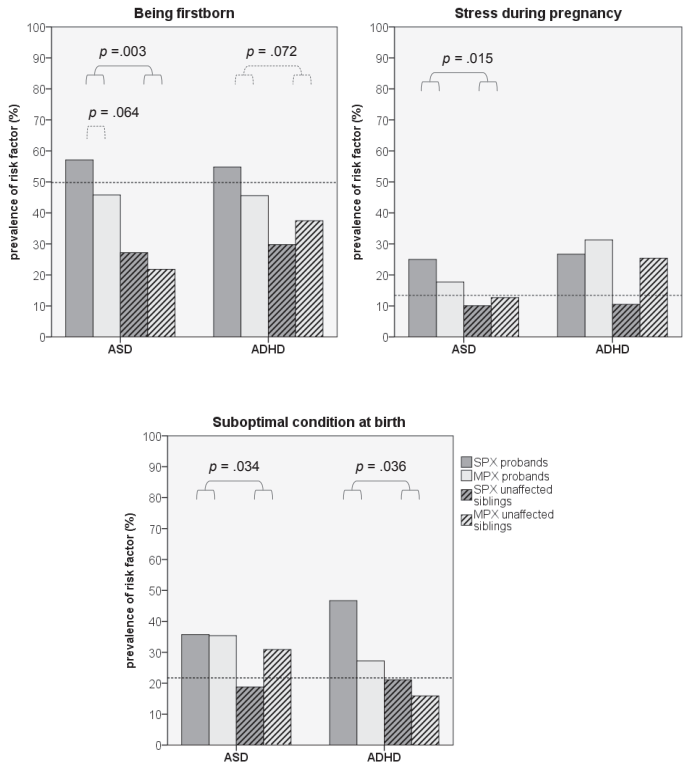

Figure 2 Comparisons between ASD and ADHD probands and their unaffected siblings, stratified for SPX and MPX families

Note. ASD = autism spectrum disorders; ADHD = attention-deficit/hyperactivity disorder; SPX = simplex; MPX = multiplex. The interpolation lines represent the percentage of control children with the risk factor present. Dotted lines indicate trend-level significant findings

families, no differences were found between ADHD-affected and unaffected siblings for any of the identified PPFs (*p*-values >.10). However, despite suboptimal condition at birth not being significantly associated with ADHD, this perinatal complication was nominally significantly more often reported in ADHD-affected compared to ADHD-unaffected offspring (χ^2 (1, N=244) = 4.39, *p*=.036, OR=2.13 [95% CI: 1.05-4.33]). Similarly, stress during pregnancy was more frequent in ASD-affected vs. ASD-unaffected children (χ^2 (1, N=286) = 5.91, *p*=.015, OR=2.19 [95% CI: 1.16-4.13]), see Figure 2.

A final step in the data analyses was the stratification into SPX vs. MPX families. This revealed a trend-level significant difference between SPX and MPX ASD families in the factor being firstborn (χ^2 (1, N=288) = 3.44, *p*=.064, OR=.51 [95% CI: .24-1.04], with SPX ASD families having proportionally more firstborns than MPX ASD families. Trend-level effects were found for SPX versus MPX ASD unaffected siblings on suboptimal condition (χ^2 (1, N=135) = 5.91, *p*=.052, OR=.44 [95% CI: .19-1.01]) and for SPX versus MPX ADHD siblings on being firstborn (χ^2 (1, N=175) = 3.35, *p*=.067, OR=.51 [95% CI: .24-1.05]). Siblings from MPX families were more likely to be firstborn (ADHD) or to have experienced a suboptimal condition at birth (ASD) than unaffected siblings from SPX families, see Figure 2.

DISCUSSION

This is the first study to test sharing and uniqueness of the involvement of PPFs for ASD and ADHD, using an approach that stratifies the sample into affected versus unaffected offspring and SPX versus MPX affected families. Our results revealed that except for large family size and more firstborns amongst affected offspring, no shared PFFs were identified for ASD and ADHD. PPFs specifically related to ASD (maternal infections and suboptimal condition at birth) were more often reported in affected offspring. PPFs associated with ADHD (low parental age, maternal diseases, maternal smoking and maternal stress) were shared between affected and unaffected siblings. Stratification into SPX and MPX revealed that SPX ASD probands were more often firstborn, but were less likely to have experienced a suboptimal condition at birth than MPX ASD probands. Firstborn-ship was also highest amongst MPX compared to SPX ADHD unaffected siblings.

Except for large family size and more firstborns amongst affected offspring, no shared PFFs were identified for ASD and ADHD. This is surprising given that cross-disorder traits were present in both ASD and ADHD affected and unaffected siblings, mainly in MPX families (see sample characteristics). This may suggest that the comorbid presence of ASD and ADHD symptoms is not likely explained by shared pre-and perinatal risk factors. The finding that affected siblings were more often firstborns than unaffected siblings concurs with previous literature (Gardener *et al.*, 2009, Marin *et al.*, 2014). This

suggests that being firstborn may increase the risk for ASD and ADHD alike, and possibly increase the risk for psychopathology in general (Feehan *et al.*, 1994, Happe, 1999). Other alternative (or additional) explanations for the increased risk of developing ASD or ADHD as a firstborn have also been suggested. For instance, becoming a parent for the first time is life-changing and puts high demands on parents. Parents are often more anxious and restrictive with the first child than with later children (Eisenman, 1992) and firstborn children (particularly boys) experience significantly higher ineffective parenting behaviors as compared to children without siblings or those with older siblings (Arim *et al.*, 2012). Interestingly, family size was larger in families including children with ASD or ADHD. Large family size has been previously associated with ADHD (Biederman *et al.*, 1995) and has been linked to negative child outcomes such as lower average educational levels (Booth and Hiau, 2009). Parents who choose to have more children might be (inherently) different from parents with fewer children, suggesting that family size may be considered a proxy for developmental risk.

Several PPFs were either associated with ASD (maternal infections and suboptimal condition at birth) or ADHD (low parental age, maternal diseases, smoking during pregnancy, and stress during pregnancy). Higher frequencies of maternal infections and suboptimal conditions at birth in ASD cases are consistent with previous findings (Gardener *et al.*, 2011, Visser *et al.*, 2013). These factors are likely to reflect immune dysfunction and hypoxia, impacting on neurodevelopment (Kolevzon *et al.*, 2007, Onore *et al.*, 2012). Young maternal age is a known risk factor for behavioral problems and has been previously linked to ADHD (Gustafsson and Kallen, 2011). The finding that maternal smoking and stress during pregnancy were significantly associated with ADHD also corroborates with previous findings (Thapar *et al.*, 2013). Some studies have reported GxE interactions between some key ADHD risk genes (DAT1, DRD4), maternal smoking (Neuman *et al.*, 2007), and maternal stress (Grizenko *et al.*, 2012), others did not (Altink *et al.*, 2009). Whether maternal smoking and stress are causal agents or proxy variables for the genetic risk to develop ADHD remains unclear (D'Onofrio *et al.*, 2013). The link between maternal tobacco use or maternal stress and offspring ADHD might be attributable to transmission of ADHD risk genes, in addition to any true environmentally mediated effect (Thapar *et al.*, 2013). Proposed mechanism linking stress during pregnancy and ADHD include a disruption in stress-response systems and prefrontal cortex development (Class *et al.*, 2014). Last, maternal diseases such as gestational diabetes mellitus (GDM) and preeclampsia have been previously associated with ADHD (Cannon and Keller, 2006, Nomura *et al.*, 2012). Maternal diseases might impact fetal brain growth, possibly resulting in greater inattention and hyperactivity (Ornoy, 2005). Our study adds importantly to the existing literature by showing that PPFs are relatively specific for ASD and ADHD. These findings might suggest that the strong overlap between the disorders is unlikely to be caused by many overlapping pre- or perinatal risk factors.

More differentiation in the role of PPFs contributing to ASD and ADHD was found when stratifying the sample into affected versus unaffected children. In ADHD, all PPFs were shared between affected and unaffected siblings. This corroborates previous findings that some PPFs in ADHD (i.e., maternal smoking and alcohol use during pregnancy) did not differ among affected and unaffected siblings (Ben Amor *et al.*, 2005). This was not the case in ASD. Here, PPFs were more prevalent in autistic children compared to their unaffected siblings (Deykin and MacMahon, 1980). This suggests that PPFs in ASD may have a unique, highly penetrant contribution to the disorder and are more likely to be true determinants, whereas in the case of ADHD, PPFs are weak risk factors that only slightly increase the overall liability for the disorder in a family. Further stratification into SPX and MPX families had some additional value in understanding the role of PPFs, especially for ASD. SPX and MPX ASD families differed with respect to birth order and suboptimal conditions at birth, pointing to potential pre-/perinatal etiological differences between SPX and MPX forms of ASD. Differences in birth rank effects between SPX and MPX ASD families were previously examined, but the results were opposite to ours (Turner *et al.*, 2011), with middle children (particularly those born second) having a higher risk of developing autism than other children in MPX families and increasing risk with each additional birth in SPX families. It was argued that the latter finding might be explained by a higher number of (possibly causative) de novo mutations due to increasing parental age. It is a challenge to explain our finding that SPX affected offspring was more often firstborn than unaffected offspring. Possibly, gene x environment interactions and purely environmental mechanisms for the development of ASD are different for the two types of ASD families. SPX and MPX ADHD could not be dissociated from each other regarding PPFs. These results suggest that SPX-MPX stratification is more suitable to differentiate effects of PPF in ASD families, but in its current form it does not provide further insight in the role of PPFs in ADHD. However, the limited sample sizes of SPX ADHD families in combination with the low exposure rates for some of the PPFs might have resulted in a lack of power. Based on our results, some risk factors appear to be more frequently shared by affected and unaffected siblings from MPX than SPX ADHD (e.g. maternal diseases and stress during pregnancy). Surely, more research is needed to confirm this hypothesis.

Of note, no evidence was found for the association of low birth weight with ASD or ADHD, which was the most promising common risk factor based on previous reports (Ben Amor *et al.*, 2005, Gardener *et al.*, 2011). A possible explanation might be that low birth weight is often associated with advanced maternal and paternal age (Shah *et al.*, 2010), and the proportion of mothers older than 35 years at conception was significantly smaller in case mothers than in control mothers in our study. We could not replicate the consistently reported finding of advanced parental age being associated with ASD in our sample (Gardener *et al.*, 2009). Also, no support was found for maternal smoking being a

shared PPF for ASD and ADHD. Previous studies reported that maternal smoking might be specifically related to PDD-NOS, but not (childhood) autistic disorder or Asperger's syndrome (Visser *et al.*, 2013). In our sample, we did not examine subgroups within ASD, which might have diluted the effect. Alternatively, among the ASDs, PDD-NOS is the most difficult subtype to distinguish from ADHD, and maternal smoking may actually be a unique (genetically driven) PPF for ADHD (Jaspers *et al.*, 2013).

A number of limitations of this study should be considered when weighing the results. First, this study should be viewed as a first step towards a better understanding of the unique and shared contribution of PPFs to the development of sporadic or common ASD and/or ADHD. However, the combination of limited sample sizes of SPX ADHD families in combination with the low exposure rates and interdependence for some of the PPFs might have resulted in decreased power to detect some true group differences. Therefore, our results should be interpreted with caution. A large majority of ADHD cases stem from MPX families, SPX ADHD families seem to be a relatively rare phenomenon in itself (15.3% in our sample). Strategic oversampling of SPX ADHD families might be necessary to increase the power of studies using the SPX-MPX stratification. A second important limitation is that our study design was not suited to test causal inference, thus caution is required in assuming that PPFs have causal effects on ASD and ADHD. As Thapar and Rutter (2009) pointed out previously, significant associations between pre-/perinatal risk factors and psychiatric disorders may arise because of postnatal risks (e.g. parent mental health problems, social adversity) or through unmeasured confounders including maternally transmitted inherited factors (Thapar and Rutter, 2009). For example, in a novel natural experimental design examining children who are genetically unrelated to their mother as a result of in vitro fertilization (IVF), Thapar and colleagues showed that the association between maternal smoking and ADHD may be genetically transmitted from mother to child rather than being a direct effect of smoking on the fetus (Rice *et al.*, 2009, Thapar *et al.*, 2009). Therefore, additional studies (indeed using natural experiments as in Rice *et al.*, 2009, Thapar *et al.*, 2009), even though highly difficult to undertake, are needed to further test whether the identified PFFs are true causal factors for ASD and ADHD. We feel that our study nonetheless adds to the current literature because to our knowledge, this is the first study that examined (a) overlapping pre-/perinatal risk factors for ASD and ADHD, and (b) whether risk factors are shared or non-shared between affected and unaffected siblings which may lead to important conclusions about the role of a risk factor in the development of a disorder. In addition, our results may potentially explain why some individuals develop ASD and others ADHD, because (a) different PPFs were associated with either disorders, and (b) in ADHD, the significant associations between disorder and PPFs might reflect inherited factors, whereas in ASD they are likely to reflect direct pre-/perinatal effects. Third, the pre- and perinatal data was collected retrospectively which could have resulted in a recall bias when comparing

cases to controls. However, previous research shows a reasonable agreement between parent report and medical records for most birth-related data (Buka *et al.*, 2004), and it is unlikely to explain our main finding of PPFs being rather specific for ASD and ADHD. Nevertheless, a more optimal design to map out the role of pre-/perinatal risk factors in ASD and ADHD would be to study these factors prospectively during different stages of pregnancy (e.g. in a longitudinal follow-up study of high-risk infant siblings). Fourth, because a shorter form of the questionnaire was administered in about half of the ADHD families, some PPFs had a substantial amount of missing values. However, we believe that insofar this has affected our results, it would likely lead to underestimation of the effect of pre- and perinatal complications on ADHD since we identified significant associations between ADHD and maternal disease, stress during pregnancy despite the high percentage of missings on these factors. Last, boys were overrepresented in the clinical samples compared to the control cohort and previous studies report that pre-/perinatal complications are more prevalent in boys (Lukkari *et al.*, 2012). Therefore we controlled for sex in our analyses.

To conclude, the findings reported here indicate that pre- and perinatal complications are more frequent in children with ASD and ADHD compared to control children. Most of the pre-and perinatal factors were uniquely related to either ASD or ADHD, suggesting that the high co-morbidity is not likely to be explained by shared pre-and perinatal risk factors. Instead, PPFs might play a crucial role in the developmental pathways discriminating the disorders on a background of shared genetic factors. Further stratification into SPX vs. MPX families appeared to detect some differences, particularly in ASD families, pinpointing to potential pre-/perinatal etiological differences between SPX and MPX forms of the disorder. These results can stimulate further research on the complex etiologies of ASD and ADHD and the role of PPFs herein.

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CHAPTER 8

Summary

SUMMARY

Chapter 2 focused on the three core cognitive domains that are proposed to underlie ASD (i.e. social cognition [SC], executive function [EF] and central coherence) and examined whether these domains co-segregated within ASD and whether they showed signs of familiality and thus might be viable endophenotype candidates for ASD. The performance of 140 children with ASD, 172 siblings and 127 controls on tasks measuring SC (face recognition, emotional prosody, and facial emotion recognition), EF (inhibition, cognitive flexibility, and verbal working memory) and local processing style was assessed. Compelling evidence was found for co-segregation of SC and EF, but not local processing style within ASD, indicating that particularly SC and EF may be fruitful domains in future family studies of the genetic contribution to ASD. Given that SC and EF are both strongly related to ASD, using the underlying shared variance of both constructs in genetic research may increase the power for detecting susceptibility genes for ASD. Furthermore, our results have shown that performances on SC and EF tasks are highly correlated within probands, suggesting that probands who perform worse on one domain are likely to display deficits in the other domain. Moreover, siblings of autistic individuals (regardless of their affected status) tend to display a similar cognitive profile as their affected brother or sister and are therefore at risk for impairments in SC and EF as well. This latter finding highlights the importance of assessing cognitive functioning in sibling risk groups, early in development to determine if SC and EF problems develop, and further on if these lead to other functional impairments (e.g., in school performance or peer relations).

In **chapter 3**, we examined whether emotion recognition is a viable endophenotypic candidate for ASD and to assess the impact of comorbid ADHD in this context. The performance of 90 children with ASD (43 with and 47 without ADHD), 79 ASD unaffected siblings and 139 controls aged 6-13 years on facial emotion and affective prosody recognition was assessed. Results revealed that the recognition of both facial emotion and affective prosody was impaired in children with ASD and aggravated by the presence of ADHD. The latter finding could only be partly explained by typical ADHD cognitive deficits, such as inhibitory and attentional problems. The performance of unaffected siblings could overall be considered at an intermediate level, performing somewhat worse than the controls and better than the ASD probands. Our findings suggest that particularly speed measures of emotion recognition might be a viable endophenotype for ASD and a fruitful target in future family studies of the genetic contribution to ASD and comorbid ADHD. Furthermore, our results suggest that children with comorbid ASD and ADHD are at highest risk for emotion recognition problems and clinicians should therefore pay special attention to these children suffering from symptoms of both spectra.

In **chapter 4** we examined whether stratification into simplex (families with only one affected individual; SPX) and multiplex (families with two or more affected individuals; MPX) families might be a promising approach for creating (etiologically) more homogeneous subgroups of patients. Second, we examined whether SPX-MPX stratification is a useful tool to detect shared etiological underpinnings for ASD and ADHD. This approach builds on the idea that polygenic and multifactorial causes of disease will increase symptom levels in most or all members of the family (mostly present in MPX families), whereas sporadic genetic and non-genetic causes will be strictly personal to the patient (mostly present in SPX families). The approach has been successful in genetic ASD research, but has rarely been used in ADHD research. This is the first study to divide ADHD families into SPX and MPX and look across ADHD and ASD SPX and MPX families at traits of both conditions, in siblings as well as parents. ASD and ADHD traits were measured in 56 ASD and 31 ADHD SPX nuclear families, 59 ASD and 171 ADHD MPX nuclear families and 203 control nuclear families using parent-, teacher-, spouse-, and self-report questionnaires. The results revealed that SPX-MPX stratification indeed detected quantitative differences in ASD families, with SPX families being less densely affected with ASD than MPX families, suggesting that stratification based on family occurrence may help parse ASD heterogeneity and that future studies examining causal factors/pathways for ASD should consider family history. In contrast, stratification did not distinguish ADHD families; SPX and MPX unaffected relatives (siblings and parents) both showed equally elevated levels of ADHD traits compared to controls. Furthermore, heightened symptom levels of both disorders, particularly in MPX unaffected relatives, indicate shared (multifactorial) underpinnings underlying ASD and ADHD. The observed higher levels of cross-disorder traits in ASD than ADHD MPX unaffected siblings might even suggest that risk factors underlying ASD overlap to a larger degree with risk factors underlying ADHD than vice versa. These findings could inform (genetic) counseling approaches and therapeutic interventions.

In **chapter 5** we examined whether the cognitive architecture underlying SPX and MPX ADHD families is different and useful for parsing the etiological heterogeneity of ADHD. It was hypothesized that cognitive impairments may be different in SPX and MPX forms of ADHD as indicated by (a) the presence of disorder-related cognitive deficits in MPX, but not SPX unaffected siblings and (b) dissimilar cognitive profiles in SPX and MPX patients. This is the first study to suggest that different causal pathways may underlie simplex (SPX) and multiplex (MPX) forms of ADHD. Tasks measuring total IQ, executive functioning, motor functioning, and time estimation were administered to 31 SPX and 264 MPX ADHD probands, 47 SPX and 123 MPX unaffected siblings, and 263 controls, aged 6-19 years. Our results showed that several cognitive domains (IQ, visual working memory and time estimation) were most impaired in SPX ADHD probands, whereas

the formerly proposed 'key' cognitive problems related to ADHD (inhibition and motor control) were not impaired in these SPX probands. Importantly, unaffected siblings of SPX families were cognitive unimpaired. This was in sharp contrast to the cognitive underpinnings in MPX families: MPX probands and their unaffected siblings showed a wide range of cognitive problems, more in line with previous studies on cognitive endophenotypes in ADHD. These findings suggest that different causal pathways may lead up to –on the surface- comparable cognitive deficits and behavioural symptoms in children with ADHD, and that SPX-MPX stratification may be a step forward in unravelling these various causal pathways. Clinically, SPX and MPX ADHD patients may have distinct prognoses and benefit from different treatment strategies.

In **chapter 6** we examined whether the cognitive architecture underlying SPX and MPX ASD families is different and useful for parsing the etiological heterogeneity of ASD. It was hypothesized that cognitive impairments may be different in SPX and MPX forms of ASD as indicated by (a) dissimilar cognitive profiles in SPX and MPX patients, a finding with implications for treatment and (b) the presence of disorder-related cognitive deficits in MPX, but not SPX unaffected siblings, a finding highly relevant to the identification of cognitive endophenotypes for genetic research. Tasks measuring intelligence, social cognition, and executive functioning were administered to 54 SPX and 91 MPX ASD probands, 77 SPX and 46 MPX unaffected siblings, and 124 controls aged 6-20 years. Our results suggest quantitative differences between SPX and MPX forms of ASD, which becomes evident when contrasting cognitive performances within families. Children with ASD and their unaffected siblings (regardless SPX or MPX) all had poorer social cognition skills. Lower IQ scores were present in MPX and SPX affected children and MPX (but not SPX) unaffected siblings. Executive functions were relatively spared in both types of families. In all, SPX affected children were more dissimilar from their non-affected sibling compared to MPX affected children from their non-affected sibling. The finding that unaffected siblings (regardless SPX or MPX) performed normal on most cognitive tasks contrasts findings in related disorders such as ADHD. These findings suggest that cognitive deficits associated with ASD may have a stronger determining effect on the disorder compared to related disorders. An important exception is affective prosody, suggesting this domain may be sensitive towards familial risk factors for ASD. These findings may help parse the etiological heterogeneity of ASD by stratifying ASD families into families with stronger versus weaker familial aggregation of ASD-related neurocognitive deficits.

Chapter 7 set out to (a) identify the pre-/perinatal antecedents associated with ASD, ADHD, or both disorders and (b) examine whether these are unique (only found in affected offspring) or common (also present in non-affected offspring siblings), using

the MPX-SPX stratification approach. Pre-/perinatal data based on retrospective parent-report were collected in 288 children from 31 SPX and 59 MPX ASD families, 476 children from 31 SPX and 171 MPX ADHD families, and 408 control children. Except for large family size and more firstborns amongst affected offspring, no shared pre-/perinatal risk factors were identified for ASD and ADHD, indicating that the co-morbidity of ASD and ADHD is not likely explained by shared pre-/perinatal risk factors. Instead, pre-/perinatal antecedents might play a crucial role in the developmental pathways leading up to the specific disorder. Pre-/perinatal factors specifically related to ASD (maternal infections and suboptimal condition at birth) were more often reported in affected than unaffected siblings. In contrast, pre-/perinatal factors associated with ADHD (low parental age, maternal diseases, smoking and stress) were shared between affected and unaffected siblings. This suggests that pre-/perinatal factors in ADHD index an increased shared risk, whereas in ASD these factors possibly have a more determining role in the disorder. SPX-MPX stratification detected some differences, particularly in ASD families, pinpointing to potential pre-/perinatal etiological differences between SPX and MPX forms of the disorder. SPX-MPX stratification provided no deeper insight in the role of such antecedents in ADHD, but the combination of limited sample sizes of SPX ADHD families with the low exposure rates and interdependence for some of the pre-/perinatal factors might have resulted in decreased power to detect some true group differences. All in all, pre- and perinatal complications were more frequent in children with ASD and ADHD compared to control children. Counseling of pregnant women should focus on decreasing maternal stress during pregnancy by helping mothers to cope with or decrease the exposure to stress, or advising pregnant women to stop smoking to reduce the risk of ADHD.

CHAPTER 9

General discussion

GENERAL AIMS OF THIS THESIS

The main aim of this thesis is to examine shared and unique mechanisms underlying ASD and ADHD by comparing pre-/perinatal antecedents and associated cognitive deficits in both disorders. An attempt was made to parse etiological heterogeneity by forming subgroups based on familial re-occurrence of the disorders, which might facilitate research into disease etiology and eventually aid in developing effective, individualized treatment for ASD and ADHD. We sought to identify viable cognitive endophenotypes for ASD (**chapters 2 and 3**), following ADHD research in which this method has been frequently and successfully applied. Additionally, we examined whether these potential endophenotype candidates are influenced by similar familial factors (**chapter 2**) and whether comorbid symptoms of ADHD impacts on the manifestation of ASD endophenotype candidates (**chapter 3**). In **chapters 4-7**, we stratified the sample into single-incidence or sporadic (SPX) and multi-incidence or familial (MPX) families and compared behavioral phenotypes, cognitive functioning, and pre-/perinatal risk factors between disorders and between family types. Here, the three specific aims described in the introduction will be discussed in light of the present results, along with other issues that emerged from this research, critical reflections on study design and methods, and suggestions for clinical practice and future research.

WHICH COGNITIVE ENDOPHENOTYPES FOR ASD CAN BE IDENTIFIED?

Social cognition endophenotypes are most promising for ASD

Results from several studies suggested that affective prosody might be a promising endophenotype candidate for ASD (chapters 2, 3 and 6) given that it meets several of the proposed criteria for endophenotypes (Bearden and Freimer, 2006, Cannon and Keller, 2006, Gottesman and Gould, 2003). Here, it was shown that affective prosody recognition was poorer in ASD-affected individuals compared to controls and thus associated with the disorder. This corroborates previous findings (Charbonneau *et al.*, 2013, Golan *et al.*, 2007, Korpilahti *et al.*, 2007, Lindner and Rosen, 2006, Philip *et al.*, 2010). Particularly effects in speed stood out: children with ASD were slower to recognize emotions in facial and vocal expressions (chapter 3). Clinically unaffected siblings displayed similar deficits as their affected brothers and sisters, fulfilling the criterion that deficits should be present in unaffected first-degree relatives (chapters 3 and 6). Moreover, affective prosody recognition deficits appeared to be familial as indicated by significant sibling correlations (chapter 2). The convergence of these results speaks to the promise of affective prosody as an endophenotype for ASD. Interestingly, impairments in affective prosody did not differ between affected and unaffected siblings from SPX and MPX ASD

families. Thus, SPX unaffected siblings also displayed poorer affective prosody recognition, despite the proposed lower genetic loading for the disorder. This might suggest that affective prosody is a primary deficit underlying multiple causal pathways leading to ASD and thus a key target in understanding etiological pathways towards ASD.

Less convincing were results regarding facial emotion recognition. Impaired facial emotion recognition is reported in a broad range of child and adolescent psychiatric disorders and is likely to contribute to enduring difficulties in social relationships that characterize all of these disorders (Collin *et al.*, 2013). This implies that factors underlying facial emotion recognition processes play a role in the development of multiple psychiatric disorders (including ASD and ADHD) and thus an important area for future research into shared and unique risk factors for these disorders. Although facial emotion recognition deficits were familial and co-segregated with prosodic impairments (chapter 2), results were inconclusive as to whether or not unaffected siblings differed significantly from controls on this measure. In chapter 3 we observed that unaffected siblings were significantly slower that controls in recognizing facial emotional expressions, whereas in chapter 6, we did not find a difference between siblings and controls. A possible explanation for this discrepancy is the difference in age range between the two studies (i.e. ages 6-13 years in chapter 3 and ages 6-20 years in chapter 6). Deficits in facial emotion recognition might be more pronounced in younger age than in older age and siblings might have a delayed (but not necessarily impaired) development of facial emotion recognition compared to controls. The impact of age and development on the relationship between ASD and cognitive functioning in affected and unaffected siblings is an under-investigated area, but we know from studies in ADHD that the endophenotype characteristics of cognitive control functioning in unaffected siblings diminishes over time (Thissen *et al.*, 2014). This suggests that we should consider a developmental approach in future studies exploring endophenotypes for ASD (and ADHD). An alternative explanation, however, might be found in the fact that stratification into SPX and MPX families decreased the power to the extent that we were not able to detect differences, with the implication that differences in facial emotion recognition between unaffected siblings and controls are subtle. Previous literature has also been inconclusive regarding the endophenotypic properties of facial emotion recognition. Some studies have reported similar deficits in unaffected first-degree relatives (Adolphs *et al.*, 2008, Kadak *et al.*, 2014, Losh *et al.*, 2009, Losh and Piven, 2007, Neves *et al.*, 2011), others did not (Buitelaar *et al.*, 1999, Castelli, 2005). Most of the studies reporting impaired functioning in unaffected relatives tested parents -instead of siblings- of autistic children, which might also partly explain different findings. This explanation however counter-argues an involvement of delayed development.

Taken together, our findings suggest that impairments in different aspects of social cognition might be qualitatively different from each other, with impaired recognition

of facial expressions only present in those with the disorder (state-dependent) or a developmental consequence of the disorder, whereas impaired recognition of affective prosody runs in ASD families regardless of the presence of a disorder and may provide an index of the multifactorial liability to ASD. Possibly, however, not a qualitative difference but a differential developmental trajectory explains the findings: children may more easily overcome their facial expression than their affective prosody recognition impairments. Clearly, more research is needed to explore the viability of facial emotion recognition as an endophenotype for ASD.

Are impairments in executive functions not primary to ASD?

Importantly, we found little support for executive functions (EF) as endophenotypes for ASD. This is remarkable given that EF deficits were found to be highly familial (as indicated by moderate to strong sibling correlations) and highly correlated with social cognition functioning. This led us to consider using the shared variance in future endophenotype studies (chapter 2). In chapter 6, however, unaffected siblings were found to be unimpaired on various EF tasks compared to controls. This strongly contrasts with studies confirming the viability of several EF domains as endophenotypes for ADHD (Rommelse *et al.*, 2008a, Rommelse *et al.*, 2011, Rommelse *et al.*, 2008b). Further, children with ASD showed impairments on some, but certainly not all EF domains. For example, ASD probands were not more inflexible than controls, which contrasts with previous studies (Corbett *et al.*, 2009, Panerai *et al.*, 2014). However, it is known from the literature that certain aspects of EF might be preserved in (family members of persons with) ASD (Losh *et al.*, 2009, Wong *et al.*, 2006), and the broader autism phenotype might not be primarily characterized by impairments in EF (Wong *et al.*, 2006). Also, some studies suggest differentiation in EF deficits within the ASD spectrum, with the EF profile of children with Pervasive Developmental Disorders – Not Otherwise Specified being less disturbed than the other groups (Verte *et al.*, 2006). We did not differentiate between these subtypes, which might have blurred results. Again, reduced statistical power due to stratification into SPX and MPX might explain the lack of significant findings, but given that with the same sample sizes we were able to detect differences in the social cognition domain, we propose that EF deficits, more so than problems in the social cognition domain, are part of the defining features of ASD (state-dependent) or a developmental consequence of the disorder, rather than being an endophenotypic trait that is observable in unaffected siblings. Additional research in larger samples is needed to test this hypothesis.

WHAT HAVE WE LEARNED ABOUT SHARED AND UNIQUE UNDERPINNINGS FOR ASD AND ADHD?

Combining cognitive findings in ASD and ADHD: Shared and unique cognitive deficits

Recognition of affective prosody appeared to be a promising endophenotype for ASD. Unfortunately, emotion recognition was not examined at the initial assessment of the ADHD cohort, therefore we were unable to examine the viability of emotion recognition as endophenotype for ADHD and compare results from the ASD and ADHD cohorts with each other. However in the follow-up assessment (NeuroIMAGE) the ADHD affected and unaffected siblings and controls completed the same facial emotion and affective prosody recognition tasks as the children from the ASD cohort. Tentative results from a recent study by our group (De Bruijn et al., in preparation) show that children with ADHD, but not their unaffected siblings had emotion recognition problems, suggesting that emotion recognition may not be a viable endophenotype for ADHD. Although the lack of significant group differences between unaffected siblings and controls might also be explained by a normalization in cognitive performance throughout adolescence in these unaffected siblings (Thissen *et al.*, 2014), these findings suggest that emotion recognition is an endophenotype for ASD, but not for ADHD. In contrast, little support was found for the viability of EF as endophenotype for ASD, whereas EF deficits were observable in the majority of ADHD cases and in unaffected siblings from MPX ADHD families (chapter 5) corroborating previous studies in independent ADHD samples (Bidwell *et al.*, 2007, Goos *et al.*, 2009, Jester *et al.*, 2009, Nigg *et al.*, 2004). This suggests that various EF domains could be considered viable cognitive endophenotypes for (MPX) ADHD, but not for ASD. Based on these findings, I propose that different cognitive constructs might play a pivotal role in the development of ASD and ADHD (i.e. social cognition is a core deficit in ASD, whereas EF in ADHD) and the presence of these specific cognitive impairments might have a determining role in the development of either disorder.

Some open questions remain that I would like to address here. First, not all cognitive domains were assessed in both ASD and ADHD cohorts and in addition, slightly different tasks were used to measure similar cognitive constructs in the separate cohorts. This prevented us from directly comparing ASD and ADHD probands with each other. Moreover, except for emotion recognition (chapter 3), we did not include combined ASD and ADHD as a separate group. Therefore, we cannot draw firm conclusion on whether or not combined ASD and ADHD is a different nosologic entity with separate cognitive processes giving rise to the comorbid presentation as suggested previously (Chantiluke *et al.*, 2014, Rommelse *et al.*, 2011). Our results so far suggest that the combined presence of both disorders aggravates cognitive deficits, but it does not allow us to draw firm conclusions on whether or not the deficits in this group are more than a simple

summary of dysfunctions found in ASD and ADHD alone. It is recommended that future family-behavioral genetic studies include four types of families: ASD only, ADHD only, ASD and ADHD, and control families, and assess multiple cognitive domains, using similar (and multiple) cognitive measures per domain to address this issue further.

Second, although effort was made to include several tasks tapping the domains of general intelligence, social cognition, executive function, and motor function, we were not able to assess all aspects of these domains, such as theory of mind and fluency. These functions have been implicated in ASD and ADHD research (Ames and White, 2011, Buitelaar *et al.*, 1999, Corbett *et al.*, 2009, Dyck *et al.*, 2001, Geurts *et al.*, 2010, Miranda-Casas *et al.*, 2013, Nyden *et al.*, 2010): some might be more pronounced in ASD than ADHD (such as theory of mind (Ames and White, 2011, Geurts *et al.*, 2010) and planning (Miranda-Casas *et al.*, 2013)), or vice versa (Corbett *et al.*, 2009), although research has been inconclusive herein. Based on the current findings we might expect to find impairments in ASD and ADHD probands and in ASD, but not ADHD siblings on theory of mind, given that it relies heavily on emotion recognition (Buitelaar and van der Wees, 1997). In contrast, various EF tasks (planning, fluency) might be impaired in ASD and ADHD probands and ADHD (but not ASD) unaffected siblings. However, this might not necessarily be so given that EF refers to a broad range of related, but distinct high level cognitive capacities.

Third, certain cognitive domains that have been proposed to be promising pleiotropic endophenotype candidates for ASD and ADHD were not tested in the cohorts, such as delay aversion, reward anticipation and sensory functioning. Delay aversion (i.e. the motivation to escape or avoid delay) and altered reward anticipation (i.e. processing the anticipation of a reward) have received a lot of research attention in the last decade and are considered (in addition to EF) prime cognitive deficits in ADHD (Bitsakou *et al.*, 2009, Furukawa *et al.*, 2014, Plichta and Scheres, 2014). Affected-unaffected sibling studies in ADHD have consistently shown that both functions are viable endophenotype candidates (Rommelse *et al.*, 2011). Very little is known about delay aversion in ASD (Rommelse *et al.*, 2011), but impairments in reward anticipation have been implicated in ASD research (Stavropoulos and Carver, 2014), stressing the potential of this domain as pleiotropic endophenotype for ASD and ADHD. Sensory dysfunctioning (i.e. problems in processing sensations from one's own body and the environment) is associated with both ASD and ADHD (Rogers and Ozonoff, 2005, Rommelse *et al.*, 2011). In the new DSM-5, more attention is focused on sensory functioning with the inclusion of the separate criterion 'hyper-or hypo-reactivity to sensory input or unusual interest in sensory aspects of environment' (APA, 2013). It is difficult to speculate whether the abovementioned domains are shared between ASD and ADHD, and if so, whether cognitive testing would reveal similar patterns in SPX and MPX ASD and ADHD affected and unaffected siblings. Future studies are needed to improve our understanding of the (pleiotropic)

endophenotypic properties of these cognitive domains for ASD and ADHD. The neurocognitive tasks used here were thus by no means representative of the full domain of neurocognitive functions and tasks relevant for ASD and ADHD. Note, however, that compared with many studies in the literature, the studies in this thesis rank very high in terms of comprehensiveness of the tasks investigated.

Co-occurrence of ASD and ADHD results in poorer emotion recognition skills

We tested the impact of the comorbid presence of ADHD on ASD-related social cognitive deficits (i.e. impaired recognition of facial expressions and affective prosody) (**chapter 3**). Our results clearly showed that children with a combined diagnosis of ASD and ADHD had poorer outcomes on both visual and auditory emotion recognition than autistic children without ADHD. This poorer performance in the comorbid ASD and ADHD group was not solely a consequence of typical ADHD-related cognitive problems (such as impaired inhibition and attention): group differences between ASD-only and ASD+ADHD children on affective prosody recognition remained present after controlling for these measures. This corroborates other studies that suggested a more pronounced impairment in children with co-diagnoses of ASD and ADHD (Tye *et al.*, 2013a, Tye *et al.*, 2013b). Whether this translates to other cognitive domains (such as EF, motor function, etcetera) was not tested in this thesis, but based on the findings by Tye and colleagues, this seems likely (Tye *et al.*, 2013a). However, dysfunctions of children with a combined ASD and ADHD diagnosis might not merely be the sum of the dysfunctions seen in children with ASD-only and with ADHD-only (Nyden *et al.*, 2010, Rommelse *et al.*, 2011). These results demonstrate that comorbidity has a not-to-be-ignored impact on cognitive functioning and the presence of comorbid psychopathology should be accounted for in future endophenotype/cognitive research. Clinicians should pay special attention to these children suffering from symptoms of both spectra given that they are at highest risk for impaired cognitive functioning.

Are pre-/perinatal antecedents specific for ASD or ADHD?

In chapter 7, we investigated whether shared pre-/perinatal risk factors might partly account for the high co-occurrence of ASD and ADHD. Since ASD or ADHD have been studied mostly in isolation, we have little knowledge about whether pre-/perinatal risk factors are shared between the disorders. Based on such previous reports, it was expected that certain pre-/perinatal risk factors (such as advanced parental age, low birth weight, stress and smoking during pregnancy) might be shared between ASD and ADHD, given that these risk factors were found to be significantly associated with both disorders. Other pre-/perinatal risk factors might be specifically related to ASD (e.g. maternal infections, and birth injury or trauma) or ADHD (e.g. exposure to alcohol or toxins, low maternal age), given that these factors were only implicated in ASD or

ADHD research (Gardener *et al.*, 2009, 2011, Langley *et al.*, 2005, Mick *et al.*, 2002, Mill and Petronis, 2008, Thapar *et al.*, 2013, Throckmorton-Belzer *et al.*, 2009, Visser *et al.*, 2013). Our study was designed to test the sharing and uniqueness of the involvement of pre-/perinatal risk factors for ASD and ADHD by directly comparing ASD and ADHD cases and their unaffected siblings from sporadic and familial case families. Much to our surprise, no pre-/perinatal risk factors were found to be significantly associated with both disorders. Instead, risk factors were relatively specific for ASD and ADHD, although this finding should be interpreted with caution given the limited sample sizes of SPX ADHD families combined with the low exposure rates and interdependence for some of the pre-/perinatal risk factors. Another important finding was that the risk factors associated with ADHD (amongst others smoking during pregnancy) appear to index an increased shared risk corroborating previous studies suggesting that the association between ADHD and maternal smoking might be an inherited or gene x environmental effect (Thapar *et al.*, 2013). In contrast, in ASD, pre-/perinatal risk factors were only present in affected offspring and thus possibly have a more determining role in the disorder.

To our knowledge, no previous studies were published that report on the specificity of pre-/perinatal risk factors for ASD and ADHD. Recently, a study was published that hypothesized that ASD and ADHD, as well as cerebral palsy (CP), are all the result of an exaggerated fetal central nervous system inflammatory response to a pre-/perinatal response (such as hypoxia, maternal infection a fetal infection, or a maternal inflammatory disease), with a suggested timing effect of the insult 32-40 weeks post-conception for ADHD and 36-48 weeks post-conception for ASD (Strickland, 2014). Strickland suggests inflammatory insults might impact on glutaminergic and GABAergic tracts (both develop during the last trimester of the pregnancy) potentially resulting in the development of one of these disorders. In addition, thalamic white matter tracts might be involved in ADHD development (Strickland, 2014). This might suggest that it is not the specific insult, but the timing of the insult that might be relevant for ASD or ADHD development. We did not investigate timing effects of the identified pre-/perinatal risk factors, but this would be an interesting addition to our current design. Notwithstanding the limitations of our study, our findings provide a novel perspective on pre-/perinatal antecedents in ASD and ADHD. They suggest that pre-/perinatal risk factors play a crucial role in the developmental pathways discriminating the disorders, potentially on a background of shared genetic factors. These results can stimulate further research on the complex etiologies of ASD and ADHD and the role of pre-/perinatal antecedents herein. A more optimal design to map out the role of pre-/perinatal risk factors in ASD and ADHD would be to study these factors (including inflammatory insults and maternal infections) prospectively during different stages of pregnancy (e.g. in a longitudinal follow-up study of high-risk infant siblings) while considering the role of gene x environmental interactions between these pre-/perinatal insults and genetic susceptibility to the disorders.

EXPLORING THE VALIDITY OF SPX-MPX STRATIFICATION

Does SPX-MPX stratification help in identifying shared and unique underpinnings of ASD and ADHD?

All considered, one could answer affirmatively. Comparing ASD traits in SPX and MPX ADHD family members (and vice versa, ADHD traits in SPX and MPX ASD families) allowed us to test whether the overlap between ASD and ADHD in affected children and their first-degree family members depends on shared or non-shared causal mechanisms underlying the primary disorder in the family. If the shared additive genetic factors proposed to explain the large portion of covariance in ASD and ADHD (Ronald *et al.*, 2008) are polygenic in nature, then we would expect to find a heightened prevalence of comorbid ASD symptoms in ADHD MPX, but not SPX families (and vice versa higher prevalence of ADHD in ASD MPX but not SPX families). This was indeed what we found. Highest levels of cross-disorder traits were found in first-degree relatives from MPX families, suggesting that these relatives were at highest risk for developing ASD and ADHD traits (compared to relatives from SPX families and normal controls). Based on our findings it might thus be hypothesized that in most cases, the genetic risk factors underlying comorbid ASD and ADHD are common polymorphisms with small effects given the higher prevalence of comorbidity in MPX families. Tentative findings suggest that these common polymorphisms may modulate ASD and ADHD severity (Gadow *et al.*, 2013). This does not preclude the possibility that strong genetic effects can also lead to comorbidity in a subset of families, as the literature on CNVs for ADHD suggests that the same rare variants (with potentially large(r) effect sizes) overlap with those for ASD (Williams *et al.*, 2012, Williams *et al.*, 2010) and in several ASD CNV studies, family members of the ASD patients also carrying the CNV had diagnoses of ADHD (Marshall *et al.*, 2008, Rommelse *et al.*, 2010, Weiss *et al.*, 2008). However, it is known that ASD greatly reduces reproductive fitness (meaning that affected individuals do not transmit the genetic defects) (Ploeger and Galis, 2011). Rare, deleterious variants underlying ASD are thus likely subjected to natural selection and therefore removed (or maintained at a low frequency) in the population (Jouan *et al.*, 2012). This also decreases the likelihood that rare genetic variants with large effect account for many comorbid ASD and ADHD cases.

Moreover, the type of diagnosis (ASD or ADHD) of the proband appeared informative for behavioral comorbidity outcomes in these children and their unaffected relatives. Unaffected siblings from families with multiple siblings with ASD were at highest risk for ASD and ADHD symptoms. The reversed pattern was not found (MPX ADHD unaffected siblings having highest levels of ASD traits), suggesting that ADHD might be seen as a milder, less severe subtype within the ASD spectrum as has been stated in the gradient overarching disorder hypothesis (Van der Meer *et al.*, 2012) or that risk factors underlying ASD may overlap to a larger degree with risk factors underlying ADHD than

vice versa (Rommelse *et al.*, 2010, van Steijn *et al.*, 2012). All in all, our findings indicate that family members of MPX families are at highest risk of displaying subthreshold ASD and/or ADHD symptoms, making these families of particular interest for future studies targeting the overlap between ASD and ADHD.

Is SPX-MPX stratification based on familial re-occurrence a promising approach to parse etiological heterogeneity in ASD and ADHD?

Overall, our findings suggest that differences exist between SPX and MPX forms of ASD and ADHD hinting at potentially different underlying causal pathways. When comparing affected children from SPX and MPX families with each other, it was found that familial (MPX) ADHD was related to a wide range of cognitive vulnerabilities, including deficits in response inhibition and motor control. In contrast, these domains were relatively spared in SPX ADHD, yet lower TIQ and impairments in visual working memory and time estimation were more pronounced in these SPX ADHD probands (chapter 5). Similarly, SPX ASD probands also displayed lowest IQ compared to MPX cases, in addition to more pronounced emotion recognition problems (chapter 6). These findings suggest that different causal pathways may lead up to –on the surface- comparable cognitive deficits in affected children, a phenomenon referred to in developmental psychopathology as equifinality (Cicchetti and Rogosch, 1996). Although the reality of equifinality is well-recognized (Nigg *et al.*, 2004, Sonuga-Barke, 2002), few solutions have been provided to tease etiological heterogeneity apart. SPX-MPX stratification may be a step forward in unravelling these various causal pathways and may thus help reduce etiological heterogeneity.

Further support for the usefulness of SPX-MPX stratification in parsing etiological heterogeneity was found when looking at the cognitive manifestations of unaffected siblings. What is often overlooked in the literature is the heterogeneous character of the unaffected sibling group. In schizophrenia, another complex, highly heritable disorder, different cognitive subtypes (normal, mixed, impaired) within the unaffected siblings group were found (Quee *et al.*, 2014) and this is expected for ASD and ADHD siblings as well given the high etiological heterogeneity of both disorders. We proposed that different modes of inheritance (i.e. sporadic vs. familial) likely explained why some unaffected siblings perform cognitively normal (siblings from SPX families), whereas others are impaired (siblings from MPX families). Our findings strongly support this hypothesis for ADHD, but less so for ASD. In ADHD, unaffected siblings from SPX ADHD families were generally unimpaired compared to controls (except for verbal working memory), whereas MPX ADHD unaffected siblings showed impairments on most cognitive domains. Thus, siblings stemming from families with non-shared (*de novo*) risk factors underlying the disorder in the proband performed normal on cognitive tasks ('the normal group'), whereas siblings stemming from families with shared (inherited)

risk factors underlying the disorder in the proband showed similar (yet milder) cognitive impairments as their affected brother or sister ('the mixed or impaired groups'). In contrast –and rather unexpectedly-, unaffected siblings from *both* SPX and MPX ASD families performed significantly poorer than controls on affective prosody recognition. Here, the difference between SPX and MPX unaffected siblings was less obvious given that the unaffected siblings from SPX ASD families were not completely clean from cognitive deficits. The finding that unaffected siblings from SPX ASD and ADHD families displayed some cognitive problems (i.e. poorer affective prosody recognition in ASD and poorer verbal working memory in ADHD), suggests that also in SPX families some risks may be shared between family members, and the distinction between MPX and SPX may be quantitative rather than qualitative. Etiological heterogeneity of ASD and ADHD may be parsed by stratifying families into families with stronger (MPX) versus weaker (SPX) familial aggregation of disorder-related neurocognitive deficits.

Do we need to subgroup children based on familiality?

These results cater to the ongoing debate on the need of subgrouping in ASD and ADHD (Grzadzinski *et al.*, 2013). The high within-disorder heterogeneity of both ASD and ADHD presents significant challenges to the search for genes and for effective treatments. Genetic and intervention studies might be more effective if 'true' etiological subgroups within ASD or ADHD could be identified for study, rather than the heterogeneous whole. Recent attempts have mostly focused on defining subgroups (beyond current DSM-5 defined subtypes) at the phenotypic or cognitive level (Charman *et al.*, 2011, Georgiades *et al.*, 2013, Nigg *et al.*, 2002, Nigg *et al.*, 2005, Sonuga-Barke, 2002, Willcutt *et al.*, 2005). For example, neuropsychological impairments characterize only a portion of the children with ASD and ADHD and do not contribute causally to the disorder in all cases, suggesting that we might need 'neuropsychologically impaired' subtypes (Nigg *et al.*, 2005). Yet, these studies do not explicitly address etiological heterogeneity. Based on our findings of differences between sporadic and familial forms of ASD and ADHD (nevertheless resulting in fairly similar behavioral and cognitive profiles in affected children from these two types of families), I propose that subgrouping ASD and ADHD based on family re-occurrence (i.e. sporadic vs. familial) is a necessary first step in parsing etiological heterogeneity, before behavioral or cognitive subtypes within these different modes of inheritance can be identified.

Issues that emerge from the SPX-MPX stratification method

Worthy of note is that the ratio between SPX and MPX families was different for ASD and ADHD. In ASD, about a third of families were SPX, a third was MPX, and a third could not be classified. In contrast, in ADHD the vast majority of families were MPX (72%), and only a small subsample (13%) could be considered SPX (another 15 % could not be classified).

This is an important finding in itself and may suggest that most ADHD cases are caused by multifactorial common genetic and non-genetic risk factors with low penetrance, whereas in ASD, a substantial proportion of causes is likely caused by non-transmitted (*de novo*) genetic or incidental environmental risk factors. This might partly explain why rare, genetic variants have received relatively little attention in ADHD research, although recently several studies were published on rare variants and *de novo* mutations in ADHD (Ben Amor *et al.*, 2005, Elia *et al.*, 2012, Lionel *et al.*, 2011, Williams *et al.*, 2012, Williams *et al.*, 2010). In any case, a small proportion of ADHD families exist in which only one individual has developed ADHD whilst the other family members are also at heightened risk (given elevated levels of ADHD traits in unaffected relatives). These families might be particularly valuable for detecting protective mechanisms decreasing the risk for developing ADHD and of particular interest for (whole-) exome sequencing studies exploring the genome for rare ADHD-related de novo mutations. That is, whole-exome sequencing has begun to shed light on the role of rare and de novo coding sequence variation, particularly in simplex ASD samples (Iossifov et al., 2012, O'Roak et al., 2011, O'Roak et al., 2012, Sanders et al., 2012). Applying these analyses in simplex ADHD families may lead to the discovery of new ADHD risk variants.

Some limitations emerged from this model that need to be addressed. First, SPX-MPX stratification is not a straightforward one. Correct classification requires valid knowledge of family history and fecundity is a major confounder (Sullivan *et al.*, 2012). Moreover, classification was based on the number of affected individuals per nuclear family, which indicates that in order to accurately classify the family, no subsequent pregnancies should follow the birth of the (then) youngest child and all individuals should carefully be phenotyped. This limits the ability of the SPX-MPX stratification in providing current generation parents with reliable risk estimates of having another child with the disorder. Including extended family data (grandparents, cousins, aunts, and uncles) might improve classification, but this requires substantial extra work, and runs into several ethical and practical issues (such as privacy regulations, deaths, lack of reliable clinical diagnosis especially for older generations). Notwithstanding the difficulties with correct classification, current clinical genetics practice uses family history to predict recurrence-risk of ASD. From the literature, it is known that recurrence rate is significantly higher in multiplex than simplex families (32.2% versus 13.5-20.1%) (Ozonoff *et al.*, 2011). Our results suggest that family history might also be informative in ADHD genetic counseling and research. Second, it should be noted that no formal diagnosis was made in parents. Instead, we based the affected status of parents on self- and spouse report questionnaires (particularly for parents of ASD families), which may have overstepped the clinical boundaries of these instruments. Future studies should consider including observational measures and (semi-)structured diagnostic interviews to improve classification. Third, a number of families could not be classified based on

current criteria, particularly for ASD. Including extended family data might help with this issue as well, but alternatively, adapting criteria (e.g. an unaffected father also counts as unaffected male relative) might be worthwhile to consider. Preventing bias, we excluded these families in our analyses, but this resulted in a loss of (a) statistical power, and (b) potentially meaningful information these families could provide about risk factors or protective factors for ASD and ADHD. Future studies should therefore improve classification.

It is important to keep in mind that SPX-MPX stratification is an overly simplified model to distinguish between different (non-)heritable forms of the disorder based on diagnostic status of the family members. Although SPX-MPX stratify is a first step forward, genetic hypotheses should ideally be directly tested by conducting genetic analyses in parent-child triads to confirm whether *de novo* mutations might underlie SPX families, and common risk factors might underlie MPX families. Still, it is unlikely that there is a simple dichotomy between SPX and MPX ASD/ADHD. Our findings that differences between SPX and MPX ASD/ADHD families are quantitative rather than qualitative (chapters 4-6), suggest that whilst factors uniquely present in the affected child (such as *de novo* mutations) might underlie ASD or ADHD in some of the SPX cases, multifactorial risk factors might still underlie the disorder in others (Krumm *et al.*, 2013, Sullivan *et al.*, 2012). Vice versa, rare transmitted mutations may play a role in familial forms of ASD and ADHD. Recently, exome sequencing in extended pedigrees with multiple ASD affected individuals was used to identify new ASD loci (Cukier *et al.*, 2014). The authors stated that since multi-incidence extended families are likely to carry ASD susceptibility loci of moderate to high effect, identity by descent (IBD) filtering in these pedigrees would permit them to isolate genes contributing to ASD pathogenesis. Using this method, the authors identified several new genes that likely play a role in ASD, some of them related to other neuropsychiatric and neurodevelopmental disorders such as intellectual disability and epilepsy (Cukier *et al.*, 2014). Although we are still far from understanding the genetic background of ADHD and ASD, stratification based on family re-occurrence in both ASD and ADHD samples might further advance genetic research and provide deeper insights in genetic risks for both disorder.

DO THE RESULTS OF THIS THESIS LEAD TO A BETTER UNDERSTANDING OF THE OVERLAP BETWEEN ASD AND ADHD?

Together, the studies presented in this thesis provided us with a deeper insight into the shared and unique underpinnings of ASD and ADHD. Both disorders are characterized by impairments in cognitive functions, but different cognitive constructs (social cognition in ASD and EF in ADHD) might play a pivotal role in the development of ASD

and ADHD, respectively. Further, the comorbid presence of both disorders aggravated the cognitive impairments, indicating that comorbidity should not be ignored in future studies exploring the role of cognition in ASD and ADHD. Our findings also suggest that high co-occurrence is likely caused by shared (pleiotropic) common genetic variants with low penetrance given that levels of comorbid pathology were highest in MPX families, particularly in ASD, which corroborates previous findings (Ronald *et al.*, 2008). MPX families thus might be of primary interest when exploring shared genetic risk factors for ASD and ADHD. These findings also suggest that risk factors of ASD may overlap to a greater extent with risk factors for ADHD than vice versa (Rommelse *et al.*, 2010, van Steijn *et al.*, 2012). Lastly, the lack of shared pre-/perinatal risk factors for ASD and ADHD indicates that the high comorbidity is not likely explained by overlapping pre- and perinatal antecedents. Instead, pre-/perinatal risk factors may play a crucial role in the developmental pathways discriminating the disorders on a background of shared genetic factors.

KEY FINDINGS

- Ø Both affected and unaffected siblings from ASD families exhibit emotion recognition problems.
- Ø Children with comorbid ASD and ADHD are slower in recognizing emotions, particularly in vocal emotional expressions than children with ASD only.
- Ø In ASD families, MPX (but not SPX) unaffected siblings display notable ASD/ADHD symptoms compared to controls. In ADHD families, both SPX and MPX unaffected siblings show higher levels of ASD/ADHD symptoms than controls.
- Ø In ASD families, unaffected siblings (regardless SPX or MPX) perform normal on most cognitive domains (IQ, face and facial emotion recognition, inhibition, working memory, and cognitive flexibility). An important exception is affective prosody recognition; both SPX and MPX unaffected siblings perform poorer than controls on this task.
- Ø In ADHD families, unaffected siblings from MPX families display deficits similar to their affected brother/sister on IQ, inhibition, working memory, set shifting, response variability and timing. In contrast, unaffected siblings from SPX families are unimpaired on these domains.
- Ø Children with a sporadic form of ASD/ADHD are cognitively more severely impaired than children with a familial form of ASD/ADHD.
- Ø Maternal infections and suboptimal condition at birth are associated with an increased risk of ASD. Low parental age, maternal diseases, and smoking and stress during pregnancy are associated with an increased risk of ADHD. Except for large

family size and more firstborns amongst affected offspring, no pre-/perinatal risk factors are significantly associated with both disorders.

- Ø In ASD families, pre-/perinatal risk factors are more often reported in affected than unaffected siblings, whereas in ADHD families these risk factors are shared between affected and unaffected siblings.
- Ø There are less SPX ADHD families than SPX ASD families.

STRENGTHS AND LIMITATIONS

The strengths of this thesis are 1) the combined use of, two large family-behavioral genetic cohorts of ASD families, ADHD families and control families, 2) administration of gold-standard, well-validated dichotomous DSM measures of ASD and ADHD to thoroughly screen all participating family members for ASD and ADHD, and 3) simultaneous study of multiple candidate endophenotypes.

Some limitations also need to be acknowledged. Discussed in more detail above were the limitations that arose from the SPX-MPX stratification and the selected study methods. Further, the small group of SPX ADHD families, and the moderate sample sizes for SPX and MPX ASD families might have reduced statistical power to detect group differences. Therefore, we calculated effect sizes to accompany statistical testing to qualify all effects and not only those that reached statistical significance. In addition, pre-/perinatal data were available for about 50% of the ADHD cohort. The combination of the large proportion of missing data, the small sample size of SPX ADHD families, and the low exposure rates and interdependence of some of the pre-and perinatal risk factors might have resulted in decreased power to detect some true group differences. This combined with the fact that we used a novel approach and were the first to directly compare pre-/perinatal risks for ASD and ADHD indicates that our studies need replication in independent, larger samples.

A second limitation was the wide age range (2-20 years). Age has a strong effect on neurocognitive performance (Paus, 2005) and is a factor in symptom presentation. Therefore, we controlled for age-effects by including age as a covariate in the analyses (chapters 2 and 3), or by regressing scores for each dependent cognitive measure on age and using the unstandardized residuals as dependent variables (chapters 5 and 6). Arguably, the study of cohorts of the same age in longitudinal designs would be the ideal design to address effects of age and give insight into developmental maturation. Inclusion of hundreds of children with a diagnosis of ADHD and ASD of the same age is, however, difficult to achieve.

Third, boys were overrepresented in the case groups (SPX and MPX ASD or ADHD probands, and SPX unaffected siblings from ASD and ADHD families), but underrepresented

in the control groups and MPX unaffected sibling groups. This was due to the fact that a) ASD and ADHD are more frequently diagnosed in males and b) the presence of male unaffected siblings was only required for SPX, but not MPX families. However, we do not believe that this has affected the results, since the effect of sex was adjusted for in each study. Again, achieving the same sex ratio in unaffected siblings as present in cases with ASD and ADHD, respectively, is very costly; it would imply to not include many available siblings in the study. In all likelihood, the loss of available information on "difficult to recruit" patients and their siblings does not outweigh the advantage that may be gained for matched sex samples.

A fourth limitation is that both ASD and ADHD samples consisted of average functioning children (IQ ≥ 70), which might limit the generalizability of our findings to the broad range of ASD and ADHD. It might be worthwhile to extend these results to lower-functioning individuals, where the impairments on the various cognitive domains might be more pronounced. Results from chapters 5 and 6 suggest that children with ASD/ADHD and lowered intelligence levels more often had SPX than MPX forms of the disorder. The inclusion of lower functioning ASD or ADHD patients might reveal different SPX-MPX ratios. Intellectual disability is frequently caused by non-hereditary genetic mutations (Hamdan *et al.*, 2011, Rauch *et al.*, 2012). Literature has also shown that children with ASD with *de novo* mutations have significantly lower non-verbal IQs than children with ASD who do not carry these mutations (Ronemus *et al.*, 2014). However, one of the difficulties that plague the literature in general is that comparable versions of tasks tapping relevant cognitive domains that can be used in lower functioning individuals are not available. This seriously hinders research in this group.

Last, only participants of European Caucasian ethnicity were included in order to minimize genetic variation between families. However, this may limit the generalization of our findings to other ethnic groups.

CLINICAL IMPLICATIONS

Although this thesis in general addresses a more theoretical - instead of a clinical - theme, results do lead to some clinical implications.

Ø Clinicians should be aware of the family history of psychopathology in diagnosis and counseling. First-degree family members of patients from MPX ASD and ADHD families are at highest risk of developing behavioral or pathology-related cognitive difficulties. This highlights the need to assess such problems in these relatives and to follow them up over time in order to track their development.

- Ø Awareness of family history might inform therapeutic interventions. Specific cognitive strengths and weaknesses profiles in SPX and MPX forms of ASD and ADHD might help tailor treatment to best suit the needs of the affected child.
- Ø Comorbidity of ADHD in ASD and vice versa should not be ignored in diagnosis, treatment and counseling. Children with a co-diagnosis of ASD and ADHD have the poorest outcomes cognitively and might benefit most from different treatment strategies compared to children diagnosed with ASD- and ADHD-only.
- Ø Pre- and perinatal complications are more frequent in children with ASD and ADHD compared to control children. Counseling of pregnant women should focus on decreasing maternal stress during pregnancy by helping mothers to cope with or decrease the exposure to stress, or help pregnant women to stop smoking as this, among multiple positive outcomes, reduces the risk for ADHD.

RECOMMENDATIONS FOR FUTURE RESEARCH

In discussing our main findings, several recommendations for future research emerged. The common thread in these recommendations was that future studies should consider family re-occurrence and comorbidity in studying the etiology of ASD and ADHD. A summary of the recommendations is provided below.

- Ø Comorbidity has a not-to-be-ignored impact on cognitive functioning and the presence of comorbid psychopathology should be accounted for in future endophenotype/cognitive research.
- Ø Given that etiologies differ between single-incidence or sporadic (SPX) and multi-incidence or familial (MPX) ASD and ADHD families, future studies should account for family re-occurrence of the disorder when examining the genetic and cognitive underpinnings, pre-/perinatal antecedents, and phenotypic manifestations of ASD and ADHD. Given the relatively low occurrence of sporadic ADHD, strategic oversampling of SPX ADHD families might be necessary to increase the power of studies using the SPX-MPX stratification.
- Ø As a next step, future studies should follow-up families with sporadic and familial ASD or ADHD to examine the behavioral and cognitive development of the affected and unaffected siblings over time. Longitudinal follow-up studies of extended families (parents, siblings, and next generation offspring) would be the ideal design to tackle these research questions.
- Ø Based on the finding that individuals with low(est) IQs were more frequently from SPX families, we hypothesize that risk factors that underlie sporadic cases of psychopathology (such as *de novo* mutations or incidental environmental factors of strong

effect) are more frequent in lower functioning patients and result in more pronounced cognitive deficits. It would be worthwhile to investigate these hypotheses by extending our findings to families of lower-functioning individuals. The inclusion of lower functioning ASD or ADHD patients might reveal different SPX-MPX ratios.

Ø Although SPX-MPX stratification is a first step forward, genetic studies should directly test parent-child triads to confirm whether *de novo* mutations might underlie SPX families, and common risk factors might underlie MPX families.

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CHAPTER 10

Samenvatting in het Nederlands

(Dutch summary)

Dankwoord

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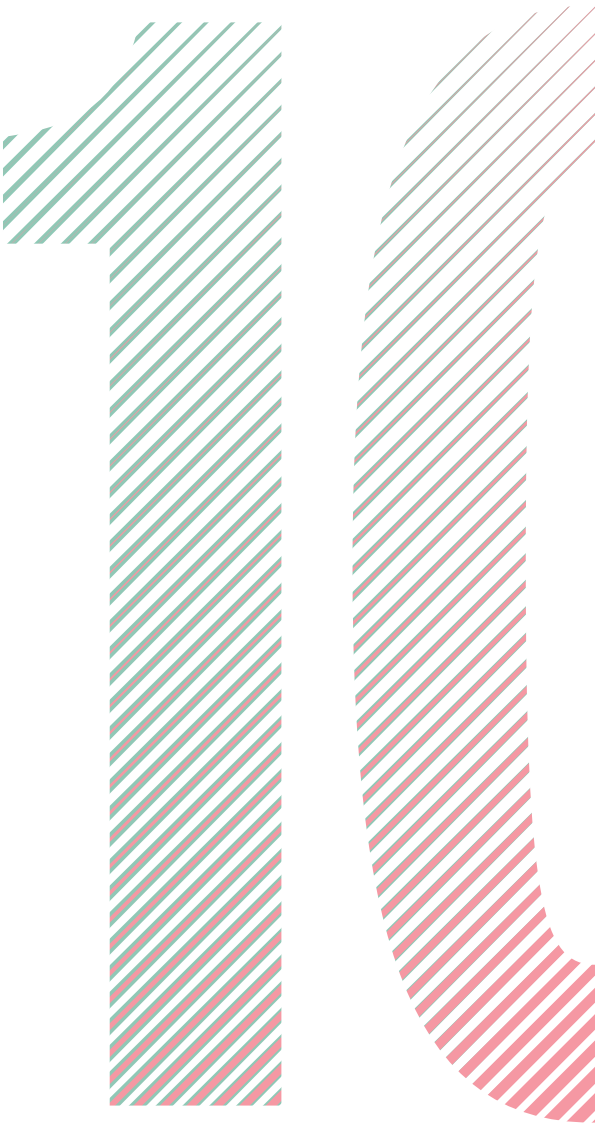
Publications

Curriculum Vitae



SAMENVATTING IN HET NEDERLANDS (SUMMARY IN DUTCH)

Autisme spectrum stoornissen (ASS) en aandachtstekort stoornis met hyperactiviteit (ADHD) zijn psychiatrische ontwikkelingsstoornissen. ASS wordt gekenmerkt door beperkingen in de sociale interactie en (non-)verbale communicatie en door een beperkt, repetitief of stereotiep gedragspatroon (APA, 2013). ADHD wordt gekenmerkt door concentratieproblemen en/of hyperactief (rusteloos) en impulsief gedrag (APA, 2013). Recente prevalentie cijfers tonen aan dat ASS bij ongeveer 1% van de populatie voorkomt en ADHD gemiddeld bij 5% van de populatie (Erskine *et al.*, 2013, Kim *et al.*, 2011, Russell *et al.*, 2014). Beide stoornissen komen vaker voor bij mannen dan bij vrouwen. Ook komen ASS en ADHD vaak samen voor: ongeveer 30-80% van de kinderen met ASS laten kenmerken zien passend bij de diagnose ADHD. Vice versa voldoet ongeveer 20-50% van de kinderen met ADHD ook aan de klinische criteria voor ASS (Rommelse *et al.*, 2011, Simonoff *et al.*, 2008). Verder zijn personen met ASS en ADHD bekend met cognitieve functie problemen. ASS wordt onder andere gekenmerkt door problemen in de sociale cognitie (zoals moeite hebben met het herkennen van emoties), executieve functies (moeite met het wisselen van gedrag), centrale coherentie (geneigd zijn om te focussen op details in plaats van op het geheel) en een lagere verbale intelligentie. ADHD wordt onder andere gekenmerkt door executieve functie problemen (bijvoorbeeld moeite met het remmen van gedrag), aandacht- en geheugenproblemen (zie voor een uitgebreid overzicht Rommelse *et al.*, 2011).

Hoewel ASS en ADHD wereldwijd vaak voorkomen, zijn de oorzaken en mechanismen die ten grondslag liggen aan de ontwikkeling van één of beide stoornissen in een individu veelal onbekend. Het onderzoek hiernaar wordt bovendien bemoeilijkt door de grote verscheidenheid (ook wel heterogeniteit genoemd) in uitingsvormen, ontwikkelingsbeloop en onderliggende etiologische factoren, die beide stoornissen kenmerkt. Geen kind met ASS of ADHD is hetzelfde als een ander, en waarschijnlijk zijn er honderden verschillende genetische en niet-genetische risicofactoren, die doorgaans in combinatie met elkaar kunnen leiden tot ASS en/of ADHD. Dit belicht de complexiteit van beide stoornissen en de enorme uitdaging voor het huidige onderzoek, om de oorzaken van ASS, ADHD of beide stoornissen vast te stellen. Door het identificeren van etiologisch meer homogene subgroepen wordt de heterogeniteit ingeperkt. Door vervolgens binnen deze subgroepen te zoeken naar de biologische oorzaken van ASS en ADHD wordt de kans op het vinden van risicofactoren mogelijk vergroot. Daarnaast worden ASS en ADHD doorgaans afzonderlijk van elkaar onderzocht, hoewel ze in de praktijk veelvuldig samen voorkomen. Hierdoor bestaat er nog veel onduidelijkheid over unieke en gedeelde factoren van beide stoornissen. Door ASS en ADHD gezamenlijk te bestuderen, kunnen nieuwe inzichten in de pathofysiologie van beide stoornissen

naar voren komen. Dit kan het onderzoek naar de onderliggende etiologie en effectieve, individuele behandelingen voor ASS en ADHD bespoedigen.

HET VERMINDEREN VAN HETEROGENITEIT

Endofenotypen

Het vinden van (cognitieve) endofenotypen zou kunnen helpen, om meer homogene subgroepen van ASS en ADHD te vinden, met een vergelijkbaar etiologisch profiel (Gottesman and Gould, 2003, Wang *et al.*, 2012). Endofenotypen zijn objectieve, meetbare kenmerken, die erfelijk bepaald zijn en in ieder geval een deel van de genetische lading met de stoornis delen (Bearden and Freimer, 2006, Cannon and Keller, 2006, Gottesman and Gould, 2003). Endofenotypen worden opgespoord door te zoeken naar vergelijkbare (sub)klinische uitingen in niet-aangedane familieleden. Dit type onderzoek wordt veelvuldig toegepast in het ADHD veld en veel publicaties beschrijven een versterkt voorkomen van ADHD trekken, comorbide gedragsproblematiek en ADHD-gerelateerde cognitieve beperkingen in niet-aangedane gezinsleden (Rommelse, 2008, Rommelse *et al.*, 2011). In ASS is onderzoek naar cognitieve endofenotypen nog minder ver ontwikkeld. Gezien de sterke invloed van erfelijke/genetische factoren op het ontstaan van de stoornis, lijkt deze aanpak echter zeer interessant voor ASS onderzoek.

SPX-MPX indeling

Hoewel het endofenotype model veelbelovend is, kleven er aan het model ook enkele nadelen. Een belangrijk nadeel is dat het model geen onderscheid maakt tussen verschillende erfelijke vormen van ASS en ADHD. Grofweg gaat het endofenotype model uit van een multifactorieel ontstaansmodel voor ASS en ADHD. Dat wil zeggen dat ervan uit wordt gegaan dat de stoornis ontstaat door verschillende risicogenen en omgevingsfactoren, die elk met een klein effect het risico op ASS of ADHD vergroten. Omdat eerstegraads gezinsleden van kinderen met ADHD gemiddeld 50% van de erfelijke variatie gemeenschappelijk hebben, is het waarschijnlijk, dat deze gezinsleden in ieder geval een deel van de ASS of ADHD risicovarianten delen. Echter, het is bekend uit voornamelijk ASS onderzoek, dat deze veronderstelling niet altijd recht doet aan de werkelijkheid. In ongeveer 10% van de personen met ASS heeft de stoornis een enkele, aanwijsbare genetische oorzaak (zoals bij het Fragiele X syndroom), en bij nog eens 7-10% blijken zeldzame, niet-erfelijke genetische veranderingen (*de novo* mutaties) een rol te spelen in de ontwikkeling van ASS. In genetisch ASS onderzoek wordt daarom al vaak onderscheid gemaakt tussen simplex ASS (één aangedaan persoon per gezin [SPX]) en multiplex ASS (meerdere aangedane personen per gezin [MPX]). De verwachting is dat in SPX ASS, vaker dan in MPX ASS, de stoornis wordt veroorzaakt door een zeldzame,

niet-erfelijke genetische factor (zoals een *de novo* mutatie), die alleen bij het aangedane kind aanwezig is. In MPX ASS, echter, zou de stoornis veroorzaakt worden door verschillende genetische risicofactoren, die gedeeld worden door ouders en kinderen omdat zij genetisch aan elkaar verwant zijn. Uit onderzoek blijkt inderdaad dat de frequentie van *de novo* mutaties veel hoger ligt in SPX ASS gezinnen (~7-10%) dan in MPX ASS gezinnen (~2-3%) en controle gezinnen (~1%) (Sebat *et al.*, 2007), en dat gezinsleden van MPX ASS gezinnen vaker en meer ASS gedragskenmerken vertonen vergeleken met familieleden uit SPX ASS of controle gezinnen (Gerdts *et al.*, 2013). Hieruit volgt dat het zoeken naar endofenotypen voor ASS in SPX ASS gezinnen waarschijnlijk minder zinvol is; naar verwachting delen de niet-aangedane gezinsleden in deze gezinnen de risicovariant(en) niet. In ADHD onderzoek is er relatief weinig aandacht voor zeldzame genetische mutaties of omgevingsfactoren (zoals medische complicaties of een laag geboortegewicht) in het ontstaan van de stoornis, hoewel er recentelijk enkele studies zijn verschenen die bewijs leveren voor een rol voor deze niet-erfelijke risico varianten in ADHD (D'Onofrio *et al.*, 2014, Lionel *et al.*, 2011, Williams *et al.*, 2012, Williams *et al.*, 2010). Deze bevindingen suggereren dat ook in ADHD, een mogelijk onderscheid bestaat tussen SPX en MPX gezinnen. Eveneens waardevol is het uitzoeken of SPX-MPX indeling behulpzaam is in het zoeken van gedeelde en unieke grondslagen van ASS en ADHD. Deze vernieuwende aanpak, die besproken wordt in hoofdstukken 4 t/m 7, levert ons hopelijk nieuwe inzichten op over de rol van cognitie, pre-/perinatale risicofactoren, en gedrag in verschillende erfelijke vormen van ASS en ADHD.

DOEL VAN DIT PROEFSCHRIFT

Dit proefschrift heeft ten doel, om de gedeelde en unieke mechanismen, die ten grondslag liggen aan ASS en ADHD, te onderzoeken, door pre-/perinatale risicofactoren en cognitieve functies in beide stoornissen met elkaar te vergelijken. Vernieuwend aan dit proefschrift is, (a) dat ASS en ADHD gezamenlijk worden bestudeerd, en (b) dat er een onderscheid is gemaakt tussen verschillende erfelijke varianten van ASS en ADHD (SPX-MPX indeling) in een poging de etiologische heterogeniteit die beide stoornissen kenmerkt in te perken. Drie specifieke onderzoeksdoelen in dit proefschrift zijn:

a) Het identificeren van cognitieve endofenotypen voor ASS (in navolging van uitgebreid endofenotype onderzoek in ADHD) (hoofdstukken 2 en 3).
b) Het onderzoeken van gedeelde en unieke grondslagen voor ASS en ADHD door het vergelijken van gedragskenmerken, cognitieve functies en pre-/perinatale risicofactoren in ASS en ADHD (hoofdstukken 4 – 7).

c) Nagaan of (de mogelijke oorzaken voor) het samen voorkomen van ASS en ADHD afhangt van of er één gezinslid of meerdere gezinsleden per gezin zijn aangedaan. Voor dit doel werden aangedane en niet-aangedane gezinsleden uit SPX en MPX ASS en ADHD gezinnen met elkaar vergeleken op het gebied van gedrag, cognitie en de vroege ontwikkeling (hoofdstukken 4 - 7).

DATA

Om bovenstaande onderzoeksdoelen te toetsen, gebruiken we data van twee grootschalige familiestudies, het Biologische Oorzaken van Autisme (BOA) project en het Internationaal Multicenter ADHD Genetica (IMAGE) project. Aan beide projecten namen kinderen met een klinische diagnose ASS (BOA) of ADHD (IMAGE), hun biologische broers/zussen en hun biologische ouders deel. Alle gezinsleden zijn uitgebreid onderzocht op de aanwezigheid van ASS en ADHD gedragskenmerken en op cognitief functioneren. Onder andere intelligentie, sociale cognitie, executieve- en motorische functies is getoetst. Tot slot is de vroege ontwikkeling (pre- en perinataal) van elk kind met de ouders besproken. Voor de studies beschreven in hoofdstukken 4 t/m 7 zijn de deelnemende gezinnen opgedeeld in gezinnen met één individu met ASS of ADHD (SPX) en gezinnen met meerdere individuen met een stoornis (MPX).

DE BELANGRIJKSTE BEVINDINGEN VAN DIT PROEFSCHRIFT

- Ø Kinderen met ASS en hun klinisch niet-aangedane broers en zussen laten vergelijkbare problemen op het gebied van emotieherkenning zien (hoofdstukken 2, 3 en 6)
- Ø Het comorbide voorkomen van ASS en ADHD heeft een niet te negeren invloed op het cognitieve functioneren van een kind. Kinderen met zowel ASS en ADHD zijn langzamer in het herkennen van emoties, en vooral in het herkennen van emoties in de stem (affectieve prosodie), vergeleken met kinderen met enkel ASS (hoofdstuk 3)
- Ø In ASS gezinnen laten MPX (maar niet SPX) niet-aangedane broers/zussen significant verhoogde ASS en ADHD symptomen zien vergeleken met controle kinderen. In tegenstelling, in ADHD gezinnen laten zowel SPX als MPX niet-aangedane broers/zussen verhoogde niveaus van ASS en ADHD symptomen zien (hoofdstuk 4)
- Ø In ADHD gezinnen laten de niet-aangedane kinderen uit MPX gezinnen vergelijkbare cognitieve beperkingen zien als hun aangedane broer/zus op IQ, inhibitie, werkgeheugen, cognitieve flexibiliteit, variabiliteit in reactie en timing. Echter, de niet-aangedane kinderen uit SPX ADHD gezinnen laten een normale cognitieve prestatie

zien vergeleken met controles, met een uitzondering voor verbaal werkgeheugen (hoofdstuk 5).

- Ø In ASS gezinnen presteren de niet-aangedane kinderen normaal op de meeste cognitieve domeinen (IQ, gezichtsherkenning, herkenning van emotionele gezichtsuitdrukkingen, inhibitie, werkgeheugen en cognitieve flexibiliteit), ongeacht of ze uit een SPX of MPX gezin komen. Een belangrijke uitzondering is affectieve prosodie; zowel SPX als MPX niet-aangedane kinderen laten een slechtere prestatie zien dan controle kinderen op deze taak, vergelijkbaar met hun aangedane broer/zus (hoofdstuk 6).
- Ø Kinderen met een sporadische (SPX) vorm van ASS of ADHD zijn cognitief gezien meer beperkt dan kinderen met een familiale (MPX) vorm van ASS of ADHD (hoofdstukken 5 en 6).
- Ø Infecties tijdens de zwangerschap en een suboptimale conditie tijdens de geboorte zijn geassocieerd met een verhoogd risico op ASS. Jong ouderschap en ziektes, roken en stress tijdens de zwangerschap zijn geassocieerd met een verhoogde kans op ADHD. Behalve een grotere gezinsomvang en meer eerstgeborenen onder aangedane kinderen, zijn er geen pre- of perinatale risicofactoren die significant geassocieerd zijn met beide stoornissen (hoofdstuk 7).
- Ø Er zijn minder SPX ADHD gezinnen dan SPX ASS gezinnen in ons cohort. Dit betekent dat ADHD in de meeste gevallen veroorzaakt zal worden door een combinatie van vele genetische en niet-genetische risicofactoren die elk het risico op ADHD een klein beetje verhogen en die deels aanwezig zijn in niet-aangedane gezinsleden. Daarentegen lijken sporadische risicovarianten een rol te spelen in een aanzienlijk aantal ASS gevallen (hoofdstukken 4 t/m 7).

WELKE COGNITIEVE ENDOPHENOTYPES VOOR ASS HEBBEN WE GEÏDENTIFICEERD?

Uit de resultaten van **hoofdstukken 2, 3 en 6** komt naar voren dat affectieve prosodie de meest veelbelovende endofenotypische kandidaat is voor ASS. Kinderen met ASS evenals hun klinische niet-aangedane broers en zussen zijn significant langzamer in het herkennen van emoties in de stem dan controle kinderen. Bovendien hangen de prestaties van broers/ zussen met en zonder ASS diagnose sterk met elkaar samen. Minder overtuigend zijn de resultaten met betrekking tot het herkennen van emoties in gezichtsuitdrukkingen. Hoewel gevonden is dat (a) kinderen met ASS problemen hebben met het herkennen van emotionele gezichtsuitdrukkingen en (b) herkenning van emotionele gezichtsuitdrukkingen sterk samenhangt met herkenning van emoties in de stem (**hoofdstuk 2**), zijn de resultaten met betrekking tot de aanwezigheid van

vergelijkbare beperkingen in niet-aangedane broers en zussen tegenstrijdig. De studie beschreven in **hoofdstuk 3** laat zien dat niet-aangedane broers/zussen significant langzamer zijn dan controle kinderen, terwijl dit verschil tussen niet-aangedane broers/zussen en controle kinderen niet meer gevonden wordt in **hoofdstuk 6**. Een mogelijke verklaring is het verschil in leeftijdsbereik tussen beide studies (6 – 13 jaar in hoofdstuk 3 en 6 – 20 jaar in hoofdstuk 6). Een alternatieve verklaring is dat indeling in SPX en MPX gezinnen onze power om verschillen te detecteren vermindert.

Opvallend genoeg is er weinig bewijs gevonden voor de rol van executieve functies als endofenotypes voor ASS. Hoewel in **hoofdstuk 2** wordt gevonden dat executieve functies sterk familiaal zijn en sterk samenhangen met sociale cognitie, blijkt uit **hoofdstuk 6** dat niet-aangedane broers en zussen normaal presteren op executieve functiematen. Kinderen met ASS laten bovendien beperkingen zien op sommige, maar lang niet alle aspecten van executief functioneren. Dit sluit aan bij eerdere studies die aantonen dat (familieleden van) personen met ASS niet op alle executieve functies problemen hebben (Losh *et al.*, 2009, Wong *et al.*, 2006).

Concluderend impliceren de bevindingen dat emotieherkenning (vooral affectieve prosodie) een veelbelovend endofenotype is voor ASS. Subgroepering gebaseerd op het al dan niet hebben van emotieherkenningsproblemen zou mogelijk kunnen helpen in het creëren van etiologisch meer homogene groepen en het identificeren van de verschillende etiologische paden naar ASS. Toekomstig onderzoek zou zich kunnen richten op het toetsen van andere cognitieve functies (zoals motorisch en sensorisch functioneren) als mogelijke cognitieve endofenotypes voor ASS en/of voor de combinatie ASS+ADHD, om daarmee meer licht te werpen op de verschillende causale paden die ten grondslag liggen aan de ontwikkeling van de stoornissen.

WAT HEBBEN WE GELEERD OVER GEDEELDE EN UNIEKE GRONDSLAGEN VOOR ASS EN ADHD?

De resultaten van onze zoektocht naar cognitieve endofenotypes voor ASS (overtuigend bewijs voor emotieherkenning, weinig bewijs voor executieve functies), lijken in scherp contrast te staan met studies die juist de geschiktheid van executieve functies als endofenotype voor ADHD onderschrijven (Rommelse *et al.*, 2008a, Rommelse *et al.*, 2011, Rommelse *et al.*, 2008b). Er is relatief weinig onderzoek gedaan naar de rol van sociale cognitie in ADHD en, naar ons weten, zijn er nog geen studies gepubliceerd die beschrijven of emotieherkenning een endofenotype is voor ADHD. Omdat emotieherkenning niet getoetst is in de oorspronkelijke meting van ons ADHD cohort, kunnen wij eveneens geen concrete uitspraken doen over de geschiktheid van emotieherkenning als endofenotype voor ADHD. Echter, uit de eerste voorlopige resultaten van een follow-

up meting van het ADHD cohort komt naar voren dat emotieherkenning waarschijnlijk geen endofenotype voor ADHD is (De Bruijn *et al.*, in voorbereiding). Op basis hiervan, concluderen wij dat verschillende cognitieve functies een centrale rol spelen in de ontwikkeling van ASS en ADHD (dat wil zeggen, sociale cognitie in ASS en executief functioneren in ADHD) en dat de aanwezigheid van deze specifieke cognitieve problemen mogelijk bepalen/voorspellen of een kind ASS ontwikkelt of ADHD.

Verder blijkt dat kinderen met een dubbele diagnose ASS en ADHD langzamer zijn in het herkennen van emoties vergeleken met kinderen met enkel ASS **(hoofdstuk 3)**. De slechtere prestatie kan niet volledig verklaard worden door de mogelijke aanwezigheid van aan ADHD gerelateerde cognitieve problemen, zoals moeite met het vasthouden van de aandacht of een verminderd vermogen om eigen gedrag te remmen. Dit sluit aan bij andere studies die beschrijven dat kinderen met een dubbele diagnose ASS en ADHD meer uitgesproken cognitieve problemen laten zien (Tye *et al.*, 2013a, Tye *et al.*, 2013b), hoewel het niet noodzakelijkerwijs de som betreft van problemen die kinderen met enkel ASS of enkel ADHD laten zien (Nyden *et al.*, 2010). Deze resultaten benadrukken dat de invloed van comorbide gedragskenmerken op cognitie niet genegeerd mag worden in vervolgonderzoek.

Tot slot blijkt dat het niet waarschijnlijk is, dat de overlap tussen ASS en ADHD verklaard kan worden door gedeelde pre-/perinatale risicofactoren. Behalve een grotere gezinsomvang en meer eerstgeborenen onder aangedane kinderen zijn er geen pre- of perinatale risicofactoren, die significant geassocieerd zijn met beide stoornissen **(hoofdstuk 7).** Dit betekent dat pre-/perinatale risicofactoren mogelijk een cruciale rol spelen in de uiteindelijke ontwikkeling van ofwel ASS ofwel ADHD tegen een achtergrond van gedeelde genetische risicofactoren. Ons onderzoeksdesign is echter niet geschikt om causaliteit aan te tonen; vervolgonderzoek is nodig om deze hypothese te toetsen

HELPT DE SPX-MPX INDELING IN DE ZOEKTOCHT NAAR UNIEKE EN GEDEELDE GRONDSLAGEN VOOR ASS EN ADHD?

Concluderend tonen de resultaten besproken in **hoofdstukken 4 t/m 7** overtuigend aan, dat er etiologische verschillen zijn tussen SPX en MPX, ASS en ADHD. Niet-aangedane familieleden uit SPX ASS gezinnen laten nauwelijks ASS en ADHD kenmerken zien, terwijl MPX ASS niet-aangedane familieleden sterk verhoogde ASS en ADHD gedragskenmerken laten zien vergeleken met controles (**hoofdstuk 4**). Op cognitief gebied is er weinig onderscheid tussen SPX en MPX ASS: niet-aangedane kinderen uit MPX, maar niet SPX ASS gezinnen, hadden een verlaagd IQ vergelijkbaar met hun aangedane broer/zus. Echter, kinderen met ASS en hun niet-aangedane broers/zussen –ongeacht SPX of MPX status- laten allemaal problemen zien bij de herkenning van emoties in

de stem. Executieve functies zijn relatief gespaard in alle kinderen (**hoofdstuk 6**). De bevindingen in ADHD gezinnen tonen een omgekeerd patroon. Dat wil zeggen, op gedragsmatig niveau is er geen verschil tussen aangedane en niet-aangedane gezinsleden uit SPX en MPX ADHD gezinnen; niet-aangedane familieleden uit zowel SPX als MPX ADHD gezinnen laten sterk verhoogde ASS en ADHD kenmerken zien (**hoofdstuk 4**). Echter, op cognitief gebied blijken beperkingen in IQ, visueel werk geheugen en timing het meest prominent te zijn bij kinderen met ADHD uit SPX ADHD gezinnen, terwijl inhibitie en motorische controle beperkingen bij kinderen met ADHD uit MPX ADHD gezinnen op de voorgrond staan (**hoofdstuk** 5). Dit wijst op een zekere specificiteit in de cognitieve beperkingen geassocieerd met SPX en MPX ADHD. Bovendien laten niet-aangedane kinderen uit SPX ADHD families normaal cognitief functioneren zien, terwijl niet-aangedane kinderen uit MPX ADHD families vergelijkbare cognitieve problemen hebben als hun broer of zus met ADHD. Tot slot wordt gevonden dat kinderen met ASS uit SPX gezinnen vaker eerstgeboren zijn dan kinderen met ASS uit MPX gezinnen (**hoofdstuk 7**). Dit wijst op een mogelijk verschillende rol van pre-/perinatale risicofactoren in de ontwikkeling van SPX en MPX vormen van ASS.

Op basis van deze bevindingen concluderen wij dat het onderscheiden van gezinnen waar één persoon is aangedaan en gezinnen waar meerdere personen zijn aangedaan een belangrijke stap is in het verminderen van heterogeniteit en het ontrafelen van de verschillende etiologische paden die ten grondslag liggen aan SPX en MPX vormen van ASS en ADHD. De implicaties van deze bevindingen voor de klinische praktijk en toekomstig onderzoek worden verderop besproken. Echter, het is belangrijk om in acht te nemen, dat de SPX-MPX indeling een sterk versimpeld model is om verschillende erfelijke en niet-erfelijke vormen van ASS of ADHD te onderscheiden. Verder genetisch onderzoek is noodzakelijk om uit te wijzen, of in SPX gezinnen inderdaad vaker niet-erfelijke genetische of omgevingsfactoren aan de ontwikkeling van de stoornis ten grondslag liggen dan in MPX gezinnen. Onze bevindingen wijzen vooral op kwantitatieve (en niet kwalitatieve) verschillen tussen SPX en MPX ASS en ADHD. Dat suggereert, dat er nog steeds enige mate van heterogeniteit in de SPX en MPX groepen zal zitten. Ook is de SPX-MPX indeling geen statisch gegeven; een nieuwe zwangerschap zou ertoe kunnen leiden, dat een voorheen SPX gezin later als MPX geclassificeerd wordt. Een laatste complicatie in ons onderzoek is dat een behoorlijk aantal gezinnen niet binnen de classificatie criteria valt, en dat slechts een klein gedeelte van de ADHD gezinnen de SPX status heeft, wat onze statistische power om eventuele (kwalitatieve) groepsverschillen te detecteren mogelijk heeft verminderd. Ondanks deze bezwaren, lijkt het stratificeren in SPX en MPX gezinnen een zinvolle aanpak om meer homogene groepen te creëren binnen de ASS en ADHD patiënten populatie. Daarmee is de indeling een waardevolle toevoeging aan huidig (genetisch) onderzoek.

WELKE RICHTING GEVEN DEZE BEVINDINGEN AAN DE KLINISCHE PRAKTIJK EN TOEKOMSTIG WETENSCHAPPELIJK ONDERZOEK?

Uit dit proefschrift zijn enkele hoofdbevindingen te herleiden, die richting kunnen geven aan de klinische praktijk en toekomstig onderzoek.

- Ø Kinderen met een dubbele diagnose ASS en ADHD hebben de slechtste uitkomsten op cognitief vlak. Mogelijk profiteren deze kinderen het meest van andere behandelstrategieën dan kinderen met een enkele diagnose ASS of ADHD. Het in kaart brengen van comorbide gedragskenmerken zou daarom een belangrijke rol moeten spelen in diagnostiek en counseling en in toekomstig onderzoek naar effectieve behandelmethoden voor ASS en ADHD.
- Ø Uit de resultaten komt duidelijk naar voren dat er verschillen bestaan tussen SPX en MPX ASS en ADHD. Deze resultaten hebben implicaties voor diagnostiek, (genetische) counseling en therapeutische interventies.
  - § Familieleden van MPX ASS en ADHD gezinnen lopen het hoogste risico op gedragsproblematiek en/of cognitieve problemen Dit onderstreept het belang van het afnemen van een familieanamnese bij een kind, waarbij het vermoeden van ASS en/of ADHD bestaat, om de familiare belasting in kaart te brengen.
  - § Het zou raadzaam zijn om de (cognitieve) ontwikkeling van niet-aangedane broers en zussen van (in het bijzonder) kinderen met een MPX vorm van ASS of ADHD in de gaten te houden, om bij eventuele problemen tijdig op te kunnen treden.
- Ø Pre-/perinatale complicaties komen vaker voorkomen bij kinderen met ASS of ADHD dan bij controle kinderen. Counseling van zwangere vrouwen zou gericht kunnen worden op het verminderen van stress tijdens de zwangerschap of het helpen om te stoppen met roken tijdens de zwangerschap, omdat dit naast andere positieve uitkomsten, het risico op ADHD kan verkleinen.
- Ø Toekomstig onderzoek naar de genetische en cognitieve grondslagen, pre-/perinatale risicofactoren en gedragskenmerken van ASS, ADHD of de combinatie van beide stoornissen, zal meer rekening moeten houden met verschillende etiologische vormen van ASS en ADHD. Het bestuderen van subgroepen met een verschillende etiologie als één totale groep leidt mogelijk tot het niet detecteren van belangrijke associaties tussen genen, cognitie en gedrag. Hieruit volgen enkele vervolgstappen, die gezet kunnen worden om tot een beter begrip te komen van de verschillen tussen SPX en MPX ASS en ADHD.
  - § Door middel van genetisch onderzoek zal getoetst moeten worden of sporadische risicovarianten inderdaad vaker voorkomen bij SPX ASS en ADHD, terwijl genetische varianten die algemeen voorkomen in de populatie, vaker aan de stoornis ten grondslag liggen in MPX gezinnen.

§ Longitudinale follow-up studies, die de gedragsmatige en cognitieve ontwikkeling van aangedane en niet-aangedane kinderen uit SPX en MPX gezinnen in kaart brengen, kunnen leiden tot nieuwe inzichten over het nut van de SPX-MPX indeling in het voorspellen van de (klinische) uitkomsten van deze kinderen over tijd.
§ Om de betrouwbaarheid van de SPX-MPX indeling te vergroten, kan men overwegen het huidige model aan te scherpen, bijvoorbeeld door data van opa's en oma's, ooms en tantes, neefjes en nichtjes toe te voegen aan de classificatie.

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Het zit erop. Na jaren van data verzamelen, analyses runnen, schrijven, nog meer analyses runnen, nog meer schrijven en tot slot alle artikelen proberen te publiceren, is het proefschrift dan toch echt af. Er wordt vaak gezegd dat een proefschrift geen levenswerk is. Hoewel dat inhoudelijk wel zal kloppen (een promovendus komt doorgaans immers net kijken in de wondere wereld van de wetenschap), is het figuurlijk gezien een treffende omschrijving van hoe ik het promotietraject heb beleefd. Het was een langdurig proces, met voor- en tegenslagen, successen en frustraties. Ik heb er veel van geleerd, over mezelf, over wetenschap beoefenen, over ASS en ADHD. Ik heb geleerd dat ik best aardige onderzoeksvragen kan bedenken, dat het begeleiden van stagiaires mij veel voldoening geeft, dat ik het werken met enorme datasets en ingewikkelde analysemethodes verrassend leuk vind, dat een voorliefde voor het lezen van literaire romans niet noodzakelijkerwijs een voorliefde voor het lezen van wetenschappelijke artikelen inhoudt, en dat het schrijven van artikelen – ondanks het enorme aantal computeruren-me toch wel ligt. Ik heb geleerd beter om te gaan met de niet aflatende stroom feedback, dat alles altijd langer duurt dan je denkt, dat aan voortschrijdend inzicht niet te tornen valt en dat pragmatisch denken noodzakelijk is (doch lastig voor iemand met een ietwat neurotische, perfectionistische inslag). Hoewel de werkdruk me bij tijd en wijlen overweldigde en ik blij ben dat het werk er nu op zit, had ik dit nooit willen missen.

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PUBLICATIONS AS PART OF THIS THESIS

Oerlemans, A.M. Droste, K., Van Steijn, D.J., De Sonneville, L.M.J., Buitelaar, J.K. & Rommelse, N.N.J. (2013). Co-segregation of social cognition, executive function and local processing style in children with ASD, their siblings and normal controls. *Journal of Autism and Developmental Disorders* **43**, 2764-2778.

Oerlemans, A.M., Van der Meer, J.M.J., Van Steijn, D.J., De Ruiter, S.W., De Bruijn, Y.G.E., De Sonneville, L.M.J., Buitelaar, J.K. & Rommelse, N.N.J. (2014) Recognition of facial emotionand affective prosody in children with ASD (+ADHD) and their unaffected siblings. *European Child & Adolescent Psychiatry* **23,** 257-271.

Oerlemans, A.M., Hartman, C.A., de Bruijn, Y.G.E., Van Steijn, D.J., Franke, B., Buitelaar, J.K. & Rommelse, N.N.J. (2014) Simplex and multiplex stratification in ASD and ADHD families: a promising approach for identifying overlapping and unique underpinnings of ASD and ADHD? *Journal of Autism and Developmental Disorders*. (epub ahead of print)

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Oerlemans, A.M., Hartman, C.A., Franke, B., Buitelaar, J.K. & Rommelse, N.N.J. Does the cognitive architecture of simplex and multiplex ASD families differ ? *(submitted for publication)*

Oerlemans, A.M., Burmanje, M.J., Franke, B., Buitelaar, J.K., Hartman, C.A. & Rommelse, N.N.J. Identifying unique versus shared pre-, and perinatal risk factors for ASD and ADHD using asimplex-multiplex stratification. *(submitted for publication)*

PUBLICATIONS OUTSIDE THIS THESIS

De Bildt, A., Oosterling, I.J., Van Lang, N.D.J., Kuijper, S., Dekker, V., Sytema, S., Oerlemans, A.M., Van Steijn, D.J., Visser, J.C., Rommelse, N.N.J., Minderaa, R.B., Van Engeland, H., Van der Gaag, R.J., Buitelaar, J.K. & De Jonge, M.V. (2013). How to use the ADI-R for classifying autism spectrum disorders? Psychometric properties of criteria from the literature in 1204 Dutch children. *Journal of Autism and Developmental Disorders* **43**, 2280-2294

Langerak, I.P.C., Oerlemans, A.M., Van der Meer, J.M.J., De Bruijn, Y.G.E., Staal, W.G., Buitelaar, J.K. & Rommelse, N.N.J. Who is most impaired? Comparing cognitive deficits in ASD patients with low, normal and high IQ. *(submitted for publication)*

Van der Meer, J.M.J., Oerlemans, A.M., Van Steijn, D.J., Lappenschaar, M.G.A., De Sonneville, L.M.J., Buitelaar, J.K. & Rommelse, N.N.J. (2012). Are ASD and ADHD different manifestations of one overarching disorder? Cognitive and symptom evidence from a clinical and population based sample. *Journal of the American Academy of Child and Adolescent Psychiatry* **51**, 1160-1172.

Van der Meer, J.M.J., Hartman, C.A., Thissen, A.J.A.M., Oerlemans, A.M., Luman, M., Buitelaar, J.K. & Rommelse, N.N.J. How 'core' are motor timing difficulties in ADHD? A latent class comparison of pure and comorbid ADHD classes. *(submitted for publication)*

Van Steijn, D.J., Oerlemans, A.M., Van Aken, M.A., Buitelaar, J.K. & Rommelse, N.N.J. (2012). Match or mismatch? Influence of parental and offspring ASD and ADHD symptoms on the parent child relationship. *Journal of Autism and Developmental Disorders* **43**, 1935-1945.

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der symptoms related to parenting styles in families with ASD (+ADHD) affected children? *European Journal of Child and Adolescent Psychiatry* **22**, 671-681.

Van Steijn, D.J., Richards, J.S., Oerlemans, A.M., De Ruiter, S.W., Van Aken, M.A.G., Franke, B., Buitelaar, J.K. & Rommelse, N.N.J. (2012). The co-occurrence of Attention Deficit/Hyperactivity Disorder and Autism Spectrum Disorder symptoms in parents of children with ASD or ASD with ADHD. *Journal of Child Psychology and Psychiatry* **53**, 954-963.

Van Steijn, D.J. Oerlemans, A.M., van Aken, M.A.G., Buitelaar, J.K. & Rommelse, N.N.J. (2013). The reciprocal relationship of ASD, ADHD, Depressive Symptoms and stress in parents of children with ASD and/or ADHD. *Journal of Autism and Developmental Disorders* **44**,1064-1076.

Van Steijn, D.J., Oerlemans, A.M., Van Aken, M.A.G., Buitelaar, J.K. & Rommelse, N.N.J. (2014) The influence of parental and offspring Autism Spectrum Disorder (ASD) and Attention Deficit/Hyperactivity Disorder (ADHD) symptoms on family climate. *Journal of Child and Family Studies*. (epub ahead of print)

ORAL AND POSTER PRESENTATIONS

Poster presentation Congres Even-waardig, 2011, Lunteren, The Netherlands

Oral and poster presentation, Spring conference Dutch Association for Psychiatry (NVvP), April 2013, Maastricht, The Netherlands. Facial emotion recognition and affective prosody in children with ASD (+ADHD) and their unaffected siblings.

Oral educational symposium presentation (organizer), International Meeting for Autism Research (IMFAR), May 2013, San Sebastian, Spain. Quantitative ASD and ADHD traits in unaffected relatives of simplex versus multiplex ASD and ADHD families: evidence for overlapping and unique underpinnings for ASD and ADHD

Poster presentation, European Network on Hyperkinetic Disorders (Eunethydis), October 2013, Prague, Czech Republic

Poster presentation, Donders Discussions, October 2013, Nijmegen, the Netherlands

Poster presentation, International Meeting for Autism Research (IMFAR), May 2014, Atlanta, Georgia, USA.

Oral and poster presentation, European Network on Hyperkinetic Disorders (Eunethydis), Istanbul, Turkey, May 2014. Cognitive impairments are different in single-incidence and multi-incidence ADHD families.

AWARDS

J-Eunethydis price – travel award + oral presentation at J-Eunethydis Conference, May 2014, Istanbul, Turkey

Poster prize for 'Cognitive impairments are different in single-incidence and multi-incidence ADHD families', Eunethydis, May 2014, Istanbul, Turkey

Poster prize for 'Identifying unique versus shared pre-, and perinatal risk factors for ASD and ADHD using a simplex-multiplex stratification', Eunethydis, May 2014, Istanbul, Turkey

CURRICULUM VITAE

Anoek Oerlemans was born in Tilburg on April 16th, 1986. She finished secondary school (Cobbenhagen College Tilburg) in 2004. Subsequently, she began studying Psychology at the Radboud University Nijmegen. In 2009, she obtained her Master's degree (cum laude) in Psychology, with a specialization in clinical neuropsychology. Furthermore, she successfully completed the Radboud Honors Program, an interdisciplinary extra curriculum for highly motivated students (2005-2007), studied Literature Sciences (2006-2010) and completed a minor in Journalism (2008).

In October 2009, she commenced with her PhD at the department of Psychiatry at the Radboud University Medical Center, Nijmegen, The Netherlands, under the supervision of Prof. J.K. Buitelaar, Prof. B. Franke and Dr. N.N.J. Lambregts-Rommelse, and later also Dr. C.A. Hartman. During her PhD she followed several courses in academic writing, presentation skills, behavioral genetics and statistics and presented her work at national and international conferences on ASD and ADHD. In addition, she supervised a handful of bachelor and master theses, several internships, and assisted in tutoring courses in developmental psychopathology and neuropsychology at the Radboud University Nijmegen. She was also the secretary for the PhD Organisation Nijmegen (PON) from March 2010 until September 2011.

Currently, Anoek is a postdoctoral researcher at the Donders Institute for Brain, Cognition and Behavior, Department of Cognitive Neuroscience, were she studies clinical outcomes of ASD traits. She is married and lives with her husband in Nijmegen, the Netherlands.