ON THE SPECTRUM

the neurobiology of childhood psychiatric symptoms in the general population

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On the Spectrum:

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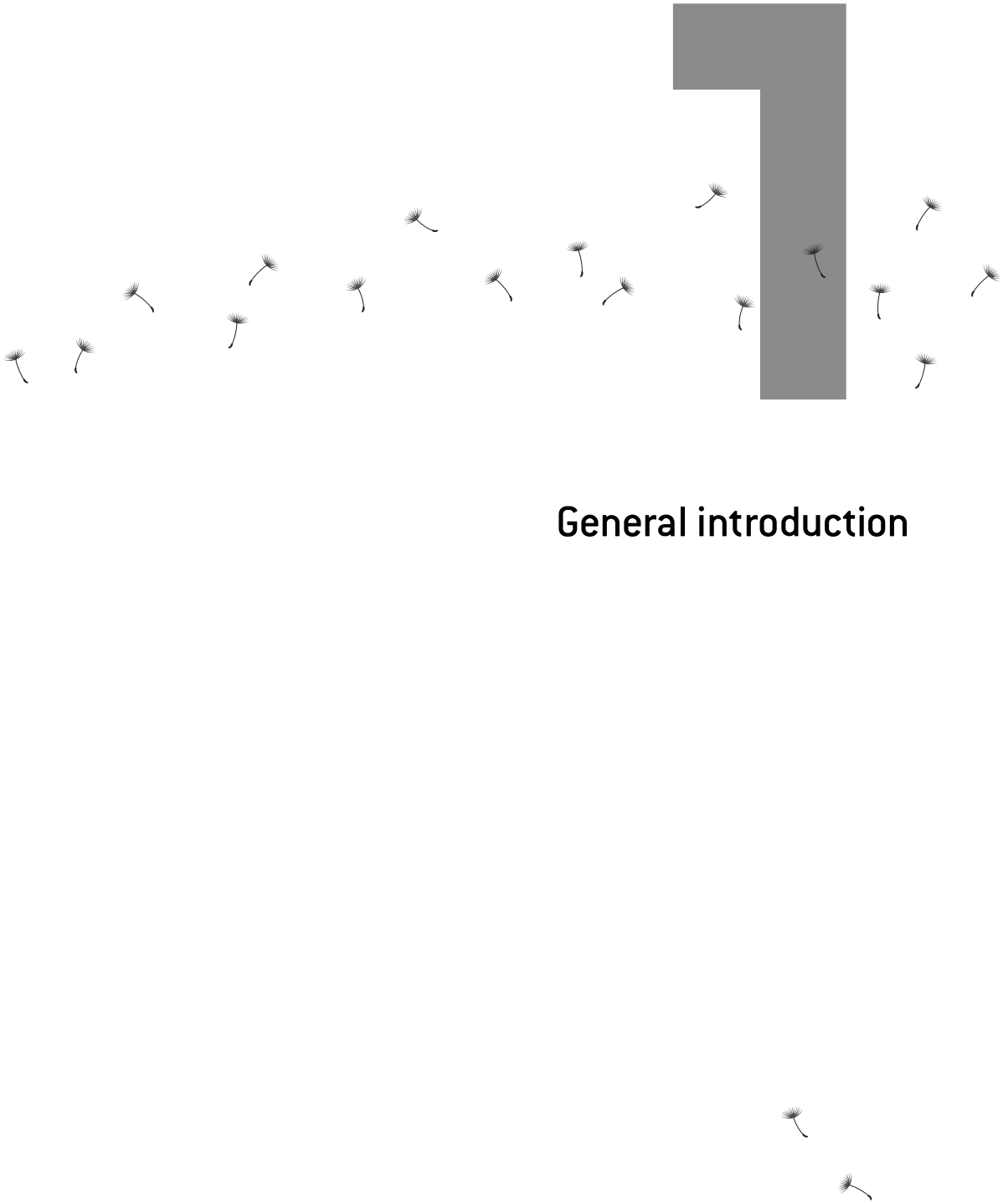

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General introduction

GENERAL RATIONALE

One of the main challenges in child and adolescent psychiatry is to accurately and meaningfully classify psychiatric symptoms. The most widely used classification instrument in clinical practice is the manual from the American Psychiatric Association, the *Diagnostic and Statistical Manual of Mental Disorders*, in its most recent version DSM-V. Within the DSM framework, psychiatric symptoms are categorized in one or more categorical disorders or subtypes. As the psychiatric symptomology and clinical presentation of children is very heterogeneous in nature, a DSM-diagnosis typically involves a list of symptoms, only a few of which are required to meet criteria for the dichotomous disorder. As a result, two people with the same diagnosis may not have overlapping symptoms (Sonuga-Barke; Cantwell 1996). Further, child psychiatry is characterized by a high level of comorbidity between different symptom types (Caron and Rutter 1991). As a result, multiple categorical, but non-exclusive diagnoses are often needed to capture all psychiatric symptoms of a child. Moreover, the level of severity of symptoms is a dimensional characteristic, with great variation between individuals, and the choice of a cut-off to determine whose problems are clinically relevant, is inherently arbitrary. In the DSM framework, subclinical scores may not always be categorized. The fact that current classifications of psychiatric symptoms are not etiologically informed is thought to be one of the factors underlying the limited success of the search for the underlying neurobiology of psychiatric disorders. While a dichotomous diagnostic construct provides a guideline for treatment, there is increasing doubt as to whether it is an appropriate framework to study the neurobiology of these disorders, as the neurobiological features may not map onto these clinical categories (Sonuga-Barke; Kendler 2013).

The search for alternative phenotypes for etiological research raises questions about the ideal context for such studies. The general population is increasingly recognized as a useful setting to study the etiology of psychiatric disorders. In the general population, there is a clear continuum in psychiatric symptoms. Most people have very few symptoms, some have a few symptoms, and some people are more affected, so that these latter individuals may meet criteria for a psychiatric diagnosis. There is potential benefit in studying those that have subclinical symptoms, but not a full-blown disorder. The inherent selection bias of clinically recruited samples can be avoided, and results obtained in this way may be generalizable to a broader population.

Despite these insights, neurobiological research still tends to focus on the most severely affected children, comparing them with children that are considered “typically developing”. Recent funding guidelines have stressed the importance of looking beyond the DSM-framework when trying to elucidate the neurobiology of these disorders (RDoC) (Insel,

Cuthbert, Garvey, Heinssen, Pine et al. 2010). One way to circumvent the problems of dichotomous classifications is to assess symptoms in a continuous manner, without using a cut-off. In this thesis, we apply this continuous approach to neurobiological studies of autism and internalizing symptoms. Interestingly, various continuously measured psychiatric symptoms are often interrelated and specific combinations of symptoms can form patterns. In this thesis, we use this phenomenon to study the etiology of internalizing and externalizing problems in the general population.

PART 1: AUTISTIC TRAITS IN THE GENERAL POPULATION

A continuum in the neurobiology of ASD

Autism spectrum disorder (ASD) is a heterogeneous condition, with central features of impairment in reciprocal social interactions, as well as restricted, stereotypical behaviors. ASD is generally recognized in early childhood and the problems are rather stable. Children that are given a diagnosis generally meet criteria for the disorder throughout their lives, although improvement of symptoms occurs in some (McGovern and Sigman 2005). ASD comes with a severe burden, both for the affected individual in terms of health loss during the lifespan, as well as for caregivers. According to the most recent estimates, ASD was the leading cause of disability in children under 5 years of age among all mental disorders, and the fourth cause between ages 5 and 14 (Baxter, Brugha, Erskine, Scheurer, Vos et al. 2015). Unsurprisingly, considerable costs are associated with ASD, affecting individuals with the disorder, their families and society as a whole (Buescher, Cidav, Knapp and Mandell 2014).

The definition of ASD has been a topic of debate during the past years, and considerable adjustments have been made over time. In the previous edition of the DSM (DSM-IV-TR, American Psychiatric Association 1994) Asperger's syndrome and Autistic Disorder were separate subcategories within the diagnosis of "Pervasive Developmental Disorders." However, the DSM-V does not highlight subcategories of a larger disorder, but rather, acknowledges an overarching concept of ASD, comprising two subscales: social communication and restricted behavior. Impairment on these subscales can differ in severity. This is in line with the important observation that symptoms of ASD, especially dimensions of social impairment, form a continuum. These traits do not occur solely in patients with the disorder. Rather, this spectrum of traits extends into the general population (Constantino 2011). ASD is conceptualized as the severe end of this spectrum.

Despite large numbers of affected individuals and great efforts of researchers, the neurobiology of ASD remains elusive. Both genetic and environmental factors are thought to contribute to the etiology (London 2000; Geschwind 2011). Generally, it is assumed that symptoms of ASD might arise from altered trajectories of neurodevelopment. In recent years,

neuroimaging techniques have greatly expanded the possibilities to study brain development *in vivo*, providing a promising route to unravelling the neurobiology of ASD. A better understanding of the neurobiology of ASD could identify novel targets for early identification and treatment of the disorder. Reconciling the phenotypic insights of a continuum in ASD with modern neuroimaging techniques could help in the quest for such targets.

ASD is thought to be associated with differences in the brain, that occur at many different levels (Parellada, Penzol, Pina, Moreno, Gonzalez-Vioque et al. 2014). In this thesis, we study various aspects of the neurobiology of ASD from a quantitative trait perspective, focusing on social impairment.

Prenatal and postnatal structural brain development

One of the most prominent theories of the underlying neurobiology of ASD involves "early brain overgrowth". Traditionally, retrospective studies of brain growth in young children with ASD have reported early brain overgrowth in the first year of life (Hazlett, Poe, Gerig, Smith, Provenzale et al. 2005). More recently, the existence and exact timing of early brain growth abnormalities have been disputed, as many studies used historic references and study samples tend to suffer from ascertainment bias (Raznahan, Wallace, Antezana, Greenstein, Lenroot et al. 2013). However, there seems to be at least some consensus that children with ASD show subtle accelerated head growth in early life, which results in a greater head circumference and brain volume by the age of two (Piven, Arndt, Bailey and Andreasen 1996; Courchesne, Karns, Davis, Ziccardi, Carper et al. 2001; Constantino, Majmudar, Bottini, Arvin, Virkud et al. 2010). While it is thought that brain abnormalities in ASD may be present before birth, less is known about prenatal head growth trajectories. The few studies available have small sample sizes, typically depend on retrospectively collected ultrasound data, after a diagnosis of ASD has been established and do not all include repeated measurements of growth.

In addition, it is unclear whether this potential 'early overgrowth' persists into later development. Postnatal brain development has been the topic of numerous studies in ASD since modern neuroimaging techniques became available in children (Amaral, Schumann and Nordahl 2008). One of the proposed underlying mechanisms of ASD involves altered growth and refinement of the cortex, as reflected by global and regional differences in the thickness of the cortex. However, studies of cortical morphology in ASD have reported mixed results. This may partly be explained by a developmental effect. Studies in older children (12 years and up) point to a thinner cortex in subjects with ASD compared to controls (Hadjikhani, Joseph, Snyder and Tager-Flusberg 2006; Wallace, Dankner, Kenworthy, Giedd and Martin 2010; Scheel, Rotarska-Jagiela, Schilbach, Lehnhardt, Krug et al. 2011), whereas studies in younger children primarily show a thicker cortex (Hardan, Muddasani, Vemulapalli, Keshavan and Minshew 2006; Schumann, Bloss, Barnes, Wideman, Carper et al. 2010; Raznahan, Lenroot, Thurm, Gozzi, Hanley et al. 2012). In addition, measures of gyrification, or the

folding of the brain into a complex pattern of gyri and sulci, provide a window into important developmental processes (White, Su, Schmidt, Kao and Sapiro 2010). The few studies examining gyrification in children and adolescents with ASD have yielded contradictory results, reporting both increased gyrification (Kates, Ikuta and Burnette 2009; Jou, Minshew, Keshavan and Hardan 2010; Wallace, Robustelli, Dankner, Kenworthy, Giedd et al. 2013), or lower gyrification (Schaer, Ottet, Scariati, Dukes, Franchini et al. 2013), or no difference in gyrification (Casanova, El-Baz, Mott, Mannheim, Hassan et al. 2009) in subjects with ASD compared to typically developing controls.

To date, both early brain growth and later characteristics of the cortex have almost exclusively been studied in relatively small samples of children with ASD, compared to matched control groups. Despite increasing support for the notion that ASD lies at the extreme end of a continuum, and that characteristics of the underlying neurobiology may form a continuum as well, there are no studies that investigate fetal or postnatal head growth across the spectrum of autistic symptom severity. In chapter 2 and 3, we related pre- and postnatal characteristics of the brain to continuously measured autistic traits.

Structural and functional connectivity

Another prominent model conceptualizes the symptoms of ASD as arising from disruptions in development of connections between different regions of the brain (Geschwind and Levitt 2007; Uddin, Supekar and Menon 2013). This model has been supported by postmortem data (Casanova, van Kooten, Switala, van Engeland, Heinsen et al. 2006), as well as by *in vivo* neuroimaging studies (Anderson 2014). Notably, long-distance connectivity in the brain appears to be decreased, alongside greater localized connectivity (Belmonte, Allen, Beckel-Mitchener, Boulanger, Carper et al. 2004). While efficient communication between spatially separated regions of the brain is in a large part facilitated by myelinated neuronal fibers, the role of white matter microstructure, or structural connectivity in ASD is not yet clear. The microstructural architecture of white matter tracts in the brain can be examined *in vivo* using Diffusion Tensor Imaging (DTI), a non-invasive imaging technique that provides a measure of water diffusion. This can be used to evaluate the integrity of white matter tracts. While there is great heterogeneity in DTI studies of children with ASD, the most replicated findings show lower integrity of tracts that facilitate long-range connections: the corpus callosum, the left uncinate fasciculus, and the left superior longitudinal fasciculus (Aoki, Abe, Nippashi and Yamasue 2013). However, it is unclear whether these findings extend to children who are less severely affected, both in terms of social impairment and intelligence. This question is addressed in chapter 4.

Another opportunity to tap into the connectivity of the brain is presented by functional MRI (FMRI). By studying regional fluctuations in blood oxygen level dependent (BOLD) signal, patterns of activity of different areas of the brain can be approximated. A specific subtype of

functional neuroimaging is resting-state functional magnetic resonance imaging (RS-FMRI), which relies on a phenomenon called intrinsic brain activity, or brain activity that is not induced by an external stimulus. In the context of RS-FMRI, connectivity between different brain regions is assessed through temporal fluctuations in the BOLD signal, where brain regions with high temporal correlation are more connected. Studies of children with ASD have repeatedly demonstrated abnormal patterns of functional connectivity, including both lower connectivity than controls, (hypoconnectivity) and increased connectivity (hyperconnectivity) (Uddin et al. 2013). However, the majority of existing resting-state functional MRI studies have made the assumption that the brain's functional connectivity is static over a period of multiple minutes. This has been shown to be a major limitation as important dynamic patterns of connectivity could be missed (Calhoun, Miller, Pearlson and Adali 2014). Recent novel analysis methods provide the opportunity to study dynamic patterns of connectivity in the context of ASD, as well as in typical development. These methods are used in chapter 5 to study whether children with ASD or traits of the disorder show different temporal patterns of connectivity, in addition to differences in static connectivity.

PART II: EMOTIONAL AND BEHAVIORAL PROBLEMS IN THE GENERAL POPULATION

Classification of internalizing and externalizing problems

Two broad categories of child behavior are distinguished in developmental research: internalizing and externalizing problems. Internalizing problems represent problems that manifest mainly within the self, such as depressed mood or anxiety, while externalizing problems are characterized by disruptive behaviors that are more likely to involve conflicts with others (Achenbach and Rescorla 2000). Internalizing problems in childhood are very predictive of later mood and anxiety disorders (Roza, Hofstra, van der Ende and Verhulst 2003), while externalizing problems often precede later diagnoses of ADHD or conduct disorders (Moffitt 1993). Internalizing and externalizing problems can be quantified in continuous dimensions using well-known questionnaires such as the Child Behavior Checklist (CBCL) (Achenbach et al. 2000). Although internalizing and externalizing symptoms are quite different in nature, they often co-occur: children that have a high level of externalizing symptoms tend to also have internalizing symptoms (Achenbach et al. 2000). This phenotypic overlap poses a challenge to the study of specific neurobiological or cognitive correlates. Such specific correlates potentially contain crucial information. Firstly, it can help lead to a better understanding of underlying mechanisms. Second, specific correlates can be of importance in predicting the time course of psychopathology. Third, knowing the specific relation of cognitive symptoms or biomarkers and specific psychiatric symptoms may be

beneficial for treatment decisions. One strategy to distinguish specific correlates of one type of pathology is to adjust for other types of pathology. However, this potentially removes variation of interest. Another strategy involves trying to parse out a group of children who mainly have problems in a single domain. This can be done using cut-points on internalizing and externalizing dimensions. However, the use of cut-points on continuous variables results in loss of information, and the choice of a specific cut-off is rather arbitrary. Person-centered methods, such as latent class analysis (LCA) can be used to identify more homogeneous groups of individuals with similar patterns of psychopathology.

GENERAL

Aims

The first aim of this thesis was to study various characteristics of the underlying neurobiology of ASD, using the continuum of traits in the general population. The second aim of this thesis was to study separate cognitive deficits of internalizing and externalizing problems and specific neurobiological correlates of internalizing problems.

Setting

The studies described in this thesis are all embedded in Generation R, a population-based prospective cohort study from fetal live onwards, in Rotterdam, the Netherlands (Jaddoe, van Duijn, Franco, van der Heijden, van Iizendoorn et al. 2012; Tiemeier, Velders, Szekely, Roza, Dieleman et al. 2012). The Generation R Study was designed to study early determinants of children's growth, development, and health. All pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. In total, 9,778 pregnant women participated in the study. At enrolment, various maternal, paternal and familial characteristics were collected. Fetal ultrasound measurements were systematically performed at prenatal visits during the three trimesters of pregnancy (Verburg, Steegers, De Ridder, Snijders, Smith et al. 2008). At several time points during the preschool period, parents reported on the development of their child via questionnaires. When children reached the age of 5 to 7 years, more detailed assessments were performed, during a visit to the research center. At this stage 8,305 children actively participated in the study. Measurements at the research center included cognitive and behavioral assessments. Parents were asked to report on children's emotional and behavioral problems and autistic traits via questionnaires. In addition, between September 2009 and July 2013, a neuroimaging substudy was conducted, in which 1,070 children, ages 6-to-10 years were scanned (White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013). Finally, between March and October 2015 medical records were obtained from the general practitioners of children who scored screen-

positive on one or more aspects of a multifaceted screening procedure for ASD in order to confirm this diagnosis.

Chapter 3 of this thesis additionally involves data collected within the Raine Study, an Australian cohort study of women recruited prior to 18 weeks gestation from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics, between May 1989 and November 1991 (Newnham, Evans, Michael, Stanley and Landau 1993). As part of a trial investigating effects of prenatal ultrasound, women underwent one or more ultrasound measurements during pregnancy. To these women, 2,868 children were born and available for postnatal follow-up, including behavioral assessments.

Outline

This thesis is divided into two parts. Part I focuses on various aspects of the neurobiology of autism, utilizing continuous trait measures. In chapter 2, we describe several characteristics of cortical morphology in children with autistic traits and in chapter 3, we focused on prenatal brain growth using ultrasound measurements. In chapter 4, we studied white matter integrity and chapter 5, we investigated dynamic characteristics of resting state connectivity in children with autistic traits.

The second part of this thesis focuses on specific cognitive and neurobiological characteristics of children with internalizing and externalizing problems. In chapter 6, we studied specific cognitive problems in children with internalizing, externalizing and dysregulation problems. In chapter 7, we studied brain morphology related to internalizing behavior in young children. Finally, in chapter 8, we discuss the main findings of these studies in the context of recent literature and we discuss methodological considerations, as well as implications of these studies for further research and clinical practice.

REFERENCES

Achenbach, T. M. and L. A. Rescorla (2000). Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT., University of Vermont, Reseach Center for Children, Youth and Families.

Amaral, D. G., C. M. Schumann and C. W. Nordahl (2008). "Neuroanatomy of autism." Trends in Neurosciences **31**(3): 137-145.

Anderson, J. S. (2014). Cortical Underconnectivity Hypothesis in Autism: Evidence from Functional Connectivity MRI. Comprehensive Guide to Autism. B. V. Patel, R. V. Preedy and R. C. Martin. New York, NY, Springer New York**:** 1457-1471.

Aoki, Y., O. Abe, Y. Nippashi and H. Yamasue (2013). "Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies." Molecular Autism **4**.

Baxter, A. J., T. S. Brugha, H. E. Erskine, R. W. Scheurer, T. Vos and J. G. Scott (2015). "The epidemiology and global burden of autism spectrum disorders." Psychol Med **45**(3): 601-613.

Belmonte, M. K., G. Allen, A. Beckel-Mitchener, L. M. Boulanger, R. A. Carper and S. J. Webb (2004). "Autism and abnormal development of brain connectivity." J Neurosci **24**(42): 9228-9231.

Buescher, A. V., Z. Cidav, M. Knapp and D. S. Mandell (2014). "Costs of autism spectrum disorders in the United Kingdom and the United States." JAMA Pediatr **168**(8): 721-728.

Calhoun, V. D., R. Miller, G. Pearlson and T. Adali (2014). "The chronnectome: time-varying connectivity networks as the next frontier in fMRI data discovery." Neuron **84**(2): 262-274.

Cantwell, D. P. (1996). "Classification of child and adolescent psychopathology." J Child Psychol Psychiatry **37**(1): 3-12.

Caron, C. and M. Rutter (1991). "Comorbidity in Child Psychopathology - Concepts, Issues and Research Strategies." Journal of Child Psychology and Psychiatry and Allied Disciplines **32**(7): 1063-1080.

Casanova, M. F., A. El-Baz, M. Mott, G. Mannheim, H. Hassan, R. Fahmi, J. Giedd, J. M. Rumsey, A. E. Switala and A. Farag (2009). "Reduced Gyral Window and Corpus Callosum Size in Autism: Possible Macroscopic Correlates of a Minicolumnopathy." Journal of Autism and Developmental Disorders **39**(5): 751-764.

Casanova, M. F., I. A. van Kooten, A. E. Switala, H. van Engeland, H. Heinsen, H. W. Steinbusch, P. R. Hof, J. Trippe, J. Stone and C. Schmitz (2006). "Minicolumnar abnormalities in autism." Acta Neuropathol **112**(3): 287-303.

Constantino, J. N. (2011). "The quantitative nature of autistic social impairment." Pediatr Res **69**(5 Pt 2): 55R-62R.

Constantino, J. N., P. Majmudar, A. Bottini, M. Arvin, Y. Virkud, P. Simons and E. Spitznagel (2010). "Infant head growth in male siblings of children with and without autism spectrum disorders." Journal of neurodevelopmental disorders **2**(1): 39-46.

Courchesne, E., C. M. Karns, H. R. Davis, R. Ziccardi, R. A. Carper, Z. D. Tigue, H. J. Chisum, P. Moses, K. Pierce, C. Lord, A. J. Lincoln, S. Pizzo, L. Schreibman, R. H. Haas, N. A. Akshoomoff and R. Y. Courchesne (2001). "Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study." Neurology **57**(2): 245-254.

Geschwind, D. H. (2011). "Genetics of autism spectrum disorders." Trends Cogn Sci **15**(9): 409-416.

Geschwind, D. H. and P. Levitt (2007). "Autism spectrum disorders: developmental disconnection syndromes." Curr Opin Neurobiol **17**(1): 103-111.

Hadjikhani, N., R. M. Joseph, J. Snyder and H. Tager-Flusberg (2006). "Anatomical differences in the mirror neuron system and social cognition network in autism." Cerebral Cortex **16**(9): 1276-1282.

Hardan, A. Y., S. Muddasani, M. Vemulapalli, M. S. Keshavan and N. J. Minshew (2006). "An MRI study of increased cortical thickness in autism." American Journal of Psychiatry **163**(7): 1290-1292.

Hazlett, H. C., M. Poe, G. Gerig, R. G. Smith, J. Provenzale, A. Ross, J. Gilmore and J. Piven (2005). "Magnetic resonance Imaging and head circumference study of brain size in autism - Birth through age 2 years." Archives of General Psychiatry **62**(12): 1366-1376.

Insel, T., B. Cuthbert, M. Garvey, R. Heinssen, D. S. Pine, K. Quinn, C. Sanislow and P. Wang (2010). "Research Domain Criteria (RDoC): Toward a New Classification Framework for Research on Mental Disorders." American Journal of Psychiatry **167**(7): 748-751.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." Eur J Epidemiol **27**(9): 739-756.

Jou, R. J., N. J. Minshew, M. S. Keshavan and A. Y. Hardan (2010). "Cortical Gyrification in Autistic and Asperger Disorders: A Preliminary Magnetic Resonance Imaging Study." Journal of Child Neurology **25**(12): 1462-1467.

Kates, W. R., I. Ikuta and C. P. Burnette (2009). "Gyrification Patterns in Monozygotic Twin Pairs Varying in Discordance for Autism." Autism Research **2**(5): 267-278.

Kendler, K. S. (2013). "What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn." Mol Psychiatry **18**(10): 1058-1066.

London, E. A. (2000). "The environment as an etiologic factor in autism: a new direction for research." Environ Health Perspect **108 Suppl 3**: 401-404.

McGovern, C. W. and M. Sigman (2005). "Continuity and change from early childhood to adolescence in autism." J Child Psychol Psychiatry **46**(4): 401-408.

Moffitt, T. E. (1993). "Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy." Psychol Rev **100**(4): 674-701.

Newnham, J. P., S. F. Evans, C. A. Michael, F. J. Stanley and L. I. Landau (1993). "Effects of frequent ultrasound during pregnancy: a randomised controlled trial." Lancet **342**(8876): 887-891.

Parellada, M., M. J. Penzol, L. Pina, C. Moreno, E. Gonzalez-Vioque, G. Zalsman and C. Arango (2014). "The neurobiology of autism spectrum disorders." Eur Psychiatry **29**(1): 11-19.

Piven, J., S. Arndt, J. Bailey and N. Andreasen (1996). "Regional brain enlargement in autism: a magnetic resonance imaging study." Journal of the American Academy of Child and Adolescent Psychiatry **35**(4): 530-536.

Raznahan, A., R. Lenroot, A. Thurm, M. Gozzi, A. Hanley, S. J. Spence, S. E. Swedo and J. N. Giedd (2012). "Mapping cortical anatomy in preschool aged children with autism using surface-based morphometry." Neuroimage Clin **2**: 111-119.

Raznahan, A., G. L. Wallace, L. Antezana, D. Greenstein, R. Lenroot, A. Thurm, M. Gozzi, S. Spence, A. Martin, S. E. Swedo and J. N. Giedd (2013). "Compared to What? Early Brain Overgrowth in Autism and the Perils of Population Norms." Biological psychiatry.

Roza, S. J., M. B. Hofstra, J. van der Ende and F. C. Verhulst (2003). "Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: a 14-year follow-up during childhood, adolescence, and young adulthood." Am J Psychiatry **160**(12): 2116-2121.

Schaer, M., M. C. Ottet, E. Scariati, D. Dukes, M. Franchini, S. Eliez and B. Glaser (2013). "Decreased frontal gyrification correlates with altered connectivity in children with autism." Frontiers in Human Neuroscience **7**.

Scheel, C., A. Rotarska-Jagiela, L. Schilbach, F. G. Lehnhardt, B. Krug, K. Vogeley and R. Tepest (2011). "Imaging derived cortical thickness reduction in high-functioning autism: Key regions and temporal slope." Neuroimage **58**(2): 391-400.

Schumann, C. M., C. S. Bloss, C. C. Barnes, G. M. Wideman, R. A. Carper, N. Akshoomoff, K. Pierce, D. Hagler, N. Schork, C. Lord and E. Courchesne (2010). "Longitudinal Magnetic Resonance Imaging Study of Cortical Development through Early Childhood in Autism." Journal of Neuroscience **30**(12): 4419-4427.

Sonuga-Barke, E. J. S. “Editorial: Distinguishing between the challenges posed by surface and deep forms of heterogeneity to diagnostic systems: do we need a new approach to subtyping of child and adolescent psychiatric disorders.”

Tiemeier, H., F. P. Velders, E. Szekely, S. J. Roza, G. Dieleman, V. W. V. Jaddoe, A. G. Uitterlinden, T. J. H. White, M. J. Bakermans-Kranenburg, A. Hofman, M. H. Van IJzendoorn, J. J. Hudziak and F. C. Verhulst (2012). “The Generation R Study: A Review of Design, Findings to Date, and a Study of the 5-HTTLPR by Environmental Interaction From Fetal Life Onward.” Journal of the American Academy of Child and Adolescent Psychiatry **51**(11): 1119-1135.

Uddin, L. Q., K. Supekar and V. Menon (2013). “Reconceptualizing functional brain connectivity in autism from a developmental perspective.” Front Hum Neurosci **7**: 458.

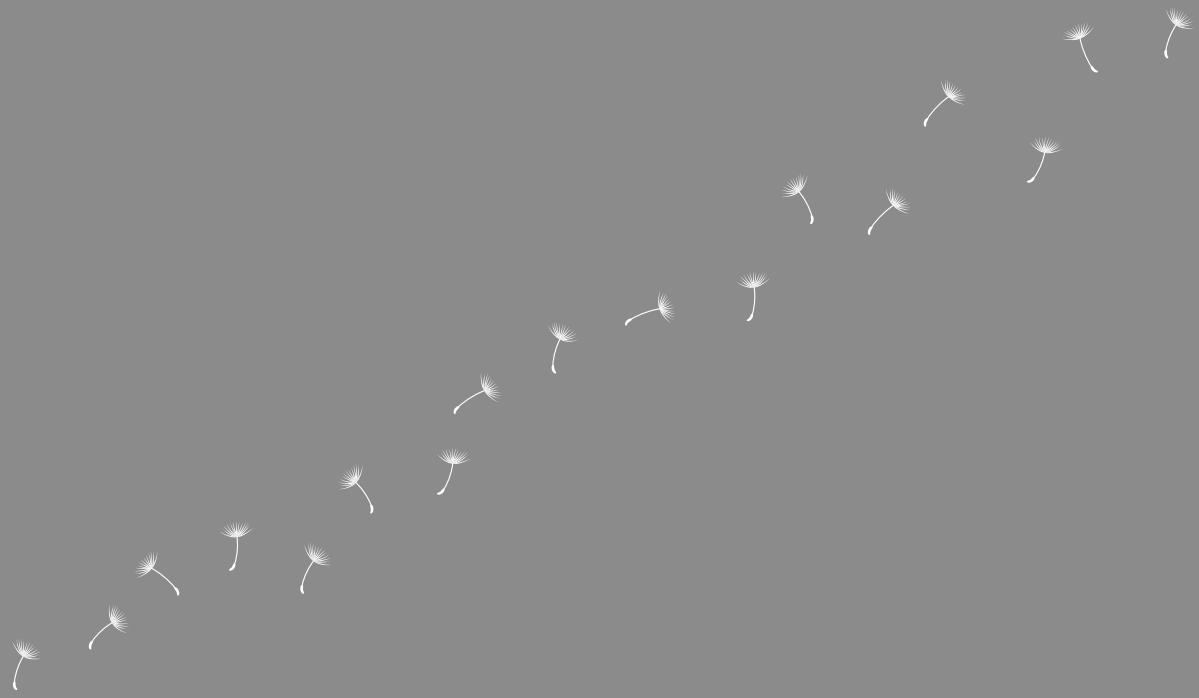
Verburg, B. O., E. A. Steegers, M. De Ridder, R. J. Snijders, E. Smith, A. Hofman, H. A. Moll, V. W. Jaddoe and J. C. Witteman (2008). “New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study.” Ultrasound Obstet Gynecol **31**(4): 388-396.

Wallace, G. L., N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2010). “Age-related temporal and parietal cortical thinning in autism spectrum disorders.” Brain **133**: 3745-3754.

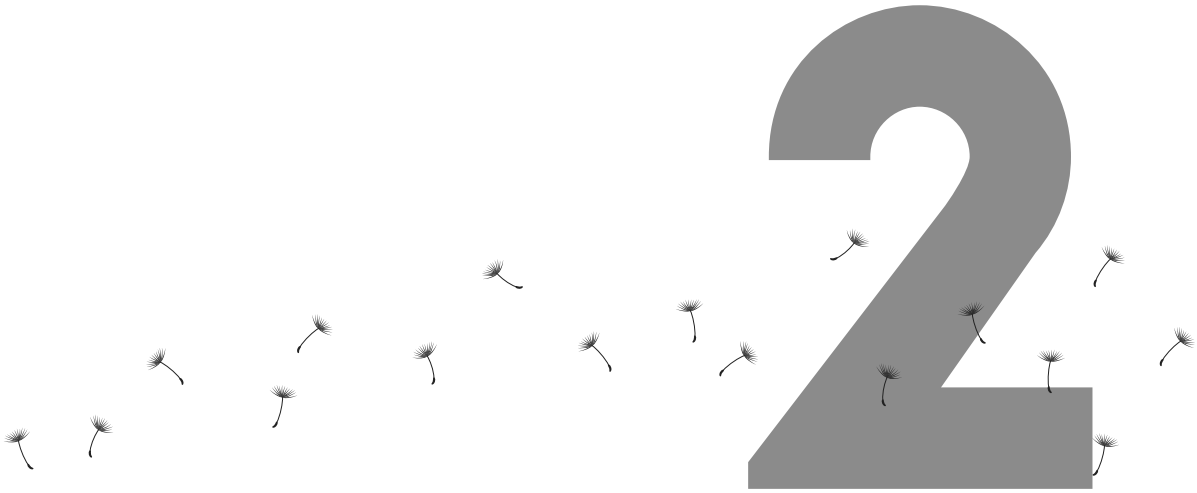
Wallace, G. L., B. Robustelli, N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2013). “Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders.” Brain **136**: 1956-1967.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. V. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). “Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology.” European Journal of Epidemiology **28**(1): 99-111.

White, T., S. Su, M. Schmidt, C. Y. Kao and G. Sapiro (2010). “The development of gyrification in childhood and adolescence.” Brain and Cognition **72**(1): 36-45.



Part I:
The neurobiology of autistic traits



Cortical morphology in 6-to-10 year old children with autistic traits – A population-based neuroimaging study

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ABSTRACT

Objective: Recent evidence suggests that symptoms of social impairment in Autism Spectrum Disorders (ASD) form a spectrum that extends into the general population. However, it is unclear if the neuroanatomy of ASD also shows a similar continuum in the general population. Therefore, the goal of this study was to investigate the relation between cortical morphology and autistic traits along a continuum in a large, population-based sample of young children.

Method: The study includes 717 children aged 6-to-10 years who are participants in the Generation R Study, a large population-based cohort. Autistic traits were measured using the Social Responsiveness Scale when children were approximately 6 years old. High-resolution MRI was obtained and morphological measures of the cortex including cortical thickness and gyrification were quantified brain-wide using FreeSurfer.

Results: Children with more autistic traits showed widespread areas of decreased gyrification. After excluding children with the highest autistic traits and confirmed ASD, the association remained present in a large cluster involving the left hemisphere temporal and precuneus regions. Finally, we found comparable effects when comparing a small sample of confirmed ASD cases to age and gender-matched controls.

Conclusion: We found differences in cortical morphology to be related to autistic traits along a continuum in a large population-based sample of school-aged children. Part of these differences remained after excluding the most severely affected children. Our findings lend support to an extension of the neurobiology of autistic traits to the general population.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a severe neurodevelopmental disorder in which social problems are a key symptom. Recent studies have suggested that these social problems are part of a spectrum of quantitative traits that extends into the general population (Constantino and Todd 2003). If true, one would expect that the neuroanatomical aspects of ASD also lie on a continuum in the general population. The majority of studies to date exploring the underlying neuroanatomy of ASD children involve case/control designs using clinically diagnosed patients with ASD. While these studies are very beneficial to help elucidate the underlying neurobiology of ASD, they do not answer the question whether autistic traits, including subclinical traits, share similar neuroanatomic features.

The optimum approach to evaluate whether the neuroanatomy of ASD fits a continuum in the general population involves three steps. The first step is to show a relationship between autistic symptoms along a continuum and neuroanatomy in the population. The second step is to show that those with high levels of autistic traits or ASD show the same neuroanatomical findings as those in the whole population. Finally, the third step is to assess whether the relationship between autistic symptoms and neuroanatomy remain after removing those with the highest level of autistic symptoms. While there have been a number of studies that have assessed one or two of the above steps, no study to date combined all three components in the same study.

There have been no studies that evaluated brain morphology of autistic traits in population-based samples. There are, however, numerous studies evaluating the neuroanatomy of children with clinical ASD versus controls (Amaral, Schumann and Nordahl 2008), although with mixed findings (e.g. both thicker and thinner cortex, as well as increased or decreased surface complexity). Despite this variability, replicated results include thicker cortex in younger children (between 1.5–8 years), supporting the hypothesis of early brain overgrowth. Thicker cortex was found in the frontal (Schumann, Bloss, Barnes, Wideman, Carper et al. 2010; Raznahan, Lenroot, Thurm, Gozzi, Hanley et al. 2012) and temporal regions (Hardan, Muddasani, Vemulapalli, Keshavan and Minshew 2006; Schumann et al. 2010) although others have reported no differences (Hazlett, Poe, Gerig, Styner, Chappell et al. 2011). Studies in older children (12 years and up) have found thinner temporal, parietal and occipital cortices in subjects with ASD (Wallace, Dankner, Kenworthy, Giedd and Martin 2010; Scheel, Rotarska-Jagiela, Schilbach, Lehnhardt, Krug et al. 2011; Zielinski, Prigge, Nielsen, Froehlich, Abildskov et al. 2014). More recently, studies of gyrification in children and adolescents with ASD have also yielded contradictory results, reporting higher gyrification in bilateral posterior (Wallace, Robustelli, Dankner, Kenworthy, Giedd et al. 2013), left inferior (Jou, Minshew, Keshavan and

Hardan 2010) and right parietal cortices (Kates, Ikuta and Burnette 2009), and also lower gyrification in the right frontal cortex (Schaer, Ottet, Scariati, Dukes, Franchini et al. 2013), or no difference in gyrification (Casanova, El-Baz, Mott, Mannheim, Hassan et al. 2009).

Two studies of ASD included subanalyses evaluating autistic symptoms in the control group. One study found a negative correlation of autistic traits and local gyrification in the right superior temporal sulcus (Wallace et al. 2013), whereas the other study reported increased gyrification in the right parietal lobe associated with more autistic symptoms (Kates et al. 2009). Only one study evaluated a continuous measure of autistic traits in typically developing youth and found thinner cortex in the right superior temporal cortex (Wallace, Shaw, Lee, Clasen, Raznahan et al. 2012).

Thus, our goal was to perform all three steps in the same study and to assess whether differences in cortical morphology are related to the variation in autistic symptoms as observed in the general pediatric population. Based on prior literature, we have centered our analyses around two features of cortical morphology that have shown differences in clinical samples of children with ASD, namely gyrification and cortical thickness. In line with studies in school-aged children, we hypothesized that a higher load of autistic traits would show a thicker cortex, particularly in frontal and temporal lobes, and less gyrification in frontal regions. Furthermore, we hypothesized that these findings would be present (i.) along a continuum in the entire cohort, (ii.) in the children with the highest level of autistic symptoms, and (iii.) after excluding children with the highest level of autistic symptoms.

Finally, considering the well-established sex differences in the prevalence of ASD (Constantino et al. 2003), the sexual dimorphism in neurodevelopment (Lenroot, Gogtay, Greenstein, Wells, Wallace et al. 2007), and since many studies evaluate only boys (Lai, Lombardo, Suckling, Ruigrok, Chakrabarti et al. 2013), we examined *a priori* whether autistic traits in boys and girls showed similar neuroanatomical correlates.

METHODS

Participants

This study is embedded in the Generation R Study, a population-based cohort study, investigating children's development from fetal life onwards (Jaddoe, van Duijn, Franco, van der Heijden, van Iizendoorn et al. 2012). The participants included 1,070 children, ages 6-to-10 years, who were scanned between September 2009 and July 2013 as part of a sub-study (White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013).

Two hundred fourteen children were excluded based on missing information on autistic traits and 105 children were excluded due to poor image quality (See Supplementary information). For 15 children, a gyrification output could not be constructed. Furthermore, for each sibling or twin pair, one sibling was excluded (n=17). Two children were excluded based on major incidental findings. The final study sample consisted of 717 6-to-10 year old children (Supplementary Figure 1).

Informed consent and assent were obtained from parents and children, respectively, after providing them with a complete description of the study. All procedures were approved by the Medical Ethics Committee of the Erasmus Medical Center.

Social Responsiveness Scale

Around age 6 years (range 4.89-8.90 years, see Table 1), the Social Responsiveness Scale was administered to obtain a measure of autistic traits (Constantino 2002). The Social Responsiveness Scale provides a valid quantitative measure of subclinical and clinical autistic traits (Constantino, Davis, Todd, Schindler, Gross et al. 2003). We utilized the 18-item short-form of the Social Responsiveness Scale, which shows correlations ranging from 0.93 and 0.99 with the full scale in three different large studies (see Supplementary section for additional information). The authors recommend cut-offs for screening in population-based settings (consistent with weighted Social Responsiveness Scale scores of 1.078 for boys 1.000 for girls) (Constantino 2002).

At approximately 7 years of age, children who scored on the top 15th percentile of the Child Behavior Checklist 1½-5 total score and those who scored on the top 2nd percentile of the PDP scale underwent a screening procedure for ASD, using the Social Communication Questionnaire (SCQ) a 40-item parent-reported screening instrument to assess characteristic autistic behavior. SCQ scores 15 or above are considered positive for screening (Berument, Rutter, Lord, Pickles and Bailey 1999). Children with scores above this threshold were invited for an Autism Diagnostic Observation Scale (ADOS) (Lord, Risi, Lambrecht, Cook, Leventhal et al. 2000) and their mothers for an Autism Diagnostic Interview–Revised (ADI-R) (Lord, Rutter and Lecouteur 1994).

Magnetic Resonance Imaging

All children were first familiarized with the MRI scanning environment during a mock scanning session. Structural MRI scans were obtained on a 3-Tesla scanner (Discovery MR750, GE Worldwide, Milwaukee, USA). Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.1 (http://surfer.nmr.mgh.harvard.edu/). Details can be found in the Supplementary material.

Statistical analysis

To investigate the relationship between cortical morphology and autistic traits, we performed vertex-wise analyses using the FreeSurfer Query, Design, Estimate, Contrast module (QDEC, www.surfer.nmr.mgh.harvard.edu), which allows users to perform inter-subject/group averaging and inference, using the General Linear Model (GLM) on the morphometric data produced by the FreeSurfer processing stream. Analyses were corrected for multiple comparisons using the built-in Monte Carlo simulation at a threshold of $p<0.05$, a cluster-wise correction that controls for the rate of false positive clusters.

In QDEC, we used a regression model with Social Responsiveness Scale score as the continuous predictor, gender as a discrete factor and age as a nuisance variable within the DODS-design matrix (Different Offset Different Slope). Due to limitations in the number of covariates in QDEC, we exported local gyrification data of each participant for the identified clusters into the Statistical Package for the Social Sciences (SPSS, IBM Corp., version 21.0) to assess whether the associations withstood correction for confounding factors. Linear regression analyses were performed with Social Responsiveness Scale score as the independent variable. All analyses were corrected for gender and age, whereas other variables were included as covariates when they changed the effect estimate (B) by 5% or more. More information on covariates is provided in the Supplementary material. We explored gender interaction in QDEC and performed a stratified analyses for boys and girls to visualize gender-specific patterns and make our results directly comparable with gender-restricted samples. In addition, we performed sensitivity analyses, in which we excluded subjects with the highest load of autistic traits and subjects with a diagnosis of ASD.

RESULTS

Sample characteristics

Child and maternal characteristics are presented in Table 1. Boys had somewhat higher Social Responsiveness Scale scores (mean difference=0.06, $t(715)=2.96$, $p=0.003$) and more attention problems (mean difference=0.70, $t(702)=4.63$, $p=0.000004$). Characteristics of the excluded sample are presented in Supplementary Table 1. Children that were excluded from the final sample were more likely to be of non-Dutch origin ($\chi^2(2, N=1070) = 44.88$, $p=1.79*10^{-10)}$, had somewhat higher Child Behavior Checklist attention problems scores (mean difference=-0.50; $t(975)=-3.23$, $p=0.001$), slightly lower IQ (mean difference=-0.07; $t(980) = 3.61$, $p=0.0003$) and (if excluded for reasons other than missing information on autistic traits) somewhat higher Social Responsiveness Scale scores (mean difference=-0.07; $t(856)=-2.09$, $p=0.038$). Mothers of excluded children were more likely to be lower educated ($\chi^2(2, N=971)=14.32$, $p=0.001$) with less income ($\chi^2(2, N=937)=44.59$, $p=2.08*10^{-10)}$.

Table 1. Participant characteristics

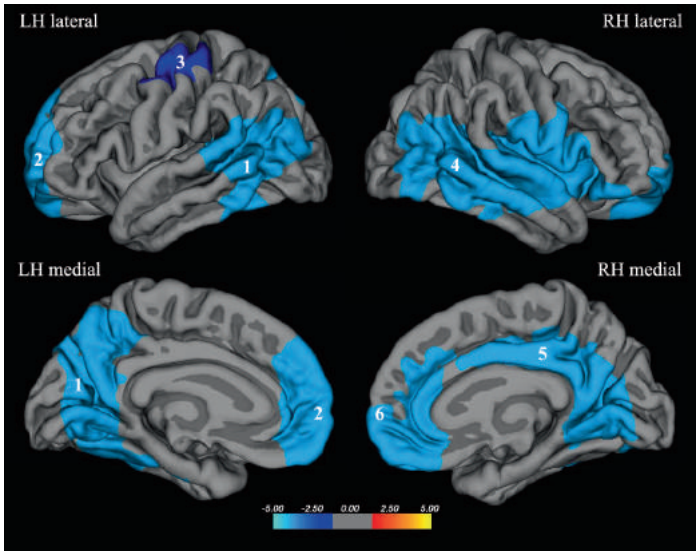
Child characteristics					
	observations				
Gender (% boy)	717	52			
Ethnicity (%)	717				
Dutch		74.3			
Other Western		6.7			
Non-Western		19.0			
		mean	**SD**	**range**	
Social Responsiveness Scale Weighted total score	717	0.27	0.29	0	3
Age at Social Responsiveness Scale (years)	717	6.17	0.47	4.89	8.90
Age at MRI (years)	717	7.97	1.00	6.12	10.70
Child Behavior Checklist					
Attention problems scale	704	1.98	2.05	0	9
Non verbal IQ	663	102.86	14.42	50	142
Handedness (% right-handed)	716	90.2			
Maternal characteristics					
Education level (%)					
High	698	58.6			
Medium		30.2			
Low		11.2			
Monthly household income (%)					
High	680	80.7			
Medium		14.3			
Low		4.7			
Alcohol use during pregnancy[a] (%)					
Never	652	34.2			
Until pregnancy was known		15.0			
Continued		50.8			
Smoking during pregnancy[b] (%)					
Never	695	77.4			
Until pregnancy was known		6.0			
Continued		16.5			

[a]Drinking > 1 drink/day varied between 0.3 and 2.9 % with the highest percentage in the first trimester.
[b]Smoking ten or more cigarettes per day fluctuated between 4.2 and 5.7 with the highest percentage in the first trimester.

Gyrification

Six regions in the brain showed significant negative correlations between Social Responsiveness Scale and gyrification (Table 2 and Figure 1), three in each hemisphere.

Figure 1. Gyrification and autistic traits in the full sample.



1 LH temporal/precuneus
2 LH frontal
3 LH pre/postcentral
4 RH temporal/frontal
5 RH cingulate
6 RH frontal/cingulate

Note. Analyses were adjusted for age and gender. Colors represent the -log10(p-value). Blue clusters represent a negative correlation with autistic traits. Numbers refer to the entire cluster. Large clusters 1 and 2 are visible both in the lateral and the medial view of the left hemisphere.

In the left hemisphere, the first cluster (LH1) included the posterior temporal cortex, covering portions of the superior, middle, and inferior temporal cortex, as well as the inferior parietal cortex and supramarginal gyrus. This cluster extended medially to include the precuneus, cuneus, pericalcarine, lingual cortex and fusiform gyrus (p=0.0001).

The second cluster (LH2) included rostral middle frontal cortex and extended into the superior frontal, medial orbitofrontal, and rostral anterior cingulate cortices (p=0.0001). The third cluster (LH3) covered part of the central sulcus, pre- and postcentral gyrus (p=0.028).

In the right hemisphere, cluster RH4 included a large part of the temporal lobe extending posteriorly into the inferior parietal cortex and supramarginal gyrus and anteriorly to the insula, supramarginal gyrus and pre- and postcentral gyrus (p=0.0001). Cluster RH5 included part of the posterior cingulate cortex and extended posteriorly to the precuneus and caudally to the lingual gyrus (p=0.0001). Cluster RH6 included the superiorfrontal cortex, the medial orbital frontal and anterior cingulate cortex.

Table 2 Gyrification and autistic traits (n=717): vertex-wise analyses (A) and regression analyses (B)

A. Vertex-wise analyses

		Cluster size (mm2)	Talairach coordinates X	Y	Z	No. of vertices within cluster	Clusterwise p-value
Left Hemisphere							
	Temporal/precuneus	15222.16	-48.7	-43.6	7.5	28452	.0001
	Frontal	5039.94	-9.5	62.7	-5.5	7569	.0001
	Pre/postcentral	2100.33	-30.6	-27.2	48.6	4993	.0275
Right Hemisphere							
	Temporal/frontal	16329.37	61.7	-7.9	0.3	34398	.0001
	Cingulate	7276.69	14.1	-61.1	1.1	14941	.0001
	Frontal/cingulate	4493.51	6.2	46.3	-19.8	7670	.0001

B. Regression analyses

			B	SE B	*p*	β
Left Hemisphere						
	Temporal/precuneus	Model 1	-0.20	0.05	.000	-0.15
		Model 2 (adjusted)	-0.15	0.11	.006	-0.11
	Frontal	Model 1	-0.14	0.04	.000	-0.14
		Model 2 (adjusted)	-0.10	0.08	.020	-0.10
	Pre/postcentral	Model 1	-0.20	0.08	.009	-0.10
		Model 2 (adjusted)	-0.12	0.17	.150	-0.06
Right Hemisphere						
	Temporal/frontal	Model 1	-0.36	0.09	.000	-0.15
		Model 2 (adjusted)	-0.28	0.19	.005	-0.12
	Cingulate	Model 1	-0.30	0.08	.000	-0.14
		Model 2 (adjusted)	-0.22	0.18	.013	-0.11
	Frontal/cingulate	Model 1	-0.11	0.04	.003	-0.11
		Model 2 (adjusted)	-0.07	0.08	.087	-0.07

Note. Local gyrification indices were residualized for age at scanning. Model 1 adjusted for age when Social Responsiveness Scale was completed and gender. Model 2 additionally adjusted for child ethnicity, maternal education, maternal alcohol use, maternal smoking, Child Behavior Checklist attention problems and non-verbal IQ.

The association remained in all but two clusters (LH 3 and RH6), after adjusting for confounding factors (Table 2B). The significant findings remained after additionally correcting for total brain volume (Supplementary Table 2). Plots of the age-residualized gyrification indices against quintiles of Social Responsiveness Scale scores are shown in Supplementary Figure 2. Adding a quadratic term did not significantly improve the model in any of the gyrification clusters.

There was no gender by gyrification interaction and gender-stratified analysis revealed similar patterns of decreased gyrification (Supplementary Figure 3). In children with a delay of less than a year between administration of the Social Responsiveness Scale and the MRI scan (n=179), the direction of the effect was similar.

Case control analyses

To assess differences in gyrification between children with a diagnosis of ASD and control subjects, we performed independent t-tests on the 6 boys in our sample with a confirmed ADI-R/ADOS diagnosis with a group of age and gender-matched controls. In general, subjects with ASD had lower gyrification indices, although this difference did not reach statistical significance (Supplementary Table 3).

Analyses without children with the highest levels of autistic traits and ASD

Next, we explored if these associations also held excluding children with high levels of autistic traits. We excluded the 6 boys who met criteria for ASD as measured by either ADI-R or ADOS. Further, boys with weighted Social Responsiveness Scale scores above 1.078 and girls with scores over 1.000 were excluded, in line with cut-offs recommended for screening in population-based settings (Constantino 2002). This resulted in a sample of 695 children. The results show effect sizes similar to those in the full sample (Table 3). After correcting for covariates, the relation between less gyrification and more autistic traits remained significant in the left hemisphere temporal/precuneus cluster (LH1) and a trend remained in the left hemisphere frontal cluster (LH2).

Specificity analyses

To explore the specificity of our results, we repeated the analyses with two other subscales of the Child Behavior Checklist as predictors (aggression and anxiety/depression). This was in addition to attention problems and non-verbal IQ, that were included as covariates in the main analysis. This yielded very small effect sizes that became insignificant after correcting for confounders.

Table 3. Autistic traits and gyrification in the sample without children with the highest levels of autistic traits and ASD (n=695)

			B	SE B	*p*	β
Left Hemisphere						
	Temporal/precuneus	Model 1	-0.21	0.07	.002	-0.12
		Model 2 (adjusted)	-0.15	0.07	.044	-0.08
	Frontal	Model 1	-0.15	0.05	.002	-0.12
		Model 2 (adjusted)	-0.10	0.06	.067	-0.08
	Pre/postcentral	Model 1	-0.17	0.10	.091	-0.06
		Model 2 (adjusted)	-0.08	0.11	.461	-0.03
Right Hemisphere						
	Temporal/frontal	Model 1	-0.34	0.12	.003	-0.11
		Model 2 (adjusted)	-0.22	0.13	.090	-0.07
	Cingulate	Model 1	-0.28	0.10	.008	-0.10
		Model 2 (adjusted)	-0.17	0.12	.152	-0.06
	Frontal/cingulate	Model 1	-0.12	0.05	.013	-0.10
		Model 2 (adjusted)	-0.07	0.06	.218	-0.05

Note. Local gyrification indices were residualized for age at scanning. Model 1 adjusted for age when Social Responsiveness Scale was completed and gender. Model 2 additionally adjusted for child ethnicity, maternal education, maternal alcohol use, maternal smoking, Child Behavior Checklist attention problems and IQ.

Cortical thickness

For the total sample, including both genders (n=717), we found no areas where thickness correlated with autistic traits and no gender interaction.

In boys only (n=373), thicker cortex in the pericalcarine area was related to more autistic traits (Supplementary Figure 4,*p*=0.011). In girls (n=344), no areas correlated with autistic traits.

Additional measures

No relation was found between autistic traits and the global hemispheric means of cortical thickness, surface area, and gyrification (Table 4). In addition, there were no associations between autistic traits and volumes of subcortical nuclei.

While cortical thickness and gyrification were the main outcomes of interest, several other measures were explored, including cortical volume, sulcal depth and surface area. Larger volume in the bilateral pericalcarine region was related to more autistic traits (Supplementary Figure 5). Similarly, decreased sulcal depth in the posterior part of the right superior temporal sulcus was associated with more autistic traits (Supplementary Figure 6). For surface area, no differences were found.

Table 4. Autistic traits and global brain measures

		B	SE B	*p*	β
Left Hemisphere					
Mean cortical thickness	Model 1	-0.01	0.04	.878	-0.04
	Model 2 (adjusted)	0.03	0.04	.379	0.03
Mean pial surface area	Model 1	-12399.11	3379.49	.000	0.02
	Model 2 (adjusted)	-1839.10	1373.26	.180	-0.02
Mean gyrification	Model 1	-0.13	0.04	.003	0.05
	Model 2 (adjusted)	-0.05	0.04	.188	-0.04
Right Hemisphere					
Mean cortical thickness	Model 1	-0.02	0.04	.709	0.01
	Model 2 (adjusted)	0.03	0.04	.409	0.03
Mean pial surface area	Model 1	-12861.23	3497.54	.000	0.01
	Model 2 (adjusted)	-1911.55	1407.19	.174	-0.02
Mean gyrification	Model 1	-0.16	0.05	.001	0.03
	Model 2 (adjusted)	-0.07	0.04	.093	-0.05

Note. Model 1 adjusted for gender and age when Social Responsiveness Scale was completed. Model 2 additionally adjusted for total brain volume.

DISCUSSION

There is emerging evidence that autistic traits fall on a continuum within the general population (Constantino et al. 2003). If this is the case, then it is likely that the neuroanatomy of autistic traits also fall along a continuum within the general population. Thus, the primary goal of this study was to evaluate the relationship between autistic traits and structural brain measures in a large population-based study of school-age children. We studied whether neurobiological findings of autistic traits are present along a continuum in a population using a three-step approach, which involved: (i.) showing that a significant linear relationship exists between the autistic traits and the neurobiological measurement and that a non linear relationship does not provide a significantly better fit; (ii.) performing a case/control evaluation of children with ASD to show that the findings are present in the cases with diagnosed ASD compared to controls; and (iii.) excluding individuals with ASD or the highest autistic symptoms to assess that the linear relationship remains.

Evaluating brain morphology across a continuum of autistic traits, we found that school-aged children show a widespread decrease in cortical gyrification with increasing autistic traits. In some regions, this relationship appeared to be driven by subjects with more autistic symptoms, suggesting that for these regions a cut-off effect may be applicable. However, there was a strong linear relationship in regions involving the left hemisphere temporal/precuneus area that remained after excluding children with the highest levels of autistic

traits and confirmed ASD. Finally, we found less gyrification with more autistic traits and a comparable –though non-significant– effect when comparing a small sample of confirmed ASD cases to age and gender-matched controls. Taken together, these findings provide supportive evidence for the existence of a continuum, at least for some regions, in the neurobiology of autistic traits in children.

While other studies of gyrification in ASD have not had the goal of evaluating whether traits are continuous in the population, they have evaluated gyrification abnormalities in ASD. Studies have found both decreased (Schaer et al. 2013) and increased (Kates et al. 2009; Wallace et al. 2013) gyrification in clinical cohorts with ASD. In a case/control study of ASD in 11 subjects (8 male) compared to typically developing controls aged 9-17 years, Schaer et al. found decreased gyrification in frontal brain regions (Schaer et al. 2013). However, using the same technique, Wallace et al. (Wallace et al. 2013) reported regions of increased gyrification in an all-male case control study of ASD (aged 12-23 years). The differences in the age and gender constellations of these studies could be responsible for the discrepancy in the findings. However, this is less intuitive since gyrification peaks early in childhood and, parallel with cortical pruning, decreases over time (White, Su, Schmidt, Kao and Sapiro 2010; Shaw, Malek, Watson, Sharp, Evans et al. 2012). Thus, factors such as heterogeneity in the features of ASD, small sample size, or sample selection differences could be responsible for the differences. Interestingly though, when Wallace et al. performed a subanalysis that included only the typically developing subjects, they found negative correlations between autistic traits and gyrification in a region showing marked overlap with our finding in the right superior temporal lobe.

Gyrification is a poorly understood phenomenon that is thought to accommodate efficient neural processing in the brain (Van Essen 1997). Decreased gyrification could point to disruptions in fetal development, since the majority of gyrification takes place in the third trimester, at a time when the brain undergoes prolific growth. However, the many primary sulci are formed before that, between 20 and 28 weeks of gestational age (Habas, Scott, Roosta, Rajagopalan, Kim et al. 2012). Following Van Essen's model (Van Essen 1997), decreased gyrification could reflect decreased short-range connectivity of the neurons within specific regions. Indeed, Schaer et al. recently found decreased gyrification in ASD to be related to decreased connectivity in the same regions (Schaer et al. 2013). In our study, decreased gyrification was not associated with decreased surface area. This decoupling of various aspects of the cortex is puzzling, but consistent with at least one other study in ASD (Wallace et al. 2013) and could potentially be understood by the fact that both features derive from distinct stages in gestation and that different aspects of the cortex are regulated by different genetic mechanisms (Panizzon, Fennema-Notestine, Eyler, Jernigan, Prom-Wormley et al. 2009). A disruption within a particular developmental window may interfere with the development of one feature, while leaving another preserved.

While we found widespread regions of decreased gyrification, specific brain regions were more affected. Interestingly, the regions we found are functionally associated with capacities that are impaired in ASD. The frontal and posterior cingulate cortices both form components of the default mode network, a resting-state functional brain network thought to be involved in self-reflection (Fair, Cohen, Dosenbach, Church, Miezin et al. 2008). Additionally, the anterior cingulate cortex, the temporal lobes and superior temporal sulcus, have been related to 'theory of mind' (Gallagher and Frith 2003). The superior temporal sulcus, in which we found less gyrification and decreased sulcal depth, is recognized as a structure crucial to perception and processing of language, auditory stimuli, and specifically social stimuli (both visual and auditory) (Zilbovicius, Meresse, Chabane, Brunelle, Samson et al. 2006). Previous research has shown decreased gray matter (Hadjikhani, Joseph, Snyder and Tager-Flusberg 2006; Scheel et al. 2011), white matter volumes (von dem Hagen, Nummenmaa, Yu, Engell, Ewbank et al. 2011), and abnormal activation of the superior temporal region in ASD (Zilbovicius et al. 2006).

We found larger volume and thicker cortex of the pericalcarine region, a region that is among the first to reach peak cortical thickness (Shaw, Kabani, Lerch, Eckstrand, Lenroot et al. 2008), around age 7 years, which falls within the age range in our study. Thicker occipital cortex has been found in school-aged children with ASD (Zielinski et al. 2014) and is in line with the early brain overgrowth hypothesis. Further, Magnetic Resonance Spectroscopy has revealed diminished levels of creatine (Levitt, O'Neill, Blanton, Smalley, Fadale et al. 2003). Thicker cortex in the pericalcarine area was exclusively found in boys, which potentially reflects gender specificity in brain development in children with autistic traits. Since females are generally underrepresented in clinical ASD samples, due to the low prevalence in females, it is possible that some findings arising from previous studies on cortical thickness are in fact driven by the males only. However, the regions where we found decreased gyrification were mostly overlapping across genders, in line with work that suggests similar brain differences in men and women with ASD (Craig, Zaman, Daly, Cutter, Robertson et al. 2007).

Our study has a number of strengths. First we imaged children from a large, population-based cohort with autistic traits across a broad spectrum. This provides the unique opportunity to test whether the underlying neurobiology of autistic traits extends into the general population. In addition, considerable information is available on potential confounding factors. Although autistic traits frequently co-occur with attention problems and lower IQ (Grzadzinski, Di Martino, Brady, Mairena, O'Neale et al. 2011), the brain correlates described here are specifically related to autistic traits independent of IQ and attention problems. Further, our results were independent of symptoms of aggression, anxiety and depression.

There are several limitations to the study. First, the measurement of autistic traits and cortical morphology was not contemporaneous. However, autistic traits are relatively stable over time (Constantino, Abbacchi, Lavesser, Reed, Givens et al. 2009) and a sensitivity

analysis only including children with a short delay between the two measurements yielded similar results. Second, as the MRI data were assessed cross-sectionally, we cannot study longitudinal trajectories of brain development associated with autistics traits. Third, while we corrected for multiple testing within each surface-based analysis, we did not perform additional correction for the different surface-based analyses. Fourth, the ADOS/ADI-R assessments were not available for all participants. In our sample, six boys met criteria for ASD on these instruments, but it is possible we would identify more cases of ASD, had we been able to perform in-depth assessments in all participants. Since this is a small group, a cautious interpretation of these findings is warranted. Fifth, there is a potential for selection effects, since children that were excluded differed from the sample on socioeconomic factors and problem scores. Thus, it is possible that our results are not representative of the general population, although we are likely missing the children with more severe autistic symptoms. Finally, in interpreting data from a data-driven analysis, there is a risk of reverse inference. However, the regions we found have been consistently and specifically associated with ASD in the literature.

To conclude, we found differences in cortical morphology to be related to autistic traits in a large population-based sample of school-aged children. Our findings lend support to an extension of the neurobiology of autistic traits to the general population.

REFERENCES

Amaral, D. G., C. M. Schumann and C. W. Nordahl (2008). "Neuroanatomy of autism." Trends in Neurosciences **31**(3): 137-145.

Berument, S. K., M. Rutter, C. Lord, A. Pickles and A. Bailey (1999). "Autism screening questionnaire: diagnostic validity." British Journal of Psychiatry **175**: 444-451.

Casanova, M. F., A. El-Baz, M. Mott, G. Mannheim, H. Hassan, R. Fahmi, J. Giedd, J. M. Rumsey, A. E. Switala and A. Farag (2009). "Reduced Gyral Window and Corpus Callosum Size in Autism: Possible Macroscopic Correlates of a Minicolumnopathy." Journal of Autism and Developmental Disorders **39**(5): 751-764.

Constantino, J. N. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Constantino, J. N., A. M. Abbacchi, P. D. Lavesser, H. Reed, L. Givens, L. Chiang, T. Gray, M. Gross, Y. Zhang and R. D. Todd (2009). "Developmental course of autistic social impairment in males." Development and Psychopathology **21**(1): 127-138.

Constantino, J. N., S. A. Davis, R. D. Todd, M. K. Schindler, M. M. Gross, S. L. Brophy, L. M. Metzger, C. S. Shoushtari, R. Splinter and W. Reich (2003). "Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the autism diagnostic interview-revised." Journal of Autism and Developmental Disorders **33**(4): 427-433.

Constantino, J. N. and R. D. Todd (2003). "Autistic traits in the general population- A twin study." Archives of General Psychiatry **60**(5): 524-530.

Craig, M. C., S. H. Zaman, E. M. Daly, W. J. Cutter, D. M. W. Robertson, B. Hallahan, F. Toal, S. Reed, A. Ambikapathy, M. Brammer, C. M. Murphyand and D. G. M. Murphy (2007). "Women with autistic-spectrum disorder: Magnetic resonance imaging study of brain anatomy." British Journal of Psychiatry **191**: 224-228.

Fair, D. A., A. L. Cohen, N. U. F. Dosenbach, J. A. Church, F. M. Miezin, D. M. Barch, M. E. Raichle, S. E. Petersen and B. L. Schlaggar (2008). "The maturing architecture of the brain's default network." Proceedings of the National Academy of Sciences of the United States of America **105**(10): 4028-4032.

Gallagher, H. L. and C. D. Frith (2003). "Functional imaging of 'theory of mind'." Trends in Cognitive Sciences **7**(2): 77-83.

Grzadzinski, R., A. Di Martino, E. Brady, M. A. Mairena, M. O'Neale, E. Petkova, C. Lord and F. X. Castellanos (2011). "Examining autistic traits in children with ADHD: does the autism spectrum extend to ADHD?" Journal of Autism and Developmental Disorders **41**(9): 1178-1191.

Habas, P. A., J. A. Scott, A. Roosta, V. Rajagopalan, K. Kim, F. Rousseau, A. J. Barkovich, O. A. Glenn and C. Studholme (2012). "Early Folding Patterns and Asymmetries of the Normal Human Brain Detected from in Utero MRI." Cerebral Cortex **22**(1): 13-25.

Hadjikhani, N., R. M. Joseph, J. Snyder and H. Tager-Flusberg (2006). "Anatomical differences in the mirror neuron system and social cognition network in autism." Cerebral Cortex **16**(9): 1276-1282.

Hardan, A. Y., S. Muddasani, M. Vemulapalli, M. S. Keshavan and N. J. Minshew (2006). "An MRI study of increased cortical thickness in autism." American Journal of Psychiatry **163**(7): 1290-1292.

Hazlett, H. C., M. D. Poe, G. Gerig, M. Styner, C. Chappell, R. G. Smith, C. Vachet and J. Piven (2011). "Early Brain Overgrowth in Autism Associated With an Increase in Cortical Surface Area Before Age 2 Years." Archives of General Psychiatry **68**(5): 467-476.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." Eur J Epidemiol **27**(9): 739-756.

Jou, R. J., N. J. Minshew, M. S. Keshavan and A. Y. Hardan (2010). "Cortical Gyrification in Autistic and Asperger Disorders: A Preliminary Magnetic Resonance Imaging Study." Journal of Child Neurology **25**(12): 1462-1467.

Kates, W. R., I. Ikuta and C. P. Burnette (2009). "Gyrification Patterns in Monozygotic Twin Pairs Varying in Discordance for Autism." Autism Research **2**(5): 267-278.

Lai, M. C., M. V. Lombardo, J. Suckling, A. N. V. Ruigrok, B. Chakrabarti, C. Ecker, S. C. L. Deoni, M. C. Craig, D. G. M. Murphy, E. T. Bullmore, S. Baron-Cohen and M. A. Consortium (2013). "Biological sex affects the neurobiology of autism." Brain **136**: 2799-2815.

Lenroot, R. K., N. Gogtay, D. K. Greenstein, E. M. Wells, G. L. Wallace, L. S. Clasen, J. D. Blumenthal, J. Lerch, A. P. Zijdenbos, A. C. Evans, P. M. Thompson and J. N. Giedd (2007). "Sexual dimorphism of brain developmental trajectories during childhood and adolescence." Neuroimage **36**(4): 1065-1073.

Levitt, J. G., J. O'Neill, R. E. Blanton, S. Smalley, D. Fadale, J. T. McCracken, D. Guthrie, A. W. Toga and J. R. Alger (2003). "Proton magnetic resonance spectroscopic imaging of the brain in childhood autism." Biological Psychiatry **54**(12): 1355-1366.

Lord, C., S. Risi, L. Lambrecht, E. H. Cook, B. L. Leventhal, P. C. DiLavore, A. Pickles and M. Rutter (2000). "The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism." Journal of Autism and Developmental Disorders **30**(3): 205-223.

Lord, C., M. Rutter and A. Lecouteur (1994). "Autism Diagnostic Interview-Revised - a Revised Version of a Diagnostic Interview for Caregivers of Individuals with Possible Pervasive Developmental Disorders." Journal of Autism and Developmental Disorders **24**(5): 659-685.

Panizzon, M. S., C. Fennema-Notestine, L. T. Eyler, T. L. Jernigan, E. Prom-Wormley, M. Neale, K. Jacobson, M. J. Lyons, M. D. Grant, C. E. Franz, H. Xian, M. Tsuang, B. Fischl, L. Seidman, A. Dale and W. S. Kremen (2009). "Distinct Genetic Influences on Cortical Surface Area and Cortical Thickness." Cerebral Cortex **19**(11): 2728-2735.

Raznahan, A., R. Lenroot, A. Thurm, M. Gozzi, A. Hanley, S. J. Spence, S. E. Swedo and J. N. Giedd (2012). "Mapping cortical anatomy in preschool aged children with autism using surface-based morphometry." Neuroimage Clin **2**: 111-119.

Schaer, M., M. C. Ottet, E. Scariati, D. Dukes, M. Franchini, S. Eliez and B. Glaser (2013). "Decreased frontal gyrification correlates with altered connectivity in children with autism." Frontiers in Human Neuroscience **7**.

Scheel, C., A. Rotarska-Jagiela, L. Schilbach, F. G. Lehnhardt, B. Krug, K. Vogeley and R. Tepest (2011). "Imaging derived cortical thickness reduction in high-functioning autism: Key regions and temporal slope." Neuroimage **58**(2): 391-400.

Schumann, C. M., C. S. Bloss, C. C. Barnes, G. M. Wideman, R. A. Carper, N. Akshoomoff, K. Pierce, D. Hagler, N. Schork, C. Lord and E. Courchesne (2010). "Longitudinal Magnetic Resonance Imaging Study of Cortical Development through Early Childhood in Autism." Journal of Neuroscience **30**(12): 4419-4427.

Shaw, P., N. J. Kabani, J. P. Lerch, K. Eckstrand, R. Lenroot, N. Gogtay, D. Greenstein, L. Clasen, A. Evans, J. L. Rapoport, J. N. Giedd and S. P. Wise (2008). "Neurodevelopmental trajectories of the human cerebral cortex." Journal of Neuroscience **28**(14): 3586-3594.

Shaw, P., M. Malek, B. Watson, W. Sharp, A. Evans and D. Greenstein (2012). "Development of Cortical Surface Area and Gyrification in Attention-Deficit/Hyperactivity Disorder." Biological Psychiatry **72**(3): 191-197.

Van Essen, D. C. (1997). "A tension-based theory of morphogenesis and compact wiring in the central nervous system." Nature **385**(6614): 313-318.

von dem Hagen, E. A., L. Nummenmaa, R. Yu, A. D. Engell, M. P. Ewbank and A. J. Calder (2011). "Autism spectrum traits in the typical population predict structure and function in the posterior superior temporal sulcus." Cereb Cortex **21**(3): 493-500.

Wallace, G. L., N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2010). "Age-related temporal and parietal cortical thinning in autism spectrum disorders." Brain **133**: 3745-3754.

Wallace, G. L., B. Robustelli, N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2013). "Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders." Brain **136**: 1956-1967.

Wallace, G. L., P. Shaw, N. R. Lee, L. S. Clasen, A. Raznahan, R. K. Lenroot, A. Martin and J. N. Giedd (2012). "Distinct Cortical Correlates of Autistic versus Antisocial Traits in a Longitudinal Sample of Typically Developing Youth." Journal of Neuroscience **32**(14): 4856-4860.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. V. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." European Journal of Epidemiology **28**(1): 99-111.

White, T., S. Su, M. Schmidt, C. Y. Kao and G. Sapiro (2010). "The development of gyrification in childhood and adolescence." Brain and Cognition **72**(1): 36-45.

Zielinski, B. A., M. B. D. Prigge, J. A. Nielsen, A. L. Froehlich, T. J. Abildskov, J. S. Anderson, P. T. Fletcher, K. M. Zygmunt, B. G. Travers, N. Lange, A. L. Alexander, E. D. Bigler and J. E. Lainhart (2014). "Longitudinal changes in cortical thickness in autism and typical development." Brain **137**: 1799-1812.

Zilbovicius, M., I. Meresse, N. Chabane, F. Brunelle, Y. Samson and N. Boddaert (2006). "Autism, the superior temporal sulcus and social perception." Trends in Neurosciences **29**(7): 359-366.

SUPPLEMENT

Covariates

Child ethnicity was defined using the ethnicity categorization of 'Statistics Netherlands' (1). Handedness of the child was obtained using the Edinburgh Handedness Inventory (2). Maternal education was defined as highest education completed (3) and household income was defined by the total net monthly income of the household. Information on maternal alcohol use and smoking during pregnancy was obtained using questionnaires from each trimester of pregnancy. Child attention problems, which are known to be highly comorbid with autistic traits (4), were measured at the age of 6 years using the Attention Problems (AP) syndrome scale of the Child Behavior Checklist (CBCL) for ages 1.5-5. Non-verbal IQ at age 6 was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence Test –Revised (5). Total brain volume was calculated by adding up the bilateral supratentorial volumes and cerebellum.

In all regression analyses in SPSS, missing values of potential confounding (family) risk factors (7.5% for IQ, 0.1% for handedness, 2.6% for maternal education, 3.9% for household income, 9.1% for alcohol use during pregnancy and 3.1% for smoking during pregnancy) were imputed using the multiple imputation (Markov chain Monte Carlo) method in SPSS with 5 imputations and 10 iterations.

The Social Responsiveness Scale

The Social Responsiveness Scale (SRS) is a 65-item questionnaire that represents the parent's observation of the child's social behavior during the past six months. Each item is scored from 0 ('never true') to 3 ('almost always true'). The SRS can be scored on a total scale and on social cognition, social communication and social mannerism subscales. Higher scores indicate more problems. The SRS covers various dimensions of interpersonal behavior, communication and repetitive/stereotypic behavior characteristics of autism spectrum disorders. When using a clinical cut-off score, the SRS was found to have high sensitivity (0.85) and moderate specificity (0.75) in a sample of 61 child psychiatric patients (6). Associations of SRS total scores with ADI-R algorithm scores for DSM-IV criterion sets were on the order or 0.7 in that same sample and in another sample of 119 children with special educational needs ADI-R total scores correlated 0.59 with SRS total scores (7).

The 18-items questionnaire in the current study contained items from the following subscales: social cognition, social communication and autistic mannerism. In the Generation R sample, the Cronbach's alpha indicated high inter-item reliability for the SRS (alpha=0.79). In a sample of 3857 children aged 4-18 years (as part of the Social Spectrum Study, a multicenter study social development in the children referred to a mental health care institution in the

South-West of the Netherlands from 2010-2012) the correlation between total scores derived from the selected 18 items (SRS short-form) and the SRS scores derived from the complete test was *r*=0.95 (p<0.001) (unpublished data). The correlation between total scores derived by the SRS short-form and the SRS in the Missouri Twin Study was 0.93 in monozygotic male twins (n=98) and 0.94 in dizygotic male twins (n=134). In a sample of 2,719 children from the Interactive Autism Network (unpublished data), the corresponding correlation was 0.99.

In this study, the Dutch version of the Social Responsiveness Scale was administered as part of a written questionnaire on the child's behavior and growth around age 6 (8). The questionnaires were mailed to the parents. In 92% of cases, the questionnaires were filled out by the biological mother. Scores of questionnaires filled out by the mother were not significantly different from those filled out by fathers (*p*=.478). For individual items contributing to the Social Responsiveness Scale scores, a maximum of 25% missing items were allowed. Total scores were weighted by the number of non-missing items. In all analyses, Social Responsiveness Scale scores were square root transformed to approach a normal distribution.

Magnetic Resonance Imaging

Structural MRI scans were obtained on a 3-Tesla scanner (Discovery MR750, GE Worldwide, Milwaukee, USA). Using an 8-channel head coil, a whole-brain high-resolution T_1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained. The scan parameters were: TR = 10.3 ms, TE = 4.2 ms, flip angle = 16º, 186 contiguous slices with a thickness of 0.9 mm, and in-plane resolution = 0.9 × 0.9 mm.

All T_1-weighted scans were rated on a 6-item scale for quality (unusable, poor, fairly good, good, very good, excellent). Scans rated as 'fairly good' or better were included. After processing by FreeSurfer, all images were again visually inspected to rate the segmentation quality. Processed data rated as unusable or poor was excluded from analyses, as well as the subjects for whom the required output could not be constructed.

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite version 5.1 (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures have been fully described in prior publications (9). Briefly, cortical thickness was calculated as the closest distance from the gray/white matter boundary of the cortex to the gray matter/cerebral spinal fluid boundary at the cortical vertex for each tessellated surface (10). Thickness maps were smoothed with a 10 mm full-width half-maximum (FWHM) Gaussian kernel prior to statistical analysis. Numerous studies using FreeSurfer in typical and atypical developing school-age children are available (11).

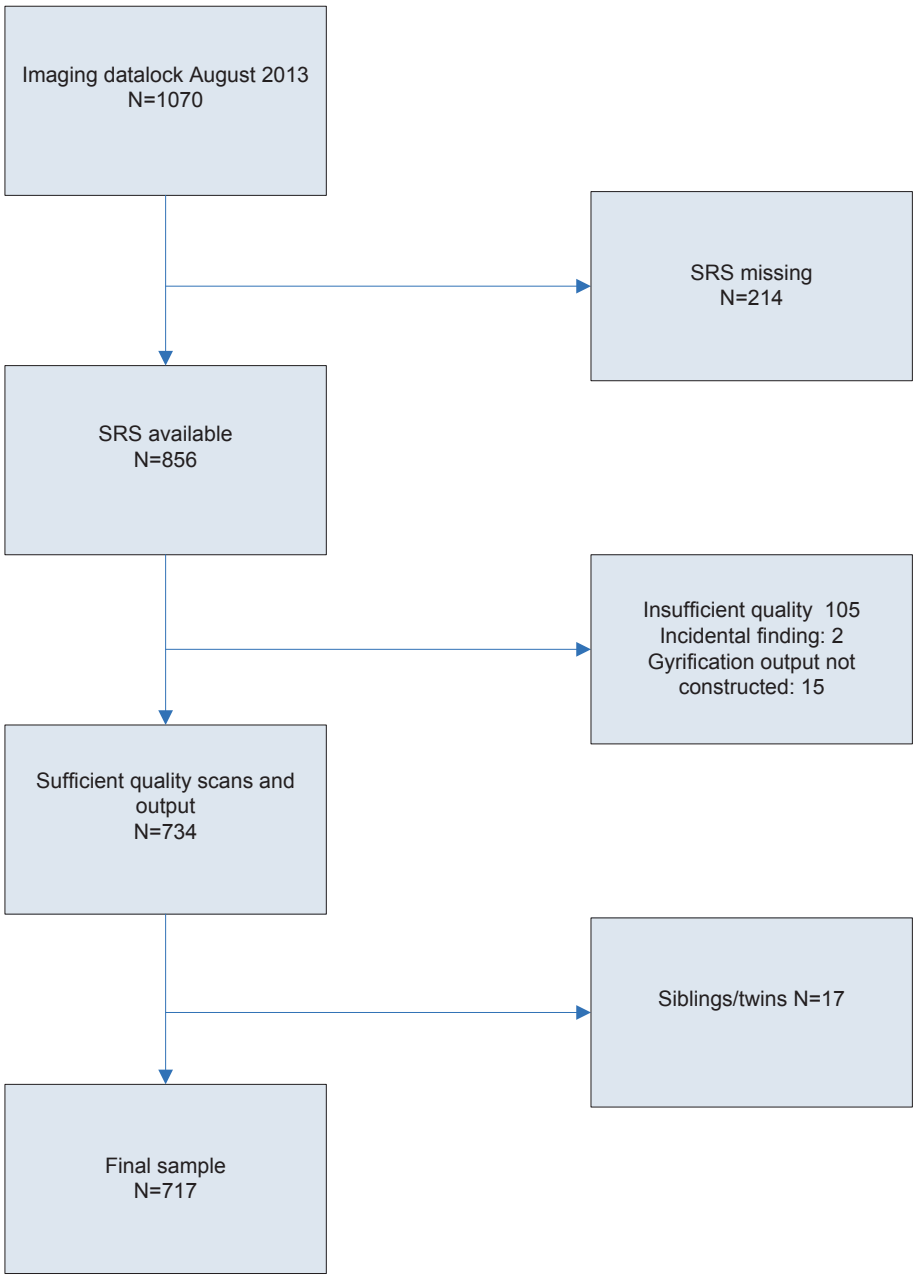
To assess the local gyrification index (LGI) we used the method of Schaer et al. (12), that is implemented in FreeSurfer. This approach provides an estimation of the local gyrification index, taking into account the three-dimensional cortical surface. Identification of the pial

and white matter surfaces against an additional surface that tightly wraps the pial surface are used to estimate the degree of cortical folding at a 25 mm spherical vertex-based region. This method has been validated and used in several studies focusing on childhood and adolescent psychopathology (13, 14). The surface based LGI maps were smoothed prior to the analyses using a 5 mm full-width half-maximum (FWHM) Gaussian kernel, consistent with several comparable studies (13).

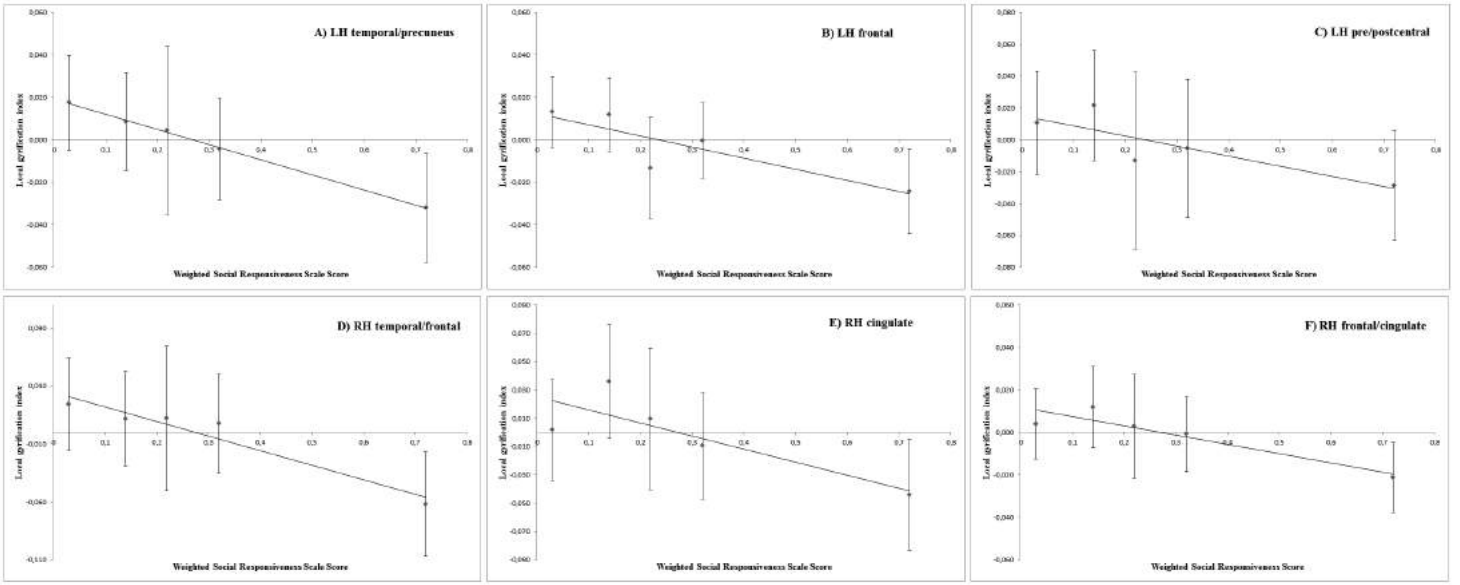
SUPPLEMENTARY REFERENCES

1. Allochtonen in Nederland. Voorburg/Heerlen: Amsterdam University Press; 2004.
2. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971;9(1):97-113.
3. Netherlands S. Standaardonderwijsindeling. Voorburg/Heerlen2004.
4. Grzadzinski R, Di Martino A, Brady E, Mairena MA, O'Neale M, Petkova E, Lord C, Castellanos FX. Examining autistic traits in children with ADHD: does the autism spectrum extend to ADHD? J Autism Dev Disord. 2011;41(9):1178-91.
5. Tellegen PJ W-WB, Laros JA. Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2.5- 7/. Amsterdam: Boom Testuitgevers; 2005.
6. Constantino JN. Social Responsiveness Scale (SRS), Manual. Los Angeles: Western Psychological services; 2002.
7. Charman T, Baird G, Simonoff E, Loucas T, Chandler S, Meldrum D, Pickles A. Efficacy of three screening instruments in the identification of autistic-spectrum disorders. Brit J Psychiat. 2007;191:554-9.
8. Roeyers H TM, Druart C, De Schryver, M, & Schittekate, M. SRS Screeningslijst voor autismespectrumstoornissen. Handleiding Amsterdam: Hogrefe; 2011.
9. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis- I. Segmentation and surface reconstruction. Neuroimage. 1999;9(2):179-94.
10. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. P Natl Acad Sci USA. 2000;97(20):11050-5.
11. Ghosh SS, Kakunoori S, Augustinack J, Nieto-Castanon A, Kovelman I, Gaab N, Christodoulou JA, Triantafyllou C, Gabrieli JD, Fischl B. Evaluating the validity of volume-based and surface-based brain image registration for developmental cognitive neuroscience studies in children 4 to 11 years of age. Neuroimage. 2010;53(1):85-93.
12. Schaer M, Ottet MC, Scariati E, Dukes D, Franchini M, Eliez S, Glaser B. Decreased frontal gyrification correlates with altered connectivity in children with autism. Frontiers in Human Neuroscience. 2013;7.
13. Wallace GL, Robustelli B, Dankner N, Kenworthy L, Giedd JN, Martin A. Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders. Brain. 2013;136:1956-67.
14. Kelly PA, Viding E, Wallace GL, Schaer M, De Brito SA, Robustelli B, McCrory EJ. Cortical Thickness, Surface Area, and Gyrification Abnormalities in Children Exposed to Maltreatment: Neural Markers of Vulnerability? Biol Psychiat. 2013;74(11):845-52.

Supplementary Figure 1. Flowchart

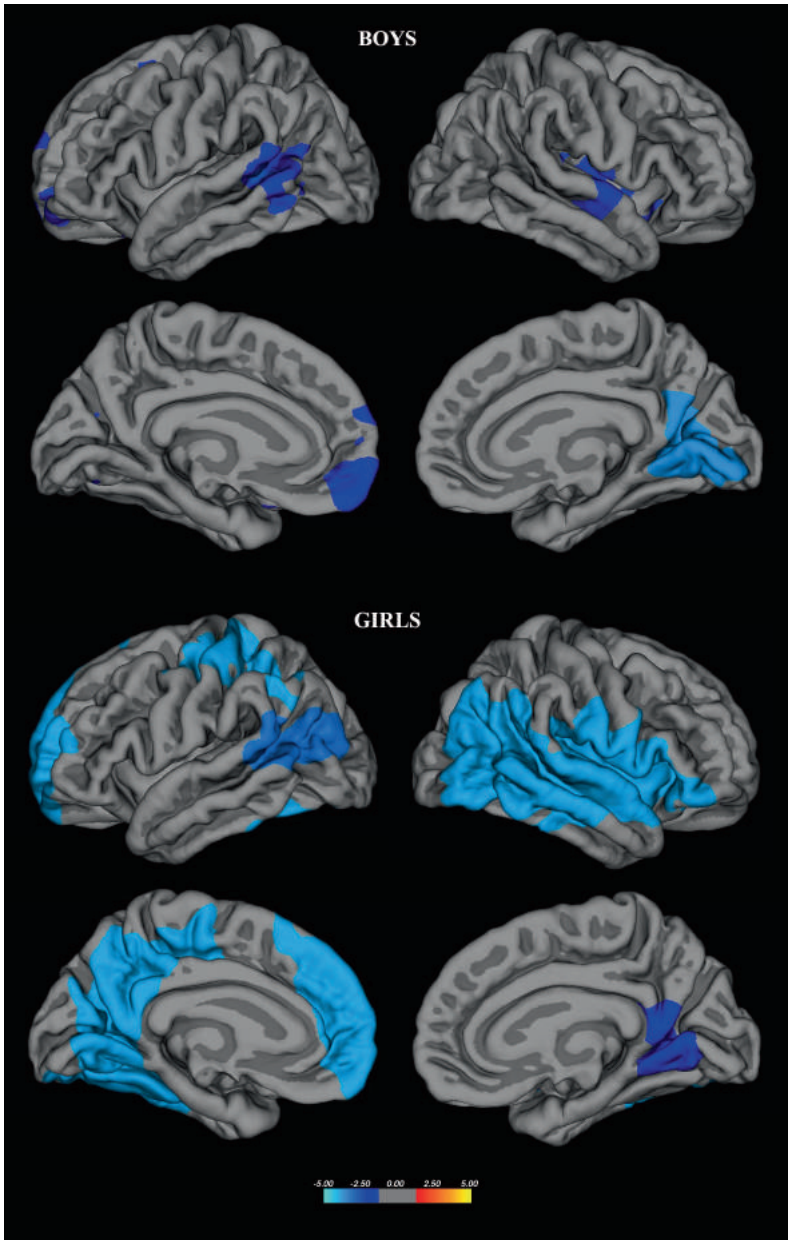


Supplementary Figure 2. Plots of age-residualized local gyrification indices against quintiles of Social Responsiveness Score

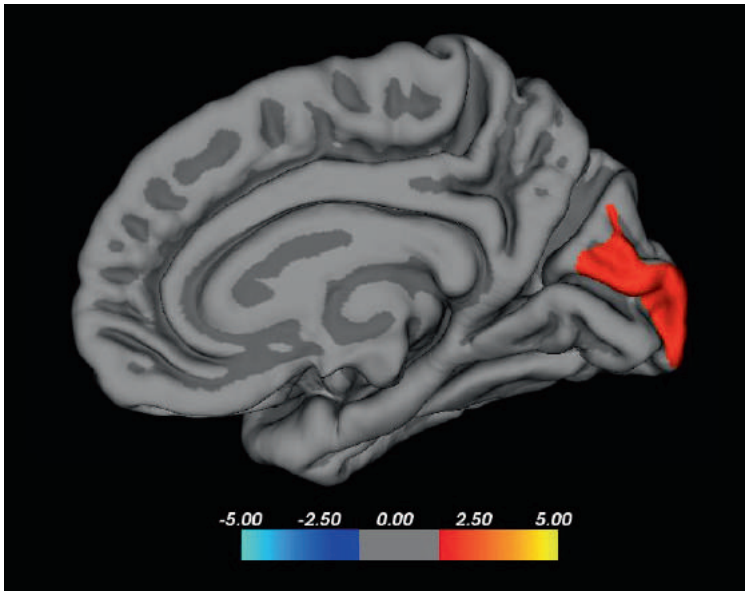


Note. Means and 95% confidence intervals of Local Gyrification Indices are plotted against mean Social Responsiveness Scores per quintile. This is why distances are not equal.

Supplementary Figure 3. Gyrification and autistic traits in the full sample, boys and girls shown separately.

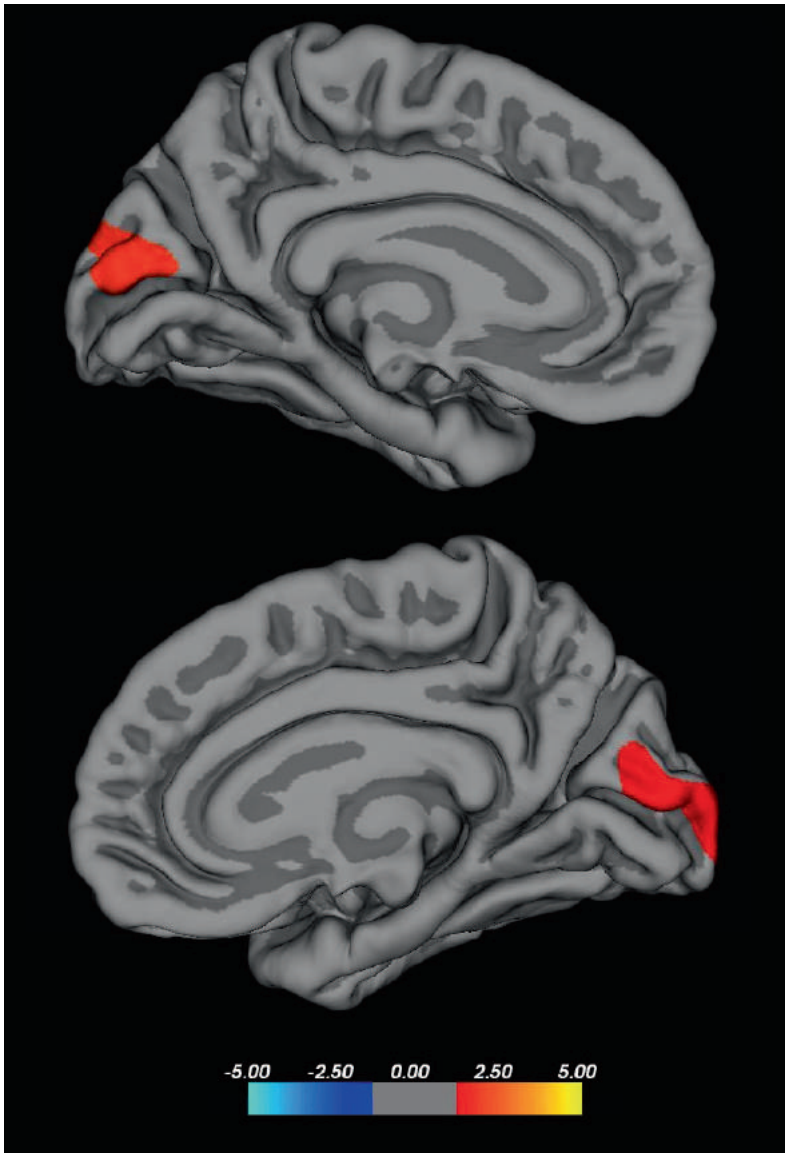


Supplementary Figure 4. Cortical thickness and autistic traits in boys (right hemisphere)



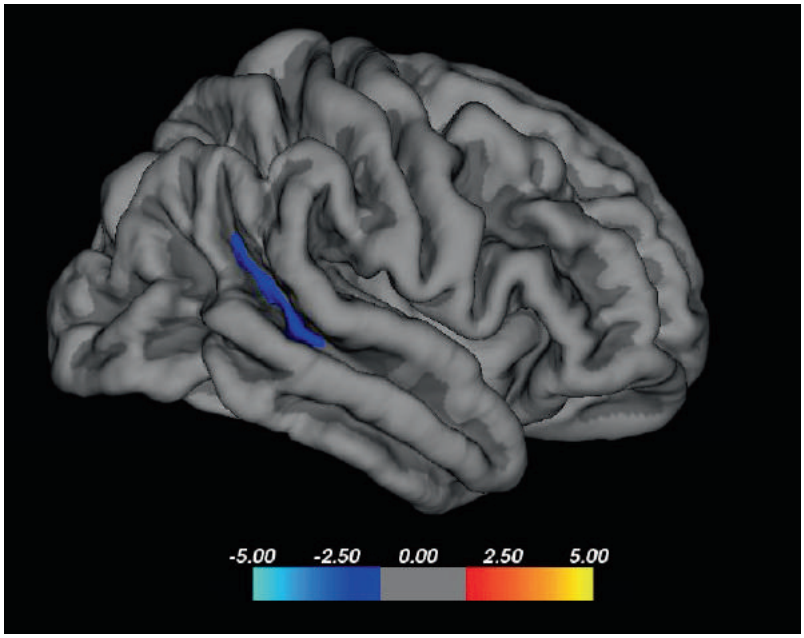
Note. Analyses were adjusted for age. Colors represent the –log(p-value). Red clusters represent a positive correlation.

Supplementary Figure 5. Cortical volume and autistic traits.



Note. Analyses were adjusted for age. Colors represent the –log(p-value). Red clusters represent a positive correlation.

Supplementary Figure 6. Sulcal depth and autistic traits.



Note. Analyses were adjusted for age and in the full sample also for gender. Colors represent the -log10(p-value). Blue clusters represent a negative correlation with autistic traits.

Supplementary Table 1. Non-response analysis

Child characteristics	**Filled out SRS questionnaire (n=5298)**					**Imaging first wave of datacollection (n=1070)**									
						Current study sample (n=717)					**Not in sample (n=353)**				
	observations					**observations**					**observations**				
Ethnicity (%)	5275					717					353				
Dutch		65.3					74.3					54.7			
Other Western		9.4					6.7					8.5			
Non-Western		25.3					19.0					36.8			
		mean	**SD**	**range**			**mean**	**SD**	**range**			**mean**	**SD**	**range**	
Social Responsiveness Scale	5043					717					139				
weighted score		0.23	0.25	0	3		0.27	0.29	0	3		0.35		0	3
Age at SRS (years)	5298	6.18	0.49	4.89	8.90	717	6.17	0.47	4.89	8.90	157	5.19	0.47	6.22	8.9
Child Behavior Checklist	5043					704					271				
attention problems score		1.46	1.69	0	10		1.98	2.05	0	9		2.48	2.48	0	9
IQ (non-verbal)	4444	102.69	14.69	50	150	663	102.86	14.42	50	142	319	99.31	14.52	56	139
Maternal characteristics															
Education level (%)	5054					698					273				
High		60.9					58.6					16.5			
Medium		28.5					30.2					38.1			
Low		10.6					11.2					16.5			
Monthly household income (%)	4784					680					257				
High		80.5					80.7					60.3			
Medium		14.1					14.3					25.7			
Low		5.4					4.7					14.0			

Supplementary Table 2. Total brain volume corrected analyses (n=717)

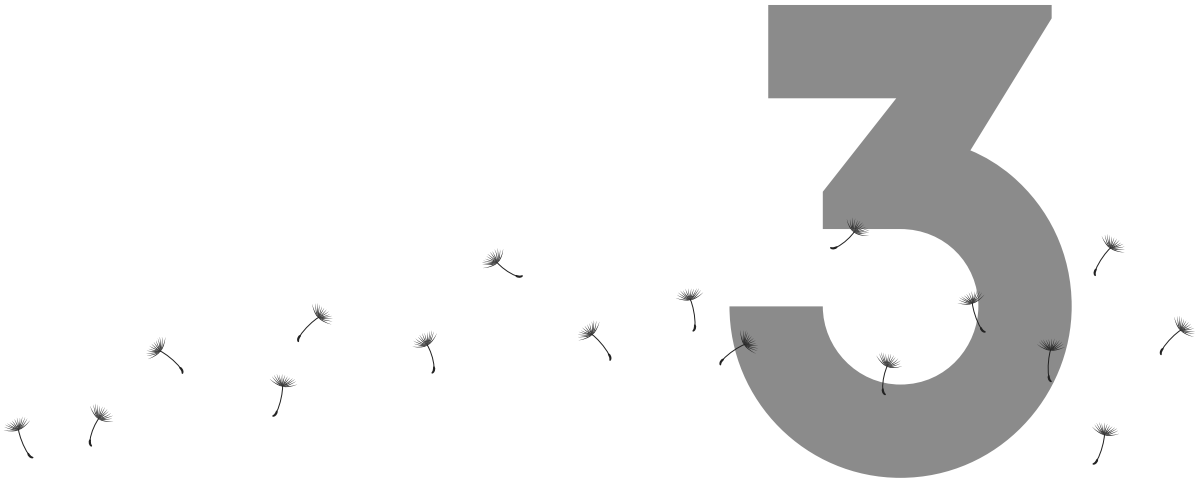
			B	SE B	*p*	β
Left Hemisphere						
	Temporal/precuneus	Model 1	-0.202	0.049	.000	-0.150
		Model 2 (adjusted)	-0.133	0.051	.009	-0.099
	Frontal	Model 1	-0.135	0.037	.000	-0.137
		Model 2 (adjusted)	-0.080	0.037	.032	-0.080
	Pre/postcentral	Model 1	-0.197	0.075	.009	-0.097
		Model 2 (adjusted)	-0.092	0.079	.243	-0.045
Right Hemisphere						
	Temporal/frontal	Model 1	-0.361	0.086	.000	-0.153
		Model 2 (adjusted)	-0.219	0.077	.004	-0.093
	Cingulate	Model 1	-0.304	0.078	.000	-0.143
		Model 2 (adjusted)	-0.204	0.087	.019	-0.096
	Frontal/cingulate	Model 1	-0.110	0.037	.003	-0.112
		Model 2 (adjusted)	-0.056	0.039	.148	-0.057

Note. Local gyrification indices were residualized for age at scanning. Model 1 adjusted for age when Social Responsiveness Scale was completed and gender. Model 2 additionally adjusted for child ethnicity, maternal education, maternal alcohol use, maternal smoking, Child Behavior Checklist attention problem scores, non-verbal IQ and total brain volume.

Supplementary Table 3. Case control analyses of ADI-R/ADOS confirmed ASD cases (n=6, all male) vs. age and gender-matched controls (n=24)

		Mean ASD	Mean control	t	*p*
Left Hemisphere					
	Precuneus	0.01	0.07	1.01	.321
	Superior Frontal	0.07	0.04	-0.70	.492
	Precentral	0.01	0.04	0.29	.776
Right Hemisphere					
	Temporal	0.01	0.11	0.81	.427
	Posterior Cingulate	0.01	0.16	0.98	.335
	Superior Frontal	0.04	0.05	0.20	.847

Note. Local gyrification indices were residualized for age at scanning in all analyses.



A Prospective Study of Fetal Head Growth in Children, Autistic Traits and Autism Spectrum Disorder

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ABSTRACT

Background: Altered trajectories of brain growth are often reported in Autism Spectrum Disorder (ASD), particularly in the first year of life. However, less is known about prenatal head growth trajectories. The few studies available have small sample sizes, typically depend on retrospectively collected ultrasound data and do not all include repeated measurements of head circumference. While there is increasing support for the hypothesis that ASD lies at the extreme end of a continuum, and that characteristics of the underlying neurobiology may also form a continuum, there are no studies that investigate the association between fetal head growth and the spectrum of autistic symptom severity.

Objectives: The objective in the current study was to prospectively evaluate prenatal head growth in children from the general population who later develop autistic traits. A second objective was to compare prenatal head growth between children with clinically diagnosed ASD and typically developing children.

Methods: This study included 5,719 children participating in two large longitudinal prenatal cohorts, comprising 90 children with a confirmed diagnosis of ASD. Fetal head circumference (HC) was measured repeatedly during pregnancy using ultrasound. Autistic traits were measured prospectively with the Social Responsiveness Scale or the Autism-Spectrum Quotient. Longitudinal HC measurements at three time points were analyzed using Latent Growth curve models to assess the relationship of prenatal growth to autistic traits measured later in life.

Results: Our results show an inverse relationship between prenatal head growth and autistic traits later in life. A lower rate of growth in prenatal HC was associated with more autistic traits. No differences in head growth were found between children with ASD and controls.

Conclusion: To our knowledge, this is the first large population-based study to measure the association between prenatal head growth and autistic traits. Our results may indicate that prenatal head growth is involved in the development of autistic traits. Future research should clarify the mechanisms involved.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a developmental condition characterized by difficulties in social communication and by restrictive and repetitive behaviors. ASD is thought to be neurodevelopmental in origin and is associated with structural and functional brain abnormalities (Minshew and Williams 2007). Traditionally, studies of early brain growth in children with ASD have reported early brain overgrowth in the first year of life (Hazlett, Poe, Gerig, Smith, Provenzale et al. 2005). More recently, the existence and exact timing of early brain growth abnormalities have been disputed, as several studies used historic references and most study samples are affected by ascertainment bias (Raznahan, Wallace, Antezana, Greenstein, Lenroot et al. 2013). However, there seems to be at least some consensus that children with ASD show subtly accelerated head growth in early life, resulting in larger head circumference (HC) and brain volume by the age of two (Piven, Arndt, Bailey and Andreasen 1996; Courchesne, Karns, Davis, Ziccardi, Carper et al. 2001; Constantino, Majmudar, Bottini, Arvin, Virkud et al. 2010; Green, Loesch and Dissanayake 2015). However, it remains unknown whether postnatal head overgrowth is the first manifestation of altered neurodevelopment, or whether changes start earlier. Postnatal overgrowth could reflect a reaction or adaptation to earlier growth patterns during prenatal life (Hobbs, Kennedy, Dubray, Bigler, Petersen et al. 2007). Very few studies have measured head growth in prenatal life in children subsequently diagnosed with ASD compared to those who develop typically. Two earlier studies identified no group-level differences in second trimester prenatal HC between children later diagnosed with ASD and controls (Hobbs et al. 2007; Whitehouse, Hickey, Stanley, Newnham and Pennell 2011). Similarly, a study of high-risk siblings of children with confirmed ASD revealed no differences in HC (Unwin, Maybery, Murphy, Lilje, Bellesini et al. 2015). However, these studies all had relatively small samples sizes (<50 participants with ASD or at high risk of ASD), and were either retrospective or did not include repeated measurements of prenatal HC.

An alternative methodology of studying the neurobiology of ASD involves the notion that symptoms of ASD lie on a severity continuum that extends into the general population (Constantino 2011). To our knowledge there have been no studies that focus on prenatal head growth related to ASD on a trait-level. Identifying the earliest manifestations of neurobiological changes in trait-level ASD may help to identify neurodevelopmental mechanisms that underlie the disorder. The aim of this study was to measure the association between prenatal head circumference growth trajectories and autistic symptoms in children. Secondly, we compared prenatal HC growth in a subset of 90 children who had a confirmed clinical diagnosis of ASD to HC growth who did not meet criteria for ASD.

To our knowledge, this is the first large-scale study to assess whether any atypical pattern in prenatal HC growth precedes the purported postnatal accelerated head growth linked to ASD. We used data from two large population-based longitudinal cohorts with prenatal

assessments, which enabled us to reduce both recruitment bias and bias associated with comparisons to standardized growth curve data (Raznahan et al. 2013). We hypothesized that alterations of fetal HC growth patterns would be related to later autistic traits. Second, we hypothesized that any atypical pattern of prenatal HC growth linked to mild autistic traits would be more pronounced in children who later meet diagnostic criteria for ASD, consistent with a continuum in the neurobiology of ASD.

METHODS

Participants

The current study involves participants from two large, population-based cohorts.

Generation R

The Generation R Study is a population-based cohort from the Netherlands from fetal life onwards (Verburg, Steegers, De Ridder, Snijders, Smith et al. 2008; Jaddoe, van Duijn, Franco, van der Heijden, van IJzendoorn et al. 2012). The total cohort includes 8,879 mothers who were recruited prior to 18 weeks gestation, with recruitment occurring between April 2002 and January 2006. The current study sample includes 4,533 children, for whom information on autistic traits and at least one measure of prenatal HC were available. The Medical Ethics Committee of the Erasmus MC approved the study and written informed consent was obtained from the mothers.

Raine

The Western Australian Pregnancy Cohort (Raine) Study is a longitudinal cohort of women recruited prior to 18 weeks gestation from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics, between May 1989 and November 1991 (Newnham, Evans, Michael, Stanley and Landau 1993). To these women, 2,868 children were born and available for postnatal follow-up. For 1,186 children, information on autistic traits was available, as well as at least one prenatal HC measurement. Participant recruitment from the study families was approved by the Human Ethics Committee at King Edward Memorial Hospital. Ethical approval for the 20-year follow-up was received from the Human Research Ethics Committee at the University of Western Australia. Participants provided written informed consent for data collection on autistic traits at approximately 20 years of age.

Prenatal head size and growth

Generation R

Fetal ultrasound measurements were performed during the first (mean age 13.5 weeks), second (mean age 20.6 weeks), and third trimester (mean age 30.5 weeks) of pregnancy (Verburg et al. 2008; Jaddoe et al. 2012). Crown-to-rump length was used for pregnancy dating until a gestational age of 12 weeks and 5 days, and biparietal diameter for pregnancy dating thereafter. Up to three HC measurements were available. The intra-observer and inter-observer reliabilities of fetal biometry in early pregnancy were excellent (all intra-class correlation coefficients greater than 0.99) (Verburg et al. 2008).

Raine

All pregnant women enrolled in the Raine Study underwent fetal ultrasound measurement at or close to 18 weeks gestation, as part of a previously described RCT (Newnham et al. 1993), about half of the participating women were randomly allocated to an intensive ultrasound scheme (including ultrasounds at approximately 18 weeks, and then at 24, 28, 34 and 38 weeks), whereas the other half followed a less intensive scheme, with one ultrasound at 18 weeks (Stoch, Williams, Granich, Hunt, Landau et al. 2012). Gestational age was calculated from the date of the last menstrual period and confirmed via ultrasound biometrics at 18 weeks. Up to 13 HC measurements were available. Ultrasound examinations were completed by a qualified sonographer using one of two General Electric 3600 machines (Milwaukee, USA) with 3.5 MHz linear array and 5 MHz sector transducers.

Autistic traits

Generation R

When the children were 6 years of age, their mothers filled out the Social Responsiveness Scale (SRS), which is a questionnaire of autistic traits for children between 4-18 years of age (Constantino, Przybeck, Friesen and Todd 2000; Constantino 2005). It represents the parent's observation of the child's social behavior during the previous six months. Each item is scored from 0 ('never true') to 3 ('almost always true'). Higher total scores indicate more autistic traits. The data collected within the Generation R Study included an abbreviated version of the SRS with a total of 18 items (Roman, Ghassabian, Bongers-Schokking, Jaddoe, Hofman et al. 2013). The correlation between total scores derived by the SRS short-form and the complete SRS in the Missouri Twin Study (Constantino and Todd 2003) was 0.93 in monozygotic male twins (n = 98) and 0.94 in dizygotic male twins (n = 134).

Raine

At the 20-year follow-up, Raine Study participants were asked to complete the Autism Spectrum Quotient (AQ). The AQ is a self-report questionnaire that provides a quantitative measure of autistic traits in the general population (Baron-Cohen, Wheelwright, Skinner, Martin and Clubley 2001). Subjects were provided with 50 statements and asked to indicate on a 4-point scale how well each statement applies to them (strongly agree, agree, disagree, strongly disagree), and scores are coded such that higher total scores indicate a greater level of autistic-like traits (Baron-Cohen et al. 2001). The total AQ is known to have good test–retest reliability (r = 0.7), and validation studies have found that scores in the general population follow a normal distribution (Baron-Cohen et al. 2001; Whitehouse, Hickey and Ronald 2011; Ruzich, Allison, Smith, Watson, Auyeung et al. 2015).

For both the SRS and the AQ, item scores were summed to obtain total scores, where a maximum of 25% missing items were allowed and scores were weighted for the number of items completed, square-root transformed to approach normality and z-scored to facilitate comparison between cohorts.

Clinical ASD diagnoses

Generation R

In the Generation R Study, medical records were examined for children who scored screen-positive for ASD in one or more of several stages of a multifaceted screening procedure. If a potential diagnosis of ASD could be confirmed through the medical records, the child was considered a clinically confirmed case of ASD. In the Netherlands, the general practitioners hold the central medical records, including information on treatment by medical specialists. A diagnosis of ASD is generally based on clinical consensus by a specialized multidisciplinary team. The diagnostic workup typically involves an extensive developmental case history obtained from parents, as well as school information and repeated observations of the child.

To the aim of checking general practitioners' records, we selected those children for which one of three sources of information signaled possible ASD. All children were formally screened with the SRS. The authors of the scale recommend cut-offs for screening in population-based settings, consistent with short-form SRS weighted scores of 1.078 for boys and 1.000 for girls (Constantino 2002). In addition, to rule out false negatives, children who scored in the top 15% on the total score of the Child Behavior Checklist (1.5-5) underwent a more specific screening using the Social Communication Questionnaire (SCQ), a 40-item parent-reported screening instrument for ASD (Berument, Rutter, Lord, Pickles and Bailey 1999). Scores of 15 or above on the SCQ were considered screen-positive (Berument et al. 1999). Further, psychiatric diagnoses and treatment were routinely assessed at contact moments between ages 6 to 9 (center visits and questionnaires). Within the Generation R study, 86 children who were screen-positive, but for whom a diagnosis could not be confirmed were excluded from

the control group. In the final sample, there were 75 children with medical-record confirmed ASD and at least one ultrasound. Children with SRS data and at least one ultrasound (n=4,409) were considered controls.

Raine

At the 5-, 8-, 10-, 13- and 16-year follow-ups of the Raine Study, parents were asked whether their child had ever received a diagnosis of ASD by a health professional.

Diagnosis of ASD in Western Australia mandates consensus by a team comprising a pediatrician, psychologist and speech-language pathologist under DSM guidelines (American Psychiatric Association 1994). Parent report indicated that 16 children in the Raine cohort had received a diagnosis of ASD, and at least one ultrasound was available for 15 of them. All other children with AQ data and at least one ultrasound (n=1,177) were considered controls.

Covariates

Covariates were carefully chosen based on factors known to influence prenatal head growth and ASD. In both studies, information on maternal age at the time of recruitment in early pregnancy, ethnicity, education, prenatal smoking and alcohol use was obtained by self-report questionnaires.

In the Generation R Study, maternal ethnicity was categorized into Dutch, other Western, and non-Western based on the country of birth of the mother's parents (Statistics 2004). Maternal educational level was categorized in three levels: primary, secondary, and higher education (Netherlands 2003). Prenatal smoking and alcohol use were categorized into 'No', 'Until pregnancy was known' and 'Continued during pregnancy', based on the information of repeated questionnaires (Roza, Verburg, Jaddoe, Hofman, Mackenbach et al. 2007).

In the Raine Study, ethnicity was categorized as Caucasian, Asian (Chinese, Indian, or Vietnamese) and Aboriginal or Pacific Islander (including Polynesian). Maternal educational level was categorized in six levels: none, trade certificate, professional registration, college diploma, university degree or other. Information on maternal alcohol use during pregnancy was categorized in zero, once a week or less and several times a week. Maternal smoking was categorized as zero, 1-10 cigarettes or more than 11 cigarettes per day.

Data processing

HC measurements were transformed to gestational-age adjusted SD scores. In the Generation R Study, we used study-specific reference curves for this purpose (Verburg et al. 2008). In the Raine Study, we used norms from the Australasian Society for ultrasound in Medicine to calculate gestational age-adjusted SD scores (2001).

Statistical analysis

Head circumference growth

We assessed the relation between repeatedly measured HC and autistic traits using latent growth curve modeling. Latent growth curve models consider change over time through underlying latent growth parameters (e.g., intercept and linear slope), and capture individual variations around these growth parameters as random effects. Within this modelling framework, trajectories of growth can be related to an outcome of interest. In these analyses, all participants with a valid measurement of autistic traits and at least one HC measurement were included. Participants in the Generation R study had a maximum of three HC measurements, whereas the Raine study had up to 13 measurements. We used up to three measurements for the Raine Study for consistency and to avoid confounding by indication introduced by extra measurements. Where we had additional measurements, we chose measurements that were closest to the Generation R trimester-mean in terms of gestational age. From the repeated measures of SD scores of fetal HC, two components of growth were estimated (an intercept, which indicates initial level and a linear slope, which denotes the growth). The time intervals between repeated ultrasound measurements were fixed at group level for each of the cohorts. Latent growth curve analyses were performed with Mplus version 7.31 for the latent growth curve analyses (Muthén and Muthén 1998-2012). To account for the skewness of the included variables, a maximum likelihood estimator with robust standard errors was used. We used full information maximum likelihood estimation in Mplus, which accommodates missing values in the analyses.

In addition to predicting autistic traits, we also examined whether the growth components predicted a diagnosis of ASD. For this analysis, intercept and slope were related to the dichotomous outcome ASD (yes/no). Any observed associations were further evaluated in a sensitivity analysis to assess whether any effect was specific to HC or indicative of overall body size. To this end, we examined 'difference scores' calculated by subtracting z-scores for femur length (FL) from HC.

Head circumference

In addition, we tested whether HC at any of three time points in pregnancy (early, mid, or late pregnancy) was associated with later autistic traits. For each time point in pregnancy, we assessed the relation between HC and the continuous measure of autistic traits, using linear regression with autistic traits as the dependent variable.

Similarly, we tested if there were differences in HC in children later diagnosed with ASD compared to those with no diagnosis. This was tested using logistic regressions models for each time point in pregnancy, with ASD (yes/no) as the dichotomous outcome. Covariates in each of these analyses were as described above. Missing values of covariates were imputed using multiple imputation, with 10 imputed datasets. The analyses and imputations were performed using SPSS version 21. (SPSS Inc., IBM Corp., Armonk, NY, USA).

Meta-analysis

Analyses were conducted separately for each cohort. Subsequently, a pooled estimate was obtained, taking into account sample size and direction of effect, using Metal (Willer, Li and Abecasis 2010).

RESULTS

Demographics

Table 1 presents characteristics of the study samples from both cohorts used for the main analyses.

Non-response analysis

In the Generation R Study, characteristics of mothers of 4,533 children included in the analyses were compared to those of mothers of 4,201 children excluded due to missing SRS data. The excluded mothers were younger (28.0 ± 5.5 years vs. 31.1 ± 4.6, t = 28.5, p < .001), less well educated (38.1% vs. 61.3% higher education; χ^2 = 221.2, p < .001) and less likely to be of Dutch origin (31.8% vs. 62.7% Dutch; χ^2 = 1002.4, p <.001) than the included mothers. In addition, the excluded mothers smoked more often during pregnancy (21.9% vs. 13.2% continued smoking in pregnancy; χ^2 = 110.9, p < .001).

Similarly, in the Raine Study, the characteristics of mothers of 1,186 children included in the analyses were compared to those of the 1,523 mothers excluded due to missing AQ data. The excluded mothers were younger (27.0 ± 5.8 years vs. 29.1 ± 5.6, t = 9.5, p <.001), less well educated (14.4% vs. 18.0% had a college diploma or degree; χ^2 = 109.2, p < .001) and somewhat less likely to be of Caucasian origin (89.7% vs. 91.9% Caucasian; χ^2 = 14.6, p =.001) than the included mothers. In addition, the excluded mothers smoked more often during pregnancy (14.9% vs. 9.5% smoked more than 11 cigarettes a day in pregnancy; χ^2 = 48.0, p <.001).

Table 1. Participant characteristics.

Child characteristics				
Generation R			**Raine**	
n=5,433			**n=1,186**	
Gender (% boy)		49.9	Gender (% boy)	52.1
Ethnicity (%)			Ethnicity (%)	
	Dutch	65.2	Caucasian	91.9
	Other Western	9.3	Asian	5.2
	Non-Western	25.5	Aboriginal/Pacific Islander	2.9
Social Responsiveness Scale			Autism Quotient	
	Weighted total score, mean (SD)	0.23 (0.25)	Weighted total score, mean (SD)	1.44 (0.09)
Age at Social Responsiveness Scale (years), mean (SD)		6.17 (0.49)	Age at Autism Quotient (years), mean (SD)	19.78 (0.73)
Number of ultrasounds (%)			Number of ultrasounds (%)	
	one	12.7	one	37.7
	two	27.0	two	24.5
	three	56.9	three	37.8
Maternal characteristics				
Generation R			**Raine**	
Age during pregnancy (years), mean (SD)		31.1 (4.6)	Age during pregnancy (years), mean (SD)	29.1 (5.6)
Education level (%)			Education level (%)	
	High	61.3	None	43.3
	Medium	28.3	Trade certificate	7.6
	Low	10.4	Professional registration	12.1
			College diploma/degree	18.0
			University degree	13.2
			other	5.6
Alcohol in pregnancy (%)			Alcohol in pregnancy (%)	
	Never	38.3	Zero	59.6
	Until pregnancy was known	14.0	Once a week or less	35.4
	Continued occasionally	37.5	Several times a week or more	5.1
	Continued frequently	10.2		
Smoking in pregnancy (%)			Smoking in pregnancy (%)	
	Never	79.2	Zero	81.0
	Until pregnancy was known	7.6	1-10 cigarettes	9.4
	Continued	13.2	11+ cigarettes	9.5

Prenatal head growth and autistic traits

We examined whether fetal HC growth trajectories were related to later autistic traits (Table 2A). This was addressed in a separate model for each of the cohorts, in which autistic trait scores were regressed on the latent growth parameters of gestational-age-adjusted SD scores of HC. Models were adjusted for sex, ethnicity of the child, maternal educational level, maternal age, maternal alcohol use and maternal smoking during pregnancy. These models had good fit indices: comparative fit index = 0.987 and root mean square error of approximation = 0.021 for the Generation R Study and comparative fit index = 0.966 and root mean square error of approximation = 0.029 for the Raine Study. We found evidence for a consistent negative association of the slope of growth (i.e., growth rate) with autistic traits when the results for both cohorts were pooled (pooled z =-2.20, p = .03).

For the Generation R Study, in the fully adjusted model, the intercept and slope of HC growth during pregnancy were negatively associated with autistic traits: children with growth trajectories characterized by a lower slope and intercept had more autistic traits at age 6 (β slope=-0.043, p = .04, β intercept=-0.049, p = .03). For the Raine Study, we found the same direction of effect for the association of the slope of growth with autistic traits, although not statistically significant (β slope=-0.075, p = .41). Since postnatal studies of children with ASD have found both larger and smaller HC, we tested whether the relation between growth and autistic traits was nonlinear. For both studies, the addition of quadratic terms of the growth parameters did not add significantly to the models. In a sensitivity analysis, we assessed whether this effect was specific to HC by examining trajectories of HC-FL difference scores. Results were broadly consistent (pooled z =-2.11, p = .04).

In separate cross-sectional analyses for each cohort, HC in mid- and late pregnancy, but not early pregnancy, were related to autistic traits (Table 2B). Pooled results indicate that children with smaller HC in mid- and late pregnancy had higher levels of autistic traits later in life (mid pregnancy: pooled z =-2.19, p = .03, late pregnancy pooled z =-2.11, p =.04). When examining difference scores, results were consistent (pooled z =-2.79, p <.01 for mid pregnancy and pooled z =-3.66, p <.001 for late pregnancy).

Prenatal head circumference growth in children with ASD

Evaluating prenatal HC growth trajectories of children who were later diagnosed with ASD, we found no association between prenatal HC growth and a diagnosis of ASD (Table 3A) (Intercept: pooled z =-0.08, p = .94, Slope: pooled z = 0.48, p = .63). Similarly, there was no association between HC at any of three time points in pregnancy and an ASD diagnosis (Table 3B) (all p-values >.05).

Table 2. Prenatal head growth and autistic traits later in life

A. Head growth	**Generation R (SRS)**	**Raine (AQ)**	**Pooled z-statistic**	**p**
	n = 4,533	n = 1,186		
Intercept (SE); p	-0.049 (0.023); .03	0.002 (0.043); .96	-1.90	.06
Slope (SE); p	-0.043 (0.021); .04	-0.075 (0.090); .41	-2.20	.03
B. Head size	**Generation R**	**Raine**	**Pooled z-statistic**	**p**
Early pregnancy	n = 3,108	n = 889		
B (SE), β	-0.024 (0.016),-0.026	0.123 (0.070), 0.059		
p	.13	.08	-0.51	.61
Mid pregnancy	n = 4,329	n = 753		
B (SE), β	-0.023 (0.014),-0.024	-0.131 (0.077),-0.063		
p	.10	.09	-2.19	.03
Late pregnancy	n = 4,407	n = 662		
B (SE), β	-0.039 (0.015),-0.038	0.067 (0.074), 0.037		
p	.01	.36	-2.11	.04

Note: A. Values are standardized regression coefficients from latent growth curve analyses on gestational age adjusted SD scores for head circumference (constructed using growth reference curves). Models were adjusted for age, sex, ethnicity of the child, maternal educational level, maternal age, maternal alcohol and maternal smoking. Fit indices for the Generation R model were RMSEA 0.021 and CFI 0.987. Fit indices for the Raine Study model were RMSEA: 0.029 and CFI 0.966.
B. Values are regression coefficients from linear regression analysis of autistic traits on gestational age adjusted SD scores for head size. Models were adjusted for ethnicity of the child, maternal educational level, maternal age, maternal alcohol and maternal smoking.

Table 3. Prenatal head growth and ASD

A. Head growth	**Generation R**	**Raine**	**Pooled z-statistic**	**p**
	n ASD/ n controls	*n ASD/ n controls*		
	75/4,409	15/1,177		
Intercept OR (95% CI)	1.04 (0.69; 1.57)	0.76 (0.30; 1.91)		
P	.86	.56	-0.08	.94
Slope OR (95% CI)	1.07 (0.80; 1.44)	0.21 (0.02; 1.90)		
P	.63	.16	0.48	.63
B. Head size	**Generation R**	**Raine**	**Pooled z-statistic**	**p**
	n ASD/ n controls	*n ASD/ n controls*		
Early pregnancy	52/3,024	13/881		
OR (95% CI)	0.86 (0.66; 1.11)	1.34 (0.43; 4.17)		
P	.25	.61	-0.81	.42
Mid pregnancy	72/4,210	11/745		
OR (95% CI)	1.16; (0.92;1.46)	0.62 (0.18; 2.10)		
P	.20	.44	0.91	.36
Late pregnancy	72/4,287	10/656		
OR (95% CI)	1.03 (0.81; 1.31)	0.62 (0.19; 2.05)		
P	.81	.43	-0.05	.96

Note: A. Values are standardized Odd's ratios from latent growth curve analyses of ASD on gestational age adjusted SD scores for head circumference. Regression coefficients and Odd's ratios are standardized for slope and intercept. Models were adjusted for sex, ethnicity of the child, maternal educational level, maternal age, maternal alcohol and maternal smoking.
B. Values are Odds ratio's from logistic regression analysis of ASD on gestational age adjusted SD scores for head size. Models were adjusted for sex, ethnicity of the child, maternal educational level, maternal age, maternal alcohol and maternal smoking.

DISCUSSION

In a study based on two large prenatal cohorts, we found that children with slower fetal HC growth rates had more autistic traits as measured postnatally. However, these findings were not replicated when we examined children with a clinical diagnosis of ASD.

Our finding of slower HC growth in children with autistic traits is consistent with several small studies reporting slightly smaller HC at birth in children later diagnosed with ASD (Courchesne, Carper and Akshoomoff 2003; Courchesne, Pierce, Schumann, Redcay, Buckwalter et al. 2007). Slower head growth prenatally may reflect aberrant early brain development consistent with later findings in childhood ASD that include altered morphology of gray and white matter, as well as aberrant connectivity (Parellada, Penzol, Pina, Moreno, Gonzalez-Vioque et al. 2014). Of note, a postmortem study of children with ASD showed local disorganization across different cortical layers suggestive of aberrant prenatal neuronal migration (Stoner, Chow, Boyle, Sunkin, Mouton et al. 2014).

Several biological mechanisms could underlie the association of prenatal HC growth with autistic traits. Environmental factors, such as maternal thyroid dysfunction or vitamin D deficiency may lead to autism-like symptoms through restriction of prenatal brain growth (Roman et al. 2013; Whitehouse, Holt, Serralha, Holt, Hart et al. 2013; Korevaar, Muetzel, Medici, Chaker, Jaddoe et al. 2016). Alternatively, autistic traits and prenatal HC growth may share a similar genetic background. Interestingly, evidence from histopathological studies showed high expression of ASD candidate genes in the fetal cortex, although the role of these genes in prenatal brain growth has not been established yet (Willsey, Sanders, Li, Dong, Tebbenkamp et al. 2013; Birnbaum, Jaffe, Hyde, Kleinman and Weinberger 2014).

Future studies should clarify whether our finding of a relation between prenatal head growth and autistic traits is indicative of a neurobiological pathway of ASD, or may reflect features of developmental delay not necessarily specific to ASD.

Considering the finding of an association between slower HC growth prenatally and increased autistic traits postnatally, we hypothesized that those children with an ASD diagnosis would also show an altered (slower) HC growth trajectory. As we did not detect such differences, our results do not fully support a continuum in the neurobiology of ASD in the context of HC, which would imply similar differences at the severe end. Such a continuum was hypothesized based on evidence across genetic (Colvert, Tick, McEwen, Stewart, Curran et al. 2015), phenotypic (Constantino et al. 2003) and neuro-imaging studies (Blanken, Mous, Ghassabian, Muetzel, Schoemaker et al. 2015). However, autistic traits and ASD affect the brain at many levels. Some brain differences related to ASD may form a dose-response-type relation with autistic traits, such that the most severely affected patients have the most marked differences, while other characteristics may not exhibit such associations. There are several alternative explanations for the absence of this finding. While this was the largest

study to date on prenatal HC growth in clinically confirmed ASD, it may still be underpowered. Differences in HC, if present, may be subtle (Raznahan et al. 2013) and likely heterogeneous across subjects with ASD. In addition, the genetic background of ASD is heterogeneous, and likely comprises several pathways, only a subset of which may involve altered HC growth (Betancur 2011). Different variants may operate through distinct pathways, so that the relation between HC and ASD symptoms may depend on the specific genetic background. For example, in a study of postnatal head growth, a positive association of autistic symptoms with HC was found only in children with ASD classified as simplex and not in children with ASD from multiplex families (Davis, Keeney, Sikela and Hepburn 2013). It is possible that the ASD group includes a mixture of effects in both directions. Postnatally, both microcephaly and macrocephaly have been reported in so-called 'syndromic' ASD (Fombonne, Roge, Claverie, Courty and Fremolle 1999). Alternatively, it is possible that ASD is not characterized by gross differences in prenatal HC growth. This is consistent with several smaller studies that did not identify a difference in prenatal HC growth and at birth (Courchesne et al. 2007; Hobbs et al. 2007; Whitehouse et al. 2011). While dysregulation of brain development in ASD is undisputed, any prenatal head growth differences present in ASD may be more regional and thus not translate into global HC differences prenatally. While ultrasound measures provide reliable measures of HC, which is an excellent proxy for fetal brain size (Cooke, Lucas, Yudkin and Pryse-Davies 1977), more advanced methods, such as 3D ultrasound scans may provide more information about prenatal growth of specific structures in the brain.

The current study has a number of strengths. To our knowledge, this is the largest study to date of prenatal HC growth trajectories in children with ASD and the first time that a trait approach has been used in this context. Two prospective population-based cohort studies were involved, in which systematic and prospective data collection began during fetal life. We were able to study HC growth trajectories prospectively, beginning in fetal life and in the context of appropriate controls, thus avoiding the bias introduced by using published normative data (Raznahan et al. 2013). Further, we combined results obtained in these two independent cohort studies using a meta-analytic approach, which builds a form of replication into the study design. The HC measures were systematically measured using standardized research-based approaches at multiple time points during pregnancy (Verburg et al. 2008). Finally, the large sample size with repeated measurements allowed us to model growth curves with greater precision and to adjust for potential confounding variables.

We also acknowledge some limitations of the study. In both studies, the first ultrasound measurement was used for dating of the pregnancy in addition to the last menstrual period, which potentially masks very early growth differences related to ASD. Further, while there were study-specific growth curves available for the Generation R Study, we had no access to Raine Study-specific reference curves. However, we used common Australian guidelines to compute standardized scores for all children, including the children with ASD and controls.

Another potential limitation concerns the differential measurement of autistic traits across the two cohorts: while the two measures of autistic traits are aimed at measuring similar constructs, the age of administration (6 vs. 20) and the reporter (mother vs. self) differ, which potentially complicates direct comparison. However, autistic traits have been found to be relatively stable across development (Robinson, Munir, Munafo, Hughes, McCormick et al. 2011; Whitehouse et al. 2011) and despite the difference in age of outcome, associations with prenatal HC growth were similar across cohorts. Finally, both study samples were more likely to include mothers from more privileged families, with several characteristics that are generally beneficial for fetal growth. This could have affected our findings.

In conclusion, we present findings on prenatal HC growth trajectories in children with autistic symptoms from two independent, prospective, longitudinal prenatal cohort studies. We found that subjects with autistic symptoms slower HC growth during prenatal life, but found no differences in prenatal head growth for children with ASD. Our results indicate that prenatal growth could be important for the development of autistic traits. Future research should clarify the mechanisms involved.

REFERENCES

Australasian Society for Ultrasound in Medicine (2001): Guidelines, Policies and Statements D7: Statement on Normal Ultrasonic Fetal Measurements.

Baron-Cohen, S., S. Wheelwright, R. Skinner, J. Martin and E. Clubley (2001). "The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians." J Autism Dev Disord **31**(1): 5-17.

Berument, S. K., M. Rutter, C. Lord, A. Pickles and A. Bailey (1999). "Autism screening questionnaire: diagnostic validity." British Journal of Psychiatry **175**: 444-451.

Betancur, C. (2011). "Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting." Brain Res **1380**: 42-77.

Birnbaum, R., A. E. Jaffe, T. M. Hyde, J. E. Kleinman and D. R. Weinberger (2014). "Prenatal expression patterns of genes associated with neuropsychiatric disorders." Am J Psychiatry **171**(7): 758-767.

Blanken, L. M. E., S. E. Mous, A. Ghassabian, R. L. Muetzel, N. K. Schoemaker, H. El Marroun, A. van der Lugt, V. W. V. Jaddoe, A. Hofman, F. C. Verhulst, H. Tiemeier and T. White (2015). "Cortical Morphology in 6-to 10-Year Old Children With Autistic Traits: A Population-Based Neuroimaging Study." American Journal of Psychiatry **172**(5): 479-486.

Colvert, E., B. Tick, F. McEwen, C. Stewart, S. R. Curran, E. Woodhouse, N. Gillan, V. Hallett, S. Lietz, T. Garnett, A. Ronald, R. Plomin, F. Rijsdijk, F. Happe and P. Bolton (2015). "Heritability of Autism Spectrum Disorder in a UK Population-Based Twin Sample." JAMA Psychiatry **72**(5): 415-423.

Constantino, J. N. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Constantino, J. N. (2011). "The quantitative nature of autistic social impairment." Pediatr Res **69**(5 Pt 2): 55R-62R.

Constantino, J. N., Gruber, CP (2005). Social responsiveness scale (SRS); Manual. Los Angeles, Western Psychological Services.

Constantino, J. N., P. Majmudar, A. Bottini, M. Arvin, Y. Virkud, P. Simons and E. Spitznagel (2010). "Infant head growth in male siblings of children with and without autism spectrum disorders." Journal of neurodevelopmental disorders **2**(1): 39-46.

Constantino, J. N., T. Przybeck, D. Friesen and R. D. Todd (2000). "Reciprocal social behavior in children with and without pervasive developmental disorders." J Dev Behav Pediatr **21**(1): 2-11.

Constantino, J. N. and R. D. Todd (2003). "Autistic traits in the general population: a twin study." Archives of general psychiatry **60**(5): 524-530.

Cooke, R. W., A. Lucas, P. L. Yudkin and J. Pryse-Davies (1977). "Head circumference as an index of brain weight in the fetus and newborn." Early Hum Dev **1**(2): 145-149.

Courchesne, E., R. Carper and N. Akshoomoff (2003). "Evidence of brain overgrowth in the first year of life in autism." JAMA **290**(3): 337-344.

Courchesne, E., C. M. Karns, H. R. Davis, R. Ziccardi, R. A. Carper, Z. D. Tigue, H. J. Chisum, P. Moses, K. Pierce, C. Lord, A. J. Lincoln, S. Pizzo, L. Schreibman, R. H. Haas, N. A. Akshoomoff and R. Y. Courchesne (2001). "Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study." Neurology **57**(2): 245-254.

Courchesne, E., K. Pierce, C. M. Schumann, E. Redcay, J. A. Buckwalter, D. P. Kennedy and J. Morgan (2007). "Mapping early brain development in autism." Neuron **56**(2): 399-413.

Davis, J. M., J. G. Keeney, J. M. Sikela and S. Hepburn (2013). "Mode of genetic inheritance modifies the association of head circumference and autism-related symptoms: a cross-sectional study." PLoS One **8**(9): e74940.

Fombonne, E., B. Roge, J. Claverie, S. Courty and J. Fremolle (1999). "Microcephaly and macrocephaly in autism." J Autism Dev Disord **29**(2): 113-119.

Green, C., D. Z. Loesch and C. Dissanayake (2015). "A review of physical growth in children and adolescents with Autism Spectrum Disorder." Developmental Review **36**.

Hazlett, H. C., M. Poe, G. Gerig, R. G. Smith, J. Provenzale, A. Ross, J. Gilmore and J. Piven (2005). "Magnetic resonance Imaging and head circumference study of brain size in autism- Birth through age 2 years." Archives of General Psychiatry **62**(12): 1366-1376.

Hobbs, K., A. Kennedy, M. Dubray, E. D. Bigler, P. B. Petersen, W. McMahon and J. E. Lainhart (2007). "A retrospective fetal ultrasound study of brain size in autism." Biol Psychiatry **62**(9): 1048-1055.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." Eur J Epidemiol **27**(9): 739-756.

Korevaar, T. I., R. Muetzel, M. Medici, L. Chaker, V. W. Jaddoe, Y. B. de Rijke, E. A. Steegers, T. J. Visser, T. White, H. Tiemeier and R. P. Peeters (2016). "Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study." Lancet Diabetes Endocrinol **4**(1): 35-43.

Minshew, N. J. and D. L. Williams (2007). "The new neurobiology of autism: cortex, connectivity, and neuronal organization." Arch Neurol **64**(7): 945-950.

Muthén, L. K. and B. O. Muthén (1998-2012). Mplus User's Guide.. Los Angeles, CA.

Netherlands, S. (2003). "Standard classification of education 2003 (Standaard onderwijsindeling 2003)." Voorburg/Heerlen http://www.cbs.nl.

Newnham, J. P., S. F. Evans, C. A. Michael, F. J. Stanley and L. I. Landau (1993). "Effects of frequent ultrasound during pregnancy: a randomised controlled trial." Lancet **342**(8876): 887-891.

Parellada, M., M. J. Penzol, L. Pina, C. Moreno, E. Gonzalez-Vioque, G. Zalsman and C. Arango (2014). "The neurobiology of autism spectrum disorders." Eur Psychiatry **29**(1): 11-19.

Piven, J., S. Arndt, J. Bailey and N. Andreasen (1996). "Regional brain enlargement in autism: a magnetic resonance imaging study." Journal of the American Academy of Child and Adolescent Psychiatry **35**(4): 530-536.

Raznahan, A., G. L. Wallace, L. Antezana, D. Greenstein, R. Lenroot, A. Thurm, M. Gozzi, S. Spence, A. Martin, S. E. Swedo and J. N. Giedd (2013). "Compared to What? Early Brain Overgrowth in Autism and the Perils of Population Norms." Biological psychiatry.

Robinson, E. B., K. Munir, M. R. Munafo, M. Hughes, M. C. McCormick and K. C. Koenen (2011). "Stability of autistic traits in the general population: further evidence for a continuum of impairment." J Am Acad Child Adolesc Psychiatry **50**(4): 376-384.

Roman, G. C., A. Ghassabian, J. J. Bongers-Schokking, V. W. Jaddoe, A. Hofman, Y. B. de Rijke, F. C. Verhulst and H. Tiemeier (2013). "Association of gestational maternal hypothyroxinemia and increased autism risk." Annals of neurology.

Roza, S. J., B. O. Verburg, V. W. Jaddoe, A. Hofman, J. P. Mackenbach, E. A. Steegers, J. C. Witteman, F. C. Verhulst and H. Tiemeier (2007). "Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study." Eur J Neurosci **25**(3): 611-617.

Ruzich, E., C. Allison, P. Smith, P. Watson, B. Auyeung, H. Ring and S. Baron-Cohen (2015). "Measuring autistic traits in the general population: a systematic review of the Autism-Spectrum Quotient (AQ) in a nonclinical population sample of 6,900 typical adult males and females." Mol Autism **6**: 2.

Statistics, N. (2004). "Migrants in the Netherlands 2004 (Allochtonen in Nederland 2004)." Voorburg/Heerlen http://www.cbs.nl.

Stoch, Y. K., C. J. Williams, J. Granich, A. M. Hunt, L. I. Landau, J. P. Newnham and A. J. Whitehouse (2012). "Are prenatal ultrasound scans associated with the autism phenotype? Follow-up of a randomised controlled trial." J Autism Dev Disord **42**(12): 2693-2701.

Stoner, R., M. L. Chow, M. P. Boyle, S. M. Sunkin, P. R. Mouton, S. Roy, A. Wynshaw-Boris, S. A. Colamarino, E. S. Lein and E. Courchesne (2014). "Patches of Disorganization in the Neocortex of Children with Autism." New England Journal of Medicine **370**(13): 1209-1219.

Unwin, L. M., M. T. Maybery, A. Murphy, W. Lilje, M. Bellesini, A. M. Hunt, J. Granich, P. Jacoby, C. Dissanayake, C. E. Pennell, M. Hickey and A. J. Whitehouse (2015). "A Prospective Ultrasound Study of Prenatal Growth in Infant Siblings of Children With Autism." Autism Res.

Verburg, B. O., E. A. Steegers, M. De Ridder, R. J. Snijders, E. Smith, A. Hofman, H. A. Moll, V. W. Jaddoe and J. C. Witteman (2008). "New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study." Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology **31**(4): 388-396.

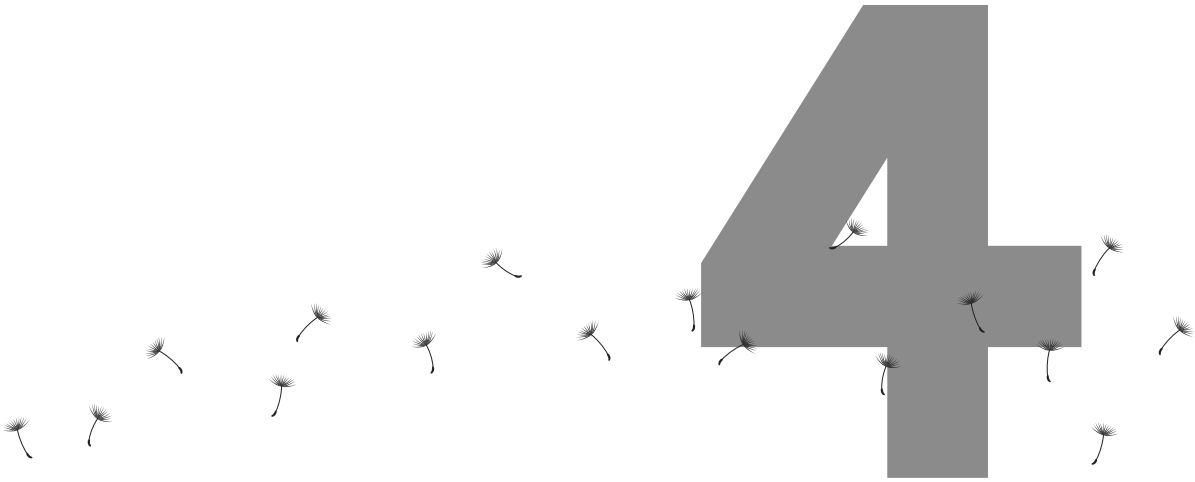
Whitehouse, A. J., M. Hickey and A. Ronald (2011). "Are autistic traits in the general population stable across development?" PLoS One **6**(8): e23029.

Whitehouse, A. J., B. J. Holt, M. Serralha, P. G. Holt, P. H. Hart and M. M. Kusel (2013). "Maternal vitamin D levels and the autism phenotype among offspring." J Autism Dev Disord **43**(7): 1495-1504.

Whitehouse, A. J. O., M. Hickey, F. J. Stanley, J. P. Newnham and C. E. Pennell (2011). "Brief Report: A Preliminary Study of Fetal Head Circumference Growth in Autism Spectrum Disorder." Journal of Autism and Developmental Disorders **41**(1): 122-129.

Willer, C. J., Y. Li and G. R. Abecasis (2010). "METAL: fast and efficient meta-analysis of genomewide association scans." Bioinformatics **26**(17): 2190-2191.

Willsey, A. J., S. J. Sanders, M. Li, S. Dong, A. T. Tebbenkamp, R. A. Muhle, S. K. Reilly, L. Lin, S. Fertuzinhos, J. A. Miller, M. T. Murtha, C. Bichsel, W. Niu, J. Cotney, A. G. Ercan-Sencicek, J. Gockley, A. R. Gupta, W. Han, X. He, E. J. Hoffman, L. Klei, J. Lei, W. Liu, L. Liu, C. Lu, X. Xu, Y. Zhu, S. M. Mane, E. S. Lein, L. Wei, J. P. Noonan, K. Roeder, B. Devlin, N. Sestan and M. W. State (2013). "Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism." Cell **155**(5): 997-1007.



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White matter microstructure in children with autistic traits

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ABSTRACT

Background: Autism spectrum disorder (ASD) is thought to arise from aberrant development of connections in the brain. Previous studies have identified differences in white matter integrity in children with ASD compared to controls, offering support to such hypotheses of disrupted connectivity. While ASD is thought to represent the severe end of a spectrum of traits, there are no studies evaluating white matter integrity in relation to autistic traits along a continuum in children from the general population.

Methods: In a sample of 604 children from the Generation R Study, a population-based cohort in Rotterdam, the Netherlands, we assessed the relation between a continuous measure of autistic traits, measured by the Social Responsiveness Scale and white matter integrity, using both probabilistic tractography and Tract-Based Spatial Statistics (TBSS).

Results: No widespread associations were observed between autistic traits and white matter integrity in these children. The tractography approach revealed a negative association between autistic traits and fractional anisotropy (FA) in the bilateral corticospinal tract but after adjusting for confounders the association disappeared. Using the TBSS approach, a small cluster in the left superior longitudinal fasciculus (SLF) was identified where FA showed a negative association with autistic traits. This association remained after adjustment for confounders and when excluding children with a confirmed diagnosis of ASD. Further, in this SLF cluster, a trend to lower FA was observed 19 children with ASD, compared to 61 matched controls.

Conclusion: We found evidence for a localized association between autistic traits on a continuum and white matter integrity in school-aged children from the general population. Autistic symptoms were associated with lower FA in the left SLF, which has been consistently reported in clinical studies in children with ASD. Thus, lower FA in the SLF could indicate a continuum of the neurobiology along the spectrum of autistic symptoms. Since we only found a very localized association, some other white matter abnormalities that are commonly reported in DTI studies in children with ASD may not relate to continuously measured social problems in the general population and may be restricted to clinically affected children.

INTRODUCTION

Children with autism spectrum disorder (ASD) show impairment in reciprocal communication, as well as patterns of restrictive and repetitive behavior. Despite major research efforts, the neurobiology of ASD remains elusive. One prominent unifying theory conceptualizes ASD as a developmental disorder characterized by aberrant connectivity between different regions of the brain (Geschwind and Levitt 2007; Uddin, Supekar and Menon 2013). Notably, connectivity involving long-distance connections in the brain appears to be reduced, accompanied by greater localized connectivity (Belmonte, Allen, Beckel-Mitchener, Boulanger, Carper et al. 2004). While efficient communication between spatially separated regions of the brain is facilitated by myelinated neuronal fibers, the role of white matter microstructure in ASD is not yet clear (Delmonte, Gallagher, O'Hanlon, McGrath and Balsters 2013; Mueller, Keeser, Samson, Kirsch, Blautzik et al. 2013; Nair, Treiber, Shukla, Shih and Muller 2013).

The microstructural architecture of white matter tracts in the brain can be examined *in vivo* using diffusion tensor imaging (DTI), a non-invasive technique that provides a measure of white matter integrity by assessing properties of water diffusion. The most commonly studied DTI metrics are fractional anisotropy (FA) and mean diffusivity (MD). FA summarizes the directionality of water diffusion in different tissues as a scalar measure between 0 and 1, where higher values indicate more unidirectional diffusion, thought to reflect higher white matter microstructural integrity. MD represents the average diffusivity across all directions, where lower values are usually associated with higher integrity of white matter. Typical white matter development is characterized by gradual increases in FA and decreases in MD (Schmithorst and Yuan 2010). Psychopathology in general is typically, but not exclusively, associated with lower FA and higher MD in various brain regions. Biologically, this can reflect a wide range of developmental and pathophysiological processes, including differences in myelination, and degree of alignment and density of axons (Beaulieu 2002; Dennis and Thompson 2013).

A number of groups have applied this technique to the study of ASD and reported abnormal white matter integrity, both in adults and children (Ameis and Catani 2015). These differences in white matter microstructure have been localized to a number of tracts, including bundles connecting the limbic system to frontal regions and in interhemispheric fibers (Ameis et al. 2015). However, considerable debate remains around the presence of such abnormalities, given the inconsistencies amongst different studies. Different studies are difficult to compare due to the heterogeneity in clinical characteristics and differences in methodologies applied to image processing and statistical analyses. Further, not all studies evaluate the same set of white matter regions. While most published reports show group differences in DTI metrics (Aoki, Abe, Nippashi and Yamasue 2013; Ameis et al. 2015), there are also studies showing little or no differences in white matter features between children with ASD and controls (Travers, Adluru, Ennis, Tromp do, Destiche et al. 2012; Ameis et al. 2015).

An important trend conceptualizes ASD as a spectrum of social communication problems that extend into the general population (Constantino 2011). Thus, it is plausible that the neurobiological underpinnings of ASD are not confined to the most severely affected individuals, but extend to those without a diagnosis. This notion is supported by evidence that white matter abnormalities in children with ASD extend to their unaffected siblings (Barnea-Goraly, Lotspeich and Reiss 2010). Further, like other neurobiological features of ASD (Di Martino, Shehzad, Kelly, Roy, Gee et al. 2009; Blanken, Mous, Ghassabian, Muetzel, Schoemaker et al. 2015), white matter integrity may also relate to levels of autistic traits in the general population. A number of studies have evaluated white matter microstructure in relation to symptom severity, mostly in samples restricted to patients with ASD. Cheon and colleagues reported higher SRS scores were related to lower FA in the right uncinate fasciculus and the right anterior thalamic radiation in a sample of 17 school-aged boys with ASD (Cheon, Kim, Oh, Park, Yoon et al. 2011). Poustka et al. reported widespread negative correlations of FA with ADOS and ADI-R symptom severity indices, and Noriuchi et al. found that lower FA in white matter near the left dorsolateral prefrontal cortex was related to higher SRS scores in a small group of children with ASD (Noriuchi, Kikuchi, Yoshiura, Kira, Shigeto et al. 2010; Poustka, Jennen-Steinmetz, Henze, Vomstein, Haffner et al. 2012). In contrast, Jou et al. found no association between white matter and SRS scores (Jou, Mateljevic, Kaiser, Sugrue, Volkmar et al. 2011). However, the majority of studies operate within a case-control design, with trait analyses restricted to symptom severity associations in children with clinical ASD (Vogan, Morgan, Leung, Anagnostou, Doyle-Thomas et al. 2016). Only a few studies have evaluated differences along a continuum of autistic traits, extending to non-clinically affected individuals (Iidaka, Miyakoshi, Harada and Nakai 2012; Jakab, Emri, Spisak, Szeman-Nagy, Beres et al. 2013; Koolschijn, Geurts, van der Leij and Scholte 2015). Two studies showed associations between autistic traits and white matter integrity in neurotypical adults (Iidaka et al. 2012; Jakab et al. 2013), while another study did not demonstrate such associations (Koolschijn et al. 2015). Iidaka et al. reported that Autism Quotient scores were positively correlated with volume of a white matter tract connecting the amygdala and the superior temporal sulcus. Jakab et al. reported a negative association between SRS scores and fractional anisotropy in the bilateral temporal fusiform and parahippocampal gyri. There have been no studies to date evaluating the relationship between autistic traits along a continuum and white matter integrity in young children from the general population. Importantly, as neurobiological findings in ASD have been shown to be highly sensitive to development (Uddin et al. 2013), the question remains whether white matter differences are present in young children with autistic traits. Previously, in this sample, we identified differences in gyrification in relation to autistic traits and it has been suggested that differences in gyrification may be driven by differences in the underlying structural connectivity (Van Essen 1997). Therefore, in this study, we examined the association between autistic traits and white matter microstructure

in six-to-ten year-old children from the general population. To evaluate this and to facilitate comparisons with the current literature, we applied two commonly used methodologies to evaluate white matter characteristics: the voxel-based approach Tract Based Spatial Statistics (TBSS) and probabilistic tractography of large, commonly studied white matter tracts.

We hypothesized that white matter microstructural abnormalities in children with autistic traits would mimic the more consistent findings in children with clinically diagnosed ASD. Thus, we expected subtle, but widespread, negative associations between autistic traits and FA, primarily in tracts that facilitate long-range connections: corpus callosum, the uncinated fasciculus (UF), and the superior longitudinal fasciculus (SLF) (Aoki et al. 2013). To assess whether associations between autistic traits and white matter were truly present on a continuum, we additionally evaluated whether they remained after excluding children with a confirmed diagnosis of ASD. In addition, any differences found along the continuum of traits were also evaluated in children with confirmed ASD, compared to a group of age, sex and IQ-matched controls.

METHODS

Participants

The current study is part of the Generation R Study, a population-based cohort of mothers and children in Rotterdam, the Netherlands (Jaddoe, van Duijn, Franco, van der Heijden, van Iizendoorn et al. 2012). When the children were approximately 6 to 8 years of age, a sub-sample of 1,070 children was recruited for MRI scanning (White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013). In this sub sample, there were 36 children who did not receive a DTI scan and 256 children with insufficient quality DTI data, leaving 778 children with usable DTI data. After excluding children with missing information on autistic traits (n=144), the study sample included 634 children. For 30 children, one of the tracts (usually the cingulum bundle) could not be reconstructed in the tractography approach and we excluded them in both approaches, after which the final study sample consisted of 604 children. The Medical Ethics Committee of the Erasmus Medical Center approved all study procedures, and parents provided written informed consent after they had received a full description of the study.

Autistic traits

At approximately 6 years of age, the Social Responsiveness Scale (SRS) was administered to parents (90.4% mothers) of all participating children to obtain a quantitative measure of autistic traits (Constantino 2002). The Social Responsiveness Scale provides a valid quantitative measure of subclinical and clinical levels of autistic traits (Constantino 2002). Parents were asked to rate their child's social behavior during the past six months. We used

the 18-item abbreviated version of the scale, which shows correlations ranging from 0.93 to 0.99 with the full scale in three different large studies (Blanken et al. 2015). Item scores were summed and weighed for the number of items completed. Higher total scores indicate more problems. Total scores were square-root transformed to approach normality.

ASD diagnosis

Identification of children with a diagnosis of ASD was a multi-step process. Children who scored in the top 15th percentile on the CBCL/1 ½-5 total score and those who scored in the upper 2% on the PDP subscale underwent a screening procedure for ASD using the Social Communication Questionnaire (SCQ), a 40-item parent-reported screening instrument for ASD (Berument, Rutter, Lord, Pickles and Bailey 1999). We contacted general practitioners of children who scored screen-positive on the SRS, SCQ or for whom the mother reported a diagnosis of ASD, in order to confirm this diagnosis based on the medical records. In the Netherlands, the general practitioner holds the central medical records, including information on treatment by (medical) specialists. Only children with a diagnosis of ASD documented in the medical records were considered ASD cases. In this study sample, we identified 19 children with a medical record-confirmed diagnosis of ASD. To create a control group, these children were subsequently matched 1:4 on age at the time of MRI, sex and non-verbal IQ. Sixty-one suitable matches were identified.

Image acquisition

Prior to MRI scanning, all children underwent a 30-minute mock scanning session in order to familiarize them with the MR-environment (White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013). Data were acquired on a 3 Tesla General Electric scanner (GE, MR750, Milwaukee, WI). Diffusion MRI data were collected with 3 b=0 volumes and 35 diffusion directions using an echo planar imaging sequence (T_R = 11,000 ms, T_E = 83 ms, Field of view = 256 mm x 256 mm, Acquisition Matrix = 128 x 128, slice thickness = 2 mm, number of slices = 77, b = 1000 s/mm^2).

Data quality assurance

Data quality assurance was a multi-step process including both visual inspection and automated software. Details on this procedure have been reported elsewhere (Muetzel, Mous, van der Ende, Blanken, van der Lugt et al. 2015). Briefly, sum of square maps from the tensor fit were visually inspected for structured patterns / artifact. Further, raw image quality was also evaluated using an automated quality control tool (DTIprep, http://www.nitrc.org/projects/dtiprep/). Probabilistic tractography and TBSS registrations to standard space were inspected for accuracy, to ensure all data were properly aligned. Further, all probabilistic tracts were visualized to ensure accurate path reconstruction.

Image preprocessing

Image preprocessing was conducted using the Functional MRI of the Brain's Software Library (FMRIB, FSL, version 5.0.5, Jenkinson, Beckmann, Behrens, Woolrich and Smith 2012) and the Camino Diffusion Toolkit (Cook, Bai, Nedjati-Gilani, Seunarine, Hall et al. 2006) via the Neuroimaging in Python Pipelines and Interfaces package (Nipype, version 0.92, Gorgolewski, Burns, Madison, Clark, Halchenko et al. 2011). Details of the image processing have been described in detail elsewhere (Muetzel et al. 2015). Briefly, non-brain tissue was removed (Jenkinson et al. 2012) and diffusion images were corrected for eddy current-induced artifacts (Haselgrove and Moore 1996) and translations/rotations resulting from head motion (Jenkinson and Smith 2001). In order to account for rotations applied to the diffusion data, the resulting transformation matrices were used to rotate the diffusion gradient direction table (Jones and Cercignani 2010). The diffusion tensor was computed using the Camino toolkit's embedded RESTORE method (Chang, Jones and Pierpaoli 2005), and common scalar metrics (e.g., FA, MD) were subsequently computed.

Probabilistic fiber tractography

Probabilistic fiber tractography was run on each subject's diffusion data using the fully automated, freely available FSL plugin, "AutoPtx" (de Groot, Ikram, Akoudad, Krestin, Hofman et al. 2015). Briefly, the Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTx) package from FSL was used to estimate the diffusion parameters at each voxel, accounting for two fiber orientations (Behrens, Berg, Jbabdi, Rushworth and Woolrich 2007). Next, a predefined set of seed and target masks, supplied by the AutoPtx software, were aligned to each subject's diffusion data using a nonlinear registration. The FSL probabilistic fiber tracking algorithm, Probtrackx, was then used to identify connectivity distributions for a number of large, commonly reported fiber bundles, based on the predefined seed and target marks. Connectivity distributions obtained in the fiber tracking process were normalized based on the number of successful seed-to-target attempts, with a threshold applied to remove voxels that were unlikely to be part of the true distribution. For each tract, average DTI scalar metrics (e.g., FA, MD), weighted by the connectivity distribution, were computed (Muetzel et al. 2015). Thus, voxels with a high probability of being part of the true white matter bundle have a higher contribution to the average DTI scalar value computed across the entire tract, compared to voxels with a lower probability. A depiction of the tracts examined in this study is provided in Supplementary Figure 1.

Tract based spatial statistics

Voxel-wise statistical analysis of the FA and MD scalar data was performed using Tract-Based Spatial Statistics (TBSS) (Smith, Jenkinson, Johansen-Berg, Rueckert, Nichols et al. 2006), which is part of FSL (Smith, Jenkinson, Woolrich, Beckmann, Behrens et al. 2004). First, FA

data were aligned into a common space (FMRIB58 FA map) using the nonlinear registration tool FNIRT (Andersson 2007; Andersson, Jenkinson and Smith 2007). The resulting non linear warp fields for the FA maps were then applied to MD maps. Next, the mean FA image was created and thresholded to create a mean FA skeleton, which is the area representing the geometric center of all tracts common to the sample. A threshold of FA > 0.2 was applied to the skeleton to include only major fiber bundles. Each subject's aligned FA/MD data was then projected onto this skeleton and the resulting data were fed into voxel-wise cross-subject statistics (see below for details).

Covariates

Covariates were chosen *a priori* based on confounding effects in a previous study of gyrification and autistic traits in this sample (Blanken et al. 2015). Sex, age at MRI scanning and age at administration of the SRS were default covariates. Maternal education and history of smoking and alcohol during pregnancy were assessed by questionnaires. The child's ethnic background was defined based on the country of birth of both parents. Maternal education was defined by the highest completed education. Maternal smoking was assessed at enrollment and in mid- and late pregnancy.

Non-verbal intelligence of the child was assessed at approximately 6 years of age using two subtests of the Snijders-Oomen Niet-verbale intelligentie Test – Revisie (SON-R 2.5–7), a non-verbal intelligence test suited for children between 2.5–7 years of age (Tellegen, Wijnberg-Williams and Laros 2005). Child attention problems, which often occur with autistic traits (Grzadzinski, Di Martino, Brady, Mairena, O'Neale et al. 2011), were assessed at age 6 using the CBCL/ 1.5-5 (Achenbach and Rescorla 2000).

Statistical analysis

Statistical analyses were conducted using the R Statistical Software version 3.1.3 (R Core Team, 2014), SPSS version 21 (IBM Corp., Armonk, NY, USA), and FSL's built-in tool "Randomise" (Winkler, Ridgway, Webster, Smith and Nichols 2014). For the tractography approach, the Lavaan package (Rosseel 2012) was used to compute global DTI measures using confirmatory factor analysis. The details of this approach have been described elsewhere (Muetzel et al. 2015). Briefly, separately for FA and MD, multiple tracts were summarized as a single latent factor, and the predicted factor scores for each subject were generated.

In TBSS, FSL's tool "Randomise" was used to perform voxel-wise analyses (Winkler et al. 2014). A total of 5000 permutations were run per analysis, and family-wise error corrected p-values were used to evaluate significant clusters (p_{FWE} <0.05). Because the Randomise tool does not allow for multiple imputation of missing data for confounders, average FA and MD values were computed for each subject within significant clusters, and fully adjusted analyses were performed in SPSS. Multiple imputation of missing data for confounders, regression analyses, and matching of children with ASD were all performed in SPSS. For associations that

remained after adjustment for a set of standard confounders, we additionally performed a sensitivity analysis adjusting for IQ and attention problems.

Additionally, we evaluated whether any associations with white matter microstructure remained after excluding children with a confirmed diagnosis of ASD. Lastly, any differences identified using the trait approach were also evaluated in the 19 children in our sample with clinically confirmed ASD, compared to a group of age, sex and IQ-matched controls. For this analysis, group status (ASD/control) was used as a predictor in linear regression models.

RESULTS

Sample characteristics

Sample characteristics are presented in Table 1.

Table 1. Participant characteristics (n=604)

Child characteristics			
		n	
Gender (% boy)		604	53.0
Ethnicity		604	
	Dutch		65.2
	Other Western		9.3
	Non-Western		25.5
Social Responsiveness Scale			
	Weighted total score	604	0.27 (0.29)
Age at Social Responsiveness Scale (years)		604	6.17 (0.44)
Age at MRI (years)		604	8.02 (1.03)
Non-verbal IQ		554	103.4 (14.1)
Maternal characteristics			
Education level (%)		591	
	High		57.3
	Medium		29.5
	Low		11.1
Alcohol in pregnancy (%)		563	
	Never		32.7
	Until pregnancy was known		14.9
	Continued occasionally		40.7
	Continued frequently		11.7
Smoking in pregnancy (%)		583	
	Never		77.4
	Until pregnancy was known		6.3
	Continued		16.3

Note. Values are mean and SD unless otherwise indicated.

Autistic traits and white matter integrity: global and specific tracts

To facilitate comparison with other studies, linear regression analyses were first performed using a minimally adjusted model with age at administration of the SRS, age at the time of scanning and sex as covariates (model 1). Subsequently, we additionally adjusted for ethnicity, maternal alcohol use and smoking during pregnancy, and maternal education. Autistic traits were not associated with global FA and MD (Table 2). To assess potential tract-specific differences that were obscured in the analyses of the global measures, we also examined FA and MD in individual tracts. In the bilateral corticospinal tract, we found a negative association with FA, where children with more autistic traits showed lower FA (Left: β=-0.08, p=0.04, and right: β=0.09, p=0.03, uncorrected for multiple comparisons). However, after adjustment for confounders, associations disappeared (Table 3).

Table 2. Autistic traits and global FA and MD.

		B (95%CI)	Beta	p
Global FA	**model 1**	-0.35 (-1.11; 0.42)	-0.04	.38
	model 2	-0.06 (-0.85; 0.74)	-0.01	.89
Global MD	**model 1**	0.00 (-0.06; 0.06)	0.00	.99
	model 2	0.00 (-0.07; 0.06)	0.00	.93

Note. Global DTI metrics from confirmatory factor analysis of multiple white matter tracts. Model 1 was adjusted for sex, age at the time of scanning and age at the time of SRS. Model 2 was additionally adjusted for ethnicity, maternal alcohol use and smoking during pregnancy, and maternal education.

Table 3. Autistic traits and tract-specific FA and MD.

		FA			MD		
		B (95%CI)	Beta	p	B (95% CI)	Beta	p
SLF L	**model 1**	-0.46 (-1.32; 0.40)	-0.04	.29	-0.01 (-0.09; 0.07)	-0.01	.80
	model 2	-0.22 (-1.12; 0.68)	-0.02	.64	-0.01 (-0.09; 0.07)	-0.01	.85
SLF R	**model 1**	-0.09 (-0.75; 0.93)	0.01	.83	-0.02 (-0.09; 0.06)	-0.02	.69
	model 2	0.34 (-0.53; 1.22)	0.03	.44	-0.03 (-0.11; 0.05)	-0.03	.49
ILF L	**model 1**	-0.32 (-0.99; 0.36)	-0.04	.36	-0.01 (-0.10; 0.07)	-0.01	.76
	model 2	-0.14 (-0.85; 0.58)	-0.02	.71	-0.02 (-0.10; 0.07)	-0.01	.75
ILF R	**model 1**	-0.53 (-1.21; 0.15)	-0.06	.13	0.06 (-0.03; 0.14)	0.05	.18
	model 2	-0.41 (-1.13; 0.31)	-0.05	.26	0.07 (-0.01; 0.16)	0.07	.10
UF L	**model 1**	-0.21 (-1.10; 0.68)	-0.02	.64	-0.01 (-0.08; 0.06)	-0.01	.79
	model 2	-0.07 (-1.01; 0.87)	-0.01	.89	-0.01 (-0.09; 0.06)	-0.02	.71
UF R	**model 1**	-0.32 (-1.04; 0.40)	-0.04	.38	-0.02 (-0.08; 0.05)	-0.02	.64
	model 2	-0.16 (-0.92; 0.60)	-0.02	.68	-0.02 (-0.09; 0.05)	-0.02	.63
CST L	**model 1**	-0.83 (-1.61;-0.05)	-0.08	.04	-0.03 (-0.08; 0.03)	-0.03	.41
	model 2	-0.37 (-1.17; 0.44)	-0.04	.37	-0.05 (-0.11; 0.01)	-0.06	.13

CST R	**model 1**	-0.86 (-1.62;-0.10)	-0.09	.03	-0.04 (-0.10; 0.02)	-0.05	.22
	model 2	-0.43 (-1.21; 0.36)	-0.04	.29	-0.05 (-0.11; 0.01)	-0.06	.13
Cingulum L	**model 1**	0.41 (-0.97; 1.80)	0.02	.56	-0.02 (-0.11; 0.07)	-0.02	.69
	model 2	0.83 (-0.63; 2.28)	0.05	.26	-0.04 (-0.14; 0.05)	-0.04	.37
Cingulum R	**model 1**	0.91 (-0.42; 2.25)	0.05	.18	0.03 (-0.06; 0.11)	0.02	.59
	model 2	1.05 (-0.35; 2.45)	0.06	.14	0.01 (-0.08; 0.10)	0.01	.83
Forceps Major	**model 1**	-0.56 (-1.65; 0.52)	-0.04	.31	-0.02 (-0.21; 0.17)	-0.01	.83
	model 2	-0.42 (-1.56; 0.71)	-0.03	.46	-0.01 (-0.21; 0.20)	0.00	.96
Forceps Minor	**model 1**	-0.01 (-0.98; 0.96)	0.00	.98	0.06 (-0.06; 0.18)	0.04	.30
	model 2	0.16 (-0.86; 1.17)	0.01	.76	0.08 (-0.04; 0.20)	0.05	.20

Note. model 1 was adjusted for sex, age at the time of scanning and age at the time of SRS. Model 2 was additionally adjusted for ethnicity, maternal alcohol use and smoking during pregnancy and maternal education.

Tract-based spatial statistics

Autistic traits were significantly associated with FA in a small cluster in the left superior longitudinal fasciculus after controlling for multiple testing. In this cluster, children with more autistic traits showed lower FA (Figure 1 and Table 4, β=-0.20, p<0.001). When examining this association in the context of additional covariates (model 2), the association remained. In a sensitivity analysis, we also assessed the association after additionally adjusting for non-verbal IQ and attention problems and results remained the same. When evaluating the association between SRS-score and FA in this cluster after excluding children with confirmed ASD, it remained highly consistent (β=-0.20, p<0.001, model fully adjusted). Lastly, case-control analyses showed that there was a trend to lower FA in this cluster in the 19 children with confirmed ASD, compared to the 61 matched controls (β=-0.20, p=0.07). We did not identify any clusters where autistic traits were associated with MD.

Figure 1. FA and autistic traits. The blue cluster in the left Superior Longitudinal Fasciculus represents a negative correlation between autistic traits and FA. Coordinates are in MNI space.

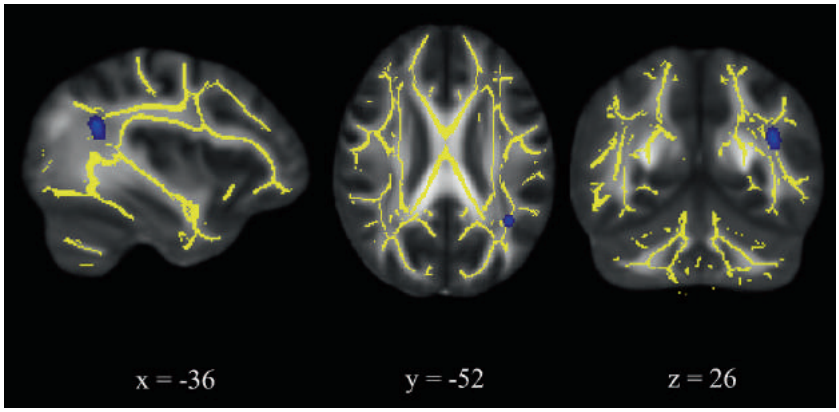


Table 4. TBSS: autistic traits and FA (n=604).

		B (95% CI)	Beta	p
Left Superior Longitudinal Fasciculus	**model 1**	-0.04 (-0.05;-0.02)	-0.20	<.001
	model 2	-0.04 (-0.06;-0.02)	-0.20	<.001

Note. model 1 was adjusted for sex, age at the time of scanning and age at the time of SRS. Model 2 was additionally adjusted for ethnicity, maternal alcohol use and smoking during pregnancy, and maternal education.

DISCUSSION

In a large sample of school-aged children we found that autistic traits were not associated with widespread differences in white matter integrity in the brain; this was consistent across two different methodologies. However, when using the TBSS approach, we identified a small cluster in the left superior longitudinal fasciculus where children with more autistic traits showed lower FA.

Our finding in the left superior longitudinal fasciculus was consistent with our hypothesis and represents one of the most replicated differences in ASD (Aoki et al. 2013). Consistent with our hypothesis that the white matter differences would show a continuum, the association remained after excluding children with a confirmed diagnosis of ASD. Further, there was a trend to a similar effect at the severe end of the spectrum, when comparing a small group of children with clinically confirmed ASD to matched controls. This supports the notion that brain features of ASD potentially extend into the general population. The superior longitudinal fasciculus is a long-range white matter tract that connects frontal areas of the brain with dorsal areas. It is implicated in a number of cognitive functions, including language, fine-motor ability and attention (Zhang, Wang, Zhao, Chen, Han et al. 2010; Urger, De Bellis, Hooper, Woolley, Chen et al. 2015). Lower microstructural white matter integrity in a long-range tract is consistent with the conceptualization of ASD as a "developmental disconnection syndrome' (Geschwind et al. 2007). Further, in the minimally adjusted tractography models, we observed an association between autistic traits and lower FA in the CST, although this association was not corrected for multiple testing. This finding is in line with other studies that have also reported lower FA in the CST in children or adolescents with ASD (Ben Bashat, Kronfeld-Duenias, Zachor, Ekstein, Hendler et al. 2007; Brito, Vasconcelos, Domingues, Hygino da Cruz, Rodrigues Lde et al. 2009). In addition, a recent study by Carper et al. found higher mean diffusivity of the corticospinal tract in adolescents with ASD (Carper, Solders, Treiber, Fishman and Muller 2015). Importantly, we did not find the same region in the TBSS approach and the effects we observed in the CST disappeared after adjusted for confounders.

While we observed a localized association in the left superior longitudinal fasciculus, our results do not support our original hypothesis of global differences in white matter microstructure. More specifically, despite the large sample size of our study, we did not find associations in all three hypothesized regions. There are several potential reasons for this. First, it is possible that some white matter differences that are commonly reported in ASD may represent more severe alterations that are restricted to more severely affected patient groups and do not, like some other neurobiological features, extend to the general population. Symptoms of children in the general population may be too mild to reveal associations in such regions. The scarcity of findings in the general population is in line with a study of Koolschijn and colleagues, who did not find any differences in white matter integrity related to the continuum of autistic traits in a large sample of neurotypical adults (Koolschijn et al. 2015). However, in contrast to this study, we did find that cortical morphology in our sample was related to autistic traits, lending support to a continuum with autistic symptoms in at least some neurobiological features (Blanken et al. 2015). Of note, the previous studies showing differences in white matter in relation to autistic traits investigated older participants (Iidaka et al. 2012; Jakab et al. 2013). Therefore, it is possible that some white matter differences occur as a consequence of long-term manifestation of autistic traits. White matter development is a dynamic process that continues into adulthood (Lebel, Walker, Leemans, Phillips and Beaulieu 2008) and some abnormalities in ASD have been shown to be specific to certain periods of development. For instance, a longitudinal study showed that children with ASD exhibited increased FA in infancy, followed by decreased FA at age 2 (Wolff, Gu, Gerig, Elison, Styner et al. 2012). Second, heterogeneity of white matter involvement may play a role. The literature of DTI studies in children with ASD is not very consistent. While there are many reports of abnormalities in various white matter tracts in subjects with ASD, there are also studies reporting no differences in those same tracts (Ameis et al. 2015). Further, additional studies with negative results may not have been published (Joober, Schmitz, Annable and Boksa 2012). Methodological differences, as well as sample characteristics, such as age, IQ or sex of the participants contribute to, but likely do not fully explain, discrepant results between studies. Heterogeneity is a central feature of ASD, and it likely does not only affect its symptomology and cognitive aspects, but also its etiology (Kendler 2013). While this issue is still poorly understood, there are likely many different etiologic pathways that lead to ASD, affecting the brain at many different levels (Happe, Ronald and Plomin 2006; Kendler 2013). At this point, there are over 100 genes associated with ASD (Betancur 2011). In some cases, the specific genetic pathway causing ASD may determine the nature and localization of white matter involvement. For instance, a study comparing white matter in children with Klinefelter syndrome to children with idiopathic ASD found differences in the localization of white matter impairments despite the fact that both groups exhibited autism-like symptoms (Goddard, van Rijn, Rombouts and Swaab 2015). This heterogeneity might complicate the identification

of group differences related to ASD or autistic traits. In addition, social impairment as measured by the SRS is only one potential endophenotype of ASD and not all features of the neurobiology may relate to this dimension of ASD.

To our knowledge, this is the first study in school-aged children that focused on white matter integrity and autistic traits along a continuum. Strengths of this study include the population-based design and the large sample size, as well as the implementation of two different methods of assessing white matter integrity. One of the factors likely contributing to the lack of consistency in DTI findings in children with ASD is that different studies typically utilize different methods. Here, we applied two commonly used approaches to assess white matter integrity, which allow us to detect global, as well as more subtle, localized differences in white matter structural integrity. There are also limitations to our study. While there was a time lag between administration of the SRS and the neuroimaging session, we do not expect that this caused our lack of findings, as we expect relative stability of this trait in this age range and we also adjusted for this statistically. Further, since this study was cross-sectional, we could not evaluate longitudinal trajectories of white matter development.

In conclusion, we found a localized association between autistic traits and white matter integrity in school-aged children drawn from the general population. In a small region in the left superior longitudinal fasciculus, we found evidence of a continuum in white matter integrity related to autistic traits. However, most of the white matter abnormalities commonly reported in children with ASD were not observed in this sample. This suggests that these differences may not form a continuum related to autistic traits in the population and may be restricted to more severely affected children.

REFERENCE

Achenbach, T. M. and L. A. Rescorla (2000). Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT., University of Vermont, Reseach Center for Children, Youth and Families.

Ameis, S. H. and M. Catani (2015). "Altered white matter connectivity as a neural substrate for social impairment in Autism Spectrum Disorder." Cortex **62**: 158-181.

Andersson, J. L. R. (2007). Non-linear optimisation.

Andersson, L. R., M. Jenkinson and S. Smith (2007). Non-linear registration, aka Spatial normalisation

Aoki, Y., O. Abe, Y. Nippashi and H. Yamasue (2013). "Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies." Molecular Autism **4**.

Barnea-Goraly, N., L. J. Lotspeich and A. L. Reiss (2010). "Similar white matter aberrations in children with autism and their unaffected siblings: a diffusion tensor imaging study using tract-based spatial statistics." Arch Gen Psychiatry **67**(10): 1052-1060.

Beaulieu, C. (2002). "The basis of anisotropic water diffusion in the nervous system- a technical review." NMR Biomed **15**(7-8): 435-455.

Behrens, T. E., H. J. Berg, S. Jbabdi, M. F. Rushworth and M. W. Woolrich (2007). "Probabilistic diffusion tractography with multiple fibre orientations: What can we gain?" Neuroimage **34**(1): 144-155.

Belmonte, M. K., G. Allen, A. Beckel-Mitchener, L. M. Boulanger, R. A. Carper and S. J. Webb (2004). "Autism and abnormal development of brain connectivity." J Neurosci **24**(42): 9228-9231.

Ben Bashat, D., V. Kronfeld-Duenias, D. A. Zachor, P. M. Ekstein, T. Hendler, R. Tarrasch, A. Even, Y. Levy and L. Ben Sira (2007). "Accelerated maturation of white matter in young children with autism: a high b value DWI study." Neuroimage **37**(1): 40-47.

Berument, S. K., M. Rutter, C. Lord, A. Pickles and A. Bailey (1999). "Autism screening questionnaire: diagnostic validity." British Journal of Psychiatry **175**: 444-451.

Betancur, C. (2011). "Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting." Brain Res **1380**: 42-77.

Blanken, L. M. E., S. E. Mous, A. Ghassabian, R. L. Muetzel, N. K. Schoemaker, H. El Marroun, A. van der Lugt, V. W. V. Jaddoe, A. Hofman, F. C. Verhulst, H. Tiemeier and T. White (2015). "Cortical Morphology in 6-to 10-Year Old Children With Autistic Traits: A Population-Based Neuroimaging Study." American Journal of Psychiatry **172**(5): 479-486.

Brito, A. R., M. M. Vasconcelos, R. C. Domingues, L. C. Hygino da Cruz, Jr., S. Rodrigues Lde, E. L. Gasparetto and C. A. Calcada (2009). "Diffusion tensor imaging findings in school-aged autistic children." J Neuroimaging **19**(4): 337-343.

Carper, R. A., S. Solders, J. M. Treiber, I. Fishman and R. A. Muller (2015). "Corticospinal tract anatomy and functional connectivity of primary motor cortex in autism." J Am Acad Child Adolesc Psychiatry **54**(10): 859-867.

Chang, L. C., D. K. Jones and C. Pierpaoli (2005). "RESTORE: robust estimation of tensors by outlier rejection." Magn Reson Med **53**(5): 1088-1095.

Cheon, K. A., Y. S. Kim, S. H. Oh, S. Y. Park, H. W. Yoon, J. Herrington, A. Nair, Y. J. Koh, D. P. Jang, Y. B. Kim, B. L. Leventhal, Z. H. Cho, F. X. Castellanos and R. T. Schultz (2011). "Involvement of the anterior thalamic radiation in boys with high functioning autism spectrum disorders: a Diffusion Tensor Imaging study." Brain Res **1417**: 77-86.

Constantino, J. N. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Constantino, J. N. (2011). "The quantitative nature of autistic social impairment." Pediatr Res **69**(5 Pt 2): 55R-62R.

Cook, P. A., Y. Bai, S. Nedjati-Gilani, K. K. Seunarine, M. G. Hall, G. J. Parker and D. C. Alexander (2006). Camino: Open-Source Diffusion-MRI Reconstruction and Processing. 14th Scientific Meeting of the International Society for Magnetic Resonance in Medicine. Seattle, WA, USA**:** 2759.

de Groot, M., M. A. Ikram, S. Akoudad, G. P. Krestin, A. Hofman, A. van der Lugt, W. J. Niessen and M. W. Vernooij (2015). "Tract-specific white matter degeneration in aging: The Rotterdam Study." Alzheimers Dement **11**(3): 321-330.

Delmonte, S., L. Gallagher, E. O'Hanlon, J. McGrath and J. H. Balsters (2013). "Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder." Front Hum Neurosci **7**: 430.

Dennis, E. L. and P. M. Thompson (2013). "Typical and atypical brain development: a review of neuroimaging studies." Dialogues Clin Neurosci **15**(3): 359-384.

Di Martino, A., Z. Shehzad, C. Kelly, A. K. Roy, D. G. Gee, L. Q. Uddin, K. Gotimer, D. F. Klein, F. X. Castellanos and M. P. Milham (2009). "Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults." Am J Psychiatry **166**(8): 891-899.

Geschwind, D. H. and P. Levitt (2007). "Autism spectrum disorders: developmental disconnection syndromes." Curr Opin Neurobiol **17**(1): 103-111.

Goddard, M. N., S. van Rijn, S. A. Rombouts and H. Swaab (2015). "White matter microstructure in a genetically defined group at increased risk of autism symptoms, and a comparison with idiopathic autism: an exploratory study." Brain Imaging Behav.

Gorgolewski, K., C. D. Burns, C. Madison, D. Clark, Y. O. Halchenko, M. L. Waskom and S. S. Ghosh (2011). "Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python." Front Neuroinform **5**: 13.

Grzadzinski, R., A. Di Martino, E. Brady, M. A. Mairena, M. O'Neale, E. Petkova, C. Lord and F. X. Castellanos (2011). "Examining autistic traits in children with ADHD: does the autism spectrum extend to ADHD?" Journal of Autism and Developmental Disorders **41**(9): 1178-1191.

Happe, F., A. Ronald and R. Plomin (2006). "Time to give up on a single explanation for autism." Nat Neurosci **9**(10): 1218-1220.

Haselgrove, J. C. and J. R. Moore (1996). "Correction for distortion of echo-planar images used to calculate the apparent diffusion coefficient." Magn Reson Med **36**(6): 960-964.

Iidaka, T., M. Miyakoshi, T. Harada and T. Nakai (2012). "White matter connectivity between superior temporal sulcus and amygdala is associated with autistic trait in healthy humans." Neurosci Lett **510**(2): 154-158.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." European journal of epidemiology **27**(9): 739-756.

Jakab, A., M. Emri, T. Spisak, A. Szeman-Nagy, M. Beres, S. A. Kis, P. Molnar and E. Berenyi (2013). "Autistic traits in neurotypical adults: correlates of graph theoretical functional network topology and white matter anisotropy patterns." PLoS One **8**(4): e60982.

Jenkinson, M., C. F. Beckmann, T. E. Behrens, M. W. Woolrich and S. M. Smith (2012). "Fsl." Neuroimage **62**(2): 782-790.

Jenkinson, M. and S. Smith (2001). "A global optimisation method for robust affine registration of brain images." Med Image Anal **5**(2): 143-156.

Jones, D. K. and M. Cercignani (2010). "Twenty-five pitfalls in the analysis of diffusion MRI data." NMR Biomed **23**(7): 803-820.

Joober, R., N. Schmitz, L. Annable and P. Boksa (2012). "Publication bias: what are the challenges and can they be overcome?" J Psychiatry Neurosci **37**(3): 149-152.

Jou, R. J., N. Mateljevic, M. D. Kaiser, D. R. Sugrue, F. R. Volkmar and K. A. Pelphrey (2011). "Structural neural phenotype of autism: preliminary evidence from a diffusion tensor imaging study using tract-based spatial statistics." AJNR Am J Neuroradiol **32**(9): 1607-1613.

Kendler, K. S. (2013). "What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn." Mol Psychiatry **18**(10): 1058-1066.

Koolschijn, P. C., H. M. Geurts, A. R. van der Leij and H. S. Scholte (2015). "Are Autistic Traits in the General Population Related to Global and Regional Brain Differences?" J Autism Dev Disord **45**(9): 2779-2791.

Lebel, C., L. Walker, A. Leemans, L. Phillips and C. Beaulieu (2008). "Microstructural maturation of the human brain from childhood to adulthood." Neuroimage **40**(3): 1044-1055.

Mueller, S., D. Keeser, A. C. Samson, V. Kirsch, J. Blautzik, M. Grothe, O. Erat, M. Hegenloh, U. Coates, M. F. Reiser, K. Hennig-Fast and T. Meindl (2013). "Convergent Findings of Altered Functional and Structural Brain Connectivity in Individuals with High Functioning Autism: A Multimodal MRI Study." PLoS One **8**(6): e67329.

Muetzel, R. L., S. E. Mous, J. van der Ende, L. M. Blanken, A. van der Lugt, V. W. Jaddoe, F. C. Verhulst, H. Tiemeier and T. White (2015). "White matter integrity and cognitive performance in school-age children: A population-based neuroimaging study." Neuroimage **119**: 119-128.

Nair, A., J. M. Treiber, D. K. Shukla, P. Shih and R. A. Muller (2013). "Impaired thalamocortical connectivity in autism spectrum disorder: a study of functional and anatomical connectivity." Brain **136**(Pt 6): 1942-1955.

Noriuchi, M., Y. Kikuchi, T. Yoshiura, R. Kira, H. Shigeto, T. Hara, S. Tobimatsu and Y. Kamio (2010). "Altered white matter fractional anisotropy and social impairment in children with autism spectrum disorder." Brain Res **1362**: 141-149.

Poustka, L., C. Jennen-Steinmetz, R. Henze, K. Vomstein, J. Haffner and B. Sieltjes (2012). "Fronto-temporal disconnectivity and symptom severity in children with autism spectrum disorder." World J Biol Psychiatry **13**(4): 269-280.

Rosseel, Y. (2012). "lavaan: An R Package for Structural Equation Modeling " Journal of Statistical Software **48**(2): 1-36.

Schmithorst, V. J. and W. Yuan (2010). "White matter development during adolescence as shown by diffusion MRI." Brain Cogn **72**(1): 16-25.

Smith, S. M., M. Jenkinson, H. Johansen-Berg, D. Rueckert, T. E. Nichols, C. E. Mackay, K. E. Watkins, O. Ciccarelli, M. Z. Cader, P. M. Matthews and T. E. Behrens (2006). "Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data." Neuroimage **31**(4): 1487-1505.

Smith, S. M., M. Jenkinson, M. W. Woolrich, C. F. Beckmann, T. E. Behrens, H. Johansen-Berg, P. R. Bannister, M. De Luca, I. Drobnjak, D. E. Flitney, R. K. Niazy, J. Saunders, J. Vickers, Y. Zhang, N. De Stefano, J. M. Brady and P. M. Matthews (2004). "Advances in functional and structural MR image analysis and implementation as FSL." Neuroimage **23 Suppl 1**: S208-219.

Tellegen, P., B. Wijnberg-Williams and J. Laros (2005). Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2.5- 7/. Amsterdam, Boom Testuitgevers.

Travers, B. G., N. Adluru, C. Ennis, P. M. Tromp do, D. Destiche, S. Doran, E. D. Bigler, N. Lange, J. E. Lainhart and A. L. Alexander (2012). "Diffusion tensor imaging in autism spectrum disorder: a review." Autism Res **5**(5): 289-313.

Uddin, L. Q., K. Supekar and V. Menon (2013). "Reconceptualizing functional brain connectivity in autism from a developmental perspective." Front Hum Neurosci **7**: 458.

Urger, S. E., M. D. De Bellis, S. R. Hooper, D. P. Woolley, S. D. Chen and J. Provenzale (2015). "The superior longitudinal fasciculus in typically developing children and adolescents: diffusion tensor imaging and neuropsychological correlates." J Child Neurol **30**(1): 9-20.

Van Essen, D. C. (1997). "A tension-based theory of morphogenesis and compact wiring in the central nervous system." Nature **385**(6614): 313-318.

Vogan, V. M., B. R. Morgan, R. C. Leung, E. Anagnostou, K. Doyle-Thomas and M. J. Taylor (2016). "Widespread White Matter Differences in Children and Adolescents with Autism Spectrum Disorder." J Autism Dev Disord **46**(6): 2138-2147.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." European journal of epidemiology **28**(1): 99-111.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. V. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." European Journal of Epidemiology **28**(1): 99-111.

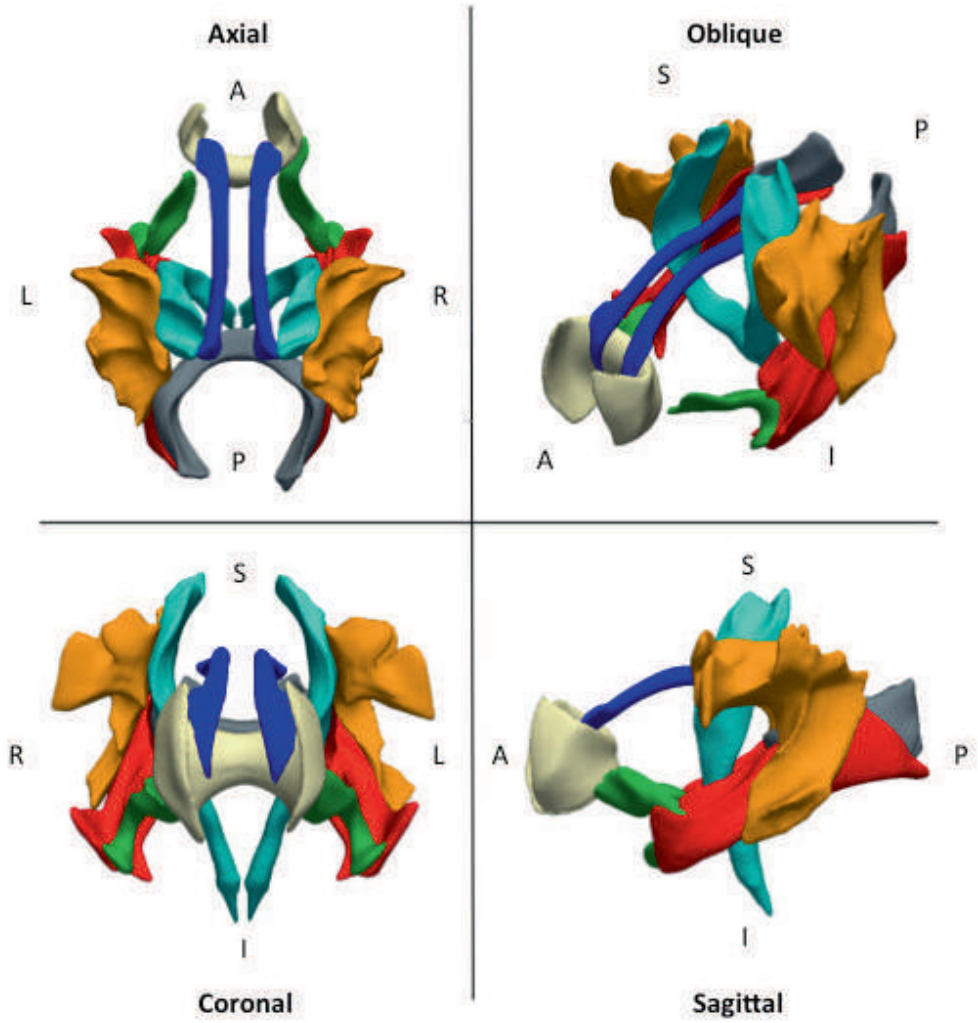
Winkler, A. M., G. R. Ridgway, M. A. Webster, S. M. Smith and T. E. Nichols (2014). "Permutation inference for the general linear model." Neuroimage **92**: 381-397.

Wolff, J. J., H. Gu, G. Gerig, J. T. Elison, M. Styner, S. Gouttard, K. N. Botteron, S. R. Dager, G. Dawson, A. M. Estes, A. C. Evans, H. C. Hazlett, P. Kostopoulos, R. C. McKinstry, S. J. Paterson, R. T. Schultz, L. Zwaigenbaum, J. Piven and I. Network (2012). "Differences in white matter fiber tract development present from 6 to 24 months in infants with autism." Am J Psychiatry **169**(6): 589-600.

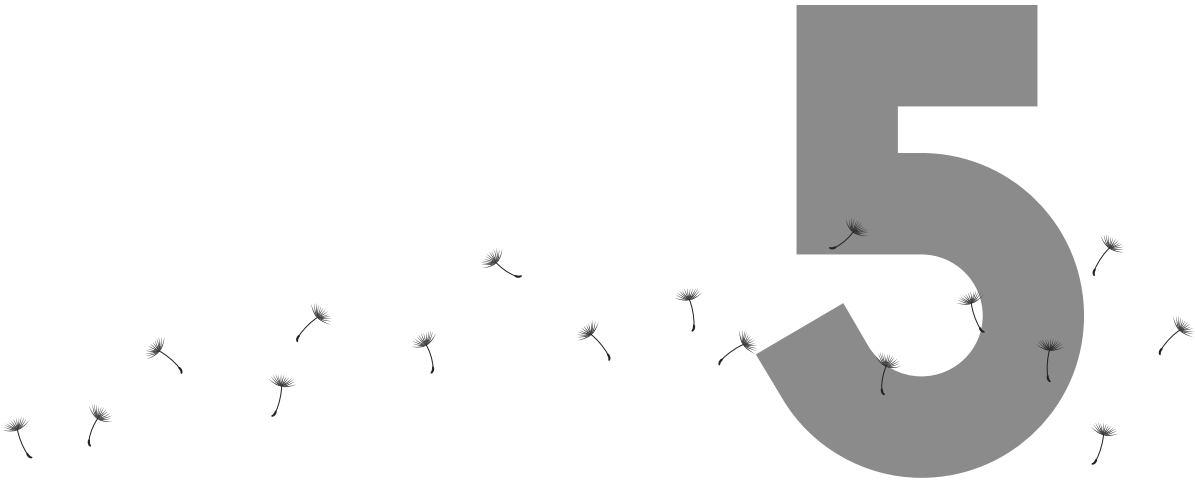
Zhang, Y., C. Wang, X. Zhao, H. Chen, Z. Han and Y. Wang (2010). "Diffusion tensor imaging depicting damage to the arcuate fasciculus in patients with conduction aphasia: a study of the Wernicke-Geschwind model." Neurol Res **32**(7): 775-778.

SUPPLEMENT

Supplementary Figure 1.



Note. Tracts are group average representations in standard coordinate space. Blue indicates the cingulum bundle, gray the forceps major, tan the forceps minor, red the inferior longitudinal fasciculus, orange the superior longitudinal fasciculus, and green the uncinate fasciculus. R = Right, L = Left, A = Anterior, P = Posterior, I = Inferior, S = Superior.



From Chronnectivity To Chronnectopathy: Connectivity Dynamics of Typical Development And Autistic Traits

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ABSTRACT

Autism spectrum disorder is often studied with little context of typical brain development. In addition, most functional connectivity studies operate under the assumption that connectivity remains static over multiple minutes. We hypothesized that relaxing this stationarity assumption would reveal novel features of both autism and typical brain development. We employed a 'chronnectomic' (recurring, time-varying patterns of connectivity) approach to evaluate transient states of connectivity using resting-state functional MRI in a sample of 774 6-to-10 year-old children. Whole-brain dynamic connectivity was evaluated using a sliding window technique, and revealed four transient states. Inter-domain connectivity increased with age in modularized dynamic states, illustrating an important pattern of connectivity associated with the developing brain. Furthermore, we demonstrate that higher levels of autistic traits were associated with more time spent in a globally disconnected state. These results provide a road map to the chronnectomic organization of the brain and also suggest that children with autistic traits exhibit delayed characteristics of functional brain maturation.

A number of developmental disorders, including autism spectrum disorder (ASD), have demonstrated abnormal functional hypoconnectivity and hyperconnectivity within the connectome (Uddin, Supekar and Menon 2013). Atypical development of neural interactions has been considered as a major basis in theoretical models of neuropsychiatric disorders (Geschwind and Levitt 2007). Evidence suggests that short-range or intra-domain connectivity is more dominant during infancy (Fransson, Skiold, Horsch, Nordell, Blennow et al. 2007, Gao, Gilmore, Giovanello, Smith, Shen et al. 2011) and decreases with age during childhood and adolescence, while long-range or inter-domain connectivity becomes more dominant in early adulthood (Fair, Cohen, Power, Dosenbach, Church et al. 2009, Dosenbach, Nardos, Cohen, Fair, Power et al. 2010). To our knowledge, no study has provided a baseline to understand how the brain's dynamic functional connectivity (i.e. chronnectivity) matures with age during childhood, and compared this baseline with the dysfunctional chronnectopathy of emerging mental illness. Likewise, the majority of existing models have made the assumption that the brain's functional connectivity is static over a period of multiple minutes. This has been shown to be a major limitation as important dynamic patterns of connectivity could be overlooked (Calhoun, Miller, Pearlson and Adali 2014). In the context of this paper, the term chronnectome (and therefore chronnectivity) refers to dynamics in the functional network connectivity among multiple brain regions. Also, in this paper, the term connectome refers to the functional network connectivity at a macro-scale, with the assumption that the functional connectivity over this period of time is relatively static. In this work, we address both of these limitations by performing a chronnectomic analysis of typical development and autistic traits.

Over the past decade, various *in vivo* techniques, including functional magnetic resonance imaging (fMRI), have been increasingly used to study neuronal connectivity in the developing brain, particularly during rest (rs-fMRI). A wide array of methods has been used to categorize the brain into functionally interconnected parcels, or intrinsic connectivity networks (ICNs), such as the default-mode network. Importantly, the majority of existing rs-fMRI analysis strategies operate under the assumption that connectivity remains stationary or static throughout the entire measurement period (static functional network connectivity (sFNC)), potentially obscuring transient patterns of connectivity at different time instances. Recent advances in analysis methods have relaxed this stationarity assumption to yield indices of dynamic functional network connectivity (dFNC) that offer unique chronnectomic information (Hutchison, Womelsdorf, Allen, Bandettini, Calhoun et al. 2013, Allen, Damaraju, Plis, Erhardt, Eichele et al. 2014) and are sensitive to neurobiological features of normal brain development (Hutchison and Morton 2015) and psychopathology (Rashid, Damaraju, Pearlson and Calhoun 2014).

Despite the presence of an extensive and expanding literature, the neurobiological etiology of severe mental disorders such as autism spectrum disorder remains elusive. ASD has traditionally been conceptualized categorically, but is increasingly recognized as the severe end of a continuum of traits that extend into the general population (Constantino and Todd 2003). Imaging studies using a phenotype of quantitative social impairment can complement case-control studies to better understand the neurobiology of ASD. Irrespective of classification approach, one of the prominent hypotheses on the origins of ASD is an aberrant development of neuronal connections throughout the brain (i.e., 'developmental disconnection syndrome', Geschwind and Levitt 2007).

Within this context, we utilized resting-state fMRI scans from a large, population-based cohort study of children (Jaddoe, van Duijn, Franco, van der Heijden, van IIzendoorn et al. 2012, White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013) to search for both underlying maturational properties of dFNC that characterize age- and sex-specific connectivity dynamics, and an underlying neurobiological substrate of autistic traits in the general population. We hypothesized the presence of distinct dynamic connectivity states in children that are similar to those already reported in adults, given many static networks are already present at a young age (Gao, Gilmore et al. 2011). Further we expect age-related correlates of dynamic connectivity to resemble adult-like patterns, where increasing age is associated with states previously reported in adults. Lastly, as previous work has shown aberrant connectivity dynamics in psychopathology, we hypothesize to see an association between aberrant dynamic connectivity and features of autism. As traits of ASD have been shown to form a continuum in the general population, we hypothesized that such connectivity features would also be present along a continuum. Results showed that multiple brain domains (comprised of sets of ICNs) that are widely recognized in studies of adults (e.g., sub-cortical, default-mode and sensorimotor) are also identified in this large group of young children. Results also reveal that the dynamic properties of connectivity vary with both age and sex. Specifically, we found increased inter-domain connectivity with age in the more mature, "adult-like" dFNC states, in which older children also spent more time compared to the younger children. Interestingly, children with autistic traits spent more time in a globally disconnected state, which resembled the connectivity dynamics observed in younger children. These results show a link between the typical and atypical development trajectories as captured by dynamic FNC, where individuals with higher levels of autistic traits show a potentially delayed transition to spending time in the globally modularized or more heavily connected states. Taken together, the present study provides a conceptual framework to support further investigations of typical and atypical brain development in the general population using novel neuroimaging methodology and clinical insight.

RESULTS

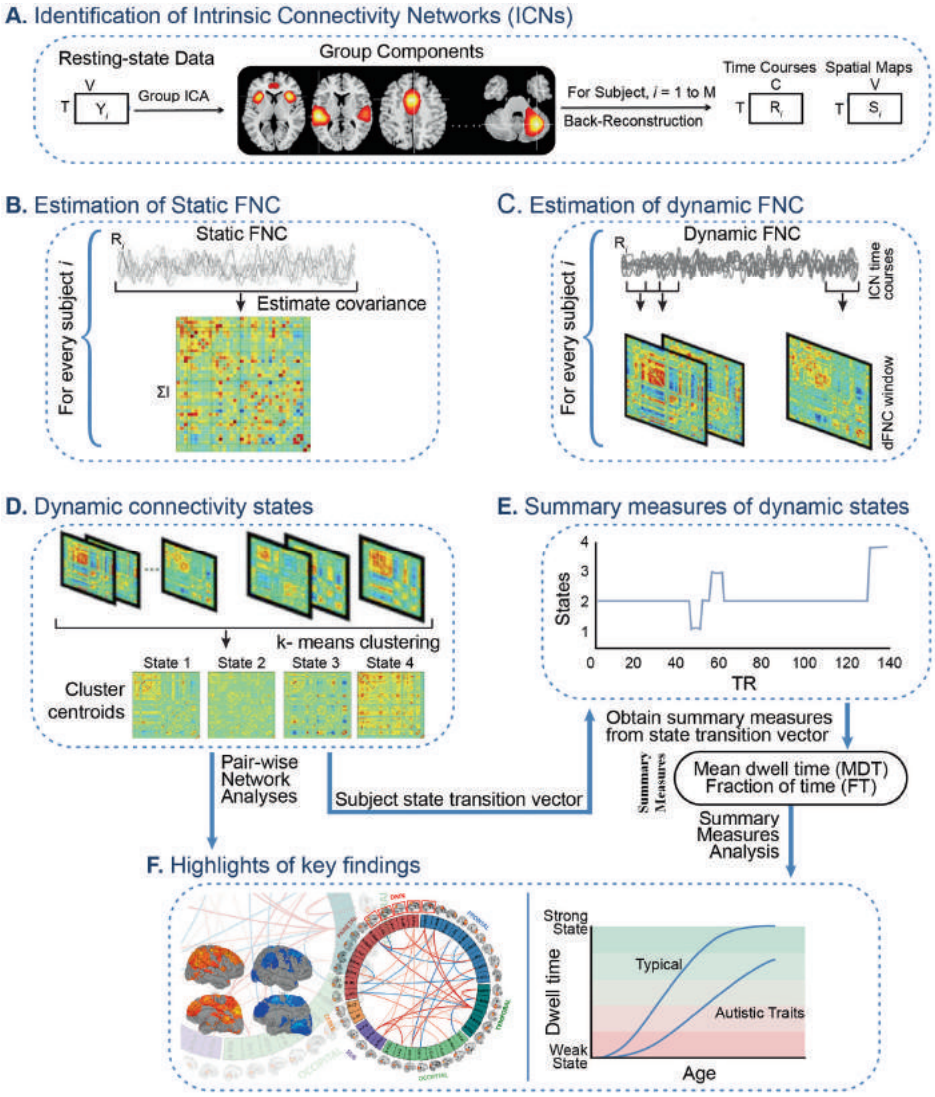
Characterizing static and dynamic functional network connectivity during development

Our first goal was to characterize the connectivity in typical development through age associations in a large sample of 774 school-age children. To do this we first evaluated the properties of both static and dynamic FNC (Figure 1(A-D)) of the developing brain using 38 ICNs (extracted from a 100 component, group independent component analysis (Calhoun, Adali, Pearlson and Pekar 2001)) grouped into brain domains according to their anatomical and functional properties (Figure 2). The static FNC of the developing brain showed similar patterns as previous large-scale analyses of adults (Allen, Damaraju et al. 2014, Damaraju, Allen, Belger, Ford, McEwen et al. 2014) for both intra- and inter-domain connectivity. The default mode network was strongly connected within itself, and less connected to other brain networks (Figure S1). The dynamic connectivity analysis (Figure 3) identified two modularized states (State-1: globally modularized, i.e. modularized FNCs were present globally in intra- and inter-domain connectivity, and State-3: default-mode modularized, i.e. strong intra-domain positive connectivity and inter-domain negative connectivity in DMN). In addition, one globally disconnected state was identified (State-2: globally loosely connected intra- and inter-domain connectivity captured, a state not previously observed in adults) and one globally hyperconnected state (State-4: high positive connectivity found globally). Previous dynamic connectivity studies with adult subjects reported occurrence of three out of these four dynamic states (Allen, Damaraju et al. 2014).

Development of dynamic FNC states

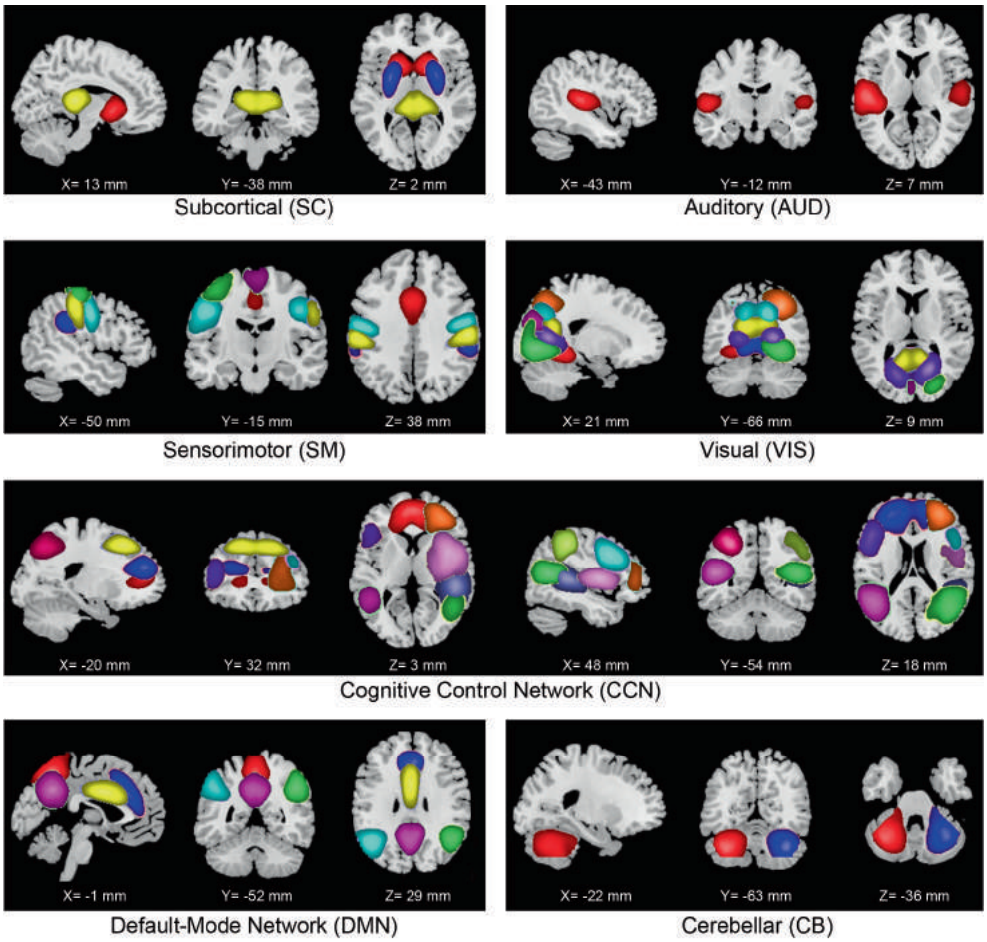
Next, we evaluated the relationship of age and sex with the discrete dynamic FNC states to evaluate the trajectory of the states from a less to a more mature representation of FNC patterns (Figure 4). The age-related associations were mostly localized in (but not limited to) State-1, the globally modularized dynamic state. In particular, positive age-related associations among frontal-temporal components, and both positive and negative age-related associations among frontal-parietal and temporal-parietal components were observed in State-1. Also, the sex-related associations were mostly localized in (but not limited to) State-3, a state characterized by a modularized DMN. This particular dynamic state showed greater connectivity among frontal-temporal and frontal-occipital components in girls, and greater connectivity between a parietal component (right angular gyrus (also a DMN component)) and a temporal component (right middle temporal gyrus) in boys. In other FNC states, the age- and sex-specific changes were mostly localized to the DMN. Specifically, the left middle cingulate cortex (MCC) DMN component showed increased inter-domain connectivity with age in all FNC states, and increased intra-domain connectivity with age in State-4. Lastly, the left MCC showed an increase in inter-domain connectivity for girls in all FNC states, and an increase in intra-domain connectivity for boys in State-3.

Figure 1. *Graphical depiction of the analysis method and key findings.*



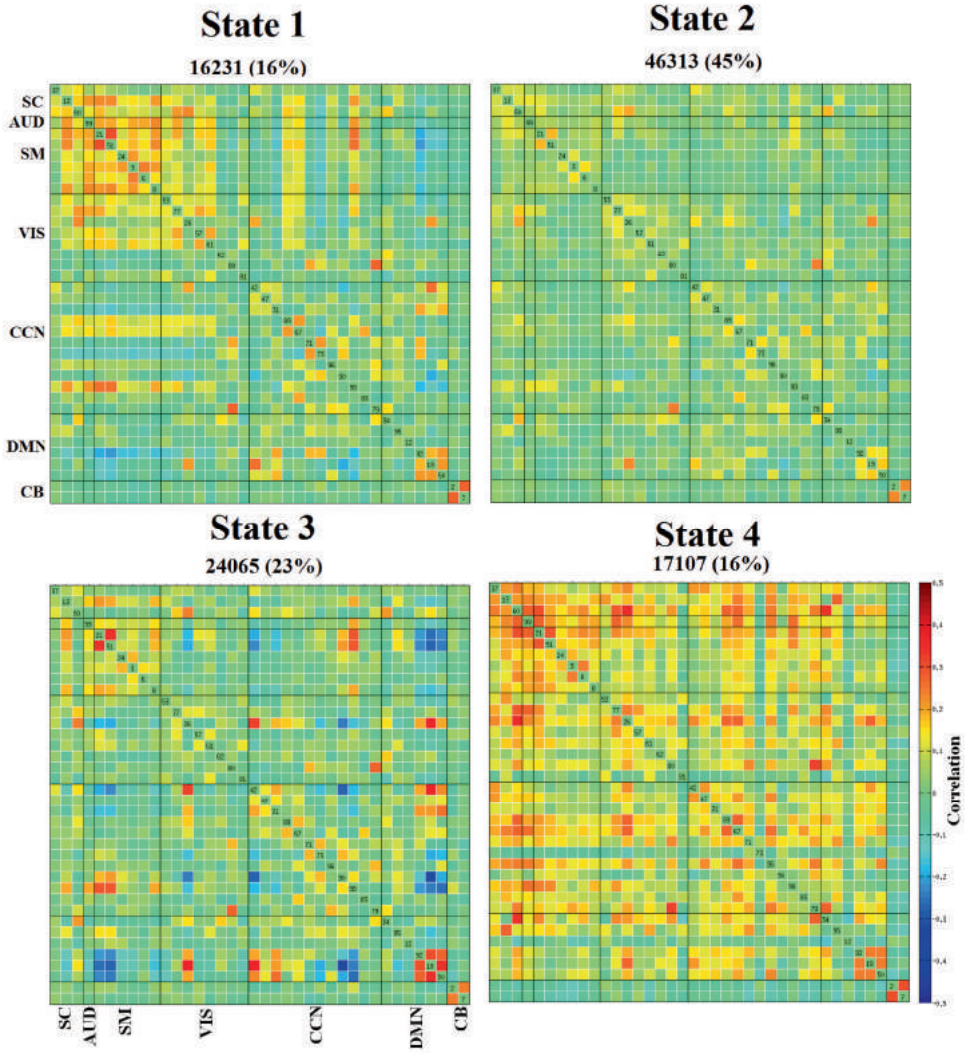
(A) The static and dynamic functional network connectivity (FNC) approach begin with group independent component analysis (ICA) to decompose resting-state fMRI data into intrinsic connectivity networks (ICNs). The group ICA approach provides a measure of the component time courses (TC) and spatial maps for each subject using the back-reconstruction technique. **(B)** Static FNC between components is estimated as the covariance of the time courses. **(C)** Dynamic FNC is estimated as the covariance from windowed portions of the time courses. **(D)** K-means clustering is used to identify discrete dynamic connectivity states. **(E)** Results obtained from k-means clustering are used to determine which state a given subject is occupying at a given time, and summary measures of dynamic states, such as, mean dwell time (MDT) and fraction of time (FT) spent in each state over the duration of the measurement period are computed. **(F)** Highlights of the key findings for pairwise network analyses and summary measures analyses in association with age, sex and autistic traits.

Figure 2. *Non-artifactual intrinsic connectivity networks (ICNs).*



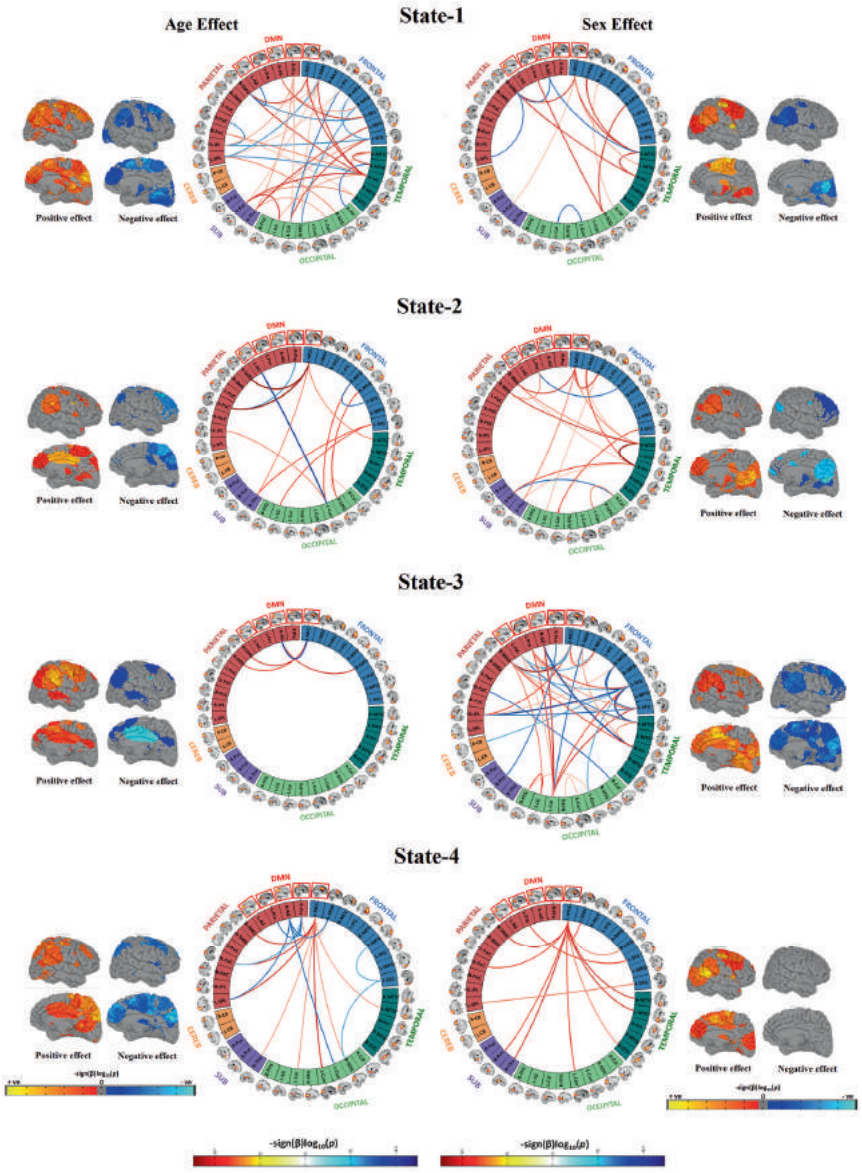
Composite maps of the 38 identified intrinsic connectivity networks (ICNs) used in static and dynamic functional network connectivity (FNC) analyses. The ICNs are divided into seven subcategories and arranged based on their anatomical and functional properties. Within each functional network, each color in the composite maps corresponds to a different ICN. Component labels and peak coordinates are provided in Table S2.

Figure 3. *Dynamic functional network connectivity (FNC) states.*



The four dynamic states represented in connectivity matrices are symmetrically grouped by functional networks, and colors represent the average strength and direction of the pairwise correlation between two components, with red-yellow indicating a position correlation, and blue indicating a negative correlation. Here, SC: subcortical, AUD: auditory, SM: sensorimotor, VIS: visual, CCN: cognitive control network, DMN: default-mode network, and CB: cerebellar network. Labels for the dynamic states include, state-1: globally modularized, state-2: globally disconnected, state-3: DMN modularized, and state-4: globally hyperconnected.

Figure 4. *Connectogram and rendering maps showing age and sex associations across the dynamic connectivity states.*



Connectograms are sorted by major brain lobes. Rendering maps are divided into average positive and negative effects. For age analyses, red lines indicate positive association between a particular pairwise connection and age, whereas blue lines indicate a negative age association. For analyses of sex, red lines indicate where female subjects showed stronger connectivity than male subjects, and blue lines indicate where male subjects showed stronger connectivity compared to female subjects. All the results presented in the connectograms survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$.

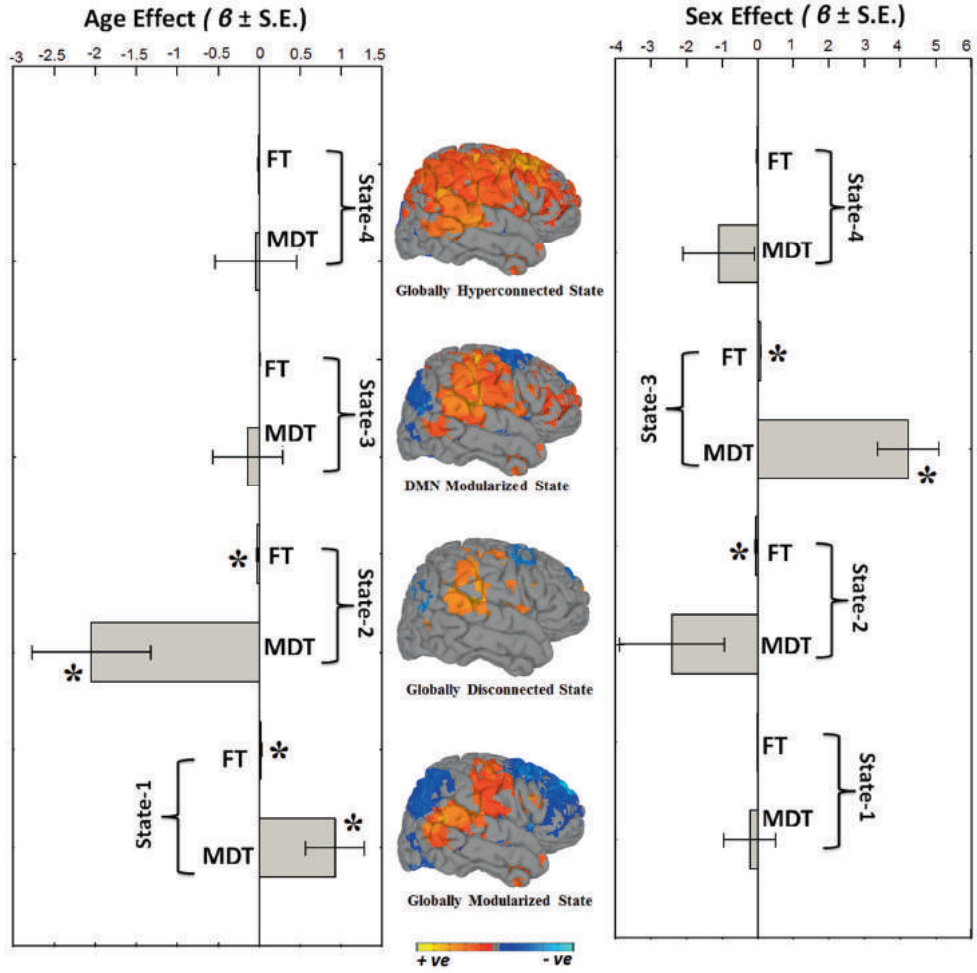
Age- and sex-related associations with time spent in dynamic states

Next, we explored how dynamic connectivity properties such as mean dwell time (MDT) and fraction of time spent in dynamic states (FT) change as functions of age and sex (Figure 5). Here, for each of the dynamic FNC states, we computed the MDT (how long an individual spends in a given state on average) and FT (total time spent in a given state). The equations for MDT and FT are given in the online method section. We found that older children showed higher MDT and FT in the globally modularized dFNC state, or State-1. Conversely, younger subjects showed higher MDT and FT in the globally disconnected state, or State-2. We also investigated the sex-related differences in MDT and FT in the dynamic states. We found that in the disconnected state (State-2), boys showed higher FT, whereas in DMN-modularized state (State-3), girls showed higher MDT and FT. The other two dynamic states, the globally modularized state (State-1) and the globally hyperconnected state (State-4) showed trend-level sex effects, where boys showed higher MDT and FT compared to girls in States-1 and-4.

Characterization of dynamic connectopathy (chronnectopathy) in autistic traits and children with ASD

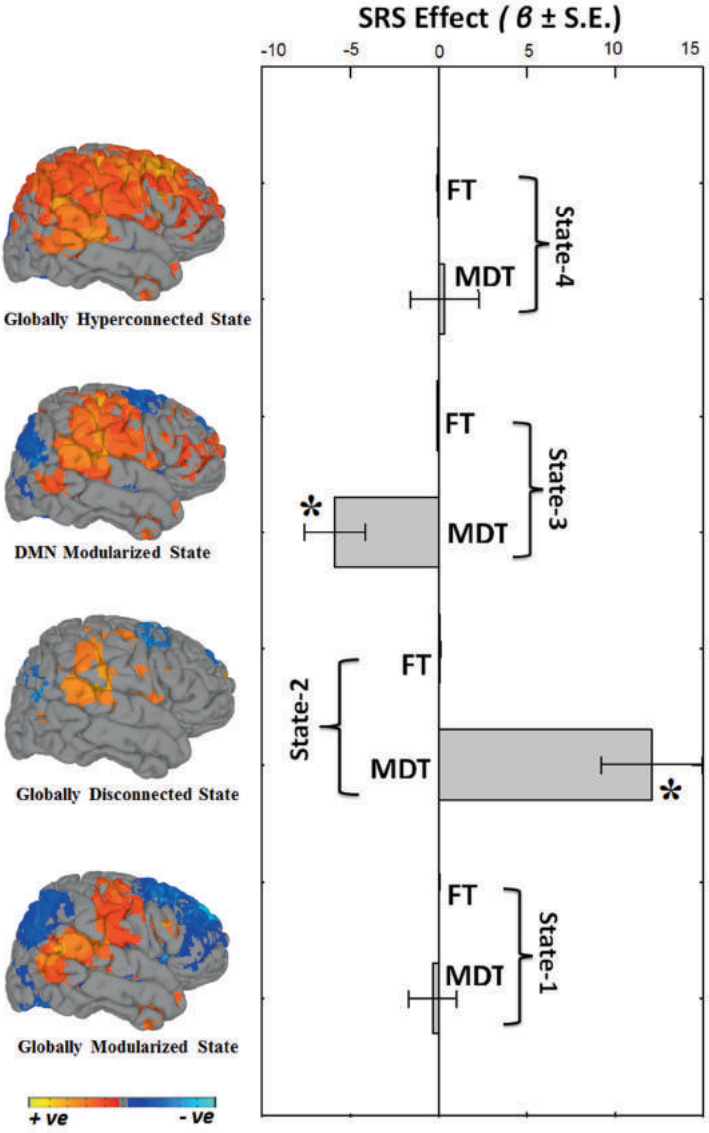
In addition to characterizing static and dynamic FNCs in typical development, we also studied the chronnectopathy, or disruption of the typical dynamic connectivity patterns, in children with autistic traits. We assessed autistic traits using the Social Responsiveness Scale (SRS) (Constantino, Davis, Todd, Schindler, Gross et al. 2003) in a subset of children (n=560). For static connectivity, one component pair (the left supplementary motor area, i.e. SMA, and the right supramarginal gyrus, i.e. SmG), showed an association with autistic traits. Specifically, children with more autistic traits showed weaker static FNC. Interestingly, for dFNC State-3, children with more autistic traits showed higher connectivity in three component pairs (right insula and left superior frontal gyrus, right SmG and left precuneus i.e. preC, and right insula and left preC) and lower connectivity in two component pairs (right-insula and right SmG, and left SMA and right SmG). Next, we assessed how MDT and FT vary with respect to autistic trait scores (Figure 6). In the globally disconnected state (State-2), autistic traits showed a positive association with MDT. In the DMN-modularized state (State-3), autistic traits were negatively associated with MDT. Thus, children with high levels of autistic traits spent more time in the globally disconnected state (State-2) and children with lower levels spent more time in the DMN-modularized state (State-3). Results remained highly consistent when models were additionally adjusted for non-verbal IQ. A similar pattern of effects was observed at the severe end of the spectrum, when 22 children with clinical ASD were compared to 88 age, sex and IQ matched controls (Figure S6). Further, in order to assess whether the above-mentioned associations are a core feature of the trait-continuum or if effects were driven by the most severely affected children, sensitivity analyses were run. In these sensitivity analyses, children with clinical ASD or an autistic traits score above the screening threshold were excluded, and results remained consistent (Figure S7).

Figure 5. *Summary metrics and age- and sex- effects.*



Summary metrics from the four dynamic connectivity states in relation to age and sex. Mean dwell time (MDT) represents how long an individual spends in a given state on average, and fraction of time (FT) is the summed total time spent in a given state over the course of the measurement period. For age associations, positive beta coefficient (β) indicates older children spend more time in that particular state whereas negative beta coefficient (β) indicates younger children spend more time in a particular state. For sex analyses, positive beta coefficient (β) indicates girls spend more time in the state relative to boys, and negative beta coefficient (β) indicates that boys spend more time in the state relative to girls. Bar graphs indicate the unstandardized beta coefficients (β) with standard error (S.E.) from regression models, and asterisks (*) indicate the results survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$. The rendering brain maps are showing modularized positive (red) and negative (blue) connectivity for the corresponding dynamic states.

Figure 6. *Summary metrics and autistic trait effects.*



Summary metrics from the four dynamic connectivity states in relation to autistic traits. Mean dwell time (MDT) represents how long an individual spends in a given state on average, and fraction of time (FT) is the summed total time spent in a given state over the course of the measurement period. Positive beta coefficient (β) indicates that higher levels of autistic traits are associated with more time spent in a particular state; whereas negative beta coefficient (β) indicate lower levels of autistic traits are associated with more time spent in a particular state. Bar graphs indicate the unstandardized beta coefficients (β) with standard error (S.E.) from regression models, and Asterisks (*) indicate the results survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$. The rendering brain maps are showing modularized positive (red) and negative (blue) connectivity for the corresponding dynamic states.

DISCUSSION

Here we apply a novel approach to the study of brain connectivity, both in typical and atypical child development. Complementing the existing static functional connectivity literature, we show age-related associations with discrete dynamic states that illustrate higher order maturational effects on the chronnectome. We also provide additional support for a disconnection construct in children with autistic traits using dynamic functional connectivity. In the context of often subjective and qualitative interpretations of static network matrices, we demonstrate the utility and potential clinical relevance of quantitative metrics that summarize large amounts of complex chronnectomic information.

The development of whole-brain dynamic connectivity in young children

In a large group of young children with a narrow age-range, we demonstrate that older children spend more time in states typically observed in healthy adults. Age-related associations with static connectivity were consistent with previous reports, including decreased segregation and increased integration of control networks (Fair, Dosenbach, Church, Cohen, Brahmbhatt et al. 2007). This validation of the existing static connectivity literature is nicely complemented with new information where assumptions of network stationarity are relaxed, and quantitative summary metrics, such as mean dwell time, are examined (Hutchison and Morton 2015). Interestingly, evidence for sexual dimorphism in FNC was also observed with girls spending more time in the modularized default-mode state and boys spending more time in the globally disconnected state. While no age-by-sex interaction was observed, given the narrow age range, this could complement existing evidence showing neuromaturational processes begin earlier in girls (Lenroot and Giedd 2006, Simmonds, Hallquist, Asato and Luna 2014).

Functional connectivity and autistic traits

Novel neuroimaging findings in combination with a characteristic early onset have brought momentum to ASD being conceptualized as a developmental disconnection syndrome (Geschwind and Levitt 2007). Previous studies of static FNC in ASD have revealed mixed patterns of increased and decreased connectivity strength (Uddin, Supekar et al. 2013). Similarly, within the discrete dynamic FNC states, we found local patterns of increased as well as reduced connection strength. We found decreased connectivity between the right supramarginal gyrus and the right insula, which is consistent with findings of lower insula activation in subjects with ASD in a large number task-based neuroimaging studies, covering a range of social processing tasks (Di Martino, Ross, Uddin, Sklar, Castellanos et al. 2009). However, we also found *hyper*connectivitity between the right insula, with the precuneus and the left superior frontal gyrus. Hyperconnectivity of the salience network, in which the

insula is a key region, is particularly well replicated in the context of childhood ASD (Uddin, Supekar, Lynch, Khouzam, Phillips et al. 2013). Our findings in children in a similar age range suggest that the hyperconnectivity of the insula may also extend beyond regions of the salience network. Further, divergent findings of hypo- and hyperconnectivity in this region across studies, which have been previously attributed to developmental differences between samples (Uddin, Supekar et al. 2013), may in fact be present at the same developmental stage, but across different dynamic states and thus only apparent when using dynamic connectivity approaches.

Here, for the first time, we demonstrate that children with higher levels of autistic traits spend more time in a globally disconnected state during rest, whereas children with lower levels of autistic traits spend more time in a globally modularized state that resembles an adult-like pattern of connectivity. Interestingly, in schizophrenia, another disorder frequently classified as a disconnection syndrome, patients also spend more time in weakly connected dynamic states compared to healthy controls (Damaraju, Allen et al. 2014, Rashid, Damaraju et al. 2014). This also potentially fits with previous work in adults showing that, at the individual level, those with ASD may have distinct, noisy patterns of connectivity that may even mask 'typical' patterns of connectivity (Hasson, Avidan, Gelbard, Vallines, Harel et al. 2009). Higher levels of autistic traits were also associated with less time spent in a default-mode modularized state; a state where nodes from the well-documented default-mode network were prominent. Despite heterogeneity in much of the functional connectivity literature, there is a growing body of evidence that the default mode network is more weakly connected in individuals with ASD (Stigler, McDonald, Anand, Saykin and McDougle 2011, Jung, Kosaka, Saito, Ishitobi, Morita et al. 2014). Interestingly, task-based data examining the effect of a cognitive load on the DMN has previously suggested the DMN does not 'deactivate' during a task in ASD (Kennedy, Redcay and Courchesne 2006). However, in the context of our findings, it is possible that rather failing to deactivate, the DMN actually fails to 'activate' in individuals with ASD; an interpretation that could be made from task-rest contrasts of BOLD activation. We also demonstrated that, in the absence of clinically relevant cases, autistic symptoms in the general pediatric population are related to dynamic aspects of network connectivity. This is further evidence that aspects of the neurobiology of autistic traits, similar to the symptomatology, indeed lie on a continuum (Constantino and Todd 2003, Di Martino, Shehzad, Kelly, Roy, Gee et al. 2009, Blanken, Mous, Ghassabian, Muetzel, Schoemaker et al. 2015). In addition to the dimensional trait approach, children with confirmed ASD were compared to a group of age- and sex-matched controls, revealing similar patterns of increased dwell time within the globally disconnected state. Thus, we show that these dynamic functional connectivity features of autistic traits are also present in the most severely affected children. We propose to label this continuum of dynamic connectivity features "chronnectopathy". However, it should be noted that, similar to the behavioral

phenotype, only the severe end is “clinical” and certainly not all tendencies to this pattern should be considered such. Interestingly, the increased mean dwell time in a less connected state observed in children with autistic traits and ASD which mimics the patterns in younger, typically developing children, potentially indicative of a delayed or halted trajectory (Di Martino, Fair, Kelly, Satterthwaite, Castellanos et al. 2014)

Additional considerations

Strengths of this study include the large, population-based sample of children in a narrow age range, enabling us to show subtle age effects during a crucial, pre-adolescent period of development. Further, the age-range included in the current study is particularly under-studied in the context of ASD (Uddin, Supekar et al. 2013). Another major strength is the use of a dynamic approach to resting state connectivity combined with an efficient and interpretable presentation of a wealth of data. While there is some consistency in the expansive static connectivity literature in ASD, it is unfortunately plagued by heterogeneity in clinical characteristics of the subjects, image acquisition, analysis strategy, and ultimately the core findings (Uddin, Supekar et al. 2013, Hernandez, Rudie, Green, Bookheimer and Dapretto 2015). The quantitative summary measures presented here could potentially aid in simplifying interpretations of complex network information, which historically are often subjectively evaluated. For instance, specific and isolated features of large (e.g., 80x80) connectivity matrices are often summarized when undoubtedly more complex patterns are present. While the present study also assigned labels to the four dynamic states, most of the interpretation comes from the quantitative MDT metric. This method may be especially suitable to study ASD, as disruptions of connectivity patterns in subjects with autism are thought to be highly idiosyncratic (Hahamy, Behrmann and Malach 2015) and this method may be more suitable to pick up group differences in the context of such individual differences. The subjects were all scanned on the same MRI scanner, which reduces vendor- and hardware-dependent differences. Finally, the study of ASD is approached dimensionally as well as from a traditional case-control perspective, revealing dynamic connectivity features of ASD that lie along a continuum in the general population. While many studies of ASD include only boys, our sample was sex-balanced and also presented in the context of typical brain development. However, some limitations deserve mention. While increased scan duration is likely to reveal the complexity of dynamic connectivity states and their temporal aspects more accurately, our rs-fMRI scan was limited to just over 5 minutes to ensure high quality data, given the scale of the study and to minimize the burden on our young participants (White, Muetzel, Schmidt, Langeslag, Jaddoe et al. 2014). Further, our study was cross-sectional and all participants were of school-age, so the interpretation of our results can not be extended to other stages of development. Longitudinal studies are warranted to reveal trajectories of dynamic connectivity in typical and atypical development.

In conclusion, our approach suggests that a hallmark of childhood is not limited to the under-development of the frontal lobe, but also about the efficient utilization of vast interconnections; in essence, younger children are less frequently tapping into the resources that they have. Also, children with higher levels of autistic traits are even less likely to efficiently use such connections and may have less capacity in this regard. This study revealed novel aspects of psychopathology and future studies should evaluate the utility of this methodology in, for example, the classification, evaluation and treatment response prediction of conditions like ASD.

ONLINE METHOD

Participants

The current study is embedded in the Generation R Study, which is a large, population-based birth cohort in Rotterdam, the Netherlands (Jaddoe, van Duijn et al. 2012). One thousand seventy children, ages 6-to-10 years, were scanned between September 2009 and July 2013 as part of a sub-study within the Generation R Study (White, El Marroun et al. 2013). General exclusion criteria for the current study include severe motor or sensory disorders (deafness or blindness), neurological disorders, moderate to severe head injuries with loss of consciousness, claustrophobia, and contraindications to MRI. Raw fMRI data from 964 subjects were available for our study, and after excluding children with bad data (e.g., motion, for details see below) 774 datasets were available for statistical analysis. Informed consent was obtained from the parents, and all procedures were approved by the Medical Ethics Committee of the Erasmus Medical Center.

Autistic Traits and Autism Spectrum Disorder

The Social Responsiveness Scale was administered when children were roughly age 6 years (range: 4.89–8.90 years) to measure autistic traits based on parental observation during the last six months (Constantino 2002). The Social Responsiveness Scale provides a valid quantitative measure of subclinical and clinical autistic traits, where higher scores indicate more symptoms related to ASD (Constantino 2002). We utilized the total score derived from the abbreviated, 18-item short-form of the scale, which shows correlations ranging from 0.93 to 0.99 with the full scale in three different large studies (Blanken, Mous et al. 2015). Cutoffs were based on recommendations for screening in population-based settings (consistent with weighted scores of 1.078 for boys and 1.000 for girls) (Constantino 2002).

At approximately age 7 years, children who scored in the top 15th percentile on the Child Behavior Checklist-1.5–5 total score and those who scored in the top 2nd percentile on the Pervasive Developmental Problems sub-scale underwent a screening procedure for ASD using the Social Communication Questionnaire (SCQ), a 40-item parent-reported screening instrument to assess characteristic autistic behavior. Social Communication Questionnaire scores ≥15 are considered positive for screening (Berument, Rutter, Lord, Pickles and Bailey 1999). We approached the general practitioners of children who scored screen-positive on the SRS, SCQ or for whom the mother reported a diagnosis of ASD in order to confirm this diagnosis with medical records. In the Netherlands, the general practitioner holds the central medical records, including information on treatment by (medical) specialists. In this sample, 22 children with usable MRI data also had a confirmed diagnosis of ASD.

MRI Data Acquisition

Magnetic resonance imaging data were acquired on a 3 Tesla scanner (Discovery 750, General Electric, Milwaukee, WI) using a standard 8-channel, receive-only head coil. A three-plane localizer was run first and used to position all subsequent scans. Structural T1-weighted images were acquired using a fast spoiled gradient-recalled echo (FSPGR) sequence (TR = 10.3 ms, TE = 4.2 ms, TI = 350 ms, NEX = 1, flip angle = 16°, matrix = 256 x 256, field of view (FOV) = 230.4 mm, slice thickness = 0.9mm). Echo planar imaging was used for the rs-fMRI session with the following parameters: TR = 2000 ms, TE = 30 ms, flip angle = 85°, matrix = 64 x 64, FOV = 230 mm x 230 mm, slice thickness = 4 mm. In order to determine the number of TRs necessary for functional connectivity analyses, early acquisitions acquired 250 TRs (acquisition time = 8min 20sec). After it was determined fewer TRs were required for these analyses, the number of TRs was reduced to 160 (acquisition time = 5min 20sec) (White, Muetzel et al. 2014). Children were instructed to stay awake and keep their eyes closed during the rs-fMRI scan. Further details on the entire scanning protocol can be found elsewhere (White, El Marroun et al. 2013).

Image Preprocessing

Data preprocessing was performed using a combination of toolboxes (AFNI, http://afni.nimh.nih.gov, SPM, http://www.fil.ion.ucl.ac.uk/spm, GIFT, http://mialab.mrn.org/software/gift), and custom scripts were written in Matlab. We performed rigid body motion correction using the INRIAlign (Freire and Mangin 2001) toolbox in SPM to correct for subject head motion followed by slice-timing correction to account for timing differences in slice acquisition. Then the fMRI data were despiked using AFNI's 3dDespike algorithm to mitigate the impact of outliers. The fMRI data were subsequently warped to a Montreal Neurological Institute (MNI) template (http://www.mni.mcgill.ca) and resampled to 3 mm^3 isotropic voxels. Then we smoothed the data with a Gaussian kernel to 5 mm full width at half maximum (FWHM).

Each voxel time course was variance normalized prior to performing group independent component analysis as this has shown to better decompose subcortical sources in addition to cortical networks. In order to limit the impact of severe head motion, we excluded subjects' data with a maximum translation of > 5 mm and/or with signal-to-noise fluctuation ratio (SFNR) <200 from our analyses, resulting in a final dataset with 774 subjects.

Group Independent Component Analysis (ICA)

After preprocessing the data, functional data were analyzed using spatial group independent component analysis (GICA) framework as implemented in the GIFT software (Calhoun, Adali et al. 2001, Calhoun and Adali 2012). Spatial ICA decomposes the subject data into linear mixtures of spatially independent components that exhibit a unique time course profile. A subject-specific data reduction step was first used to reduce 160 time point data into 100 directions of maximal variability using principal component analysis. Then subject-reduced data were concatenated across time and a group data PCA step reduced this matrix further into 100 components along directions of maximal group variability. One hundred independent components were obtained from the group PCA reduced matrix using the infomax algorithm (Bell and Sejnowski 1995). To ensure stability of estimation, we repeated the ICA algorithm 20 times in ICASSO (http://www.cis.hut.fi/projects/ica/icasso), and aggregated spatial maps were estimated as the modes of component clusters (Himberg, Hyvarinen and Esposito 2004). Subject specific spatial maps (SMs) and time courses (TCs) were obtained using the spatiotemporal regression back reconstruction approach (Calhoun, Adali, Pearlson and Pekar 2001, Erhardt, Rachakonda, Bedrick, Allen, Adali et al. 2011) implemented in GIFT software.

Post-ICA processing

Subject specific SMs and TCs underwent post-processing as described in our earlier work (Allen, Damaraju et al. 2014). Briefly, we obtained one sample t-test map for each SM across all subjects and thresholded these maps to obtain regions of peak activation clusters for that component; we also computed mean power spectra of the corresponding TCs. We identified a set of components as intrinsic connectivity networks (ICNs) if their peak activation clusters fell on gray matter and showed less overlap with known vascular, susceptibility, ventricular, and edge regions corresponding to head motion. We also ensured that the mean power spectra of the selected ICN time courses showed higher low frequency spectral power. This selection procedure resulted in 38 ICNs out of the 100 independent components obtained.

The subject specific TCs corresponding to the ICNs selected were detrended, orthogonalized with respect to estimated subject motion parameters, and then despiked. The despiking procedure involved detecting spikes as determined by AFNI's 3dDespike algorithm and replacing spikes by values obtained from third order spline fit to neighboring clean portions

of the data. The despiking process reduces the impact/ bias of outliers on subsequent FNC measures (see Supplemental Fig. 1 in (Allen, Damaraju et al. 2014)). Lastly, subject-level rs-fMRI data were denoised using automated artifact removal (Tohka, Foerde, Aron, Tom, Toga et al. 2008, Rodriguez, Adali, Li, Correa and Calhoun 2010). Thus, signals attributed noise/ artifact, including those related to motion, were removed from the data.

Static Functional Network Connectivity (sFNC)

We computed functional network connectivity (FNC), defined as pairwise correlation between ICN time courses, as a measure of average connectivity among different ICNs during the scan duration. In this work, the FNC computed using the whole ICN time courses is referred to as stationary or static FNC (sFNC). Since correlation among brain networks is primarily shown to be driven by low frequency fluctuations in BOLD fMRI data (Cordes, Haughton, Arfanakis, Carew, Turski et al. 2001), we band pass filtered the processed ICN time courses between [0.01–0.15] Hz using 5th order Butterworth filter prior to computing FNC between ICNs. The mean sFNC matrix was computed over subjects. The mean sFNC matrix was initially organized into modular partitions using the Louvain algorithm of the brain connectivity toolbox (https://sites.google.com/site/bctnet). The modular partitions obtained from the algorithm were slightly rearranged to match the order of sFNC matrix rows to our recent work (Allen, Damaraju et al. 2014). After this reordering, the rows of sFNC matrix were partitioned into sub-cortical (SC), auditory (AUD), visual (VIS), sensorimotor (SM), a broad set of regions involved in cognitive control (CCN) and attention, default-mode network (DMN) regions, and cerebellar (CB) components as shown in Figure S1.

Dynamic Functional Network Connectivity (dFNC)

As recent studies both in animals and humans have highlighted the nonstationary nature of functional connectivity in BOLD fMRI data (Chang and Glover 2010, Hutchison, Womelsdorf, Gati, Everling and Menon 2013), we sought to determine whether the observed sFNC differences were primarily driven by certain connectivity configurations (Hutchison, Womelsdorf et al. 2013). Following our recent work (Allen, Damaraju et al. 2014), dynamic FNC (dFNC) between two ICA time courses was computed using a sliding window approach with a window size of 22 TR (44 s) in steps of 1 TR (Figure 1). As in our earlier work, the window constituted a rectangular window of 22 time points convolved with Gaussian of sigma 3 TRs to obtain tapering along the edges. Since estimation of covariance using time series of shorter length can be noisy, we estimated covariance from regularized inverse covariance matrix (ICOV) (Varoquaux, Baronnet, Kleinschmidt, Fillard and Thirion 2010, Smith, Miller, Salimi-Khorshidi, Webster, Beckmann et al. 2011) using the graphical LASSO framework (Friedman, Hastie and Tibshirani 2008). We imposed an additional L1 norm constraint on the inverse covariance matrix to enforce sparsity. The regularization parameter was optimized

for each subject by evaluating the log-likelihood of unseen data of the subject in a cross-validation framework. After computing dFNC values for each subject, these covariance values were Fisher-Z transformed.

Clustering and Dynamic States Detection

Based on our observation that patterns of dFNC connectivity reoccur within subjects across time and also across subjects, we used a k-means algorithm to cluster these dynamic FNC windows, partitioning the data into a set of separate clusters so as to maximize the correlation within a cluster to the cluster centroid. Instead of clustering all of the dFNC windows across all subjects, initial clustering was performed on a subset of windows from each subject, called subject exemplars hereafter, corresponding to windows of maximal variability in correlation across component pairs. To obtain the exemplars, we first computed variance of dynamic connectivity across all pairs at each window. We then selected windows corresponding to local maxima in this variance time course. The optimal number of centroid states was estimated using the elbow criterion, defined as the ratio of within cluster to between cluster distances. A k of 4 was obtained using this method in a search window of k from 2 to 9. The correlation distance metric was chosen as it is more sensitive to the connectivity pattern irrespective of magnitude (although choosing other distance functions such as cityblock, cosine and L1-norm did not make any difference in observed results). These sets of initial group centroids were used as a starting point to cluster all of the dFNC windows from all subjects.

Also, summary measures such as mean dwell time (MDT) and fraction of time (FT) were computed from the state transition vector. Using the following equations (i) and (ii), we computed MDT and FT for each subject:

$$MDT^{state\ (k)} = mean(TR_{end} - TR_{start}) \quad \ldots\ldots\ldots\ldots\ldots (i)$$

where,

TR_{start} =count (difference($state\_vector_{subject(i)}$, state_number) == 1)
TR_{end} =count (difference($state\_vector_{subject(i)}$, state_number) == -1)

and,

$$FT^{state\ (k)} = \frac{sum(state\_vector^{subject(i)} == 1)}{Total\ number\ of\ TR} \quad \ldots\ldots\ldots\ldots\ldots (ii)$$

Statistical Analyses

Statistical analyses were carried out in Matlab (version R2011b) using the statistics toolbox and linear model class. Multiple linear regression was used to examine associations with connectivity metrics. Two separate models were used to investigate associations with sFNC, dFNC and summary metrics from dFNC such as MDT and FT: first model where age, sex and age-sex interaction were entered as independent (predictor) variables and main effects for each were examined, and a second model where autistic traits (SRS) was entered as the independent variable and age, sex and age-sex interaction were added as covariates. All of the results reported correspond to a false discovery rate multiple comparison correction threshold $q < 0.05$.

The following models were used for investigating associations with sFNC. We also checked the effects of interaction between age-sex and SRS-sex (in **Model-1**: $\beta_l age_i * sex_i$; in **Model-2**: $\beta_l SRS_i * sex_i$).

Model-1$_{sFNC}$: $sFNC_i \sim \beta_0 + \beta_1 age_i + \beta_2 sex_i + \varepsilon_i$
Model-2$_{sFNC}$: $sFNC_i \sim \beta_0 + \beta_1 SRS_i + \beta_2 age_i + \beta_3 sex_i + \varepsilon_i$

For dFNC analyses, we computed a subject median (computed element-wise) for each partition from the subject windows that were assigned to that partition as a representative pattern of connectivity of the subject for that state. To investigate if the observed effects of age, sex and SRS on sFNC are primarily driven by certain dynamic FNC states, we used these subject medians for each state, as well as the summary matrices for each state, and evaluated the associations using two separate models as mentioned above.

The following models were used for investigating associations with dFNC. We also checked the effects of interaction between age-sex and SRS-sex (in **Model-3**: $\beta_l age_i * sex_i$; in **Model-4**: $\beta_l SRS_i * sex_i$).

Model-3$_{dFNC}$: $dFNC_i^{state\,(k)} \sim \beta_0 + \beta_1 age_i + \beta_2 sex_i + \varepsilon_i$
Model-4$_{dFNC}$: $dFNC_i^{state\,(k)} \sim \beta_0 + \beta_1 SRS_i + \beta_2 age_i + \beta_3 sex_i + \varepsilon_i$

The following models were used for investigating associations with summary matrices of dFNC (MDT and FT). We also checked the effects of interaction between age-sex and SRS-sex (in **Model-5** and **Model-7**: $\beta_l age_i * sex_i$; in **Model-6** and **Model-8**: $\beta_l SRS_i * sex_i$).

Model-5$_{MDT}$: $MDT_i \sim \beta_0 + \beta_1 age_i + \beta_2 sex_i + \varepsilon_i$
Model-6$_{MDT}$: $MDT_i \sim \beta_0 + \beta_1 SRS_i + \beta_2 age_i + \beta_3 sex_i + \varepsilon_i$

Model-7$_{FT}$: $FT_i \sim \beta_0 + \beta_1 age_i + \beta_2 sex_i + \varepsilon_i$
Model-8$_{FT}$: $FT_i \sim \beta_0 + \beta_1 SRS_i + \beta_2 age_i + \beta_3 sex_i + \varepsilon_i$

Pair-wise associations from the above mentioned models are depicted in connectivity matrices and in connectograms (Langen, White, Ikram, Vernooij and Niessen 2015).

Several sensitivity analyses were run in order to ensure results were not influenced various confounding factors, and are reported in the Supplemental Data section. First, to ensure behavioral problems did not influence age- and sex-related associations, analyses were run where children with high levels of behavioral problems were excluded. Similarly, to test whether continuous associations between autistic traits and connectivity were truly along a continuum and not driven by extreme cases, analyses were run after excluding children scoring highest on the SRS.

REFERENCES

Allen, E. A., E. Damaraju, S. M. Plis, E. B. Erhardt, T. Eichele and V. D. Calhoun (2014). "Tracking whole-brain connectivity dynamics in the resting state." Cereb Cortex **24**(3): 663-676.

Bell, A. J. and T. J. Sejnowski (1995). "An information-maximization approach to blind separation and blind deconvolution." Neural Comput **7**(6): 1129-1159.

Berument, S. K., M. Rutter, C. Lord, A. Pickles and A. Bailey (1999). "Autism screening questionnaire: diagnostic validity." British Journal of Psychiatry **175**: 444-451.

Blanken, L. M. E., S. E. Mous, A. Ghassabian, R. L. Muetzel, N. K. Schoemaker, H. El Marroun, A. van der Lugt, V. W. V. Jaddoe, A. Hofman, F. C. Verhulst, H. Tiemeier and T. White (2015). "Cortical Morphology in 6-to 10-Year Old Children With Autistic Traits: A Population-Based Neuroimaging Study." American Journal of Psychiatry **172**(5): 479-486.

Calhoun, V. D. and T. Adali (2012). "Multisubject independent component analysis of fMRI: a decade of intrinsic networks, default mode, and neurodiagnostic discovery." IEEE Rev Biomed Eng **5**: 60-73.

Calhoun, V. D., T. Adali, G. Pearlson and J. J. Pekar (2001). Group ICA of functional MRI data: separability, stationarity, and inference. Proc. Int. Conf. on ICA and BSS San Diego, CA.

Calhoun, V. D., T. Adali, G. D. Pearlson and J. J. Pekar (2001). "A method for making group inferences from functional MRI data using independent component analysis." Hum Brain Mapp **14**(3): 140-151.

Calhoun, V. D., R. Miller, G. Pearlson and T. Adali (2014). "The chronnectome: time-varying connectivity networks as the next frontier in fMRI data discovery." Neuron **84**(2): 262-274.

Chang, C. and G. H. Glover (2010). "Time-frequency dynamics of resting-state brain connectivity measured with fMRI." Neuroimage **50**(1): 81-98.

Constantino, J. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Constantino, J. N., S. A. Davis, R. D. Todd, M. K. Schindler, M. M. Gross, S. L. Brophy, L. M. Metzger, C. S. Shoushtari, R. Splinter and W. Reich (2003). "Validation of a brief quantitative measure of autistic traits: Comparison of the social responsiveness scale with the autism diagnostic interview-revised." Journal of Autism and Developmental Disorders **33**(4): 427-433.

Constantino, J. N. and R. D. Todd (2003). "Autistic traits in the general population: a twin study." Arch Gen Psychiatry **60**(5): 524-530.

Cordes, D., V. M. Haughton, K. Arfanakis, J. D. Carew, P. A. Turski, C. H. Moritz, M. A. Quigley and M. E. Meyerand (2001). "Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data." AJNR Am J Neuroradiol **22**(7): 1326-1333.

Damaraju, E., E. A. Allen, A. Belger, J. M. Ford, S. McEwen, D. H. Mathalon, B. A. Mueller, G. D. Pearlson, S. G. Potkin, A. Preda, J. A. Turner, J. G. Vaidya, T. G. van Erp and V. D. Calhoun (2014). "Dynamic functional connectivity analysis reveals transient states of dysconnectivity in schizophrenia." Neuroimage-Clinical **5**: 298-308.

Di Martino, A., D. A. Fair, C. Kelly, T. D. Satterthwaite, F. X. Castellanos, M. E. Thomason, R. C. Craddock, B. Luna, B. L. Leventhal, X. N. Zuo and M. P. Milham (2014). "Unraveling the miswired connectome: a developmental perspective." Neuron **83**(6): 1335-1353.

Di Martino, A., K. Ross, L. Q. Uddin, A. B. Sklar, F. X. Castellanos and M. P. Milham (2009). "Functional brain correlates of social and nonsocial processes in autism spectrum disorders: an activation likelihood estimation meta-analysis." Biol Psychiatry **65**(1): 63-74.

Di Martino, A., Z. Shehzad, C. Kelly, A. K. Roy, D. G. Gee, L. Q. Uddin, K. Gotimer, D. F. Klein, F. X. Castellanos and M. P. Milham (2009). "Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults." Am J Psychiatry **166**(8): 891-899.

Dosenbach, N. U. F., B. Nardos, A. L. Cohen, D. A. Fair, J. D. Power, J. A. Church, S. M. Nelson, G. S. Wig, A. C. Vogel, C. N. Lessov-Schlaggar, K. A. Barnes, J. W. Dubis, E. Feczko, R. S. Coalson, J. R. Pruett, D. M. Barch,

S. E. Petersen and B. L. Schlaggar (2010). "Prediction of Individual Brain Maturity Using fMRI." Science **329**(5997): 1358-1361.

Erhardt, E. B., S. Rachakonda, E. J. Bedrick, E. A. Allen, T. Adali and V. D. Calhoun (2011). "Comparison of multi-subject ICA methods for analysis of fMRI data." Hum Brain Mapp **32**(12): 2075-2095.

Fair, D. A., A. L. Cohen, J. D. Power, N. U. F. Dosenbach, J. A. Church, F. M. Miezin, B. L. Schlaggar and S. E. Petersen (2009). "Functional Brain Networks Develop from a "Local to Distributed" Organization." Plos Computational Biology **5**(5).

Fair, D. A., N. U. Dosenbach, J. A. Church, A. L. Cohen, S. Brahmbhatt, F. M. Miezin, D. M. Barch, M. E. Raichle, S. E. Petersen and B. L. Schlaggar (2007). "Development of distinct control networks through segregation and integration." Proc Natl Acad Sci U S A **104**(33): 13507-13512.

Fransson, P., B. Skiold, S. Horsch, A. Nordell, M. Blennow, H. Lagercrantz and U. Aden (2007). "Resting-state networks in the infant brain." Proceedings of the National Academy of Sciences of the United States of America **104**(39): 15531-15536.

Freire, L. and J. F. Mangin (2001). "Motion correction algorithms may create spurious brain activations in the absence of subject motion." Neuroimage **14**(3): 709-722.

Friedman, J., T. Hastie and R. Tibshirani (2008). "Sparse inverse covariance estimation with the graphical lasso." Biostatistics **9**(3): 432-441.

Gao, W., J. H. Gilmore, K. S. Giovanello, J. K. Smith, D. Shen, H. Zhu and W. Lin (2011). "Temporal and spatial evolution of brain network topology during the first two years of life." PLoS One **6**(9): e25278.

Geschwind, D. H. and P. Levitt (2007). "Autism spectrum disorders: developmental disconnection syndromes." Current Opinion in Neurobiology **17**(1): 103-111.

Hahamy, A., M. Behrmann and R. Malach (2015). "The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder." Nat Neurosci **18**(2): 302-309.

Hasson, U., G. Avidan, H. Gelbard, I. Vallines, M. Harel, N. Minshew and M. Behrmann (2009). "Shared and idiosyncratic cortical activation patterns in autism revealed under continuous real-life viewing conditions." Autism Res **2**(4): 220-231.

Hernandez, L. M., J. D. Rudie, S. A. Green, S. Bookheimer and M. Dapretto (2015). "Neural signatures of autism spectrum disorders: insights into brain network dynamics." Neuropsychopharmacology **40**(1): 171-189.

Himberg, J., A. Hyvarinen and F. Esposito (2004). "Validating the independent components of neuroimaging time series via clustering and visualization." Neuroimage **22**(3): 1214-1222.

Hutchison, R. M. and J. B. Morton (2015). "Tracking the Brain's Functional Coupling Dynamics over Development." J Neurosci **35**(17): 6849-6859.

Hutchison, R. M., T. Womelsdorf, E. A. Allen, P. A. Bandettini, V. D. Calhoun, M. Corbetta, S. Penna, J. H. Duyn, G. H. Glover, J. Gonzalez-Castillo, D. A. Handwerker, S. Keilholz, V. Kiviniemi, D. A. Leopold, F. Pasquale, O. Sporns, M. Walter and C. Chang (2013). "Dynamic functional connectivity: Promise, issues, and interpretations." Neuroimage **80**: 360-378.

Hutchison, R. M., T. Womelsdorf, J. S. Gati, S. Everling and R. S. Menon (2013). "Resting-state networks show dynamic functional connectivity in awake humans and anesthetized macaques." Human Brain Mapping **34**(9): 2154-2177.

Jaddoe, V. W. V., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van IIzendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. P. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." European Journal of Epidemiology **27**(9): 739-756.

Jung, M., H. Kosaka, D. N. Saito, M. Ishitobi, T. Morita, K. Inohara, M. Asano, S. Arai, T. Munesue, A. Tomoda, Y. Wada, N. Sadato, H. Okazawa and T. Iidaka (2014). "Default mode network in young male adults with autism spectrum disorder: relationship with autism spectrum traits." Mol Autism **5**: 35.

Kennedy, D. P., E. Redcay and E. Courchesne (2006). "Failing to deactivate: resting functional abnormalities in autism." Proc Natl Acad Sci U S A **103**(21): 8275-8280.

Langen, C. D., T. White, M. A. Ikram, M. W. Vernooij and W. J. Niessen (2015). "Integrated Analysis and Visualization of Group Differences in Structural and Functional Brain Connectivity: Applications in Typical Ageing and Schizophrenia." PLoS One **10**(9): e0137484.

Lenroot, R. K. and J. N. Giedd (2006). "Brain development in children and adolescents: insights from anatomical magnetic resonance imaging." Neurosci Biobehav Rev **30**(6): 718-729.

Rashid, B., E. Damaraju, G. D. Pearlson and V. D. Calhoun (2014). "Dynamic connectivity states estimated from resting fMRI Identify differences among Schizophrenia, bipolar disorder, and healthy control subjects." Front Hum Neurosci **8**: 897.

Rodriguez, P., T. Adali, H. Li, N. Correa and V. D. Calhoun (2010). Phase correction and denoising for ICA of complex FMRI data. IEEE International Conference on Acoustics, Speech and Signal Processing. Dallas, TX.

Simmonds, D. J., M. N. Hallquist, M. Asato and B. Luna (2014). "Developmental stages and sex differences of white matter and behavioral development through adolescence: a longitudinal diffusion tensor imaging (DTI) study." Neuroimage **92**: 356-368.

Smith, S. M., K. L. Miller, G. Salimi-Khorshidi, M. Webster, C. F. Beckmann, T. E. Nichols, J. D. Ramsey and M. W. Woolrich (2011). "Network modelling methods for FMRI." Neuroimage **54**(2): 875-891.

Stigler, K. A., B. C. McDonald, A. Anand, A. J. Saykin and C. J. McDougle (2011). "Structural and functional magnetic resonance imaging of autism spectrum disorders." Brain Res **1380**: 146-161.

Tohka, J., K. Foerde, A. R. Aron, S. M. Tom, A. W. Toga and R. A. Poldrack (2008). "Automatic independent component labeling for artifact removal in fMRI." Neuroimage **39**(3): 1227-1245.

Uddin, L. Q., K. Supekar, C. J. Lynch, A. Khouzam, J. Phillips, C. Feinstein, S. Ryali and V. Menon (2013). "Salience Network-Based Classification and Prediction of Symptom Severity in Children With Autism." Jama Psychiatry **70**(8): 869-879.

Uddin, L. Q., K. Supekar and V. Menon (2013). "Reconceptualizing functional brain connectivity in autism from a developmental perspective." Frontiers in Human Neuroscience **7**.

Varoquaux, G., F. Baronnet, A. Kleinschmidt, P. Fillard and B. Thirion (2010). "Detection of brain functional-connectivity difference in post-stroke patients using group-level covariance modeling." Med Image Comput Comput Assist Interv **13**(Pt 1): 200-208.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. V. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." European Journal of Epidemiology **28**(1): 99-111.

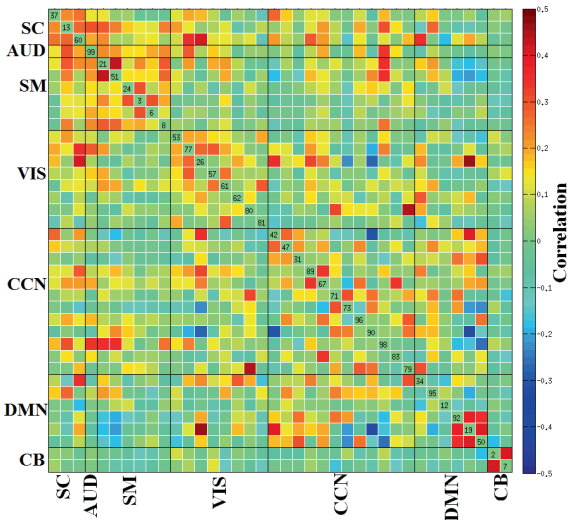
White, T., R. Muetzel, M. Schmidt, S. J. Langeslag, V. Jaddoe, A. Hofman, V. D. Calhoun, F. C. Verhulst and H. Tiemeier (2014). "Time of acquisition and network stability in pediatric resting-state functional magnetic resonance imaging." Brain Connect **4**(6): 417-427.

SUPPLEMENT

1. Static connectivity results

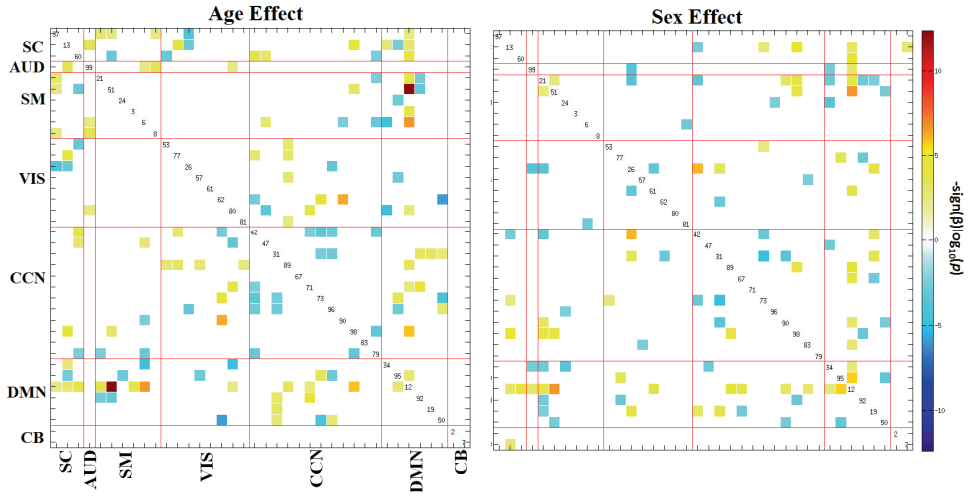
To assess how the static FNC develops and forms the adult-like connectivity patterns, we searched for age- and sex-specific static FNC profiles. Both positive and negative associations between connection strength and age were found in the static FNC. In particular, for the cognitive control network (CCN) components, the within-network connectivity showed decreasing patterns with age, and between-network connectivity mostly showed increasing patterns with age. We specifically focused on the default-mode connectivity as functions of age and sex. In the DMN, the average between-network connectivity were increasing with age for DMN components left middle cingulate cortex (MCC), right angular gyrus and left precuneus (preC), and decreasing with age for DMN components right precuneus and left angular gyrus (AG) (Figure S2). Also, both male and female dominated connectivity patterns were found in static FNC. The average between-network connectivity were greater for girls for DMN components left middle cingulate cortex and left precuneus, and greater for boys for DMN components right angular gyrus, right precuneus and left angular gyrus (Figure S2).

Figure S1. Mean static functional network connectivity (sFNC) map for 774 subjects



Black lines partition the FNC maps into the seven subcategories depicted in Figure 2. The seven subcategories into which the ICNs are partitioned into are: sub-cortical (SC), auditory (AUD), visual (VIS), sensorimotor (SM), cognitive control (CCN), default-mode network (DMN), and cerebellar (CB) components.

Figure S2. Age- and sex-related associations in static FNC.

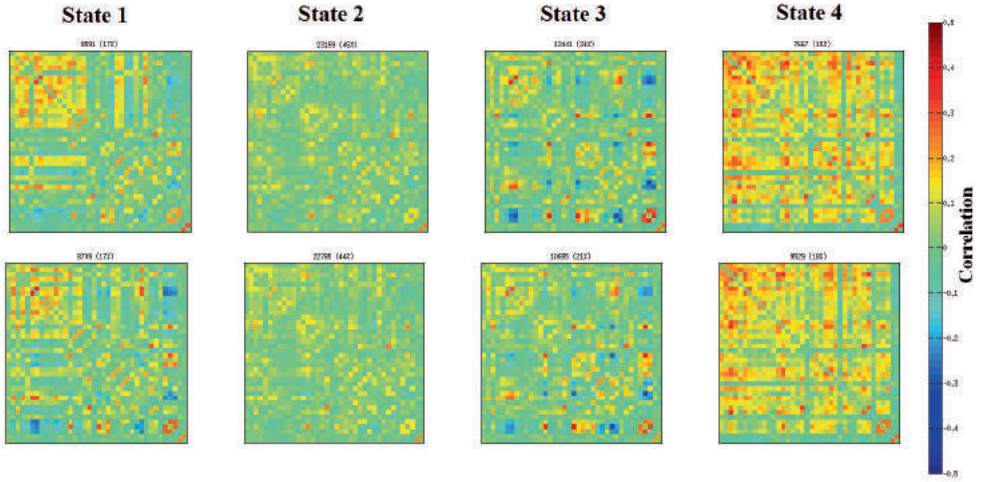


For age analyses, red indicates positive association between that particular pairwise connection and age, whereas blue indicates a negative age association. For analyses of sex, red indicates where female subjects showed stronger connectivity than male subjects, and blue indicate where male subjects showed stronger connectivity compared to female subjects. All the results presented here survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$.

2. Validation framework for connectivity measures

2.1 Reproducibility of clusters

Figure S3. Reproducibility of clusters was established via non-overlapping split-half samples of subjects.



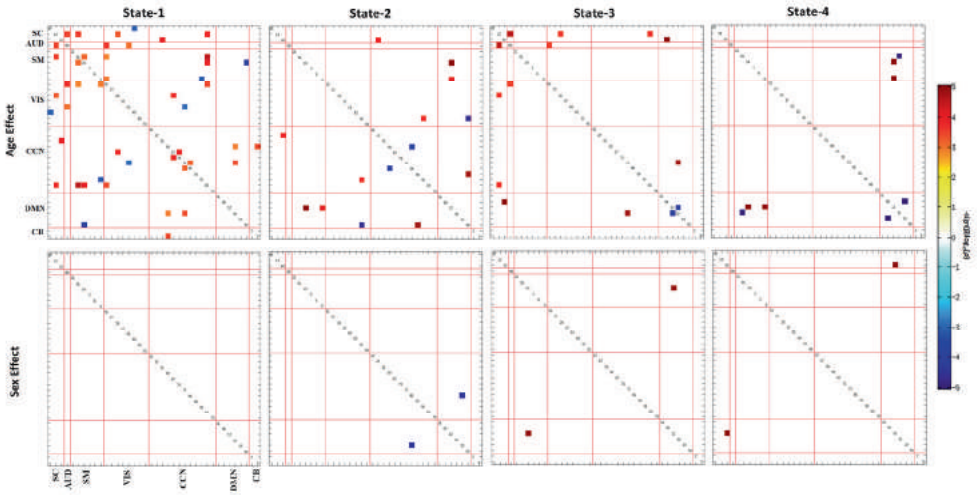
For half-split cross-validation, the subjects were split into two groups with equal number of subjects, and the k-means algorithm was applied with 500 repetitions to the subject exemplars in that group (~1500 instances). The total number and percentage of occurrences is listed above each centroid.

3. Sensitivity analyses of dynamic connectivity findings

3.1 Sensitivity analysis based on behavioral problems

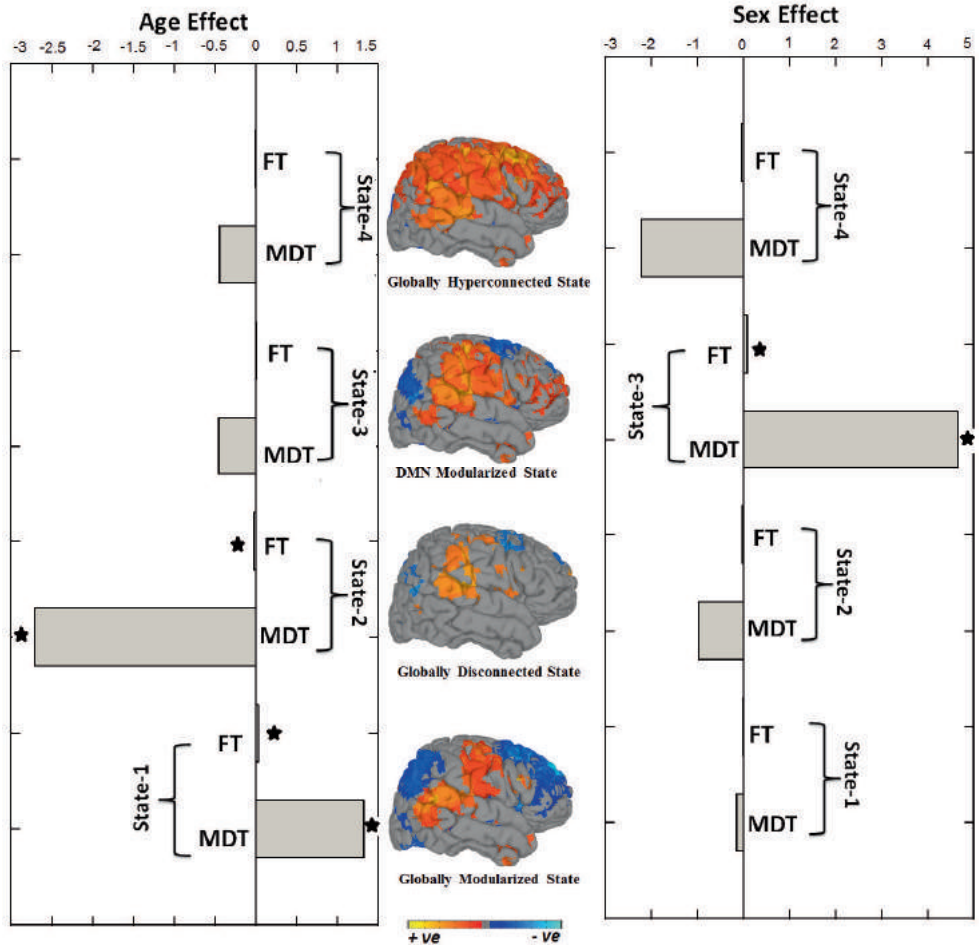
In order to ensure that the results were not driven by subjects with higher levels of behavioral problems as measured by child behavior check list (CBCL) scores, sensitivity analyses were run. To perform the sensitivity analysis, we excluded all the subjects showing any child behavioral problem using CBCL scores from the original dataset (after exclusion, number of subjects=531). We then computed the age- and sex-specific effects on dynamic FNC (Figure S4) and summary measures of the dynamic FNC such as MDT and FT (Figure S5). These results also produced the same direction of effects for each of these connectivity measures, as found with the whole dataset.

Figure S4.



Results from age- and sex-related associations across dynamic connectivity states after excluding subjects with higher levels of behavioral problems. For age analyses, red indicates positive association between that particular pairwise connection and age, whereas blue indicates a negative age association. For analyses of sex, red indicates where female subjects showed stronger connectivity than male subjects, and blue indicate where male subjects showed stronger connectivity compared to female subjects. All the results presented here survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$.

Figure S5.

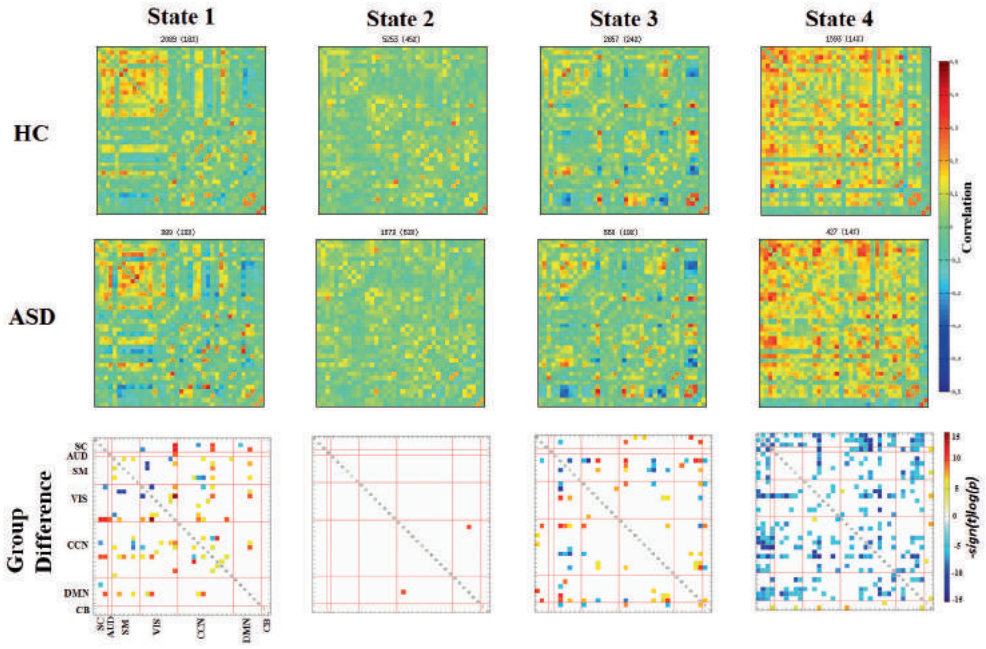


Summary metrics from the four dynamic connectivity states in relation to age and sex after excluding subjects with higher levels of behavioral problems. For age associations, positive beta coefficient (β) indicates older children spend more time in that particular state whereas negative beta coefficient (β) indicates younger children spend more time in a particular state. For sex analyses, positive beta coefficient (β) indicates girls spend more time in the state relative to boys, and negative beta coefficient (β) indicates that boys spend more time in the state relative to girls. Bar graphs indicate the unstandardized beta coefficients (β) with standard error (S.E.) from regression models, and Asterisks (*) indicate the results survived the false discovery rate (FDR) multiple comparison correction threshold of $p_{FDR} = 0.05$. The rendering brain maps are showing modularized positive (red) and negative (blue) connectivity for the corresponding dynamic states.

3.2 Case-control study for autism

We also designed a case-control study for subjects with autism spectrum disorder (ASD) and autistic traits, where we had age, sex and IQ matched 88 healthy subjects and 22 subjects with autistic traits and ASD. We assessed the difference in dynamic FNC states between healthy control (HC) and autistic traits and ASD groups (Figure S6). Note that, these results are showing group differences between HC and ASD in state-1 and state-4, two of the dynamic states that did not capture any SRS effects (effects of autistic trait) in terms of mean dwell time (MDT) and fraction of time (FT) for the original analyses with 774 subjects.

Figure S6.

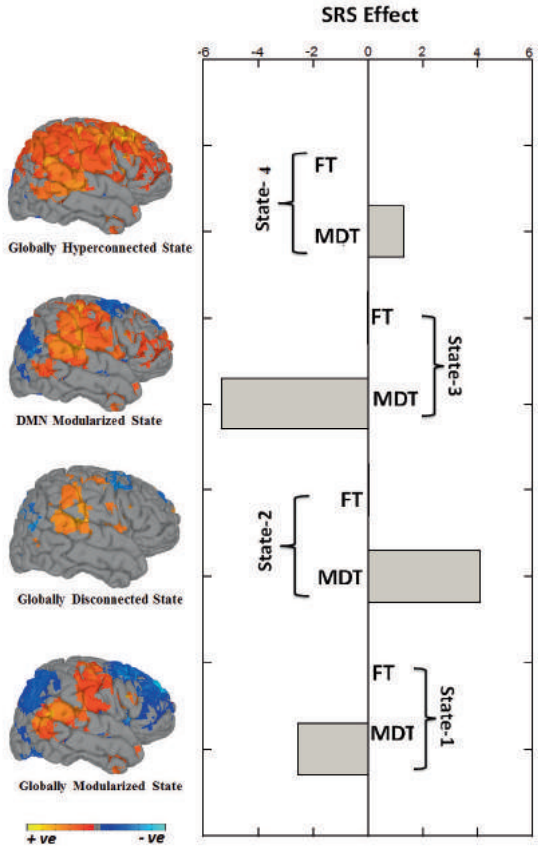


The medians of cluster centroids by state for HC (top) and ASD (middle) along with the count of subjects that had at least one window in each state are shown. The bottom row shows the FDR-corrected (indicate $p < 0.05$) results of two-sample t-test performed across subject median dFNC maps by state.

3.3 Sensitivity analysis based on autistic trait and autism

We also performed a sensitivity analysis to evaluate the effects of SRS scores on summary measures of the dynamic states (MDT and FT). We removed the subjects who are diagnosed with ASD, as well as the subjects with SRS scores above the screening cut-off. Using this subset of the subjects (after exclusion, number of subjects=528), we performed the analyses for SRS score effects on MDT and FT (Figure S7). The SRS sensitivity analysis did not reveal any FDR-corrected effect of autistic traits. However, the direction of the effects remained the same as the original analysis (with 774 subjects).

Figure S7: *Summary metrics and autistic trait effects.*



Summary metrics from the 4 dynamic connectivity states in relation to autistic traits after removing subjects with autistic traits and ASD. Positive beta coefficient (β) indicates that higher levels of autistic traits are associated with more time spent in a particular state, whereas negative beta coefficient (β) indicate lower levels of autistic traits are associated with more time spent in a particular state. Bar graphs indicate the unstandardized beta coefficients (β) from regression models. The rendering brain maps are showing modularized positive (red) and negative (blue) connectivity for the corresponding dynamic states.

3.4 IQ – adjusted analyses

As ASD is often accompanied by deficits in cognition, it was important to also rule out that any observed associations between autistic traits and dynamic connectivity were not simply a reflection of general intellectual ability. Analyses associating autistic traits with dynamic connectivity remained largely unchanged after adjusting for non-verbal IQ. Specifically, for whole-matrix associations in the four dynamic states, the general pattern of association remained. For the summary measure MDT, regression coefficients did not change more than 5%, suggesting the association is not confounded by IQ.

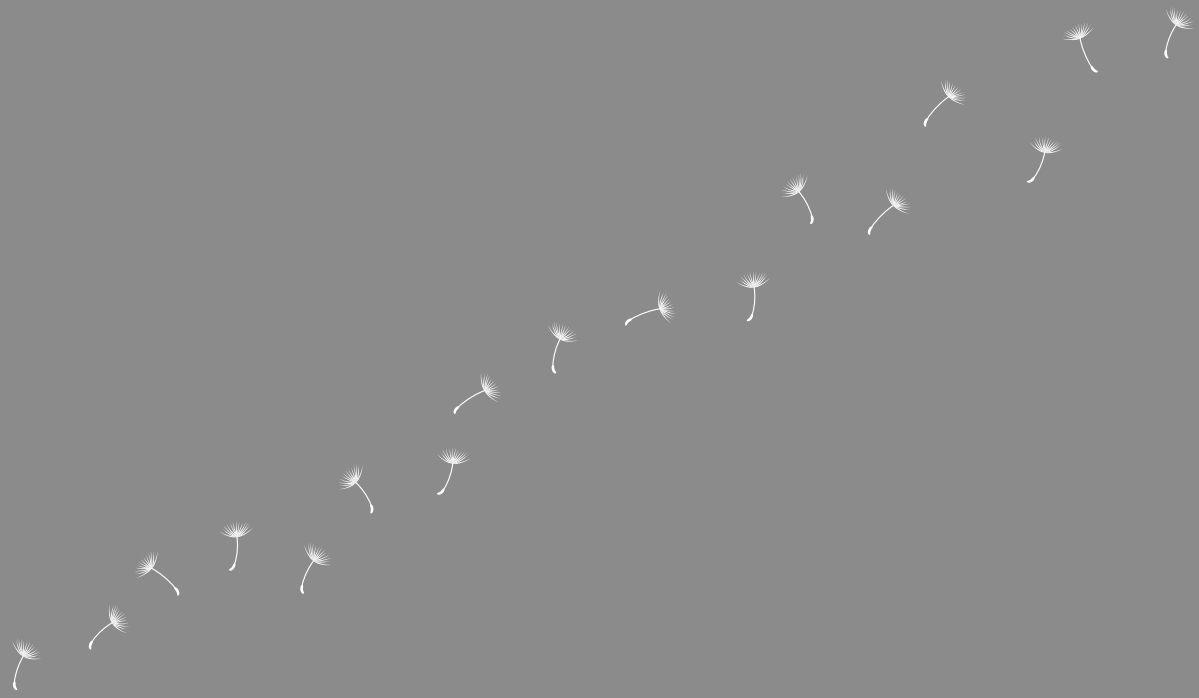
Table S1. Participant characteristics

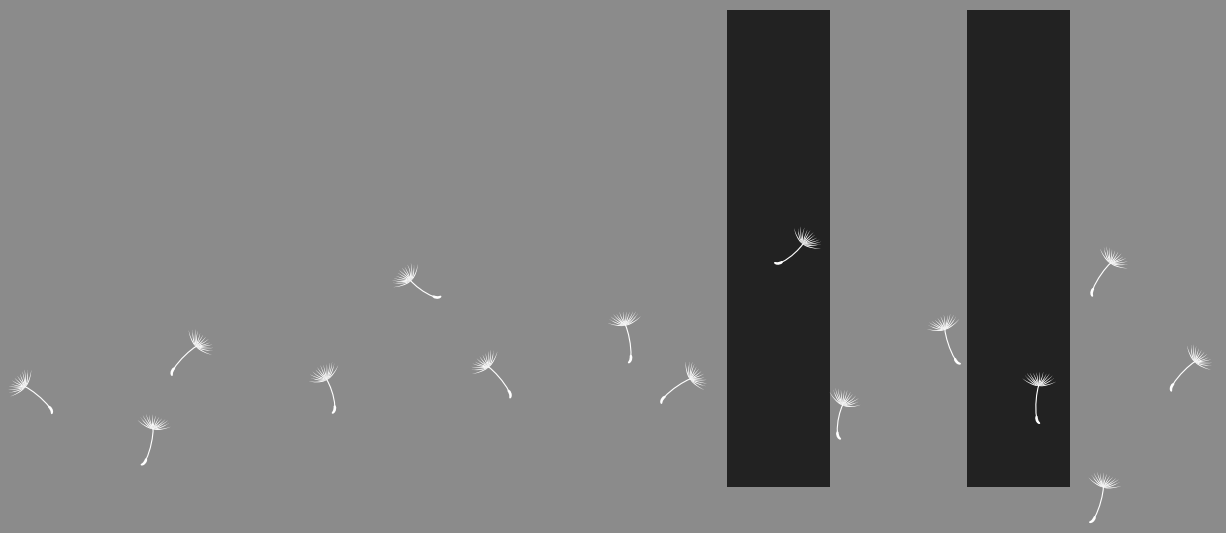
Child characteristics (n=774)	
Age at MRI	7.99±1.01
Gender (% boy)	52.1
Ethnicity (%)	
Dutch	71.8
Other Western	6.5
Non-Western	21.7
Social Responsiveness Scale weighted total score	0.27±0.31
Age (years) at Social Responsiveness Scale assessment	6.2±0.46
Non-verbal IQ	102.0±14.5

Table S2. Peak activations of ICN spatial maps. Coordinate = max coordinate (mm) in MNI space, following LPI convention.

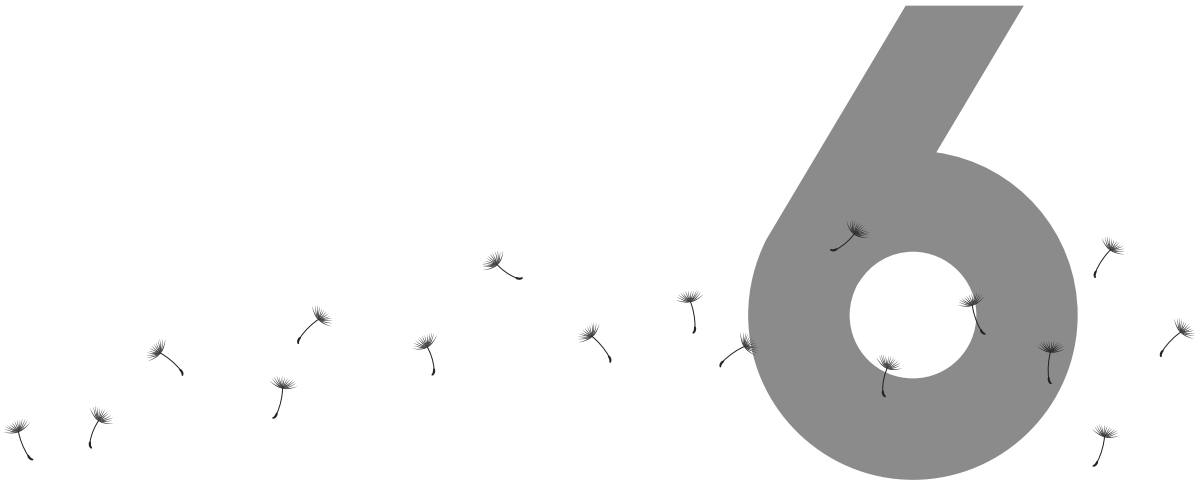
ICN regions	**Peak (mm)** **X Y Z**
SUB-CORTICAL (SC)	
IC: 37	
Right Putamen	[18 13 -6]
IC: 13	
Left Putamen	[-27 0 3]
Right Putamen	[27 3 3]
IC: 60	
Right Thalamus	[12-30 9]
AUDITORY (AUD)	
IC:99	
Left Superior Temporal Gyrus	[-51-27 9]
Right Superior Temporal Gyrus	[60-18 9]
VISUAL (VIS)	
IC:21	
Left SMA	[-3 6 48]
IC: 51	
Right SupraMarginal Gyrus	[54-33 27]
IC: 24	
Right SupraMarginal Gyrus	[-58-24 41]
IC: 3	
Left Precentral Gyrus	[-36-24 57]
IC: 6	
Right Paracentral Lobule	[6-30 66]
IC: 8	
Right Postcentral Gyrus	[54 -9 33]
Left Postcentral Gyrus	[-51-12 33]
SENSORIMOTOR (SM)	
IC: 53	
Right Fusiform Gyrus	[27-45-12]
Left Fusiform Gyrus	[-24-48 -9]
IC: 77	
Left Lingual Gyrus	[-9-57 0]
IC: 26	
Left Calcarine Gyrus	[-12-60 18]
IC: 57	

Right Fusiform Gyrus	[30-78 -6]
IC: 61	
Left Cuneus	[3-84 24]
IC: 62	
Left Cuneus	[12-72 36]
IC: 80	
Right Superior Occipital Gyrus	[30-66 45]
IC: 81	
Right Superior Occipital Gyrus	
COGNITIVE CONTROL (CCN)	
IC:42	
Left Superior Medial Gyrus	[-6 55 9]
IC: 47	
Left Middle Frontal Gyrus	[-24 48 25]
IC: 31	
Left Superior Frontal Gyrus	[-18 22 53]
IC: 89	
Right Middle Temporal Gyrus	[45-60 12]
IC: 67	
Left Middle Temporal Gyrus	[-48-57 12]
IC: 71	
Right Middle Frontal Gyrus	[42 7 40]
IC: 73	
Right Middle Frontal Gyrus	[33 45 12]
IC: 96	
Left Inferior Frontal Gyrus	[-48 15 27]
IC: 90	
Right Inferior Parietal Lobule	[48-39 48]
IC: 98	
Right Insula Lobe	[45 3 6]
IC: 83	
Right Middle Temporal Gyrus	[51-39 6]
IC: 79	
Left Superior Parietal Lobule	[-30-54 48]
DEFAULT-MODE (DMN)	
IC: 34	
Right Precuneus	[3-65 55]
IC: 95	
Right Middle Cingulate Cortex	[6 30 30]
IC: 12	
Left Middle Cingulate Cortex	[0 0 33]
IC: 92	
Right Angular Gyrus	[48-57 39]
IC: 19	
Left Precuneus	[0-57 33]
IC: 50	
Left Angular Gyrus	[-45-60 36]
CEREBELLAR (CB)	
IC: 2	
Left Cerebellum	[-33-66-42]
IC: 7	
Right Cerebellum	[36-63-39]





Part II:
Brain morphology and cognition in internalizing and externalizing symptoms



Cognitive functioning in children with internalizing, externalizing and dysregulation problems: a population-based study

Laura M.E. Blanken, Tonya White, Sabine E. Mous, Maartje Basten, Ryan L. Muetzel, Vincent W.V. Jaddoe, Marjolein Wals, Jan van der Ende, Frank C. Verhulst, Henning Tiemeier

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ABSTRACT

Background: Psychiatric symptoms in childhood are closely related to neurocognitive deficits. However, it is unclear whether internalizing and externalizing symptoms are associated with general or distinct cognitive problems.

Methods: We examined the relation between different types of psychiatric symptoms and neurocognitive functioning in a population-based sample of 1,177 school-aged children. Internalizing and externalizing behavior was studied both continuously and categorically. For continuous, variable-centred analyses, broadband scores of internalizing and externalizing symptoms were used. However, these measures are strongly correlated, which may prevent identification of distinct cognitive patterns. To distinguish groups of children with relatively homogeneous symptom patterns, a latent profile analysis of symptoms at age 6 yielded four exclusive groups of children: a class of children with predominantly internalizing symptoms, a class with externalizing symptoms, a class with co-occurring internalizing and externalizing symptoms, that resembles the CBCL Dysregulation profile and a class with no problems. Five domains of neurocognitive ability were tested: attention/executive functioning, language, memory and learning, sensorimotor functioning, and visuospatial processing.

Results: Consistently, these two different modelling approaches demonstrated that children with internalizing and externalizing symptoms show distinct cognitive profiles. Children with more externalizing symptoms performed lower in the attention/executive functioning domain, while children with more internalizing symptoms showed impairment in verbal fluency and memory. In the most severely affected class of children with internalizing and externalizing symptoms, we found specific impairment in the sensorimotor domain.

Conclusions: This study illustrates the specific interrelation of internalizing and externalizing symptoms and cognition in young children.

INTRODUCTION

In child development, cognitive functioning and psychopathology are closely intertwined. The school-age years are a period of abundant neurodevelopment, characterized by refinement of cognitive skills while, in some children, psychiatric symptoms emerge. Often, a disruption in one area of development is accompanied by impairment in the other, which may reflect a common underlying neurodevelopmental problem (Andreasen 1997). The relation of cognition and psychopathology is particularly well illustrated by developmental disorders, such as ADHD and ASD (Pennington and Ozonoff 1996), that are often characterized by lower intelligence or even intellectual impairment. While IQ provides a good measure of general cognitive ability, cognition is a broad construct with various domains, each of which can be selectively impaired or intact. There is increasing attention to assess which specific aspects of cognition are impaired in child psychiatric disorders. Such cognitive impairment can be shared across different disorders, but it may also be distinct for different types of psychopathology. Internalizing and externalizing disorders are two presentations of psychopathology at a young age, that are thought to emerge from partly distinct pathways, both in terms of genetics (Kendler, Prescott, Myers and Neale 2003) and underlying brain correlates (Chabernaud, Mennes, Kelly, Nooner, Di Martino et al. 2012) and predispose for different types of psychopathology later in life (Roza, Hofstra, van der Ende and Verhulst 2003; Petty, Rosenbaum, Hirshfeld-Becker, Henin, Hubley et al. 2008). However, it is less clear whether distinct cognitive patterns exist for internalizing and externalizing symptoms at a young age.

Cognitive problems in psychopathology have been studied particularly in the context of executive functioning, a broad construct of different abilities to regulate behavior, such as the ability to pay attention or to inhibit responses. Externalizing disorders such as ADHD and disruptive behavioral disorders have been conceptualized as arising from a set of primarily frontally mediated executive function deficits, including attention, planning, working memory and response inhibition (Sergeant, Geurts and Oosterlaan 2002; Frazier, Demaree and Youngstrom 2004; Van der Meere, Marzocchi and De Meo 2005; Willcutt, Doyle, Nigg, Faraone and Pennington 2005; Crosbie, Arnold, Paterson, Swanson, Dupuis et al. 2013). There is more debate about the specific deficits in anxiety and mood disorders, that are primarily related to neural circuitry linking limbic structures to frontal regions (Price and Drevets 2010). Neuropsychological impairment of executive functioning has been reported (Emerson, Mollet and Harrison 2005), most notably in visual and working memory in paediatric or adolescent depression (Lauer, Giordani, Boivin, Halle, Glasgow et al. 1994; Matthews, Coghill and Rhodes 2008), while differences in processing speed have also been reported (Favre, Hughes, Emslie, Stavinoha, Kennard et al. 2009). Attention has also been implicated in pediatric depression (Brooks, Iverson, Sherman and Roberge 2010). While some childhood anxiety disorders, like

OCD in children, occur with impairments of executive functioning abilities like mental set-shifting (Shin, Choi, Kim, Hwang, Kim et al. 2008), or full-scale IQ (Davis, Ollendick and Nebel-Schwalm 2008) they have also been related to impairment in verbal processing (Toren, Sadeh, Wolmer, Eldar, Koren et al. 2000). However, it is unclear whether these differences reflect specific lingual processes or aspects of executive functioning, such as impaired attention or working memory. In addition, some studies focused on the neurocognitive implications of co-occurring high levels of internalizing and externalizing symptoms, for instance in children with ADHD with comorbid internalizing symptoms. These studies show inconsistent results, that vary between better test performance (Yurtbasi, Aldemir, Teksin Bakir, Aktas, Ayvaz et al. 2015), no difference (Peyre, Speranza, Cortese, Wohl and Purper-Ouakil 2015), to worse performance in tasks of attention, response inhibition and working memory (Schatz and Rostain 2006) than children with ADHD only.

In general, there is considerable heterogeneity in the literature relating cognition to child psychopathology and studies in young children are relatively scarce. Yet, it is especially important to study younger children, as patterns of emerging psychopathology and impaired cognition can provide more insight in the etiology relatively unobscured by chronicity of symptoms or treatment effects. Further, the close relation between cognition and psychopathology in young age provides a potentially powerful treatment target for early intervention. So far, many clinical studies tend to focus on a limited range of cognitive domains within small samples of children that have one or more clinically diagnosed psychiatric disorders. Importantly, within this framework, the question of specificity of cognitive impairments cannot be answered by design. Child psychopathology is characterized by a high level of comorbidity between different symptom types, crossing the boundaries of diagnoses. Children that have a high level of externalizing symptoms, such as aggression tend to also have internalizing symptoms, such as anxiety or depressed mood (Achenbach and Rescorla 2000). Knowledge about specific patterns of cognitive impairment per symptom type could point to specific genetic or neurobiological pathways (Crosbie et al. 2013) and help in targeted treatment decisions and predictions of the clinical course of specific symptoms.

An alternative to the case-control framework is provided by studying psychopathology on the symptom level, focusing on continuous trait phenotypes (McGrath, Braaten, Doty, Willoughby, Wilson et al. 2015). However, various continuously measured psychiatric symptoms are also interrelated. Although the associated cognitive problems may in fact be distinct for each symptom type, the strong correlation between internalizing and externalizing symptoms can obscure any potential specificity of associations. Therefore, in the current study, we also used a different approach to address the relation of internalizing and externalizing symptoms and cognition, that added an element of specificity. To this aim, we complemented the traditional variable-based approach with a person-centred approach that allows to distinguish between different symptom profiles in case of heterogeneity (Muthen

and Muthen 2000). Previously, we applied a Latent Profile Analysis (LPA) to quantitative behavioral and emotional symptom data of more than 6,000 children to identify four broad, but exclusive classes with different patterns of symptoms (Basten, Althoff, Tiemeier, Jaddoe, Hofman et al. 2013). Three of these classes included children with problem behavior: a class of children with predominantly externalizing symptoms, a class with predominantly internalizing symptoms and a small class with both internalizing and externalizing symptoms that bears a resemblance to the CBCL Dysregulation Profile, a phenotype of high comorbidity that is associated with a broad range of later psychopathology (Althoff, Verhulst, Rettew, Hudziak and van der Ende 2010). In this population-based cohort, the majority of children belonged to a class without psychopathology. The three classes of problem behavior have so far only been related to a general, global measure of non-verbal intelligence (Basten, van der Ende, Tiemeier, Althoff, Rijlaarsdam et al. 2014). Here, we aimed to further specify these differences by assessing more specific cognitive sub-domains using an extensive neuropsychological test battery that covers five domains of neurocognitive ability: attention/executive functioning, language, memory, sensorimotor functioning and visuospatial ability.

In the current study, we assessed the relation between cognition and psychiatric symptoms in more than 1,000 school-aged children. In line with recommendations of the RDoC initiative, we used continuous measures of internalizing and externalizing symptoms to capture the full spectrum of symptom severity, including subclinical symptoms (Insel, Cuthbert, Garvey, Heinssen, Pine et al. 2010). However, these measures were strongly correlated, so to identify unique patterns of impairment across different symptom types, we used the previously identified problem classes representing more homogeneous groups in terms of symptomatology. Based on the literature, we hypothesized that children with externalizing symptoms show poorer performance on the attention/executive functioning domain. Further, we hypothesized that children with internalizing symptoms would show moderately impaired test performance in the domains of language, memory and attention. In the class of children with high levels of both internalizing and externalizing symptoms, we expected widespread impairment, since they likely reflect the most severely affected group.

Additionally, we tested if any impairments were independent of demographic and maternal factors or autistic symptoms. Finally, we explored whether any observed differences reflect global cognitive impairment or more specific deficits by adjusting for IQ.

METHODS

Participants

This study included a subgroup of children from the Generation R Study, a multi-ethnic population-based cohort, investigating children's health, growth and development from

fetal life onwards in Rotterdam, the Netherlands. An overview of the Generation R Study design and population is provided elsewhere (Jaddoe, van Duijn, Franco, van der Heijden, van IJzendoorn et al. 2012).

As part of a previously described sub-study (White, El Marroun, Nijs, Schmidt, van der Lugt et al. 2013), 1,307 participants completed a neuropsychological test battery. In this sub-study, children with specific traits (including autistic traits and externalizing disorders), were oversampled (see Supplementary Fig. 1 for a consort diagram). Oversampling of children with problem behavior increased the variability, which improved power of the analyses and helped in achieving a more normal distribution of psychopathology symptoms, which are generally strongly right skewed in the general population.

One hundred thirty children had missing information on problem behavior and were excluded, resulting in a study sample of 1,177 children.

The study was approved by the Medical Ethics Committee (METC) of the Erasmus Medical Centre. Written informed consent was obtained from the parents of all participants.

Internalizing and externalizing symptoms

When the children were approximately 6 years of age, mothers of 6,131 children completed the Child Behavior Checklist (CBCL/1.5-5). The CBCL is a widely used instrument has been shown to have good reliability and validity (Achenbach 2000) and is generalizable across 23 societies (Ivanova, Achenbach, Rescorla, Harder, Ang et al. 2010). It measures childhood psychiatric symptoms quantitatively, both in the clinical and non-clinical range and thereby captures the full range of severity. It contains internally consistent Internalizing and Externalizing broadband scales that globally correspond to mood and anxiety disorders and disruptive behavior disorders, respectively (Petty et al. 2008). The Internalizing and Externalizing broadband scales are able to measure broad behavioral constructs in early childhood that have been shown to predict later, more specific psychopathology (Mesman and Koot 2001; Kroes, Kalff, Steyaert, Kessels, Feron et al. 2002). The Internalizing scale consists of the following four scales: Emotionally Reactive; Anxious/Depressed; Somatic Complaints; and Withdrawn. The Externalizing scale contains two scales: Attention Problems and Aggressive Behavior (Basten et al. 2013). In our first approach, we related the continuous broadband scores to cognitive functioning. Secondly, to explore specific cognitive problems of internalizing and externalizing symptoms, we defined four classes of children with distinct patterns of behavioral and emotional symptoms that were obtained by a latent profile analysis performed on T-scores of CBCL syndrome scales that constitute the internalizing and externalizing broadband scales. These included a class of children without problems, a class with predominantly internalizing symptoms; a class with externalizing symptoms and emotional reactivity, further referred to as ‘externalizing’; and a class with high scores on both the internalizing and externalizing scales. This class is referred to as the dysregulation

class. Details on the full modelling strategy and fit indices of models including 1 to 5 classes are described by Basten et al (Basten et al. 2013). The model with four classes provided good fit measures, and the most meaningful distinction of qualitatively different profiles.

The most likely class memberships derived from this analysis were used in this study (see Table 1 for percentages). This was justified by the high entropy (0.98) of the latent class model (Clark 2009; Basten et al. 2013). The intrinsic relation of internalizing and externalizing symptoms, and scores of children in the four classes on these broadband scales are illustrated in Supplementary Figure 2.

Importantly, the classes were not based on symptom severity thresholds. However, for the interpretation of the profiles, mean T-scores are provided (Supplementary Figure 3).

Cognitive functioning

Cognitive functioning was measured using the NEPSY-II-NL, an official and validated Dutch translation and adaptation of the North American NEPSY-II battery, that can be used to assess neuropsychological functioning in 5- to 12-year-old children (Brooks, Sherman and Strauss 2009). Tasks are categorized to cover several theoretically derived domains of cognition, including attention/executive functioning, language, memory and learning, sensorimotor functioning, and visuospatial processing. The task battery is sensitive to interindividual differences, not only in clinical groups but also in the general population (Brooks, Sherman and Iverson 2010). Acceptable to good reliability and validity have been reported for the NEPSY-II (Korkman, Kirk and Kemp 2010). Due to time constraints, a selection of tests from the NEPSY was chosen such that five areas of cognitive ability were measured: attention/executive functioning, language, memory and learning, sensorimotor functioning, and visuospatial processing. The battery was administered by trained research assistants and took approximately 55 minutes.

As the NEPSY-II-NL does not provide domain-specific summary scores, a data reduction technique was used to derive them empirically. Summary scores for four NEPSY-II-NL test domains (attention/executive functioning, language, memory and learning, and visuospatial processing) were derived using a principal component analysis (PCA) on all test scores belonging to that domain. The first unrotated factor score was selected as the summary score for each cognitive domain. For the sensorimotor domain, this procedure was slightly different, as described below. The different subtestscores that contributed to each domainscore are described in the Supplementary material. In Supplementary Table 1, the correlation with the corresponding domain scores that they contributed to is provided.

The term ‘cognitive problems’ refers to the continuum of problems that a child may have and does not imply a severity threshold.

Selected tasks for each of the domains of the NEPSY-II-NL

Attention and Executive Functioning

The first task of this domain was the Auditory Attention and Response Set Task. In the Auditory Attention component of this task the children were presented recordings of words and asked to selectively respond to the word 'Red' by touching the red circle on the sheet in front of them. The sheet also contains a blue, black, yellow and red circle, but these had to be ignored, as well as all non-color words. Touching the right circle within 2 seconds indicates a correct response.

Following the Auditory Attention component, Response Set was performed, which taps into response inhibition and working memory. In this task, children are asked to respond to the word 'Red' by touching the yellow circle, respond to 'Yellow' by touching the red circle and lastly, respond to the word 'Blue' by touching the blue circle. All the other colors should be ignored. Touching the right circle within 2 seconds equals a correct response, whereas touching another circle or a delayed response (>2 seconds) are incorrect. Performance in both components of the Auditory Attention and Response Set task was measured using four summary scores per component: the total score of correct responses and the total number of commission, omission, and inhibition errors. Omission errors indicate that the child failed to respond. Commission errors are delayed or incorrect responses. Inhibitory errors occur when the child responds to a color word when no response was warranted. The second task in this domain is the Statue task. This task requires a child to maintain a 'statue-like' body position for a period of 75 seconds, while ignoring environmental distractors. Summary measures from the Statue task include the total number of body movements, eye openings, sound productions, and a total score.

Language

The language skills domain involves a test of verbal fluency, the Word Generation task. This task measures how many words a child can generate within 60 seconds in two semantic categories: animals and food or drinks. The total semantic score is the sum of the total number of unique, existing words for both categories.

Memory and Learning

The memory and learning domain entailed the Memory for Faces task, with an immediate and delayed memory component. During this task the child is first presented with multiple series of three faces, after which the child has to identify the face it has previously seen, out of another series of three faces. The delayed recall component of this task was assessed after a delay period of 15 to 25 minutes. A total correct score was calculated for both the immediate and delayed recall.

The verbal memory task that we assessed is the Narrative Memory task. This task measures immediate free recall, cued recall, and (passive) recognition of verbal information. In this task, children were presented a short story after which they were asked to provide as many details as they could remember. Subsequently, children were asked specific questions about the story (cued recall), and finally questions that only required yes and no answers were provided (recognition). The Narrative Memory task provides a total correct score for the free and cued recall combined, the free recall only, and for recognition.

Sensorimotor Functioning

In the paper-and-pencil task Visuomotor Precision, the child is asked to draw a line as quickly and as accurately as possible in between the boundaries of a paper path. For this task, two separate scores were derived. Due to the fact that different summary scores in this task may reflect distinct strategies (e.g., fast with many errors vs. slow but more accurate), it was not possible to derive a single meaningful sensorimotor factor out of the separate scores. Therefore, two independent scores were derived. The primary sensorimotor score is a speed-accuracy trade-off score, based on the product of the standardized time and number of errors in this task, while the secondary sensorimotor score is based on the number of compensatory pencillifts while performing the task.

Visuospatial Processing

The visuospatial processing domain consisted of three different tasks. The Arrows task measures the child's ability to judge the direction of an arrow by asking the child to select the arrow(s) that point(s) to centre of a target from a set of arrows. The summary score for the Arrows task is the total number of correct responses. The Geometric Puzzles task measures mental rotation, visuospatial working memory, and attention to detail. This task requires a child to discriminate which abstract figures in a set match those within a grid containing multiple abstract figures. Figures in the grid are often rotated and thus appear different than the example figure. Finally, the Route Finding task was administered, which measures visuospatial relations, orientation, and direction. The child uses a skeleton map of a specific route to translate this route onto another map. The summary score obtained from this task is the total correct score from a series of 10 maps.

Covariates

Several covariates were considered, based on previously described associations of sociodemographic factors and prenatal exposures with child psychopathology and cognitive functioning (Huizink and Mulder 2006; Dennis, Francis, Cirino, Schachar, Barnes et al. 2009; Raver, Blair, Willoughby and Investigat 2013). Intelligence was not taken into account as a default covariate, as this carries the risk of overadjustment in the context of developmental

psychopathology (Dennis et al. 2009). However, we explored whether the differences reflected global or specific cognitive deficits, by additionally correcting the fully adjusted analyses for non-verbal IQ. Non-verbal intelligence of the child was assessed at approximately 6 years of age using two subtests of the Snijders-Oomen Niet-verbale intelligentie Test – Revisie (SON-R 2.5–7), a non-verbal intelligence test suited for children between 2.5–7 years of age (Tellegen, Wijnberg-Williams and Laros 2005): Mosaics (which assesses spatial visualization abilities), and Categories (which assesses abstract reasoning abilities). Raw scores from these two subtests were standardized to reflect a mean and standard deviation of the Dutch norm population age 2½- 7 years and subsequently converted into SON-R IQ score using age-specific reference scores provided in the SON-R 2½ - 7 manual (mean=100, SD=15). Child ethnicity was defined according to the ethnicity categorization of Statistics Netherlands (Netherlands 2004). Children with both parents born in the Netherlands were considered Dutch and children were classified as non-Dutch (further categorized as 'other Western' or 'other non-Western') if one parent was born outside the Netherlands. Household income was defined by the total net monthly income of the household and categorized into three categories: income below <1200 Euros per month (below social security level), 1200-2200 Euros (low income), and >2200 Euros (modal income and above). Prenatal smoking and alcohol use were categorized into 'No', 'Until pregnancy was known' and 'Continued during pregnancy', based on the information of repeated questionnaires during pregnancy. For continued alcohol use, there were two categories: 'Continued occasionally' and 'Continued frequently'. Autistic traits were assessed using the 18-item short form of the Social Responsiveness Scale (SRS), a parent-reported questionnaire about the child's social behavior during the past six months. The Social Responsiveness Scale provides a quantitative measure of autistic traits. The authors recommend cut-offs for screening in population-based settings (consistent with weighted Social Responsiveness Scale scores of 1.078 for boys 1.000 for girls) (Constantino 2002).

Data analysis

To examine the relation between internalizing and externalizing symptoms and cognition, we performed linear regression analyses using the NEPSY-II-NL domain scores as the dependent variable. In our first approach, the CBCL broadband scales were used as the independent variable in two separate regression analyses. Scores that showed moderate negative skew were square root transformed to approach normality. In a second approach, most likely class membership was used as the independent variable. Class membership was dummy coded, with the no problems class as the reference.

All analyses were adjusted for gender and age at the time of the CBCL/1.5-5, as well as age at time of the NEPSY-II NL. In a second model, other variables were included as covariates if they changed the effect estimate (unstandardized regression coefficient B) by 5% or more.

Missing values of covariates (max 13.4%) were imputed. We computed 5 imputed datasets.

While IQ was not a default covariate, a second set of regression analyses was performed after adding it as a covariate, to distinguish between a global intellectual problem or impairment of a specific neurocognitive domain.

A sensitivity analysis was conducted to further examine the association between the classes and NEPSY-II-NL performance. We excluded those that screened positive on a questionnaire of autistic symptoms, to study whether class differences in autistic traits explained the observed results. In the classes resulting from our latent profile analysis, there is not one class that specifically identifies children with ASD traits (although they are likely overrepresented in the dysregulation class). Further, autistic traits are quite common in our population-based sample, as children with autistic symptoms were specifically targeted in the recruitment for this sub-study (see Supplementary Figure 1 and (White et al. 2013)).

All analyses were conducted using SPSS (IBM SPSS Statistics version 21.0).

RESULTS

Participant characteristics

Child and maternal characteristics for each of the four classes are presented in Table 1. As expected, the dysregulation and externalizing classes included the highest proportion of boys. Non-verbal intelligence levels were lower in the problem classes if compared to the reference class, in line with earlier reported differences (Basten et al. 2014). The dysregulation class scored 8.0 points lower on non-verbal IQ (95%CI:-11.9;-4.0, p<.001) than the reference class, while the internalizing and externalizing classes showed 4.2 and 4.4 points lower, respectively (95%CI:-7.3;-1.1, p=.008 and 95%CI:-6.9;-1.9, adjusted for age and gender). A non-response analysis is presented in the Supplement. The latent class approach distinguishes children with little symptoms from children with mostly internalizing or mostly externalizing symptoms, while in the dysregulation class, no meaningful distinction can be made between the two symptom types (Supplementary Figure 2).

Due to oversampling of children with specific traits (including autistic traits and externalizing disorders, see Supplementary Fig. 1 for a consort diagram), the prevalence of each of the problem classes was higher than in the original, larger sample (Basten et al. 2013). In this sample, 8.9% of children were part of the internalizing class, 14.5% were part of the externalizing class, 5.4% belonged to the dysregulation class and the other children (71.2%) were part of the reference class.

Table 1. Participant characteristics (n=1,177)

Child characteristics	**Dysregulation n=63**	**Internalizing problems n=105**	**Externalizing problems n=171**	**No problems group n=838**	***p* value**
Gender (% boy)	65.1	46.7	65.5	51.3	.001
Ethnicity (%)					
Dutch	49.2	54.3	60.2	74.0	<.001
Other Western	9.5	5.7	8.2	7.9	
Non-Western	41.3	40.0	31.6	18.1	
Age at CBCL (years)	6.0 (0.4)	6.0 (0.4)	6.0 (0.3)	6.0 (0.4)	.812
range	5.0-7.9	5.3-7.7	5.3- 7.4	4.9- 7.9	
Age at NEPSY-II NL (years)	7.6 (0.9)	8.0 (1.0)	8.0 (1.1)	7.9 (1.0)	.017
range	6.3- 9.6	6.1- 10.7	6.1- 10.7	6.1- 10.4	
IQ (non verbal)	95.3 (15.0)	99.0 (14.1)	98.9 (15.4)	103.2 (14.0)	<.001
range	67- 135	61- 127	50- 135	50- 142	
Maternal characteristics					
Monthly household income (%)					<.001
High	60.3	59.0	75.2	79.5	
Medium	21.0	27.0	14.9	15.4	
Low	17.7	14.0	9.9	5.1	
Alcohol use during pregnancy (%)					.174
Never	31.0	45.5	41.1	35.4	
Until pregnancy was known	15.5	11.4	16.4	14.4	
Continued occasionally	46.6	33.0	37.7	38.4	
Continued frequently	6.9	10.2	4.8	11.8	
Smoking during pregnancy (%)					.054
Never	65	76.6	69.0	78.0	
Until pregnancy was known	6.7	8.5	7.7	6.4	
Continued	28.3	14.9	23.2	15.5	

Note. Values are mean and SD unless otherwise indicated.

CBCL broadband scales

As expected, the correlation between internalizing and externalizing symptoms was strong (r(1,175)=.73, p<.001). Associations between the CBCL internalizing and externalizing broadband scales with cognitive domains are presented in Supplementary Table 2. After adjustment for confounders, internalizing symptoms were associated with lower performance in the domains of attention/executive functioning, language and memory and learning (B=-0.11, 95%CI(-0.12;-0.03], p=.001, B=-0.06, 95%CI[-0.10,-0.03], p=.001) and (B=-0.07, 95%CI[-0.11,-0.03], p<.001), respectively). Externalizing symptoms were associated with lower scores in attention/executive functioning, as well as secondary sensorimotor domainscores after adjustment for confounders (B=-0.07, 95%CI[-0.11,-0.03], p<.001) and (B=-0.05, 95%CI[-0.09,-0.01], p=.02), respectively.

Cognitive functioning across the four classes

To distinguish distinct cognitive problems of internalizing, externalizing and dysregulation symptoms, we compared performance of children in the problem classes to the reference class in all neuropsychological subdomains (Figure 1). In line with most other studies, analyses are adjusted for age and gender only. Children in the externalizing class scored lower than the reference class in the attention/executive functioning domain (B=-0.28, 95%CI[-0.43,-0.12], p<.001) and in the visuospatial domain (B=-0.23, 95%CI[-0.38,-0.08], p=.003). Contrary to the broadband score approach, children with externalizing symptoms did not have lower secondary sensorimotor domain scores.

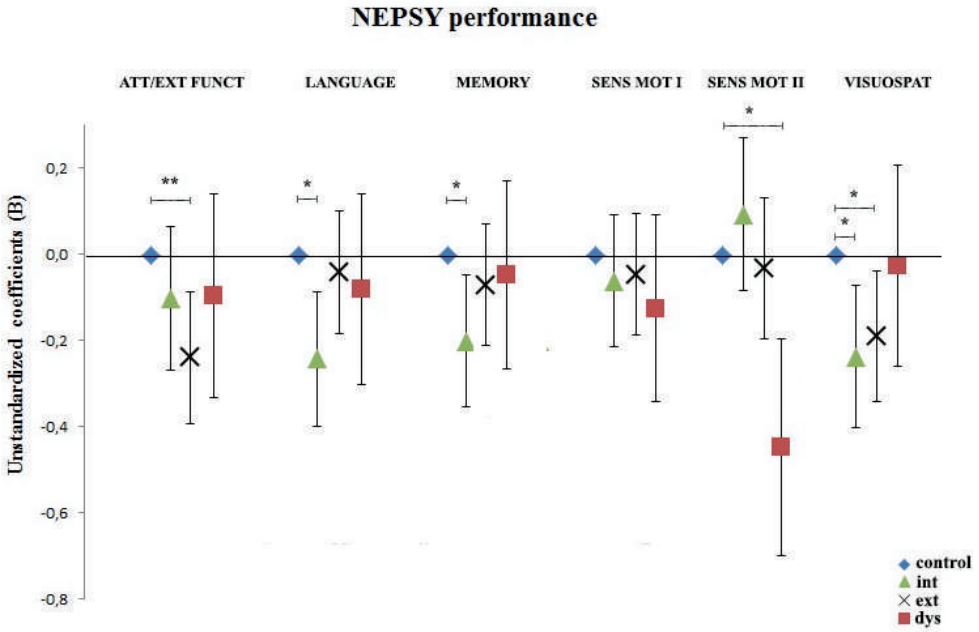
Children in the internalizing class performed worse in the language and memory domains (B=-0.30, 95%CI [-0.47,-0.12], p=.001 and B=-0.25, 95%CI[-0.42,-0.08], p=.004). Children in the internalizing class also performed worse in the visuospatial domain (B=-0.29, 95%CI[-0.48,-0.11], p=.002). In contrast to the broadband score approach, children with internalizing symptoms did not show lower performance in the attention/executive functioning domain. Children in the dysregulation class had lower secondary sensorimotor domain scores compared to the control class (B=-0.48, 95%CI [-0.74,-0.23], p=<.001), while there were no differences in the performance scores in other domains. Generally, children in the dysregulation class had the greatest SD-scores (data not shown).

In a second model, we explored if the differences in performance withstood additional adjustment for demographic and maternal factors. Fully adjusted models are presented in Table 2 (adjusted for age, gender, ethnicity, income, alcohol and smoking during pregnancy). The patterns of performance remained similar. However, there were no longer any differences in visuospatial functioning between either the internalizing or the externalizing classes and the reference class. Children in the internalizing class showed lower performance in the language and memory domains.

We explored whether the differences reflected global or specific cognitive deficits, by additionally correcting the analyses for non-verbal intelligence. In these most stringent analyses, major differences remained. The externalizing class had lower performance in attention/executive functioning and the dysregulation class had a lower secondary sensorimotor domain score (pencillifts) domain (B=-0.23, 95%CI[-0.38,-0.07], *p*=.004 and B=-0.48,95%CI [-0.73,-0.23], *p*=<.001, respectively).

The internalizing class showed poorer performance in the language and memory domains, independent of IQ (B=-0.18,95%CI[-0.35,-0.00], *p*=.048 and B=-0.18,95%CI[-0.35,-0.01], *p*=.043).

Fig. 1. Associations between the internalizing class, the externalizing class and the dysregulation class and performance on domains of the NEPSY-II-NL (n=1,177)



Note. The no problems class (n=838) is the reference. There were 171 children in the externalizing class, 105 children in the internalizing class and 63 children in the dysregulation class. Regression model was adjusted for gender, age at the time of the CBCL/1.5-5, and age at the NEPSY-II NL. *p<.01, **p<.001. Error bars represent 95% confidence intervals of the regression coefficients. The no problems class is the reference and has no error bars.

Table 2. Association between the dysregulation profile, the internalizing profile and the externalizing profiles and performance on domains of the NEPSY-II NL (n=1,177)

	Internalizing n=105		**Externalizing n=171**		**Dysregulation n=63**	
	B (95% CI)	***p***	**B (95% CI)**	***p***	**B (95% CI)**	***p***
Attention and executive functioning	-0.10 (-0.29; 0.19)	.300	-0.25 (-0.40;-0.09)	**.002**	-0.10 (-0.34; 0.14)	.417
Language	-0.19 (-0.36;-0.01)	**.035**	-0.02 (-0.16; 0.12)	.755	-0.02 (-0.23; 0.20)	.893
Memory and Learning	-0.19 (-0.37;-0.02)	**.029**	-0.07 (-0.21; 0.07)	.347	-0.06 (-0.27; 0.16)	.607
Sensorimotor, primary score	-0.06 (-0.24; 0.11)	.482	-0.06 (-0.21; 0.08)	.378	-0.14 (-0.36; 0.08)	.210
Sensorimotor, secondary score	0.09 (-0.11; 0.30)	.365	-0.07 (-0.23; 0.10)	.419	-0.48 (-0.73;-0.22)	**<.001**
Visuospatial	-0.15 (-0.33; 0.03)	.104	-0.12 (-0.27; 0.03)	.113	0.06 (-0.17; 0.29)	.601

Note. The no problems group (n=838) is the reference. The model was adjusted for age at CBCL and age at NEPSY-II-NL, gender, ethnicity, household income, alcohol and smoking during pregnancy. Significant p-values are in bold.

Sensitivity analysis excluding children with ASD

In a sensitivity analysis, we explored whether the differences in performance were dependent on the presence of a probable diagnosis of ASD. In 86.0% of this sample the Social Responsiveness Scale was available. After excluding the children with possible ASD (n=35) and children without information on autistic traits (n=164), we observed a very similar pattern of cognitive performance (Supplementary Figure 4), indicating that autistic traits did not explain the results. Similarly, a second sensitivity analysis excluding children with a non-verbal intelligence below 70 revealed the same patterns of differences.

DISCUSSION

In this study, we found a relation between neurocognitive impairment and internalizing and externalizing symptoms in children from the general population. Despite the high overlap between continuous internalizing and externalizing symptom scores we were able to test the specificity of the associations, by using empirically derived and exclusive classes based on the children's behavioral and emotional symptoms. These included a class with predominantly internalizing symptoms; a class with externalizing symptoms; and a separate class with high scores on both the internalizing and externalizing scales, which we labelled a dysregulation class. Our approach of evaluating both continuous and categorical measures revealed distinct patterns of cognitive impairment in children with predominantly internalizing and externalizing symptoms. Both approaches yielded comparable neurocognitive patterns. However, the class approach can help disentangle the specific cognitive problems of each symptom group by adjusting (without problems of collinearity). The specific relation of the externalizing class with performance in the attention/executive functioning domain clearly illustrates this. Further, the main findings were independent of IQ, which indicates a specific relation of internalizing and externalizing symptoms and these domains, independent of general cognitive ability.

We found that children with mostly externalizing symptoms showed impairment of the attention/executive functioning domain only. This difference remained after adjustment for a global measure of intelligence, indicating a specific relation between behavior and cognitive impairment, unrelated to global intelligence. This is in line with findings of specific cognitive impairment in ADHD and disruptive behavioral disorders, notably in the domain of executive function (Frazier et al. 2004), although global impairment has also been reported (Loge, Staton and Beatty 1990). Our finding is consistent with an influential theory of ADHD, that states that both the cognitive and the behavioral aspects of it reflect a core impairment in inhibitory control. This leads to lower ability to internally regulate behavior (Barkley 1997). Internal regulation and executive functions like sustained attention are thought to be modulated

by the prefrontal cortex and its striatal and parietal connections. Altered connectivity in networks involving frontal regions is thought to be central to the neurobiology of ADHD (De La Fuente, Xia, Branch and Li 2013).

In contrast, children with mostly internalizing symptoms showed impairment in verbal fluency and memory. These processes have been shown to be interrelated. Formation of memories is partly mediated by verbal processes. Next to a large active vocabulary, it is essential to be able to remember new words (Cain, Oakhill and Bryant 2004). Also, in learning disabilities such as Specific Language Disorder or dyslexia, impairments of both aspects have been reported (Hick, Botting and Conti-Ramsden 2005). Unsurprisingly, children with such learning disabilities also tend to have internalizing symptoms (Mugnaini, Lassi, La Malfa and Albertini 2009). However, since we only measured verbal fluency in our study, it is unclear whether the observed impairment of word generation in children with internalizing symptoms would be observed in other areas of language. In addition, it is also possible that it reflects an impairment in aspects of executive functioning, as has been suggested by previous reports (Hurks, Hendriksen, Vles, Kalff, Feron et al. 2004; Shao, Janse, Visser and Meyer 2014). In this study, we did not find evidence for impaired executive functioning in children with internalizing problems; moreover, the results remained after adjusting for IQ. This indicates that our findings may be specific for verbal processes.

There are several possible neurobiological mechanisms that could underlie these specific cognitive impairments in children with internalizing symptoms. Early internalizing symptoms have been characterized by disruptions in development of brain regions implicated in memory, such as the hippocampus (Koolschijn, van IJzendoorn, Bakermans-Kranenburg and Crone 2013). Disruption of the HPA-axis, with prolonged overproduction of glucocorticoids causing damage to hippocampal neurons, has been proposed to underlie memory deficits in paediatric depression (Charmandari, Kino, Souvatzoglou and Chrousos 2003; Hulvershorn, Cullen and Anand 2011). Another mechanism thought to underlie both anxiety and depression is suboptimal cortical regulation of the limbic system, including the amygdala and the insula (Phelps, Delgado, Nearing and LeDoux 2004). Altered functioning of such cortical regulatory regions could potentially also affect memory deficits. For instance, a study of major depressive disorder found memory dysfunction to be related to blood flow in the prefrontal cortex and anterior cingulate cortex (Dolan, Bench, Brown, Scott and Frackowiak 1994). Another potential neurobiological pathway is provided in the parietal-frontal integration theory, that poses that connectivity in a network involving parietal and frontal regions is crucial for intelligence and abilities like working memory (Olesen, Nagy, Westerberg and Klingberg 2003; Langeslag, Schmidt, Ghassabian, Jaddoe, Hofman et al. 2013). It is possible that connectivity in such regions is impaired in children with internalizing symptoms. This also meshes well with the decreased word production that we observed in these children, as verbal fluency is associated with activity of several frontal areas, including the left inferior

frontal gyrus and the dorsolateral prefrontal cortex (Gaillard, Hertz-Pannier, Mott, Barnett, LeBihan et al. 2000).

Decreased performance in language tasks has also been reported previously in anxiety disorders (Toren et al. 2000). Children in the internalizing class scored high on the scales of anxiety and depression, but also on withdrawn behavior. This constellation of symptoms may reflect a phenotype of more 'inhibited' or shy behavior. Inhibited children are less inclined to talk and have lower scores on tasks that require their spontaneous verbal response, such as the 'word generation' task in the NEPSY-II-NL. If the suboptimal performance of these children is a result of their shyness, or general task anxiety, these results are an indicator of their emotional symptoms. Alternatively, their emotional symptoms may limit their social interaction and impede with the development of highly training-dependent cognitive abilities such as language. Another mechanism proposed for lower cognitive performance is that these children are mentally 'occupied' by other cognitive processes such as extensive worrying that may engage their working memory (Davis et al. 2008). Problems in sustained attention and disruption of the resting-state functional MRI attention network have also been reported in children with anxiety and depression (Sylvester, Barch, Corbetta, Power, Schlaggar et al. 2013) and could underlie poorer test performance, and disrupt acquisition and consolidation of new information that is necessary for learning. Although internalizing broadband scores were associated with lower attention domainscores, this was likely a result of overlap with externalizing symptoms. We did not find this relation using the class approach, which indicates that this relation was likely due to confounding by comorbid externalizing symptoms. Likewise, a study in children with anxiety disorders showed that inattention did not mediate the relation between anxiety disorders and intellectual ability (Davis et al. 2008). Of note, internalizing problems in young children may not represent the same construct as internalizing problems at a later age. For instance, the prevalence of depression in such young children is thought to be extremely low, and any internalizing problems, especially in the general population, may primarily reflect anxiety and withdrawn behavior. However, internalizing problems at this age are very predictive of internalizing disorders at a later age (Roza et al. 2003).

In children with co-occurring internalizing and externalizing symptoms, we expected to observe widespread impairment. Children in this highly problematic class showed problems across a variety of scales and resemble the CBCL Dysregulation profile (Althoff et al. 2010). In a previous study, we found that these children had an 11 point lower non-verbal intelligence score than those without problems (Basten et al. 2014). However, contrary to our hypothesis, we did not observe widespread impairment. Possibly, the heterogeneity of the behavioral symptoms is reflected in the neurocognitive profiles. These children had the highest variability in performance across the domains (see SD). Children in this smallest class (n=63) had higher mean performance scores than the internalizing and externalizing classes,

which suggests that at least some children performed above our expectation. Decoupling of intelligence and other aspects of neurocognitive performance has been reported before (Barron-Linnankoski, Reinvall, Lahervuori, Voutilainen, Lahti-Nuuttila et al. 2015). Further, selection effects could have occurred in our study. The dysregulation class showed slightly less impaired non-verbal intelligence than previously reported (8 versus 11 points difference). Additionally, there is some evidence that children with ADHD and comorbid anxiety perform better in some cognitive tasks than children with only ADHD (Schatz et al. 2006; Yurtbasi et al. 2015). Interestingly, in the sensorimotor domain, we observed impairment. Children with dysregulation lifted their pencil more often while quickly drawing lines through different tracks. This may indicate more compensatory movements. Possibly, this group comprised children with high-functioning ASD. Often, children with ASD show rather peculiar cognitive patterns, with relatively more severe sensorimotor impairment, compared to other cognitive domains (Piek and Dyck 2004). Thus, we performed a sensitivity analysis excluding children that scored above the population screening threshold on an ASD questionnaire. After exclusion of these children, associations were attenuated but the differences in IQ between the dysregulation class and the reference class (data not shown) remained. This suggests that children with characteristics of ASD were partly responsible for the low IQ scores attributed to the dysregulation class in our previous study (Basten, van der Ende, Tiemeier, Althoff, Rijlaarsdam et al. 2014).

However, even after excluding these autistic-like children from the current analyses, the sensorimotor differences remained. It cannot be ruled out that the remaining children in the dysregulation class also had syndromes with motor clumsiness as a central feature, such as developmental coordination disorder (Piek et al. 2004).

The present study has several strengths. We used a large population-based sample and distinguished children with internalizing and externalizing symptoms using an empirical person-centred classification based on a broad range of behavioral and emotional symptoms. Importantly, our non-problematic class was representative of the general population in the sense that it was not restricted to children without any symptoms at all. The problem classes were not dependent on clinical cut-offs or DSM-criteria. Rather, these classes captured patterns of commonly co-occurring symptoms that reflect the true heterogeneity in child psychopathology. Additionally, information on potential confounding factors was available. Adjusting associations with neuropsychological performance for IQ can be helpful in elucidating specific relations between psychopathology and neurocognitive domains. Further, our neurocognitive test battery encompassed five different neurocognitive domains and provided a broad observational measure of cognition.

This study also has some limitations. First, our sample showed a slight tendency to more privileged families, so we are likely missing children with a higher risk for both cognitive problems and psychopathology. We can only carefully speculate that if the relation between

cognition and psychopathology is particularly prominent in these children, as is suggested from clinical studies, our result could represent an underestimation of the true effects.

Second, there was a delay between identification of the behavioral classes and the neuropsychological testing (mean delay 1.85 years). However, correlations between repeated measures of neurocognitive performance during childhood development have been shown to be substantial (Polderman, Posthuma, De Sonneville, Stins, Verhulst et al. 2007). In addition, internalizing and externalizing symptoms at young age correlate strongly with later symptoms (Achenbach et al. 2000). Another limitation is the relatively small sample of children with dysregulation (n=63), although this is unsurprising considering the population-based nature of the study. While there may be subgroups within this class, our study is not powered to explore those. Further, due to the cross-sectional nature of this study, we cannot infer the causal direction of these associations. Additionally, although our task battery measures a wide range of domains, it does not capture all the different constructs of each cognitive domain. This was not feasible for reasons of time and subject burden. For instance, the language domain measures verbal fluency, but does not capture other expressive and receptive aspects of language. Finally, adding measures of cognitive functioning to the latent class analyses could have added to the descriptive validity of the classes. However, this would make the classes less generalizable and cognition could not be tested as a correlate anymore, but would be an intrinsic part of the classification.

In conclusion, the current study shows specific relations between internalizing and externalizing symptoms and cognitive impairment. First, this information facilitates a better understanding of the underlying etiology. Internalizing symptoms may share neurobiological pathways with verbal fluency and memory impairments, while externalizing symptoms appear to be more specifically related to attention and executive functioning. Second, knowledge of the specific cognitive implications of psychopathological symptoms can help clinicians characterize the range of symptoms of an individual child. This is essential in determining the prognosis. Third, our results can potentially help in making informed treatment decisions, particularly if the clinical picture is characterized by comorbidity. For example, to specifically target executive functioning problems in a child that presents with a mixture of symptoms, it is helpful to treat the externalizing component of the psychopathology. Finally, a better understanding of cognitive endophenotypes could help identify novel targets for therapeutic intervention.

REFERENCES

Achenbach, T. M. and L. A. Rescorla (2000). Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT., University of Vermont, Reseach Center for Children, Youth and Families.

Achenbach, T. M. R., L.A. (2000). Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT., University of Vermont, Reseach Center for Children, Youth and Families.

Althoff, R. R., F. C. Verhulst, D. C. Rettew, J. J. Hudziak and J. van der Ende (2010). "Adult outcomes of childhood dysregulation: a 14-year follow-up study." J Am Acad Child Adolesc Psychiatry **49**(11): 1105-1116.

Andreasen, N. C. (1997). "Linking mind and brain in the study of mental illnesses: A project for a scientific psychopathology." Science **275**(5306): 1586-1593.

Barkley, R. A. (1997). "Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD." Psychological Bulletin **121**(1): 65-94.

Barron-Linnankoski, S., O. Reinvall, A. Lahervuori, A. Voutilainen, P. Lahti-Nuuttila and M. Korkman (2015). "Neurocognitive performance of children with higher functioning Autism Spectrum disorders on the NEPSY-II." Child Neuropsychology **21**(1): 55-77.

Basten, M., J. van der Ende, H. Tiemeier, R. R. Althoff, J. Rijlaarsdam, V. W. Jaddoe, A. Hofman, J. J. Hudziak, F. C. Verhulst and T. White (2014). "Nonverbal intelligence in young children with dysregulation: the Generation R Study." Eur Child Adolesc Psychiatry.

Basten, M., J. van der Ende, H. Tiemeier, R. R. Althoff, J. Rijlaarsdam, V. W. Jaddoe, A. Hofman, J. J. Hudziak, F. C. Verhulst and T. White (2014). "Nonverbal intelligence in young children with dysregulation: the Generation R Study." Eur Child Adolesc Psychiatry **23**(11): 1061-1070.

Basten, M. M. G. J., R. R. Althoff, H. Tiemeier, V. W. V. Jaddoe, A. Hofman, J. J. Hudziak, F. C. Verhulst and J. van der Ende (2013). "The Dysregulation Profile in Young Children: Empirically Defined Classes in the Generation R Study." Journal of the American Academy of Child and Adolescent Psychiatry **52**(8): 841-850.

Brooks, B. L., G. L. Iverson, E. M. Sherman and M. C. Roberge (2010). "Identifying cognitive problems in children and adolescents with depression using computerized neuropsychological testing." Appl Neuropsychol **17**(1): 37-43.

Brooks, B. L., E. M. Sherman and G. L. Iverson (2010). "Healthy children get low scores too: prevalence of low scores on the NEPSY-II in preschoolers, children, and adolescents." Arch Clin Neuropsychol **25**(3): 182-190.

Brooks, B. L., E. M. S. Sherman and E. Strauss (2009). "NEPSY-II: A Developmental Neuropsychological Assessment, Second Edition." Child Neuropsychology **16**(1): 80-101.

Cain, K., J. Oakhill and P. Bryant (2004). "Children's reading comprehension ability: Concurrent prediction by working memory, verbal ability, and component skills." Journal of Educational Psychology **96**(1): 31-42.

Chabernaud, C., M. Mennes, C. Kelly, K. Nooner, A. Di Martino, F. X. Castellanos and M. P. Milham (2012). "Dimensional brain-behavior relationships in children with attention-deficit/hyperactivity disorder." Biol Psychiatry **71**(5): 434-442.

Charmandari, E., T. Kino, E. Souvatzoglou and G. P. Chrousos (2003). "Pediatric stress: hormonal mediators and human development." Horm Res **59**(4): 161-179.

Clark, S., & Muthen, B. (2009). "http://www.statmodel.com/download/relatinglca.pdf."

Constantino, J. N. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Crosbie, J., P. Arnold, A. Paterson, J. Swanson, A. Dupuis, X. Li, J. Shan, T. Goodale, C. Tam, L. J. Strug and R. J. Schachar (2013). "Response Inhibition and ADHD Traits: Correlates and Heritability in a Community Sample." Journal of Abnormal Child Psychology **41**(3): 497-507.

Davis, T. E., T. H. Ollendick and M. Nebel-Schwalm (2008). "Intellectual ability and achievement in anxiety-disordered children: A clarification and extension of the literature." Journal of Psychopathology and Behavioral Assessment **30**(1): 43-51.

De La Fuente, A., S. Xia, C. Branch and X. Li (2013). "A review of attention-deficit/hyperactivity disorder from the perspective of brain networks." Front Hum Neurosci **7**: 192.

Dennis, M., D. J. Francis, P. T. Cirino, R. Schachar, M. A. Barnes and J. M. Fletcher (2009). "Why IQ is not a covariate in cognitive studies of neurodevelopmental disorders." J Int Neuropsychol Soc **15**(3): 331-343.

Dolan, R. J., C. J. Bench, R. G. Brown, L. C. Scott and R. S. Frackowiak (1994). "Neuropsychological dysfunction in depression: the relationship to regional cerebral blood flow." Psychol Med **24**(4): 849-857.

Emerson, C. S., G. A. Mollet and D. W. Harrison (2005). "Anxious-depression in boys: an evaluation of executive functioning." Arch Clin Neuropsychol **20**(4): 539-546.

Favre, T., C. Hughes, G. Emslie, P. Stavinoha, B. Kennard and T. Carmody (2009). "Executive Functioning in Children and Adolescents with Major Depressive Disorder." Child Neuropsychology **15**(1): 85-98.

Frazier, T. W., H. A. Demaree and E. A. Youngstrom (2004). "Meta-analysis of intellectual and neuropsychological test performance in attention-deficit/hyperactivity disorder." Neuropsychology **18**(3): 543-555.

Gaillard, W. D., L. Hertz-Pannier, S. H. Mott, A. S. Barnett, D. LeBihan and W. H. Theodore (2000). "Functional anatomy of cognitive development: fMRI of verbal fluency in children and adults." Neurology **54**(1): 180-185.

Hick, R. F., N. Botting and G. Conti-Ramsden (2005). "Short-term memory and vocabulary development in children with Down syndrome and children with specific language impairment." Dev Med Child Neurol **47**(8): 532-538.

Huizink, A. C. and E. J. H. Mulder (2006). "Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring." Neuroscience and Biobehavioral Reviews **30**(1): 24-41.

Hulvershorn, L. A., K. Cullen and A. Anand (2011). "Toward dysfunctional connectivity: a review of neuroimaging findings in pediatric major depressive disorder." Brain Imaging Behav **5**(4): 307-328.

Hurks, P. P., J. G. Hendriksen, J. S. Vles, A. C. Kalff, F. J. Feron, M. Kroes, T. M. van Zeben, J. Steyaert and J. Jolles (2004). "Verbal fluency over time as a measure of automatic and controlled processing in children with ADHD." Brain Cogn **55**(3): 535-544.

Insel, T., B. Cuthbert, M. Garvey, R. Heinssen, D. S. Pine, K. Quinn, C. Sanislow and P. Wang (2010). "Research Domain Criteria (RDoC): Toward a New Classification Framework for Research on Mental Disorders." American Journal of Psychiatry **167**(7): 748-751.

Ivanova, M. Y., T. M. Achenbach, L. A. Rescorla, V. S. Harder, R. P. Ang, N. Bilenberg, G. Bjarnadottir, C. Capron, S. S. De Pauw, P. Dias, A. Dobrean, M. Doepfner, M. Duyme, V. Eapen, N. Erol, E. M. Esmaeili, L. Ezpeleta, A. Frigerio, M. M. Goncalves, H. S. Gudmundsson, S. F. Jeng, P. Jetishi, R. Jusiene, Y. A. Kim, S. Kristensen, F. Lecannelier, P. W. Leung, J. Liu, R. Montirosso, K. J. Oh, J. Plueck, R. Pomalima, M. Shahini, J. R. Silva, Z. Simsek, A. Sourander, J. Valverde, K. G. Van Leeuwen, B. S. Woo, Y. T. Wu, S. R. Zubrick and F. C. Verhulst (2010). "Preschool psychopathology reported by parents in 23 societies: testing the seven-syndrome model of the child behavior checklist for ages 1.5-5." J Am Acad Child Adolesc Psychiatry **49**(12): 1215-1224.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." Eur J Epidemiol **27**(9): 739-756.

Kendler, K. S., C. A. Prescott, J. Myers and M. C. Neale (2003). "The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women." Arch Gen Psychiatry **60**(9): 929-937.

Koolschijn, P. C. M. P., M. H. van IJzendoorn, M. J. Bakermans-Kranenburg and E. A. Crone (2013). "Hippocampal volume and internalizing behavior problems in adolescence." European Neuropsychopharmacology **23**(7): 622-628.

Korkman, M., U. Kirk and S. Kemp (2010). Technische handleiding NEPSY-II-NL [Clinical and interpretive scoring manual NEPSY-IINL]. Enschede, The Netherlands, Ipskamp.

Kroes, M., A. C. Kalff, J. Steyaert, A. G. Kessels, F. J. Feron, J. G. Hendriksen, T. M. van Zeben, J. Troost, J. Jolles and J. S. Vles (2002). "A longitudinal community study: do psychosocial risk factors and child behavior checklist scores at 5 years of age predict psychiatric diagnoses at a later age?" J Am Acad Child Adolesc Psychiatry **41**(8): 955-963.

Langeslag, S. J. E., M. Schmidt, A. Ghassabian, V. W. Jaddoe, A. Hofman, A. van der Lugt, F. C. Verhulst, H. Tiemeier and T. J. H. White (2013). "Functional Connectivity between Parietal and Frontal Brain Regions and Intelligence in Young Children: The Generation R Study." Human Brain Mapping **34**(12): 3299-3307.

Lauer, R. E., B. Giordani, M. J. Boivin, N. Halle, B. Glasgow, N. E. Alessi and S. Berent (1994). "Effects of Depression on Memory Performance and Metamemory in Children." Journal of the American Academy of Child and Adolescent Psychiatry **33**(5): 679-685.

Loge, D. V., R. D. Staton and W. W. Beatty (1990). "Performance of Children with Adhd on Tests Sensitive to Frontal-Lobe Dysfunction." Journal of the American Academy of Child and Adolescent Psychiatry **29**(4): 540-545.

Matthews, K., D. Coghill and S. Rhodes (2008). "Neuropsychological functioning in depressed adolescent girls." J Affect Disord **111**(1): 113-118.

McGrath, L. M., E. B. Braaten, N. D. Doty, B. L. Willoughby, H. K. Wilson, E. H. O'Donnell, M. K. Colvin, H. L. Ditmars, J. E. Blais, E. N. Hill, A. Metzger, R. H. Perlis, E. G. Willcutt, J. W. Smoller, I. D. Waldman, S. V. Faraone, L. J. Seidman and A. E. Doyle (2015). "Extending the 'cross-disorder' relevance of executive functions to dimensional neuropsychiatric traits in youth." J Child Psychol Psychiatry.

Mesman, J. and H. M. Koot (2001). "Early preschool predictors of preadolescent internalizing and externalizing DSM-IV diagnoses." J Am Acad Child Adolesc Psychiatry **40**(9): 1029-1036.

Mugnaini, D., S. Lassi, G. La Malfa and G. Albertini (2009). "Internalizing correlates of dyslexia." World Journal of Pediatrics **5**(4): 255-264.

Muthen, B. and L. K. Muthen (2000). "Integrating person-centered and variable-centered analyses: Growth mixture modeling with latent trajectory classes." Alcoholism-Clinical and Experimental Research **24**(6): 882-891.

Netherlands, S. (2004). Allochtonen in Nederland. Voorburg/Heerlen, Amsterdam University Press.

Olesen, P. J., Z. Nagy, H. Westerberg and T. Klingberg (2003). "Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in a fronto-parietal network." Brain Res Cogn Brain Res **18**(1): 48-57.

Pennington, B. F. and S. Ozonoff (1996). "Executive functions and developmental psychopathology." J Child Psychol Psychiatry **37**(1): 51-87.

Petty, C. R., J. F. Rosenbaum, D. R. Hirshfeld-Becker, A. Henin, S. Hubley, S. LaCasse, S. V. Faraone and J. Biederman (2008). "The child behavior checklist broad-band scales predict subsequent psychopathology: A 5-year follow-up." J Anxiety Disord **22**(3): 532-539.

Peyre, H., M. Speranza, S. Cortese, M. Wohl and D. Purper-Ouakil (2015). "Do ADHD children with and without child behavior checklist-dysregulation profile have different clinical characteristics, cognitive features, and treatment outcomes?" J Atten Disord **19**(1): 63-71.

Phelps, E. A., M. R. Delgado, K. I. Nearing and J. E. LeDoux (2004). "Extinction learning in humans: role of the amygdala and vmPFC." Neuron **43**(6): 897-905.

Piek, J. P. and M. J. Dyck (2004). "Sensory-motor deficits in children with developmental coordination disorder, attention deficit hyperactivity disorder and autistic disorder." Human Movement Science **23**(3-4): 475-488.

Polderman, T. J., D. Posthuma, L. M. De Sonneville, J. F. Stins, F. C. Verhulst and D. I. Boomsma (2007). "Genetic analyses of the stability of executive functioning during childhood." Biol Psychol **76**(1-2): 11-20.

Price, J. L. and W. C. Drevets (2010). "Neurocircuitry of mood disorders." Neuropsychopharmacology **35**(1): 192-216.

Raver, C. C., C. Blair, M. Willoughby and F. L. P. K. Investigat (2013). "Poverty as a Predictor of 4-Year-Olds' Executive Function: New Perspectives on Models of Differential Susceptibility." Developmental Psychology **49**(2): 292-304.

Roza, S. J., M. B. Hofstra, J. van der Ende and F. C. Verhulst (2003). "Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: a 14-year follow-up during childhood, adolescence, and young adulthood." Am J Psychiatry **160**(12): 2116-2121.

Schatz, D. B. and A. L. Rostain (2006). "ADHD with comorbid anxiety: a review of the current literature." J Atten Disord **10**(2): 141-149.

Sergeant, J. A., H. Geurts and J. Oosterlaan (2002). "How specific is a deficit of executive functioning for attention-deficit/hyperactivity disorder?" Behav Brain Res **130**(1-2): 3-28.

Shao, Z., E. Janse, K. Visser and A. S. Meyer (2014). "What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults." Front Psychol **5**: 772.

Shin, M. S., H. Choi, H. Kim, J. W. Hwang, B. N. Kim and S. C. Cho (2008). "A study of neuropsychological deficit in children with obsessive-compulsive disorder." Eur Psychiatry **23**(7): 512-520.

Sylvester, C. M., D. M. Barch, M. Corbetta, J. D. Power, B. L. Schlaggar and J. L. Luby (2013). "Resting State Functional Connectivity of the Ventral Attention Network in Children With a History of Depression or Anxiety." Journal of the American Academy of Child and Adolescent Psychiatry **52**(12): 1326-1336.

Tellegen, P., B. Wijnberg-Williams and J. Laros (2005). Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2.5- 7/. Amsterdam, Boom Testuitgevers.

Toren, P., M. Sadeh, L. Wolmer, S. Eldar, S. Koren, R. Weizman and N. Laor (2000). "Neurocognitive correlates of anxiety disorders in children: A preliminary report." Journal of Anxiety Disorders **14**(3): 239-247.

Van der Meere, J., G. M. Marzocchi and T. De Meo (2005). "Response inhibition and attention deficit hyperactivity disorder with and without oppositional defiant disorder screened from a community sample." Dev Neuropsychol **28**(1): 459-472.

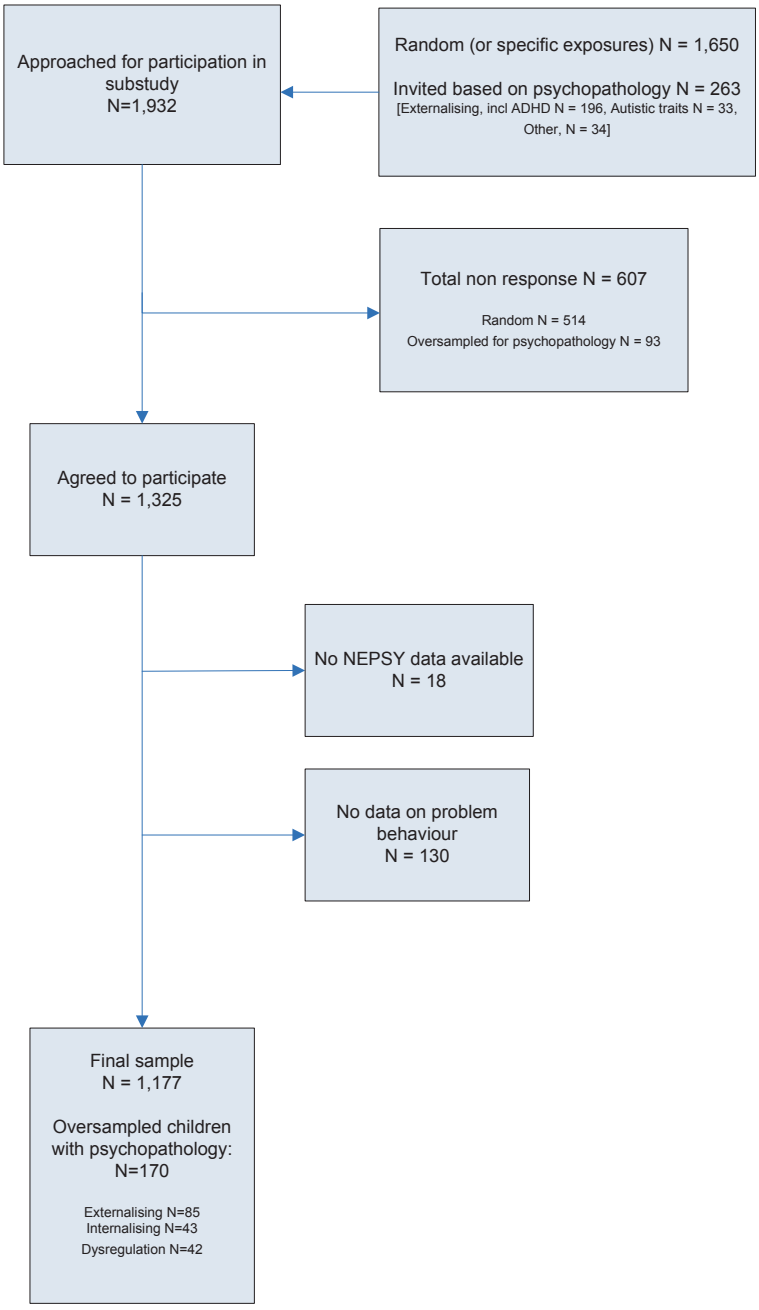
White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. V. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." European Journal of Epidemiology **28**(1): 99-111.

Willcutt, E. G., A. E. Doyle, J. T. Nigg, S. V. Faraone and B. F. Pennington (2005). "Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review." Biol Psychiatry **57**(11): 1336-1346.

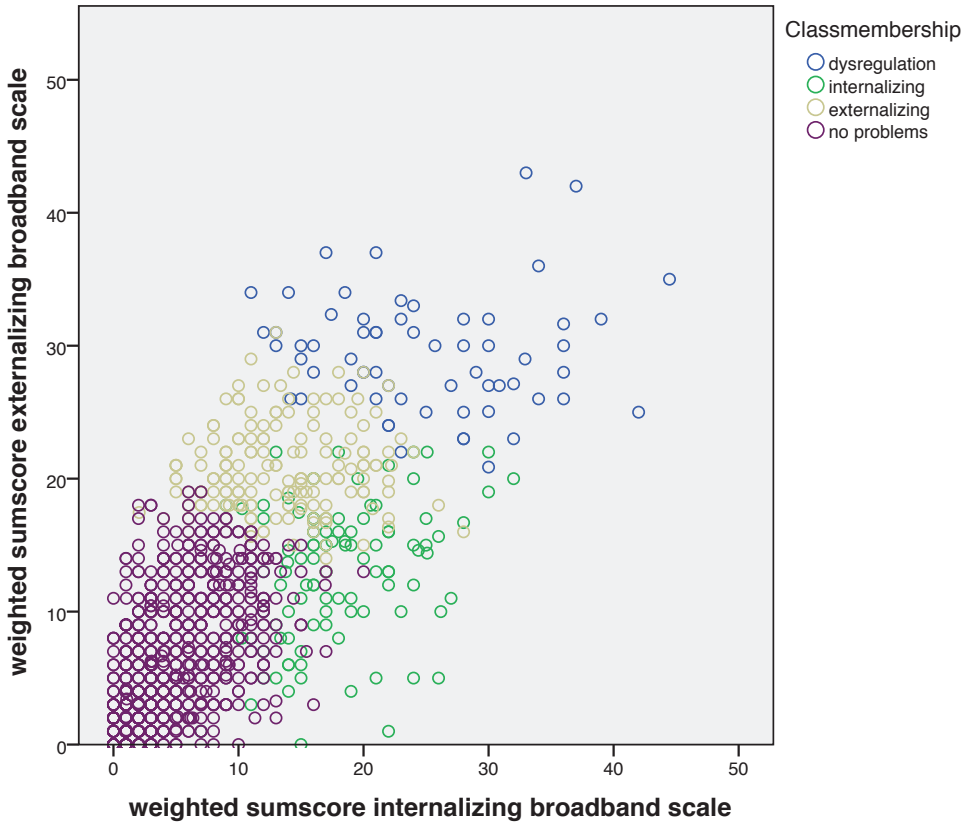
Yurtbasi, P., S. Aldemir, M. G. Teksin Bakir, S. Aktas, F. B. Ayvaz, S. Pistav Satilmis and K. Munir (2015). "Comparison of Neurological and Cognitive Deficits in Children With ADHD and Anxiety Disorders." J Atten Disord.

SUPPLEMENT

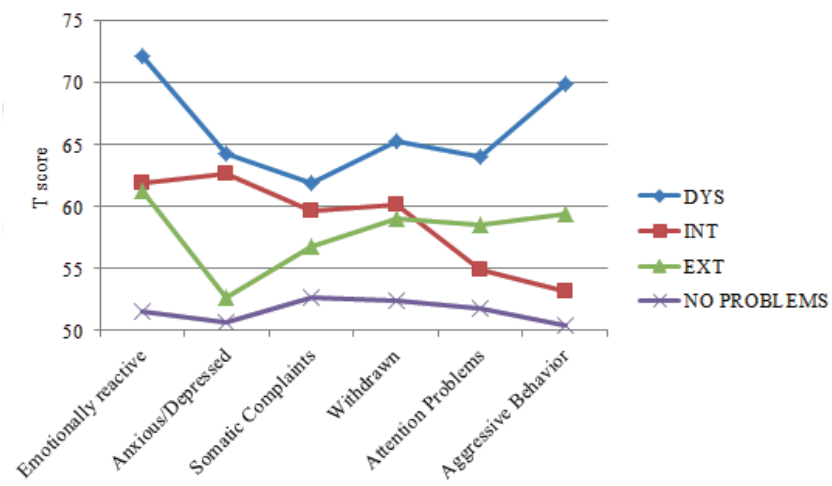
Supplementary Fig. 1 Consort diagram.



Supplementary Fig. 2 Internalizing and externalizing broadband scores in the in the internalizing, externalizing and dysregulation classes.

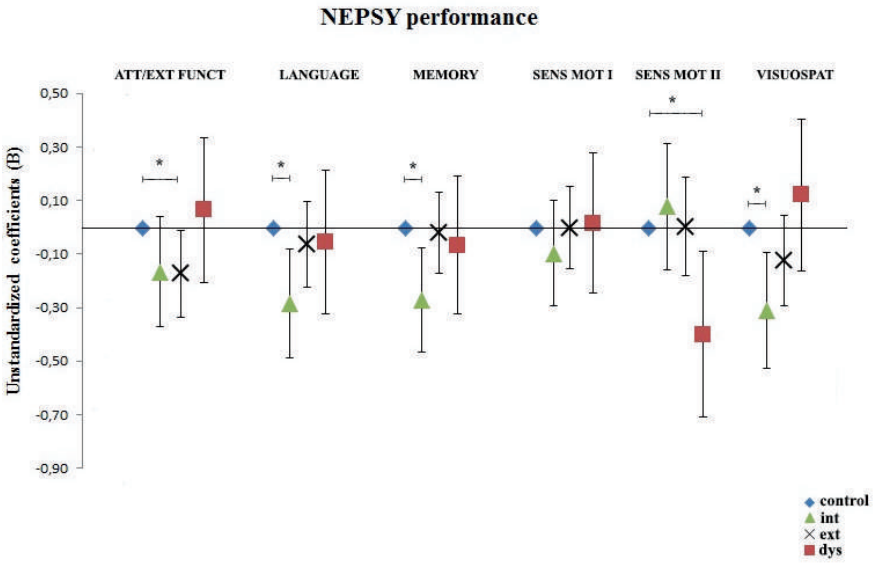


Supplementary Fig. 3 Mean T-scores of dysregulation class, the internalizing class and the externalizing class



Note. T-scores around 65 and higher were considered high, T-scores around 60 were considered moderate (in line with mean T-scores of 57–62 that were found for children referred to a mental health institution (Achenbach and Rescorla 2000) and T-scores around 55 were considered to be mild problem scores. In comparison, a matched group of non-referred children from the general population had mean T-scores of 54 (Achenbach et al. 2000).

Supplementary Fig. 4 NEPSY-II-NL performance in the internalizing, externalizing, and dysregulation classes in a sample excluding children with probable ASD (n=978)



Note. The no problems class (n=741) is the reference. Externalizing (n=125), internalizing (n=76), dysregulation (n=36). Model was adjusted for age and gender. *p<.05. Error bars represent 95% confidence intervals of the regression coefficients. The no problems class is the reference and has no error bars.

Supplementary Table 1. Spearman correlations NEPSY-II-NL subtestscores and corresponding domainscore (*n*=1,307)

	Attention and Executive Functioning domain score	Language domain score	Memory and Learning domain score	Sensorimotor Functioning I: speed accuracy interaction score	Sensorimotor Functioning II: pencillifts	Visuospatial Processing domain score
Auditory Attention						
Total score	0.61**					
Commission errors	-0.42**					
Omission errors	-0.61**					
Inhibition errors	-0.29**					
Response Set						
Total score	0.80**					
Commission errors	-0.68**					
Omission errors	-0.80**					
Inhibition errors	-0.53**					
Statue						
Total score	0.48**					
Total movements	-0.42**					
Total sounds	-0.32**					
Total eye openings	-0.41**					
Word Generation						
Total of correct words Animals		0.85**				
Total of correct words Foods/Drinks		0.87**				
Memory for Faces						
Total score			0.52**			
Memory for Faces – delayed						
Total score			0.50**			

Narrative Memory				
Total score free and cued recall	0.82**			
Total score free recall	0.84**			
Total score recognition	0.56**			
Visuomotor Precision				
Total speed accuracy interaction score		-0.96**		
Total pencillifts			-0.99**	
Arrows				
Total score				0.83**
Geometric Puzzles				
Total score				0.63**
Route Finding				
Total score				0.75**

Note. NEPSY-II-NL = neuropsychological assessment. ** $p<0.01$

Supplementary Table 2. Associations between internalizing problems, externalizing problems and performance on domains of the NEPSY-II NL

INTERNALIZING, square root transformed				
		B (95% CI)	**β**	***p***
Outcomes, SD score				
Attention and executive functioning	Age and gender	-0.08 (-0.12;-0.04)	-0.11	**<.001**
	Fully adjusted	-0.07 (-0.12;-0.03)	-0.10	**.001**
Language	Age and gender	-0.10 (-0.14;-0.06)	-0.13	**<.001**
	Fully adjusted	-0.06 (-0.10;-0.03)	-0.09	**.001**
Memory and Learning	Age and gender	-0.08 (-0.12;-0.05)	-0.11	**<.001**
	Fully adjusted	-0.07 (-0.11-;-0.03)	-0.09	**<.001**
Sensorimotor Primary	Age and gender	-0.04 (-0.08; 0.00)	-0.05	**.042**
	Fully adjusted	-0.03 (-0.08; 0.01)	-0.04	.132
Sensorimotor Secondary	Age and gender	-0.02 (-0.06; 0.03)	-0.02	.412
	Fully adjusted	-0.01 (-0.07; 0.04)	-0.02	.544
Visuospatial	Age and gender	-0.07 (-0.11;-0.03)	-0.09	**.001**
	Fully adjusted	-0.02 (-0.07; 0.03)	-0.03	.322
EXTERNALIZING, square root transformed				
		B (95% CI)	**β**	***p***
Outcomes, SD score				
Attention and executive functioning	Age and gender	-0.08 (-0.12;-0.04)	-0.11	**<.001**
	Fully adjusted	-0.07 (-0.11;-0.03)	-0.10	**<.001**
Language	Age and gender	-0.05 (-0.08;-0.01)	-0.07	**.009**
	Fully adjusted	-0.02 (-0.05; 0.02)	-0.03	.332
Memory and Learning	Age and gender	-0.03 (-0.07; 0.01)	-0.04	.102
	Fully adjusted	-0.02 (-0.05; 0.02)	-0.02	.435
Sensorimotor Primary	Age and gender	-0.04 (-0.08;-0.01)	-0.06	**.023**
	Fully adjusted	-0.03 (-0.07; 0.02)	-0.05	.066
Sensorimotor Secondary	Age and gender	-0.05 (-0.09;-0.01)	-0.07	**.014**
	Fully adjusted	-0.05 (-0.09;-0.01)	-0.07	**.018**
Visuospatial	Age and gender	-0.06 (-0.10;-0.02)	-0.08	**.003**
	Fully adjusted	-0.02 (-0.07; 0.03)	-0.03	.315

Note. The first model was adjusted for age at NEPSY-II-NL, age at CBCL and gender. The second model was additionally adjusted for ethnicity, household income, drinking during pregnancy and smoking during pregnancy. β indicates the standardized change in performance per unit change in the square root transformed CBCL broadband score transformed CBCL broadband score. Significant p-values are shown in bold.

Non-response analysis

A non-response analysis comparing subjects that were excluded because of missing information on class (n=130) with the study sample (n=1,177) showed that excluded children were more likely to be of non-Dutch origin ($\chi^2(2,N=1,307)=13.61,p=.001$), had lower IQ (mean difference=-5.35;$t(1,180)=3.91,p<.0001$), lower family income ($\chi^2(2,N=1132)=8.38,p=.015$), and had mothers with lower education levels ($\chi^2(2, N=1188)=8.02,p=.018$).

REFERENCE

Achenbach, T. M. and L. A. Rescorla (2000). Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT., University of Vermont, Reseach Center for Children, Youth and Families.

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Brain morphology and internalizing problems in young children: a population-based study

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ABSTRACT

Objectives: In childhood, internalizing and externalizing problems are highly comorbid and this can obscure specific brain-behavior associations. We explored brain morphological correlates of internalizing problems in young children, accounting for externalizing comorbidity.

Methods: In 801 children at age six years, internalizing problems were defined using two approaches: continuously (by the Child Behavior Checklist Internalizing Broadband score) and categorically (by a latent profile analysis of syndrome scales). The latter approach empirically defined a group of children with predominantly internalizing problems without substantial externalizing behavior. At eight years of age, brain volumetric measures and cortical thickness were assessed using magnetic resonance imaging. We examined the association of global volumetric measures, cortical thickness, and subcortical regions of interest with internalizing problems.

Results: There was no association between continuous internalizing scores and brain morphological measures, adjusted for externalizing problems. Categorical analyses suggested that children with predominantly internalizing problems had a smaller right amygdala than children with no problems. In whole brain vertex-wise analyses, compared to children with no problems, children with internalizing problems had thinner cortex in several regions of the right hemisphere: inferior frontal, a caudal middle region of the frontal cortex, occipital, and anterior temporal.

Conclusions: Using empirically defined classes of behavior, we showed that internalizing problems in children aged six-to-ten years are related to thinner cortex mostly in temporal but also in frontal regions. These findings suggest that structural differences observed in young children with internalizing problems might be similar to the brain regions implicated in depression or anxiety in older populations.

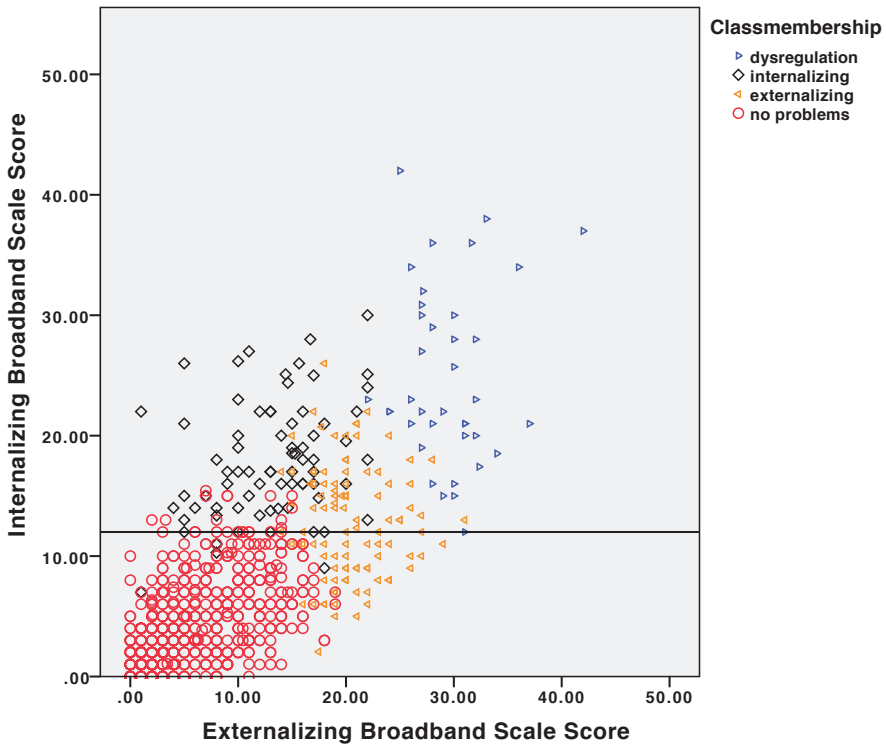
INTRODUCTION

Childhood internalizing problems are very common, impose a high burden on societies and families, and often predispose the children for adult disorders and impairment (Roza, Hofstra et al. 2003, Riglin, Petrides et al. 2014). Neuroimaging studies in adolescents and adults with depression or anxiety disorders consistently suggest the involvement of two overlapping and interconnected brain networks in emotional behavior: the medial prefrontal cortico-striato-pallido-thalamic network and the amygdalo-striato-pallido-thalamic network (Price and Drevets 2010). Ventromedial prefrontal cortex (vmPFC) and anatomically connected limbic structures including the amygdala, hippocampus, striatum, and medial thalamus are implicated in the symptomatology of mood and anxiety disorders. Areas in ventrolateral prefrontal cortex (vlPFC), which are closely related to the medial and orbital prefrontal cortex, are also involved in emotion regulation and cognitive appraisal of negative affect (Price and Drevets 2012, Schmaal, Hibar et al. 2016). While neurobiological underpinnings of psychopathology are shown to be sensitive to development (Zahn-Waxler, Klimes-Dougan et al. 2000), findings on neuroanatomical correlates of internalizing problems in young children are limited. Moreover, few studies to date have investigated brain structural correlates of internalizing problems in population-based samples, which are advantageous for examining the full spectrum of behavior (Koolschijn, van et al. 2013, Ducharme, Albaugh et al. 2014, Vulser, Lemaitre et al. 2015).

Clinical neuroimaging studies of children with anxiety or depression generally investigate small and heterogeneous samples of children with symptoms of depression, anxiety, social phobia and general anxiety disorder (Marrus, Belden et al. 2015, Strawn, Hamm et al. 2015). While such psychopathology most likely exists on a continuum in the population, the comparison of a heterogeneous group of children with anxiety or depression and controls (case-control design) does not capture the neurobiology of the full spectrum of severity. Moreover, in young children, internalizing problems –that comprise problems that manifest mainly within the self, such as sad mood or anxiety– are often comorbid with externalizing problems (Zahn-Waxler, Klimes-Dougan et al. 2000). Studying children with coexisting conditions has been a topic of interest; nonetheless, it remains challenging to identify neuroimaging abnormalities specific to internalizing problems, independent of externalizing problems. One option is covarying the internalizing problems for externalizing problems (Achenbach, Ivanova et al. 2016). This approach can be used to examine if internalizing problems are specifically associated with brain morphological abnormalities. However, because of a high correlation between internalizing and externalizing problems, covarying one for the other might change the associations with other variables (Achenbach, Ivanova et al. 2016). In this study, we additionally explored using an alternative method to examine specific morphological correlates of internalizing problems. We previously described a latent

profile analysis performed on various dimensionally assessed behaviors to delineate four groups of children: 1) a group who predominantly had externalizing problems, 2) a group who predominantly had internalizing problems, 3) a group who showed both externalizing and internalizing symptoms, and 4) a group including children with no problems (Basten, Althoff et al. 2013). Figure 1 illustrates that this person-centered approach results in groups of children with more homogeneous patterns of behavioral and emotional symptoms. The internalizing group that is derived from this approach provides an opportunity to study internalizing problems in young children in the general population more specifically.

Figure 1 Empirically defined classes of behavior and the CBCL/1½–5 Internalizing and Externalizing Broadband Scale scores



This method classifies children in one of four classes: no problem behavior (n=581), with predominantly internalizing problems (n=73), with predominantly externalizing problems (n=102), and with both internalizing and externalizing symptoms (n=38). The horizontal line indicates the borderline cut-off for internalizing problems (based on a Dutch norm sample).

Here, using magnetic resonance imaging (MRI), we aimed to identify cortical and subcortical brain morphological correlates of internalizing problems in a group of six-to-ten year old children from the general population. In addition to the assessment of internalizing

problems across the continuum of internalizing broadband scale scores (derived from the Child Behavior Checklist for toddlers (CBCL/1½–5) (Achenbach and Rescorla 2000)), we used the above mentioned method to characterize a distinct group of children with predominantly internalizing problems (Basten, Althoff et al. 2013). Using these two approaches, we first explored the association between cortical thickness and internalizing problems. Next, we tested the relationship of internalizing problems with global brain volumetric measures and specific subcortical regions of interest highly implicated in emotional behaviors (i.e. amygdala and the hippocampus) (Yap, Whittle et al. 2008, Koolschijn, van et al. 2013). We hypothesized that children with primarily internalizing problems would have thinner cortex in regions previously associated with clinical depression and anxiety, including the vmPFC. We also hypothesized that children with internalizing problems would have smaller volumes in the amygdala and hippocampus. We also tested for sex interactions, as patterns of cortical development have been shown to be distinct in boys and girls (Sowell, Peterson et al. 2007).

METHODS

Participants

Children were participants of the Generation R Study, a population-based cohort investigating children's growth and development from fetal life onwards (Jaddoe, van Duijn et al. 2012). In total, 1,070 six-to-ten year old children were recruited between September 2009 and July 2013 as part of a neuroimaging sub-study. The details of this sub-study have been described previously (White, El Marroun et al. 2013). For 845 children, data on internalizing behavior and good quality structural imaging data were available. In this sample, if participants were siblings (n=19 pairs), we excluded one sibling of each pair. In addition, children with a confirmed diagnosis of autism spectrum disorder by specialist medical records were excluded (n=22) (Blanken, Mous et al. 2015). Three children with incidental findings in their brain MRI were also excluded, which left 801 children for further analyses. Informed consent was obtained from parents and children, after a complete description of the study was provided. Erasmus Medical Center Medical Ethics Committee approved all the procedures.

Internalizing problems

When the children were six years of age, the Child Behavior Checklist/1½–5 (CBCL/1½–5) was administered to obtain a standardized parental rating of the child's emotional and behavioral problems (Achenbach and Rescorla 2000). The CBCL/1½–5 consists of 99 problem items and provides two broad groupings of syndromes: internalizing (anxiety/depressed, emotionally-reactive, somatic complaint, and withdrawn) and externalizing (attention problems and aggressive behavior). The good reliability and validity of the Dutch version of CBCL/1½–5

have been demonstrated previously (Tick, van der Ende et al. 2007). In this study, we applied two different approaches to study internalizing problems using the CBCL/1½–5. In our first approach, we used the internalizing broadband scale score (Achenbach and Rescorla 2000). In our second approach, we used four previously defined classes with distinct patterns of internalizing problems that were obtained by a latent profile analysis performed on T-scores of the above mentioned CBCL/1½–5 syndrome scales (Basten, Althoff et al. 2013). This method classifies children in one of four classes: children with no problem behavior (n=581), children with predominantly internalizing problems (n=73), children with predominantly externalizing problems (n=102), and a class with both internalizing and externalizing symptoms (n=38) (Althoff, Verhulst et al. 2010). Children were assigned to a class based on the most likely class memberships. This was justified by the high entropy (0.98) of the latent class model. Figure 1 represents the four classes of children and their CBCL/1½–5 internalizing and externalizing broadband scores.

Neuroimaging

Structural MRI scans were obtained on a 3-T scanner (Discovery MR750, General Electric Worldwide, Milwaukee) (White, El Marroun et al. 2013). The high-resolution, T1-weighted image was collected using an inversion recovery fast spoiled gradient recalled sequence with the following parameters: repetition time = 10.3 msec, echo time = 4.2 msec, inversion time = 350 msec, number of excitations = 1, flip angle = 16°, readout bandwidth = 20.8 kHz, matrix 256 × 256, imaging acceleration factor of 2, and an isotropic resolution of 0.9 × 0.9 × 0.9 mm3. All children underwent a mock scanning session prior to the scanning procedure.

Image processing

Image quality assurance is described in the supplementary information. Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite 5.1 (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in a prior publication (Reuter, Schmansky et al. 2012). Briefly, this process included the removal of non-brain tissue, automated Talairach transformation into standard space, and segmentation of the subcortical white and gray matter volumetric structures (including amygdala and the hippocampus), intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation. Once the cortical models were complete, the images underwent surface inflation (Fischl, Sereno et al. 1999), registration to a spherical atlas (Fischl, Sereno et al. 1999), and the parcellation of the cerebral cortex into units based on gyral and sulcal structure (Desikan, Segonne et al. 2006). Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex on the tessellated surface (Fischl and Dale 2000).

Covariates

Confounders were selected *a priori* (Basten, van der Ende et al. 2014, Ducharme, Albaugh et al. 2014). Information on birth date and sex was obtained from registries. Maternal age, education, ethnicity, and history of smoking or drinking were assessed by questionnaires. A child's ethnic background was defined based on the country of birth of both parents. Maternal education was defined by the highest completed education. Maternal smoking and alcohol consumption habits were assessed at enrollment and in mid- and late pregnancy. We used the Brief Symptom Inventory, a validated self-report questionnaire, to measure maternal psychopathology during pregnancy (de Beurs 2004). Handedness of the child was obtained using the Edinburgh Handedness Inventory on the day of the scan (Oldfield 1971). Non-verbal intelligence of the child was assessed at approximately 6 years of age using a shortened version of the Snijders-Oomen Niet-verbale Intelligentie Test–Revisie (SON-R 2.5–7), a non-verbal intelligence test suited for children between 2.5–7 years of age (Tellegen, Wijnberg-Williams et al. 2005).

Statistical Analysis

First we ran the analyses using the CBCL/1½–5 internalizing broadband score as the independent variable. Internalizing broadband scores were square root transformed for normality. We examined the association of internalizing scores with global volumetric measures, i.e., brain volume, cortical volume, cortical gray matter volume, cortical white matter volume and subcortical regions of interests, i.e., the hippocampus, amygdala (dependent variables), using linear regressions. Models were adjusted for maternal age, education, history of smoking and drinking in pregnancy, maternal history of psychopathology in pregnancy, and child age at behavioral assessment, ethnicity, intelligence, handedness, and externalizing scores. In an additional step, associations with the amygdala and hippocampal volumes were adjusted for total brain volume. To investigate the relationship between internalizing broadband scale scores and cortical thickness, whole-brain vertex-wise statistics were run using the Generalized Linear Model within the 'mri_glmfit' tool provided by FreeSurfer (Fischl 2012). We used a linear regression model with internalizing scores as the independent variable, adjusting for a child's sex, age, and externalizing broadband scores. To visualize patterns of confounding, we exported local thickness data for each participant for the identified clusters into SPSS (version 22.0; IBM, USA). Using linear regression analyses, we assessed whether the associations withstood correction for a range of confounding factors including maternal age, education, history of smoking and drinking in pregnancy, maternal history of psychopathology in pregnancy, and child age at behavioral assessment, ethnicity, intelligence, handedness and total brain volume. In an additional step, we adjusted the models for a child's total brain volume. Analyses were corrected for multiple comparisons using the built-in Monte Carlo simulation at a threshold set at a p value <0.05, a cluster-wise correction that controls for the rate of false positive clusters.

Next, we examined associations of empirically derived internalizing problems with brain cortical and subcortical measures. We ran the analyses using dummy-coded class memberships as independent variables (internalizing class, externalizing class and dysregulation class) and estimated the effect estimate for brain measures in children with internalizing problems when compared to the reference group of children with no problems. In this step, vertex-wise analyses were adjusted for a child's sex and age. Subsequent steps were identical to analyses with internalizing broadband scores.

In the 801 children with behavioral and imaging data, the percentage of missing in covariates was below 10% except for maternal psychopathology in pregnancy (15%). Missing values in covariates were imputed using multiple imputations. Continuous variables with skewed distribution were transformed for normal distribution before imputation. Ten copies of the original data set were generated, with missing values replaced by values randomly generated from the predictive distribution on the basis of the correlation between the variable with missing values and other variables.

In a sensitivity analysis, we defined a group of children with internalizing problems using the CBCL/1½–5 borderline cut-off (based on the Dutch norm population (Tick, van der Ende et al. 2007)) and compared their brain morphology to the children who had scores below this cut-off. We further examined if any sex interaction exists in the association of internalizing problems and brain morphology.

RESULTS

Table 1 shows the characteristics of study participants. Compared to children with no problems, children with internalizing problems were more often girls (56.2% vs. 49.1%), were more often from a non-Western ethnic background (41.1% vs. 17.2%), had a lower non-verbal intelligence score (98.9 vs. 103.9), and had more comorbid externalizing problems (mean score 13.0 vs. 6.2). Also, mothers of children with internalizing problems at six years had lower levels of education (46.6% with higher education vs. 58.5) and a higher psychopathology score in pregnancy (mean score 0.25 vs. 0.14).

We observed no association between children's internalizing broadband scores and cortical and subcortical volumetric measures (Supplementary Table 1). Table 2 summarizes the results of the analyses relating children's internalizing problems (defined as children assigned to the internalizing class compared to the group with no problems) and brain volumetric measures. In univariate analyses, children's internalizing problems were associated with total brain volume, total gray volume, and cortical gray volume. After adjustment for possible confounders (Model 2), the associations became non-significant. We found that children within the internalizing problem class had a smaller right amygdala volume compared to

children with no problems. Additional adjustment for total brain volume attenuated this association (B=-54.6, 95%CI:-116.9, 7.8).

Table 1 Participant characteristics.

Child characteristics	**No problems N=581**	**Internalizing problems N=73**
Sex (% boy)	296 (50.9)	32 (43.8)
Ethnicity (%)		
Dutch	438 (75.4)	38 (52.1)
Other Western	43 (7.4)	5 (6.8)
Non-Western	100 (17.2)	30 (41.1)
Age at behavior assessment, yr	6.0 (0.4)	6.1 (0.5)
Non-verbal intelligence score	103.9 (14.3)	98.8 (13.7)
Internalizing broadband scale score	4.5 (3.5)	17.9 (4.8)
Externalizing broadband scale score	6.2 (4.6)	13.0 (5.1)
Maternal characteristics		
Age, yr	31.2 (4.5)	29.5 (5.4)
Maternal educational level (%)		
Primary school or lower vocational	59 (10.2)	19 (26.0)
Intermediate vocational	182 (31.3)	20 (27.4)
Higher education or university	340 (58.5)	34 (46.6)
Alcohol use during pregnancy (%)		
Never	191 (32.9)	34 (46.6)
Until pregnancy was known	86 (14.8)	8 (11.0)
Continued occasionally	236 (40.6)	22 (30.1)
Continued frequently	68 (11.7)	9 (12.3)
Smoking during pregnancy (%)		
Never	457 (78.7)	58 (79.5)
Until pregnancy was known	35 (0.1)	6 (8.2)
Continued	89 (15.3)	9 (12.3)
Psychopathology score in pregnancy	0.14 (0.25)	0.25 (0.51)

Values are mean (standard deviation) for continuous variables with normal distribution, median (interquartile range) for continuous variable with skewed distribution and categories (percentage) for categorical variables.
Internalizing problems was defined using a latent profile analysis performed on all Child Behavior Checklist/1½–5 syndrome scales

Table 2 Brain morphology and Internalizing Problems at 6 years (n=794).

Internalizing problem class vs. no problems	**Model 1**	**Model 2**	**Model 2**
Brain morphology	**B (95%CI)** **P value**	**B (95%CI)** **P value**	**B (95%CI)** **P value**
Total Brain Volume, cm^3	-31.5 (-58.2,-4.8) 0.02	-16.7 (-43.5, 10.2) 0.22	
Total Gray Volume, cm^3	-23.6 (-40.7,-6.5) 0.01	-12.7 (-29.9, 4.5) 0.15	
Cortical Gray Volume, cm^3	-20.7 (-35.8,-5.5) 0.02	-11.7 (-26.9, 3.5) 0.13	
Cerebral White Matter Volume, cm^3	-7.3 (-17.4, 2.9) 0.16	-3.7 (-14.0, 6.6) 0.48	
Subcortical volumes			
Right amygdala volume, mm^3	-71.5 (-149.7,-7.0) 0.03	-69.5 (-135.6,-3.5) 0.04	-54.6 (-116.9, 7.8) 0.09
Left amygdala volume, mm^3	-52.0 (-109.4, 5.4) 0.08	-41.7 (-100.1, 16.8) 0.16	-27.2 (-80.5, 26.1) 0.32
Right hippocampus, mm^3	-42.7 (-145.7, 60.3) 0.42	-11.8 (-117.0, 93.5) 0.83	19.3 (-72.9, 111.4) 0.68
Left hippocampus, mm^3	11.7 (-89.9, 113.2) 0.82	49.4 (-53.9, 152.6) 0.35	76.1 (-13.3, 165.5) 0.10

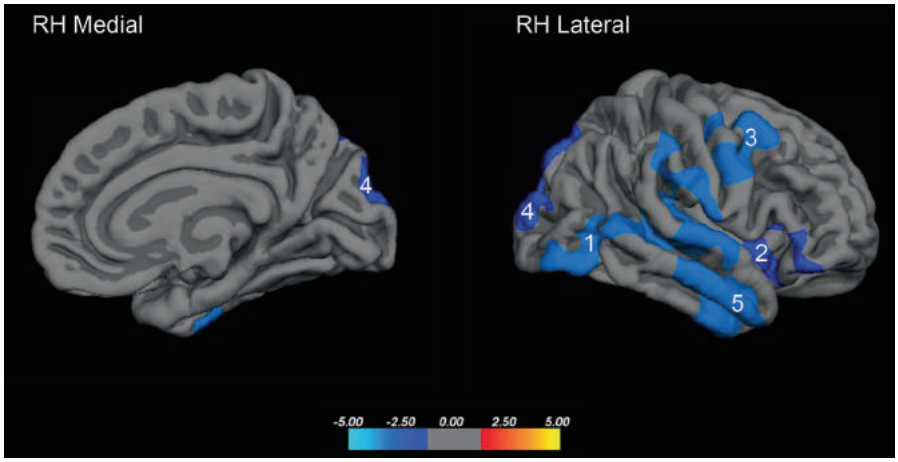
Model 1 adjusted for sex and age at scanning
Model 2 additionally adjusted for maternal age, education, history of smoking and drinking in pregnancy, maternal history of psychopathology in pregnancy, and child age at behavioral assessment, ethnicity, and intelligence
Model 3 additionally adjusted for total brain volume
Internalizing problems was defined using a latent profile analysis performed on all Child Behavior Checklist/1½–5 syndrome scales(Basten, Althoff et al. 2013)
*Bs reflect differences in brain morphological measures in children with internalizing problems vs. children with no problems.

Surface-based vertex-wise cortical analyses with internalizing broadband scale score resulted in no clusters when adjusted for a child's sex, age, and externalizing broadband scores. Figure 2 illustrates the results of surface-based vertex-wise cortical analyses with the empirically defined internalizing problems class adjusted for sex, age, and dummy-coded problem classes (children with no problems were the reference). Children with internalizing problems had thinner cortex in several brain areas in the right hemisphere including inferior frontal (pars orbitalis), middle frontal/precentral, occipital/cuneus, and temporal cortices when compared to children with no problems. Further adjustment for confounders and total brain volume did not change the results (Table 3). As children in the internalizing class have some degree of externalizing problems, which may not fully be captured by adjusting for the externalizing class dummy variable, we reran these analyses additionally adjusted for CBCL/1½–5 externalizing broadband scores. As presented in Supplementary Figure 1, we found thinner cortex in clusters which largely overlapped with those of the main analysis, including temporal lobe and inferior frontal gyrus (pars opercularis/triangularis).

Analysis with the CBCL/1½–5 borderline cut-off (based on the Dutch norm population) revealed no significant association with brain volumetric measures or cortical thickness.

There was no significant sex interaction (p for interaction >0.05); however stratified analyses for sex revealed that girls with internalizing problems had smaller right and left amygdala volumes than girls with no problems (B=-79.1, 95%CI:-162.3, 4.2, p=0.06 and B=-66.5, 95%CI:-132.0,-1.1, p=0.05, respectively). There was no association between amygdala volume and the internalizing class of behavior in boys.

Figure 2 Cortical thickness and Internalizing Problems at 6 years



Internalizing problems were defined using a latent profile analysis performed on all Child Behavior Checklist/1½–5 syndrome scales(Basten, Althoff et al. 2013)
Clusters: (1) Temporal, (2) Inferior frontal cortex (Pars Orbitalis) (3) Middle Frontal/Precentral (4) Occipital/Cuneus (5) Anterior Temporal.
RH: Right Hemisphere

Table 3 Cortical thickness and Internalizing Problems at 6 years (n=794).

Internalizing problem class vs. no problems	**Model 1**	**Model 2**	**Model 3**
Hemisphere and Region Right Hemisphere	**B (95%CI)** **P value**	**B (95%CI)** **P value**	**B (95%CI)** **P value**
Temporal	-0.130 (-0.169,-0.090) <0.001	-0.117 (-0.157,-0.077) <0.001	-0.114 (-0.152,-0.075) <0.001
Inferior frontal cortex (Pars Orbitalis)	-0.130 (-0.175,-0.085) <0.001	-0.119 (-0.165,-0.073) <0.001	-0.115 (-0.159,-0.071) <0.001
Middle Frontal/Precentral	-0.142 (-0.197,-0.086) <0.001	-0.131 (-0.188,-0.074) <0.001	-0.125 (-0.179,-0.072) <0.001
Occipital/Cuneus	-0.126 (-0.181,-0.070) <0.001	-0.103 (-0.159,-0.046) <0.001	-0.099 (-0.154,-0.044) <0.001
Anterior Temporal	-0.171 (-0.246,-0.096) <0.001	-0.167 (-0.244,-0.090) <0.001	-0.161 (-0.236,-0.087) <0.001

Cortical thickness clusters were residualized for age at scanning.
Model 1 adjusted for sex
Model 2 additionally adjusted for maternal age, education, history of smoking and drinking in pregnancy, maternal history of psychopathology in pregnancy, and child sex, age at behavioral assessment, ethnicity, and intelligence
Model 3 additionally adjusted for total brain volume
Internalizing problems was defined using a latent profile analysis performed on all Child Behavior Checklist/1½–5 syndrome scales(Basten, Althoff et al. 2013)
*Bs reflect differences in brain morphological measures in children with internalizing problems vs. children with no problems

DISCUSSION

In this large population-based study of young children, we found no association between continuous internalizing scores and brain morphological measures. However, categorical analyses on a distinct group of children with predominantly internalizing problems showed that these children had a smaller right amygdala compared to children with no problems. This association with amygdala volume was attenuated after adjustment for total brain volume. In whole brain vertex-wise analyses, children with internalizing problems had thinner cortex in right hemisphere inferior frontal, a caudal middle region of the frontal cortex, occipital, and anterior temporal compared to children with no problems.

We found no association between continuous internalizing scores and cortical and subcortical brain morphology, when adjusting for continuous externalizing scores. This is likely because covarying for externalizing scores took away the variation of interest in internalizing scores (due to high correlations between two scores) (Achenbach, Ivanova et al. 2016). This observation highlights the necessity of different methods to assess correlates specific to internalizing problems. Identifying a relatively homogeneous group of young children with internalizing problems is challenging in neuroimaging studies. In the general population, while the majority of children show no problems (Figure 1), there is a small group of children who present high levels of co-occurring internalizing and externalizing problems (previously labeled as dysregulation group) (Basten, Althoff et al. 2013). In addition, we identified two groups of children who showed subthreshold symptoms of either externalizing or internalizing dimensions. As illustrated in Figure 1, conventional methods of assessing internalizing problems, e.g. using continuous internalizing scores or categorization based on a predefined cut-off on the internalizing scores, very likely capture children who also have high scores of externalizing problems (about half of children in the externalizing class had high internalizing scores and could simply be identified as having internalizing problems using conventional methods, Figure 1). The empirically defined class of children with internalizing problems enabled us to identify children with internalizing problems while they did not score high on externalizing problems (Table 1). Therefore, we were able to establish brain cortical and subcortical differences specific to internalizing problems. Importantly, even in the relatively homogeneous group of children with internalizing problems, some degree of externalizing problem coexists (Figure 1). To ensure that the comorbid externalizing problems in children within the internalizing class did not affect the association observed with cortical morphology, we adjusted the analysis for continuous externalizing scores. This analysis revealed overlapping clusters of thinner cortex with findings of the analysis not adjusted for continuous scores in children with internalizing problems compared to children with no problems.

Our finding was suggestive that young children with internalizing problems had a smaller right amygdala volume compared to children with no problems. The results suggested a sex-specific association between amygdala volume and internalizing problems (smaller right and left amygdala volume in girls only). Abnormalities in the amygdala are implicated in the pathophysiology of clinical depression and anxiety (De Bellis, Casey et al. 2000). However, evidence regarding the amygdala volume in children with internalizing problems is conflicting. For example, in an at-risk population, adolescent boys with a smaller right amygdala had more depressive symptoms (Yap, Whittle et al. 2008). In contrast, van der Plas et al. showed that a larger amygdala volume was positively correlated with fearfulness in 7-17 year old girls with a family history of depression (van der Plas, Boes et al. 2010). A smaller amygdala volume in girls with internalizing problems within a non-clinical population provides further evidence for the pivotal role of the amygdala in the pathophysiology of internalizing problems. In the present population-based sample of six-to-ten year old children, we found no relationship between hippocampal volume and internalizing problems. The hippocampus is involved in learning and spatial and episodic memory (Burgess, Maguire et al. 2002), and some authors have postulated that deficits in the structure and function of the hippocampus accompany symptoms of depression or anxiety (Videbech and Ravnkilde 2015). It is possible that the differences in the hippocampal volume between children with and without internalizing problems may not yet be present at a young age. Another explanation is that the structural abnormalities in the hippocampus may only be present in individuals with severe symptoms of depression or anxiety.

Similar to a previous report in adolescents (Vulser, Lemaitre et al. 2015), we observed smaller global brain volumetric measures in association with internalizing problems, if analyses were only adjusted for a child's sex and age at scanning. However, when we additionally controlled for various sociodemographic characteristics, there were no differences in global brain volumetric measures between children with internalizing problems and those with no problems. This finding confirms the notion that there are no global morphological differences in children with internalizing problems compared to children with no problems (Price and Drevets 2012).

However, we showed that the children with internalizing problems had thinner cortex in several brain regions, including regions in frontal/precentral cortex and orbital cortex (shown in Figure 1). We also observed thinner cortex in the right anterior temporal cortex in children with internalizing problems. Existing evidence in clinical and community-based studies points to the involvement of vmPFC and the anterior cingulate cortex in children with anxiety and depression (Marrus, Belden et al. 2015, Vulser, Lemaitre et al. 2015). Most of these studies were performed in adolescents, but other studies indicated that young children with depression or subthreshold symptoms of anxiety/depression show similar cortical thinning in right vmPFC (Ducharme, Albaugh et al. 2014, Marrus, Belden et al. 2015). Furthermore, recent

evidence indicates the involvement of areas in the dorsolateral prefrontal cortex (dlPFC) in the neurobiology of depression (Pizzagalli 2011, Shad, Muddasani et al. 2012). Frontal regions are thought to be involved in regulation of the limbic system, including the amygdala, subgenual cingulate cortex and the insula. Similar to ours, some of the previous studies indicated the laterality of brain correlates of anxiety and depressive symptoms, showing predominantly differences in the right hemisphere (De Bellis, Keshavan et al. 2002). Furthermore, decreased gray matter volume in the postcentral gyrus and cuneus has been previously reported in children with anxiety disorders (Strawn, Hamm et al. 2015). Interestingly, considering that the cuneus is thought to be involved in memory (Cavanna and Trimble 2006), we previously showed impaired memory function in these children (Blanken, White et al. 2016). At the same time, our finding is in contrast with report of structural studies in pediatric populations with clinical diagnoses of depression or anxiety which showed thicker cortex in these patients compared to healthy controls (De Bellis, Keshavan et al. 2002, Fallucca, MacMaster et al. 2011). A smaller study (22 adolescents with depression and 22 controls) in adolescents, which examined the gray matter volume in the temporal lobe, showed smaller volumes of the right middle and superior temporal gyrus and greater volume in the subgyral temporal lobe of adolescents with depression compared to healthy controls (Shad, Muddasani et al. 2012). Projections from superior temporal regions into the amygdala are highly involved in anxiety disorders and structural abnormalities are reported in young patients with generalized anxiety disorders (De Bellis, Keshavan et al. 2002). Comparisons of the previous studies with ours should be made with caution since we examined cortical thickness (as opposed to gray matter volume in some of the previous studies) in a non-clinical sample of young children with internalizing problems. The overlap between areas with thinner cortex in children with internalizing problems observed in this study and studies of older children or adolescents with depression and anxiety indicates a continuum in cortical morphological abnormalities related to internalizing problems into childhood and non-clinical populations.

The present study had several strengths including a large sample size and utilizing the same scanner and protocol for all children which eliminated the possibility of bias due to acquisition factors. To date, few studies examined brain structural abnormalities associated with internalizing problems in non-clinical samples of young children, in particular children younger than eight years. We adjusted the analyses for several confounders, e.g. maternal psychopathology and child intelligence, as suggested in the literature (Connell and Goodman 2002). Nonetheless, our study faced some limitations. First, MRI scans were performed once and on average 1.6 years after the collection of the behavioral assessment. Therefore, no inferences can be made on the temporal direction of the association or trajectories of development. Second, internalizing behavior was parent-rated and a clinical diagnostic work-up for depression or anxiety was not available in all these children. Third, cortical segmentation of the anterior temporal lobe in FreeSurfer was suboptimal in the anterior temporal regions, so the interpretation of findings in the anterior temporal should be done with caution.

Using empirically defined classes of behavior, we showed that internalizing problems in children aged six-to-ten years are related to a thinner cortex mostly in temporal but also in frontal regions of the brain. These findings suggest that structural differences observed in young children with internalizing problems are similar to structural abnormalities in the brain regions implicated in depression or anxiety in older populations. Longitudinal studies of young children with internalizing problems have shown that these children, even though not severely affected, are more likely to develop depression and anxiety in adolescence and adulthood and reach poor educational attainment (Riglin, Petrides et al. 2014). Brain structural differences associated with subthreshold depression or anxiety in childhood might help as novel targets for early detection and intervention.

REFERENCES

Achenbach, T. M., M. Y. Ivanova, L. A. Rescorla, L. V. Turner and R. R. Althoff (2016). "Internalizing/Externalizing Problems: Review and Recommendations for Clinical and Research Applications." Journal of the American Academy of Child & Adolescent Psychiatry.

Achenbach, T. M. and L. A. Rescorla (2000). Manual for ASEBA Preschool Forms & Profiles. Burlington, VT, University of Vermont, Research Center for Children, Youth, & Families.

Althoff, R. R., F. C. Verhulst, D. C. Rettew, J. J. Hudziak and J. van der Ende (2010). "Adult outcomes of childhood dysregulation: a 14-year follow-up study." J Am Acad Child Adolesc Psychiatry **49**(11): 1105-1116.

Basten, M., J. van der Ende, H. Tiemeier, R. R. Althoff, J. Rijlaarsdam, V. W. Jaddoe, A. Hofman, J. J. Hudziak, F. C. Verhulst and T. White (2014). "Nonverbal intelligence in young children with dysregulation: the Generation R Study." Eur Child Adolesc Psychiatry **23**(11): 1061-1070.

Basten, M. M., R. R. Althoff, H. Tiemeier, V. W. Jaddoe, A. Hofman, J. J. Hudziak, F. C. Verhulst and J. van der Ende (2013). "The dysregulation profile in young children: empirically defined classes in the Generation R study." J Am Acad Child Adolesc Psychiatry **52**(8): 841-850 e842.

Blanken, L. M., S. E. Mous, A. Ghassabian, R. L. Muetzel, N. K. Schoemaker, H. El Marroun, A. van der Lugt, V. W. Jaddoe, A. Hofman, F. C. Verhulst, H. Tiemeier and T. White (2015). "Cortical morphology in 6- to 10-year old children with autistic traits: a population-based neuroimaging study." Am J Psychiatry **172**(5): 479-486.

Blanken, L. M., T. White, S. E. Mous, M. Basten, R. L. Muetzel, V. Jaddoe, J. Van der Ende, F. Verhulst and H. Tiemeier (2016). "Cognitive functioning in children with internalising, externalising and dysregulation problems: a population-based study." European Child and Adolescent Psychiatry under review.

Burgess, N., E. A. Maguire and J. O'Keefe (2002). "The human hippocampus and spatial and episodic memory." Neuron **35**(4): 625-641.

Cavanna, A. E. and M. R. Trimble (2006). "The precuneus: a review of its functional anatomy and behavioural correlates." Brain **129**(Pt 3): 564-583.

Connell, A. M. and S. H. Goodman (2002). "The association between psychopathology in fathers versus mothers and children's internalizing and externalizing behavior problems: a meta-analysis." Psychol Bull **128**(5): 746-773.

De Bellis, M. D., B. J. Casey, R. E. Dahl, B. Birmaher, D. E. Williamson, K. M. Thomas, D. A. Axelson, K. Frustaci, A. M. Boring, J. Hall and N. D. Ryan (2000). "A pilot study of amygdala volumes in pediatric generalized anxiety disorder." Biol Psychiatry **48**(1): 51-57.

De Bellis, M. D., M. S. Keshavan, H. Shifflett, S. Iyengar, R. E. Dahl, D. A. Axelson, B. Birmaher, J. Hall, G. Moritz and N. D. Ryan (2002). "Superior temporal gyrus volumes in pediatric generalized anxiety disorder." Biol Psychiatry **51**(7): 553-562.

de Beurs, E. (2004). Brief Symptom Inventory, handleiding. Leiden, the Netherlands, PITS.

Desikan, R. S., F. Segonne, B. Fischl, B. T. Quinn, B. C. Dickerson, D. Blacker, R. L. Buckner, A. M. Dale, R. P. Maguire, B. T. Hyman, M. S. Albert and R. J. Killiany (2006). "An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest." Neuroimage **31**(3): 968-980.

Ducharme, S., M. D. Albaugh, J. J. Hudziak, K. N. Botteron, T. V. Nguyen, C. Truong, A. C. Evans and S. Karama (2014). "Anxious/depressed symptoms are linked to right ventromedial prefrontal cortical thickness maturation in healthy children and young adults." Cereb Cortex **24**(11): 2941-2950.

Fallucca, E., F. P. MacMaster, J. Haddad, P. Easter, R. Dick, G. May, J. A. Stanley, C. Rix and D. R. Rosenberg (2011). "Distinguishing between major depressive disorder and obsessive-compulsive disorder in children by measuring regional cortical thickness." Arch Gen Psychiatry **68**(5): 527-533.

Fischl, B. (2012). "FreeSurfer." Neuroimage **62**(2): 774-781.

Fischl, B. and A. M. Dale (2000). "Measuring the thickness of the human cerebral cortex from magnetic resonance images." Proc Natl Acad Sci U S A **97**(20): 11050-11055.

Fischl, B., M. I. Sereno and A. M. Dale (1999). "Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system." Neuroimage **9**(2): 195-207.

Fischl, B., M. I. Sereno, R. B. Tootell and A. M. Dale (1999). "High-resolution intersubject averaging and a coordinate system for the cortical surface." Hum Brain Mapp **8**(4): 272-284.

Jaddoe, V. W., C. M. van Duijn, O. H. Franco, A. J. van der Heijden, M. H. van Iizendoorn, J. C. de Jongste, A. van der Lugt, J. P. Mackenbach, H. A. Moll, H. Raat, F. Rivadeneira, E. A. Steegers, H. Tiemeier, A. G. Uitterlinden, F. C. Verhulst and A. Hofman (2012). "The Generation R Study: design and cohort update 2012." Eur J Epidemiol **27**(9): 739-756.

Koolschijn, P. C., I. M. H. van, M. J. Bakermans-Kranenburg and E. A. Crone (2013). "Hippocampal volume and internalizing behavior problems in adolescence." Eur Neuropsychopharmacol **23**(7): 622-628.

Marrus, N., A. Belden, T. Nishino, T. Handler, J. T. Ratnanather, M. Miller, D. Barch, J. Luby and K. Botteron (2015). "Ventromedial prefrontal cortex thinning in preschool-onset depression." J Affect Disord **180**: 79-86.

Oldfield, R. C. (1971). "The assessment and analysis of handedness: the Edinburgh inventory." Neuropsychologia **9**(1): 97-113.

Pizzagalli, D. A. (2011). "Frontocingulate dysfunction in depression: toward biomarkers of treatment response." Neuropsychopharmacology **36**(1): 183-206.

Price, J. L. and W. C. Drevets (2010). "Neurocircuitry of mood disorders." Neuropsychopharmacology **35**(1): 192-216.

Price, J. L. and W. C. Drevets (2012). "Neural circuits underlying the pathophysiology of mood disorders." Trends Cogn Sci **16**(1): 61-71.

Reuter, M., N. J. Schmansky, H. D. Rosas and B. Fischl (2012). "Within-subject template estimation for unbiased longitudinal image analysis." Neuroimage **61**(4): 1402-1418.

Riglin, L., K. V. Petrides, N. Frederickson and F. Rice (2014). "The relationship between emotional problems and subsequent school attainment: a meta-analysis." J Adolesc **37**(4): 335-346.

Roza, S. J., M. B. Hofstra, J. van der Ende and F. C. Verhulst (2003). "Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: a 14-year follow-up during childhood, adolescence, and young adulthood." Am J Psychiatry **160**(12): 2116-2121.

Schmaal, L., D. P. Hibar, P. G. Samann, G. B. Hall, B. T. Baune, N. Jahanshad, J. W. Cheung, T. G. van Erp, D. Bos, M. A. Ikram, M. W. Vernooij, W. J. Niessen, H. Tiemeier, A. Hofman, K. Wittfeld, H. J. Grabe, D. Janowitz, R. Bulow, M. Selonke, H. Volzke, D. Grotegerd, U. Dannlowski, V. Arolt, N. Opel, W. Heindel, H. Kugel, D. Hoehn, M. Czisch, B. Couvy-Duchesne, M. E. Renteria, L. T. Strike, M. J. Wright, N. T. Mills, G. I. de Zubicaray, K. L. McMahon, S. E. Medland, N. G. Martin, N. A. Gillespie, R. Goya-Maldonado, O. Gruber, B. Kramer, S. N. Hatton, J. Lagopoulos, I. B. Hickie, T. Frodl, A. Carballedo, E. M. Frey, L. S. van Velzen, B. W. Penninx, M. J. van Tol, N. J. van der Wee, C. G. Davey, B. J. Harrison, B. Mwangi, B. Cao, J. C. Soares, I. M. Veer, H. Walter, D. Schoepf, B. Zurowski, C. Konrad, E. Schramm, C. Normann, K. Schnell, M. D. Sacchet, I. H. Gotlib, G. M. MacQueen, B. R. Godlewska, T. Nickson, A. M. McIntosh, M. Papmeyer, H. C. Whalley, J. Hall, J. E. Sussmann, M. Li, M. Walter, L. Aftanas, I. Brack, N. A. Bokhan, P. M. Thompson and D. J. Veltman (2016). "Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group." Mol Psychiatry.

Shad, M. U., S. Muddasani and U. Rao (2012). "Gray Matter Differences Between Healthy and Depressed Adolescents: A Voxel-Based Morphometry Study." Journal of Child and Adolescent Psychopharmacology **22**(3): 190-197.

Sowell, E. R., B. S. Peterson, E. Kan, R. P. Woods, J. Yoshii, R. Bansal, D. Xu, H. Zhu, P. M. Thompson and A. W. Toga (2007). "Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age." Cereb Cortex **17**(7): 1550-1560.

Strawn, J. R., L. Hamm, D. A. Fitzgerald, K. D. Fitzgerald, C. S. Monk and K. L. Phan (2015). "Neurostructural abnormalities in pediatric anxiety disorders." J Anxiety Disord **32**: 81-88.

Tellegen, P., B. Wijnberg-Williams and J. Laros (2005). Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2.5- 7/. Amsterdam, Boom Testuitgevers.

Tick, N. T., J. van der Ende, H. M. Koot and F. C. Verhulst (2007). "14-year changes in emotional and behavioral problems of very young Dutch children." J Am Acad Child Adolesc Psychiatry **46**(10): 1333-1340.

van der Plas, E. A. A., A. D. Boes, J. A. Wemmie, D. Tranel and P. Nopoulos (2010). "Amygdala volume correlates positively with fearfulness in normal healthy girls." Social Cognitive and Affective Neuroscience **5**(4): 424-431.

Videbech, P. and B. Ravnkilde (2015). "Hippocampal volume and depression: a meta-analysis of MRI studies." American Journal of Psychiatry.

Vulser, H., H. Lemaitre, E. Artiges, R. Miranda, J. Penttila, M. Struve, T. Fadai, V. Kappel, Y. Grimmer, R. Goodman, A. Stringaris, L. Poustka, P. Conrod, V. Frouin, T. Banaschewski, G. J. Barker, A. L. Bokde, U. Bromberg, C. Buchel, H. Flor, J. Gallinat, H. Garavan, P. Gowland, A. Heinz, B. Ittermann, C. Lawrence, E. Loth, K. Mann, F. Nees, T. Paus, Z. Pausova, M. Rietschel, T. W. Robbins, M. N. Smolka, G. Schumann, J. L. Martinot and M. L. Paillere-Martinot (2015). "Subthreshold depression and regional brain volumes in young community adolescents." J Am Acad Child Adolesc Psychiatry **54**(10): 832-840.

White, T., H. El Marroun, I. Nijs, M. Schmidt, A. van der Lugt, P. A. Wielopolki, V. W. Jaddoe, A. Hofman, G. P. Krestin, H. Tiemeier and F. C. Verhulst (2013). "Pediatric population-based neuroimaging and the Generation R Study: the intersection of developmental neuroscience and epidemiology." Eur J Epidemiol **28**(1): 99-111.

Yap, M. B., S. Whittle, M. Yucel, L. Sheeber, C. Pantelis, J. G. Simmons and N. B. Allen (2008). "Interaction of parenting experiences and brain structure in the prediction of depressive symptoms in adolescents." Arch Gen Psychiatry **65**(12): 1377-1385.

Zahn-Waxler, C., B. Klimes-Dougan and M. J. Slattery (2000). "Internalizing problems of childhood and adolescence: prospects, pitfalls, and progress in understanding the development of anxiety and depression." Dev Psychopathol **12**(3): 443-466.

Supplement Table 1 Brain morphology and Internalizing Broadband scores at age 6 years (n=801).

Internalizing broadband score	**Model 1**	**Model 2**	**Model 3**	**Model 4**
Brain morphology	**B (95%CI)** **P value**	**B (95%CI)** **P value**	**B (95%CI)** **P value**	**B (95%CI)** **P value**
Total Brain Volume, cm^3	-10.0 (-15.9,-4.2) 0.001	-5.6 (-11.8, 0.7) 0.08	5.3 (-3.1, 13.8) 0.22	
Total Gray Volume, cm^3	-6.7 (-10.5,-3.0) <0.001	-3.3 (-7.3, 0.7) 0.11	3.8 (-1.7, 9.2) 0.17	
Cortical Gray Volume, cm^3	-5.7 (-9.0,-2.4) 0.001	-2.9 (-6.4, 0.7) 0.11	3.3 (-1.5, 8.1) 0.18	
Cerebral White Matter Volume, cm^3	-2.9 (-5.1,-0.7) 0.01	-2.0 (-4.4, 0.4) 0.11	1.4 (-1.8, 4.7) 0.39	
Subcortical volumes				
Right amygdala volume, mm^3	-11.2 (-25.4, 2.9) 0.12	-12.4 (-28.1, 3.2) 0.12	15.0 (-6.0, 36.0) 0.16	11.2 (-8.6, 31.0) 0.27
Left amygdala volume, mm^3	-14.0 (-26.5,-1.4) 0.03	-11.6 (-25.3, 2.1) 0.10	-9.6 (-23.9, 4.8) 0.19	3.1 (-13.9, 20.0) 0.72
Right hippocampus, mm^3	-14.9 (-37.1, 7.3) 0.19	-6.2 (-30.4, 18.0) 0.62	-18.4 (-14.6, 51.4) 0.27	9.3 (-19.7, 38.2) 0.53
Left hippocampus, mm^3	-21.2 (-43.4, 1.1) 0.06	-10.3 (-34.4, 13.8) 0.40	17.1 (-15.9, 50.2) 0.31	4.6 (-24.1, 33.2) 0.76

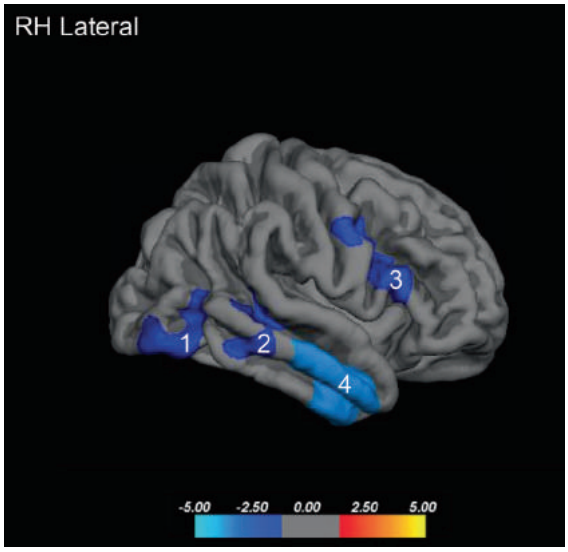
Model 1 adjusted for sex and age at scanning
Model 2 additionally adjusted for maternal age, education, history of smoking and drinking in pregnancy, maternal history of psychopathology in pregnancy, and child age at behavioral assessment, ethnicity, intelligence, and handedness
Model 3 additionally adjusted Child Behavior Checklist/1½–5 Externalizing Broadband Scale score
Model 4 additionally adjusted for total brain volume
Internalizing Broadband scores were defined using the Child Behavior Checklist/1½–5

SUPPLEMENT

Image processing

Image quality assurance was performed in two steps. The first step was a visual inspection of the image quality of the T1 sequence prior to preprocessing the data. All images were rated on a six-point scale (unusable to excellent). The next step of quality assurance took place after the images were processed through the FreeSurfer pipeline (http://surfer.nmr.mgh.harvard.edu/) and consisted of a visual inspection of the segmentation quality of the data. All images were rated on a six-point scale (not constructed to excellent). The T1 data that were rated as unusable or poor were not used nor were the data from the children whose FreeSurfer output was not constructed or were rated as poor for both hemispheres. In addition, amygdala and hippocampal segmentation quality were rated as usable or unusable. Scans with unusable hippocampal or amygdala segmentation quality (n=58) were excluded from the amygdala and hippocampal analyses only.

Supplementary Figure 1 Cortical thickness and Internalizing Problems at 6 years, covarying for externalizing scores



Clusters:

1) Lateral occipital

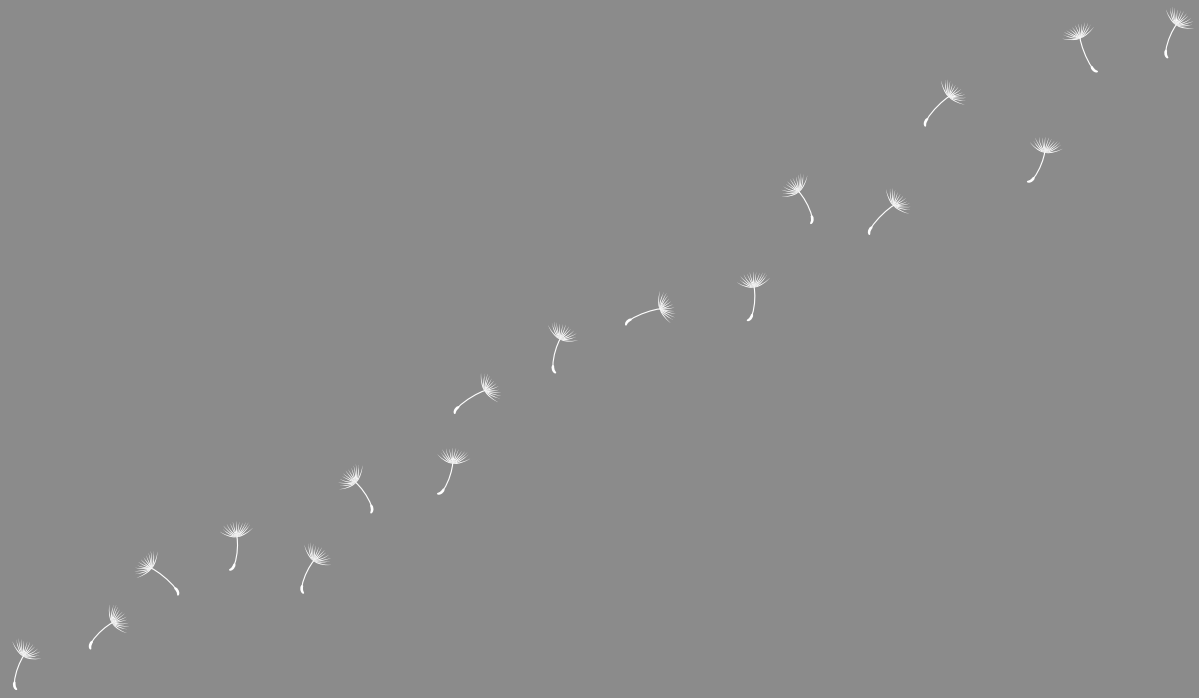
2) Temporal

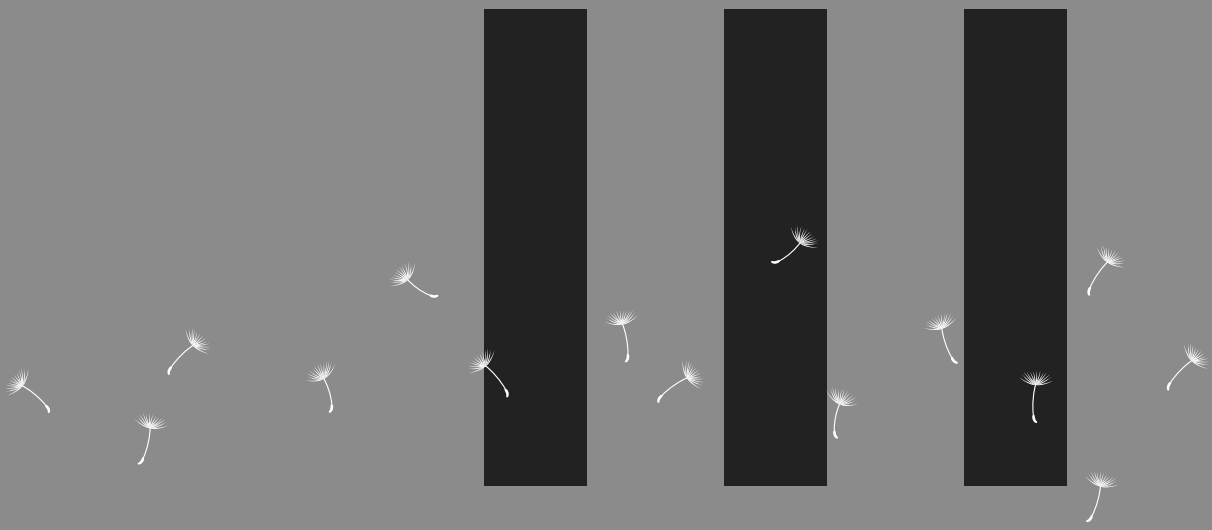
3) Pars opercularis/triangularis

4) Anterior Temporal

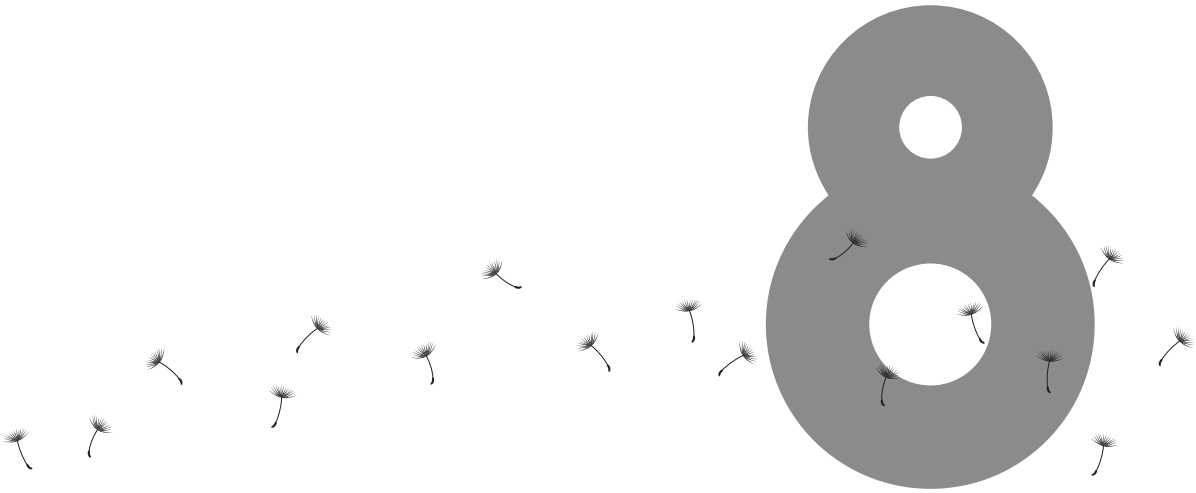
RH: Right Hemisphere

Internalizing problems were defined using a latent profile analysis performed on all Child Behavior Checklist/1½–5 syndrome scales[13]





Part III



General discussion

RATIONALE

Phenotypic studies point to a dimensional structure of child psychiatry (Insel, Cuthbert, Garvey, Heinssen, Pine et al. 2010). However, neuroimaging studies are still primarily designed as case-control studies. In this thesis, we explored the neurobiology of various dimensions of child psychopathology, primarily focusing on autistic traits. In addition, we studied internalizing and externalizing symptoms in children. Instead of using the traditional case-control framework, we used alternative approaches to define the phenotypes of interest. In the context of ASD, we utilized the fact that the social impairment in autism can be conceptualized as part of a continuum, at which ASD represents the severe end. Further, we studied internalizing and externalizing symptoms in children, using both continuous and categorical approaches. Here, we will describe the main findings of this thesis and discuss them in the context of the most recent literature. Moreover, we will address some methodological considerations that are of interest for this field of research. Finally, we will discuss the implications for future research and clinical practice.

Social responsiveness on a continuum as endophenotypes of ASD

Unaffected relatives of people with ASD often show milder expressions of the same traits. Observations of this phenomenon date back to Kanner's first case descriptions, where he observed that that some parents of children with autistic impairment were 'late talkers' and seemed 'uninterested in people' (Kanner 1968). The existence of a spectrum for ASD was initially studied by assessing the severity distribution within affected individuals. Later, the observation that there are people that do not quite meet criteria for a DSM-disorder, but do have traits, broadened that spectrum to the so called 'Broader Autism Phenotype' (Sucksmith, Roth and Hoekstra 2011). Finally, the spectrum was extended even further by the observation that people in the general population showed traits as well (Constantino 2011). Instruments were developed to measure traits of ASD, including the Social Responsiveness Scale (Constantino 2002) and the Autism Quotient (Baron-Cohen, Wheelwright, Skinner, Martin and Clubley 2001) and it was found that these traits are continuously distributed in the population. Such scales, representing the full spectrum of subclinical to severe traits can be used an endophenotype of ASD (Gottesman and Gould 2003). Endophenotypes are "components" of psychiatric disorders that can be helpful in the identification of the underlying neurobiology. Initially, they were mostly used in the context of genetics. However, this approach can be applied in neuroimaging as well. The use of continuous trait phenotypes in the general population is especially beneficial, as they increase the power of the analyses and findings can be more broadly extrapolated. In terms of symptom severity, the majority of people in the general population with such traits clearly do not meet criteria for a clinical diagnosis of ASD. However, their symptoms can still be disabling, can hamper the building of

stable relationships, and affect their ability to function in school or in a professional career (Constantino 2011).

Endophenotypes in the broader sense can be behavioral, cognitive, neurobiological or genetic (Sucksmith et al. 2011). It is important to comment here that there are many potential substrates that could serve as dimensional measures of ASD in the context of neuroimaging research and that different endophenotypes could lead to identification of different neurobiological markers. However, in this thesis, we consistently focused on the social dimension of autistic traits, which is thought to be the central feature of impairment.

In this thesis, against the background of the continuous nature of ASD, we assessed whether the brain correlates of ASD are present on a continuum as well. Symptoms of ASD are thought to be associated with altered development of the brain, in terms of structural development and growth, but also in functional development. Appreciating that traits of ASD form a severity continuum, it is actually quite intuitive to assume that the underlying brain characteristics are not just present in the most severely affected individuals but may form a continuum in the population as well. We addressed this question tapping into several stages of brain development, investigating various different characteristics of the brain.

The neurobiology of autistic traits throughout the lifespan

Symptoms of ASD become apparent early in life, and although symptoms are not always recognized immediately, retrospective developmental histories of patients usually point to symptoms appearing in the first year of life (Ozonoff, Heung, Byrd, Hansen and Hertz-Picciotto 2008). There is some evidence that environmental exposures during pregnancy, such as prenatal infections and medication use of the mother contribute to the risk of ASD (Brown 2012; El Marroun, White, van der Knaap, Homberg, Fernandez et al. 2014). A seminal postmortem study in children diagnosed with ASD suggested abnormal development of cortical architecture (Stoner, Chow, Boyle, Sunkin, Mouton et al. 2014). Interestingly, histopathological studies point to increased expression of ASD candidate genes in the fetal cortex, although the role of these genes in prenatal brain growth has not been established yet (Willsey, Sanders, Li, Dong, Tebbenkamp et al. 2013; Birnbaum, Jaffe, Hyde, Kleinman and Weinberger 2014). Taken together, this points to a crucial role of the prenatal period, as the brain differentiates from the ectoderm early in gestation to form a vastly complex organ by birth (Andersen 2003). In utero, the size of the skull, that can be assessed during routine ultrasound measurements, provides a good measure of brain size (Cooke, Lucas, Yudkin and Pryse-Davies 1977). In chapter 3, we evaluated prenatal trajectories of head growth in relation to later autistic symptoms. We found that children with slower prenatal head growth showed more traits of ASD later in life. This is supportive of the notion that prenatal brain growth differences may be involved in the neurobiology of ASD. However, we did not see this same pattern in children with clinically diagnosed ASD. Speculatively, the heterogeneity

in etiologies and resulting growth abnormalities could be greater at the severe end of the autistic trait spectrum, and may, as a result, not form a continuous extension of the association observed in relation to milder traits. Of note, both microcephaly and macrocephaly have been implicated in ASD (Fombonne, Roge, Claverie, Courty and Fremolle 1999). Perhaps, only a subset of etiologic pathways to clinical ASD involve macroscopic prenatal brain growth changes. Likewise, while many children with ASD do not show differences in head circumference at birth, it is thought that only a subset of children may show abnormally large head size (Green, Loesch and Dissanayake 2015). It is possible that such pathways are less involved in autistic traits in the general population.

Postnatal brain characteristics: structure and function

Postnatal development of the cortex is a dynamic process of several phases. There are different aspects of the cortex, each originating from different stages in neurodevelopment and regulated by at least partly independent genetic mechanisms (Shaw, Kabani, Lerch, Eckstrand, Lenroot et al. 2008; Panizzon, Fennema-Notestine, Eyler, Jernigan, Prom-Wormley et al. 2009; Raznahan, Shaw, Lalonde, Stockman, Wallace et al. 2011). Cortical thickness is defined as the area between the white matter surface and the gray matter surface and is likely related to the number of neurons and neuronal density (Herculano-Houzel, Watson and Paxinos 2013). Surface-based methods have enabled us to study this measure quite precisely at a large number of locations in the cortex (Fischl and Dale 2000). Development of cortical thickness has traditionally been conceptualized as an inverted-U shaped process, with initial expansive growth in early childhood, up to a local maximum, after which the cortex becomes thinner through a functionally selective process of "pruning" (Giedd, Blumenthal, Jeffries, Castellanos, Liu et al. 1999). However, more recently, it has been suggested that cortical thickness generally follows a simple linear decline in most cortical areas by age 5, and all areas by age 8 (Ducharme, Albaugh, Nguyen, Hudziak, Mateos-Perez et al. 2016). Altered development of the cortex is implied in a variety of neuropsychiatric disorders, including ADHD and ASD (Wallace, Dankner, Kenworthy, Giedd and Martin 2010; Shaw, Malek, Watson, Sharp, Evans et al. 2012). In chapter 2, we found that boys with more autistic traits had thicker cortex in the pericalcarine area, indicating gender specificity in morphological differences in ASD, consistent with observations in adults (Lai, Lombardo, Suckling, Ruigrok, Chakrabarti et al. 2013). Speculatively, thicker cortex in this age range may point to a developmental delay.

One other aspect of cortical morphology is gyrification, or the folding of the brain into a complex pattern of sulci and gyri. This is a poorly understood phenomenon that is thought to accommodate efficient neural processing in the brain (Van Essen 1997). The majority of gyrification takes place in the third trimester of pregnancy, at a time when the brain undergoes prolific growth. However, the many primary sulci are formed before that, between 20 and 28 weeks of gestational age (Habas, Scott, Roosta, Rajagopalan, Kim et al. 2012) and

gyrification changes in postnatal life as well (White, Su, Schmidt, Kao and Sapiro 2010). In this thesis, we found widespread reductions in gyrification to be associated with autistic traits and we found a trend toward the same effect in a small subset of children that met criteria for ASD according to the golden standard instruments. This association held in a subset of regions when we excluded the most severely affected children, indicating a true continuum in the neurobiology. Our finding of reduced gyrification is in line with some studies of young children with ASD (Schaer, Ottet, Scariati, Dukes, Franchini et al. 2013; Bos, Merchan-Naranjo, Martinez, Pina-Camacho, Balsa et al. 2015). However, there have also been studies that found the opposite (Kates, Ikuta and Burnette 2009; Wallace, Robustelli, Dankner, Kenworthy, Giedd et al. 2013). This discrepancy can partly be understood from differences in methodology, as well as gender, age and IQ of the study sample. However, a recent clinical study in children of a similar age range, using the same methodology reported the opposite finding in children with ASD (Yang, Beam, Pelphrey, Abdullahi and Jou 2016). Considering this heterogeneity, we cannot be sure of the nature of gyrification differences in young children with ASD.

One of the theories of gyrification suggests that it reflects connection strength between spatially separated regions of the brain (Van Essen 1997), and two previous studies that combined gyrification and white matter measurements revealed white matter disruptions alongside reduced gyrification in children with ASD (Schaer et al. 2013; Bos et al. 2015). Based on our previous findings of reduced gyrification, we hypothesized a continuum in white matter microstructure related to autistic traits, so that white matter differences related to autistic traits would mimic those most commonly reported in children with ASD. Studies of children with ASD show alterations in white matter microstructure, most notably in tracts that facilitate long-range connections: corpus callosum, the left uncinated fasciculus, and the left superior longitudinal fasciculus (Aoki, Abe, Nippashi and Yamasue 2013). We detected a localized association between autistic traits and lower white matter integrity in a small region in the superior longitudinal fasciculus. In this long-range white matter tract, we found a continuous association with autistic traits, that remained after excluding children with ASD. However, we did not find major differences in all of the hypothesized long-range white matter connections in these children. White matter integrity in some of these tracts may not relate to this quantitative measure of social impairment, thus disruptions may occur more exclusively at the severe end of the spectrum. Alternatively, associations may only be revealed in samples where children generally have more severe symptoms.

Our findings in functional connectivity offer more support for the conceptualization of ASD as a 'disconnection syndrome', where symptoms are thought to arise from disturbances in neural circuitry (Geschwind and Levitt 2007). Using a novel type of resting state fMRI analysis, where assumptions of stationarity of functional connectivity over time were relaxed, we found that children with more autistic traits showed alterations in these dynamic connectivity characteristics. Specifically, they spent less time in the more heavily connected,

adult-like states. These features were present on a continuum in relation to autistic traits. The association remained when the most severely affected children were excluded, and it was also observed in a subgroup of children with clinically confirmed ASD.

Does the neurobiology of ASD fall on a continuum?

In this thesis, we studied whether different aspects of the neurobiology of ASD can be described in a continuous framework. We found that some aspects of the neurobiology fall onto a continuum, while others may not. We found evidence for a continuum in gyrification. However, this was less evident in white matter differences and prenatal head growth. Importantly, autistic traits and ASD affect the brain at many levels. Some brain differences related to autistic traits may form a dose-response-type relation, such that the most severely affected patients have the most marked differences, while other characteristics may not exhibit such associations. The brain differences that form a continuum can be identified through studying continuous trait phenotypes in the general population. Some brain differences identified in the general population are mimicked in the affected patient group (Segovia, Holt, Spencer, Gorriz, Ramirez et al. 2014). However, that is not always the case for the various correlates of the Social Responsiveness Scale that we identified throughout this thesis. One potential reason is that previous clinical studies, as well as the nested case-control portions of the studies described in this thesis, were not powered to study such differences. However, there are now also clinical studies of substantial sample size, that may be better equipped to detect neurobiological associations than our population-based study. It is important to consider that, while continuous analyses are powerful, an important portion of power depends on the severity of symptoms at the extreme end of the distribution. The absence of certain hypothesized findings in our population-based sample can be reflective of the fact that subjects typically show milder impairment, and subtle effects may thus not be detected. While clinical studies are usually much smaller, symptoms of patients are more severe. In a true continuous association between traits and neurobiology, the variation of the modestly affected group adds significantly to the association. In a continuously assessed phenotype, for example a phenotype quantified with the SRS, lower scores may lack precision. Although higher scores clearly point to the autistic phenotype, these moderate scores may be somewhat less specific for ASD and thus less powerful to reveal ASD-specific associations. In addition, it is crucial to acknowledge that the spectrum of social impairment is only one of several potential phenotypes and that the absence of an association does not rule out that a continuous brain-behavior relation exists. Other phenotypes that focus on different quantitative aspects of the phenotype, may also be of use in the identification of a continuum in neurobiological characteristics. Alternatively, a subset of the brain differences observed in children with ASD may only occur in the most severely affected children. Some brain abnormalities may be specific to a distinct

genetic or environmental pathway. Other patterns may be cumulative and related to chronicity of ASD-like symptoms. While early abnormalities in brain development may initially give rise to autistic symptoms, it is likely that in the case of persistent patterns of autistic behavior, the subsequent interaction of a child with their environment also shapes the brain.

As mentioned, some of our findings are inconsistent with the existing literature in ASD. However, here it is important to note that the clinical literature itself is very inconsistent. Heterogeneity is a central feature of the neurobiology of ASD and it has complicated research in this area. As is, most "consistent" neurobiological correlates of ASD are surrounded by controversy. So-called 'hallmark findings' of ASD are usually not widely replicated, for instance the 'early brain overgrowth hypothesis', the 'amygdala theory', or even the 'disconnectivity hypothesis' are not consistently replicated. Throughout the neurobiological literature of ASD, there are inconsistencies in findings of ASD. In the presence of many small studies, the meaning of a "consistent" finding is somewhat inflated: almost any finding is supported by another study that reported a similar difference in the same structure. However, the number of refuting studies is often much larger. It is unclear whether all these inconsistent findings should be understood from differences in age, symptom severity or intelligence of the participants, or differences in methodology. While all of these factors undoubtedly play a role, there are likely also many different etiologies, so that many of these inconsistent findings can coexist: ASD can be characterized by increased or decreased gyrification, by hyper- or hypo-connectivity, by larger or smaller brains. In the following section, we will address different sources of such heterogeneity and discuss how they contribute to inconsistent findings in neurobiological research in ASD. We will cover the underpinning genetics, the phenotypic heterogeneity and the issue of comorbidity.

Genetics of ASD

ASD is a highly heritable disorder, with heritability estimates between 60 and 90% (Ronald, Happe, Price, Baron-Cohen and Plomin 2006; Colvert, Tick, McEwen, Stewart, Curran et al. 2015), although environmental factors are also involved (Mandy and Lai 2016). It has long been noted that ASD runs in families, and siblings or other family members of children with ASD often have traits of the disorder as well, even if they do not meet criteria for the disorder (Constantino, Lajonchere, Lutz, Gray, Abbacchi et al. 2006). The genetic background of ASD has long been a topic of interest and a multitude of genetic variants have been associated with ASD (Betancur 2011). Traditionally, a distinction has been made between families with only one case of ASD, so-called 'simplex cases' or 'multiplex families', in which more people are affected. It is assumed that rare variants with larger structural changes, including so-called 'copy number variants' (CNVs) play a role (Sebat, Lakshmi, Malhotra, Troge, Lese-Martin et al. 2007; Sanders, Murtha, Gupta, Murdoch, Raubeson et al. 2012), while there is also evidence that additive risk load of multiple common variants (SNPs) can be involved (Robinson, St

Pourcain, Anttila, Kosmicki, Bulik-Sullivan et al. 2016). It is estimated that around 10-20% of cases with ASD are so-called "syndromic cases", where a known single-gene mutation can be established (Folstein and Rosen-Sheidley 2001; Abrahams and Geschwind 2008). While the genetics of ASD are very complicated and not very well understood yet, some genetic findings lend support to the framework of ASD as a continuously distributed trait in the general population.

According to the so-called quantitative locus theory, ASD may be polygenic, and there are many different genes, each with a very modest effect size, that influence the entire range of variation in the autistic phenotype (Plomin, Haworth and Davis 2009). Within this framework, it is hypothesized that risk genes for ASD also give rise to autistic traits. There is a number of studies that found evidence for this, either by using heritability estimates (Ronald, Happe, Bolton, Butcher, Price et al. 2006; Robinson, Koenen, McCormick, Munir, Hallett et al. 2011; Lundstrom, Chang, Rastam, Gillberg, Larsson et al. 2012) or specific genetic ASD risk variants (St Pourcain, Wang, Glessner, Golding, Steer et al. 2010). Taken together, this work provides evidence for a related genetic etiology of autistic traits at the extreme end and in the rest of the population. In other words, genetic factors that underlie individual differences in autistic traits show considerable overlap with genetic influences on clinically diagnosed ASD (Constantino 2011). Recently, this body of evidence was strengthened by an important novel study by Robinson and colleagues that involved several large ASD consortia and population-based samples. They found that both inherited and *de novo* variants for ASD influence a continuum of autistic traits, as well as other behavioral and developmental traits. At the severe tail of this continuum, there are people with either ASD or other psychiatric disorders, such as schizophrenia (Robinson et al. 2016).

Importantly, the notions of rare structural changes and quantitative risk scores are not mutually exclusive. It is thought that most of the genetic risk factors for ASD are not rare, but common variants, that can be found in many unaffected people as well (Gaugler, Klei, Sanders, Bodea, Goldberg et al. 2014). Quite strikingly, there are several studies that show that phenotypic penetrance of chromosomal deletions, traditionally assumed to operate through a classical "hit-or-miss" mechanism, are in fact modulated by the background genetic ASD risk load (Hanson, Bernier, Porche, Jackson, Goin-Kochel et al. 2015; Moreno-De-Luca, Evans, Boomer, Hanson, Bernier et al. 2015).

It is likely that genetic aspects play an important role in the heterogeneity of neurobiological findings in ASD. There is a large group of genetic pathways that all lead to a globally overlapping behavioral phenotype, although the levels at which the brain is affected could differ profoundly (Kendler 2013). Since there are different forms of genetic liability for ASD, that probably operate at least partly in distinct ways in the brain, it is unlikely that they together point to one underlying mechanism, or that there is even such a unifying mechanism at all (Kendler 2013). While this is probably true to some degree in almost all

diseases, according to the unique disease principle (Ogino, Lochhead, Chan, Nishihara, Cho et al. 2013), it is probably even more true in ASD. ASD is especially heterogeneous in its genetic background, likely forming a mixture of polygenic and monogenic causes, both common and very rare. To some extent, these diverse genetic backgrounds may converge in overlapping brain endophenotypes, while some other pathways may be completely distinct. The brain networks underlying social functioning are so complex that a plethora of disruptions, caused by different genes and acting on different levels, could likely lead to ASD-like phenotypes. Small neuroimaging studies of patients with ASD are difficult to compare, as they likely sample these different genetic causes of ASD in various proportions. This mixture of pathways could partly underlie the inconsistency in results between such neuroimaging studies, through a phenomenon called 'molecular confounding' (Nishihara, VanderWeele, Shibuya, Mittleman, Wang et al. 2015), or bias caused by unmeasured subtypes of the disease. In many cases, this cannot be tested, as the specific genetic background of ASD is often unknown on the individual level. However, some studies of brain development have considered genetic background. For example, in the case of brain growth in the first few postnatal years, it has been shown that the growth patterns differ between children from simplex and multiplex families: a positive association of autistic symptoms with HC was found only in children with ASD classified as simplex and not in children with ASD from multiplex families (Davis, Keeney, Sikela and Hepburn 2013). Similarly, another study showed that children with autistic traits in the context of Klinefelter syndrome show different localizations of white matter abnormalities compared to children with idiopathic ASD (Goddard, van Rijn, Rombouts and Swaab 2015). Ultimately however, it is highly unlikely that studies of ASD will ever be fully stratified by distinct and homogeneous genetic background, considering the sheer multitude of variants and genetic interactions that can cause ASD in an individual. Clinical studies of sufficiently large sample size, while undoubtedly comprising different pathways to ASD, can still be useful in identifying common brain pathways.

Phenotypical heterogeneity

In the previous section, we explored the genetic heterogeneity of ASD. However, there is also clear heterogeneity on the phenotypic level. For instance, children with ASD can show normal intelligence, but ASD also frequently occurs in combination with intellectual disability. It is rather intuitive to assume that this phenotypic heterogeneity could also be an obstacle to finding one underlying neurobiology, as children with different characteristics may show distinct differences in their brains. A way to deal with this problem involves the identification of phenotypical subtypes with similar clinical characteristics. Indeed, many attempts have been made at this. Most notably, the DSM-IV included a sub-classification that included "Asperger syndrome", a group of individuals with good language ability and normal to above-normal IQ, despite initial developmental delays. However, after intensive

study, it has proven difficult to make a meaningful and reproducible distinction between Asperger syndrome and high-functioning autism (Witwer and Lecavalier 2008). And even while some classifications may seem meaningful on the behavioral level, they do not always lead to better results in neurobiological research. Notably, a genetic study performed in the Simons Simplex Collection sample showed that reducing phenotypic heterogeneity, based on IQ and symptom profiles, did not have much impact on genetic homogeneity and did not aid the discovery of genetic risk variants of ASD (Chaste, Klei, Sanders, Hus, Murtha et al. 2015). Of note, identification of more homogeneous sub-classifications may also refer to symptom dimensions within a continuously defined phenotype. A recent study pointed to an intrinsic latent structure of continuous measures of autistic traits, comprising a systemizing and an empathizing factor (Grove, Baillie, Allison, Baron-Cohen and Hoekstra 2013). It would be worthwhile to assess whether the distinction of such continuous factors contributes to the neurobiological understanding of ASD.

Another source of behavioral heterogeneity is presented by comorbidity. This alludes to the concept of symptoms or diagnoses that are co-occurring with the symptom dimension of interest. The Social Responsiveness Scale has been criticized for showing associations with other types of behavioral symptoms, such as aggression or internalizing problems (Hus, Bishop, Gotham, Huerta and Lord 2013). These can be interpreted as potential confounding factors, suggesting that any associations with the Social Responsiveness Scale in fact involve 'non-ASD-specific factors'. A way to deal with that would be to statistically adjust for other symptom scores. However, since comorbidity is often indicative of severity, this would potentially adjust for true autistic trait variation of interest (Constantino and Frazier 2013). In fact, the term 'comorbidity' may be somewhat misleading as it suggests that different conditions or symptoms are etiologically distinct entities, while they may in fact show considerable overlap and represent different aspects of the same phenotype (Lilienfeld, Sauvigne, Lynn, Cautin, Latzman et al. 2015). If ASD symptoms are indeed closely related to other, less specific impairments, then controlling for such symptoms results in an artificial, non-life-like phenotype. This phenomenon is reminiscent of the discussion of adjusting for general intelligence as a confounder in the relation between early psychopathology and cognitive functioning. As discussed in chapter 6, this should probably only be done to assess more *specific* relations between psychopathology and cognitive subdomains. In the context of developmental psychopathology, it is often unclear what came first: the cognitive impairments or the behavioral symptoms. In fact, they may well belong to a common underlying etiology. As such, general intelligence should not be a default covariate in the relation between developmental psychopathology and cognition. However, it should be noted that IQ can influence associations between symptoms of autism and the brain. Highly intelligent people with ASD likely have more resources to compensate their social difficulties. This may lead to more beneficial patterns of interaction, while subjects with low cognitive

ability may be even more susceptible to overall impaired functioning as a result of their social impairment. Such differences can ultimately shape the brain as well.

OTHER METHODOLOGICAL CONSIDERATIONS

Autistic traits: what exactly are we measuring?

Using continuous measures of social responsiveness to study the neurobiology of ASD is not without controversy. Some have suggested that dimensions of social capacity in the general population may not represent exactly the same construct as social impairment in ASD (de la Marche, Noens and Steyaert 2015). The Social Responsiveness Scale is a sensitive instrument, but children with a different developmental delay or lower general intelligence tend to have more problematic scores as well (Bolte, Poustka and Constantino 2008). Another potential critique points to the influence of the reporter. In our studies, we used parent-reported measures of autistic traits. Alternatives include self-report or school-report. Importantly, the children we studied were likely too young to reliably and validly report on their own social skills. However, more precise measurement from ASD, especially in the fine-grained differences of continuous traits, could potentially have been obtained by using multiple informants. Throughout this thesis, we have primarily used parental (in most cases maternal) report of autistic symptoms. However, this may not give the entire picture. Since we know that many parents of children with autistic traits have similar traits (Lyall, Constantino, Weisskopf, Roberts, Ascherio et al. 2014), they may not have the same capacity in reflecting on and describing the intricate nuances of social behavior in their child. One of the settings that we have not included is the school. As school is a high-demand social environment, particular aspects of autistic traits may be more obvious or impairing at school than at home. Future studies should ideally also include teacher report of autistic symptoms. Teachers observe how the child interacts with other children and they have the opportunity to compare the behavior of the child with that of many other similarly aged children, which may allow them to better distinguish between typical and atypical behavior (Constantino, Lavesser, Zhang, Abbacchi, Gray et al. 2007; Duvekot, van der Ende, Verhulst and Greaves-Lord 2015). In the study of prenatal brain growth, a self-report measure of autistic traits was included, obtained in the Raine Study when subjects were around age 20. Reflecting on one's own behavior is a nontrivial task, probably even more so for those who have autistic traits, as poor self-reflection is described as one of the characteristics. This could lead to people rating their own behavior as more appropriate than it might really be. However, to avoid this, the AQ includes many questions that ask about the person's preferences, rather than only asking them to judge their own behavior (Baron-Cohen et al. 2001). It is likely that subjects with autistic traits are able to report reliably on such items. Still, self-reported scores carry the risk of underestimating the true quantity of autistic traits.

CLINICAL IMPLICATIONS

In this thesis, we studied the neurobiology of alternative conceptualizations of child psychiatry, beyond the traditional DSM-framework. Traditional dichotomous categorizations do not do justice to intermediate phenotypes. Imposing a cut-off always leads to the exclusion of a group of sub-threshold-level affected children and may lead to misclassification. However, the clinician, in order to make clinical decisions, often needs a categorical answer. Perversely, the financial support for any psychiatric treatment may depend on it (Coghill and Sonuga-Barke 2012). To the patient, the doctor and to society, the notion of a continuous structure of autistic impairment comes with conceptual consequences. Kendell and Jablensky argued that diagnostic categories are valid only if they can be viewed as truly discrete entities with natural boundaries (Kendell and Jablensky 2003). With the phenotypical and neurobiological boundaries of autism blurring, the question arises whether this is the case for ASD, and whether we can define a meaningful cut-off. A commonly heard phrase nowadays describes people as "on the spectrum". However, in the context of a truly continuous distribution, it becomes hard to define where this "spectrum" begins. Importantly, categorical and dimensional approaches do not have to be mutually exclusive, as inclusive approaches can take the best of both worlds. For instance, a recent study showed the existence of meaningful sub-dimensions, within a continuously defined autistic phenotype (Grove et al. 2013). Person-centered statistical identification of latent subgroups, where children are categorized based on their overall, dimensional pattern of problem scores rather than severity of symptoms in a single domain, can be useful method for neuroscientific research, as we showed in chapter 6 and 7. In the context of phenotypic heterogeneity, it may facilitate identification of more specific correlates. However, it cannot be translated to clinical practice, as the application of these methods requires statistical manipulation of data that is not feasible in clinical settings. To the clinician, child psychiatry will always have to be dichotomous to some extent, as many treatment decisions require a dichotomous answer. Severity cut-offs present the most practical solution to obtain that. Accordingly, Kendell and Jablensky suggest that diagnoses that do technically not have "validity" can still have "utility". However, clinicians can also benefit from embracing a continuous approach in child psychiatry, in better understanding the full complexity of the clinical presentation of a child. Of note, subclinical symptoms may cause disability and warrant treatment. Subclinical symptoms may also interact with other diagnoses and affect the prognosis. In addition, continuous symptom severity measures can help to monitor treatment effects.

In chapters 6 and 7, we searched for distinct cognitive correlates of internalizing and externalizing correlates of behavior. This information facilitates a better understanding of the underlying etiology. Considering our findings, internalizing symptoms may share neurobiological pathways with language and memory impairments, while externalizing

symptoms could be more specifically related to attention and executive functioning. These results can potentially help in determining the prognosis of a child and making informed treatment decisions, particularly if the clinical picture is characterized by comorbidity. For example, to specifically target executive functioning problems in a child that presents with a mixture of symptoms, it could be helpful to treat the externalizing component of the psychopathology. Finally, specific patterns of symptoms may respond better to particular interventions. For instance, children with comorbid internalizing and externalizing problems appeared to benefit more from a family-centered intervention than children with problems in a single domain (Connell, Bullock, Dishion, Shaw, Wilson et al. 2008).

In this thesis, we sought neurobiological correlates, mostly of autistic traits in young children, using a range of MRI methodologies. Neurobiological research in psychiatry has been criticized for being very descriptive in nature, and, despite huge monetary investments, its failure to lead to very consistent biomarkers or large-scale breakthroughs in the diagnosis and treatment of psychiatric disorders. Neurobiology currently plays little to no role in clinical practice: it is not used in diagnostics, nor does it play a role in deciding which patient is assigned to what treatment. Perhaps adding to the suspicion that some clinicians have against neuroimaging research is the fact that historically, diseases like dementia were transferred from the psychiatrist's to the neurologist's treatment responsibility once the neurobiological or 'organic' nature of the disease became apparent. However, nowadays the classical dichotomy between functional and organic psychiatric disorders is deemed obsolete and this should not deter psychiatrists to pursue the search of neurobiological mechanisms that could ultimately help find better treatment (Rosenberg 2000). Using the words of Bullmore and colleagues, psychiatrists these days cannot afford to be neurophobic (Bullmore, Fletcher and Jones 2009). Eventually, replication of neurobiological findings and careful description of the corresponding phenotype can lead to development of novel treatments. In line with the RDoC initiative, there are now some efforts to incorporate neurobiological measures as outcome measures to evaluate treatment effects (Van Hecke, Stevens, Carson, Karst, Dolan et al. 2015).

RECOMMENDATIONS FOR RESEARCH

ASD likely comprises a cluster of many different disorders, with different neurobiological pathways. While the complexity of this collection of pathways presents researchers with an immense challenge, it will be crucial to understand these pathways better in order to develop specific treatment targets. Much like other fields in medicine, this has the potential to lead to so-called 'personalized' or 'precision' medicine. The notion that ASD may involve a diverse set of pathways has far-reaching consequences. Although ASD, with a prevalence of 1-3% is not

considered a particularly rare disorder, it may in fact be a collection of rather rare disorders anyway. To some extent, an emerging field in research that has been named 'molecular pathological epidemiology' could address this. Considering specific etiological pathways may reconcile seemingly paradoxical findings (Nishihara et al. 2015). This could be applied to the many genetic backgrounds of ASD. In order to facilitate this type of research, large-scale data sharing efforts are needed to create well powered studies. Examples of this include the ABIDE database (Martino, Strejilevich, Marengo, Ibanez, Scapola et al. 2014), the Autism Inpatient Collection (Siegel, Smith, Mazefsky, Gabriels, Erickson et al. 2015) or the Simons Simplex Collection (Fischbach and Lord 2010). Ideally, such datasets will include in-depth phenotypic, genetic and neuroimaging data. Clinically, more routine referral of children with ASD to a clinical geneticist could facilitate more rapid identification of genetic etiologies and such information could be used in future MRI research as well. While likely more monogenic forms of ASD could be identified this way, this is unlikely to be successful for polygenic forms, where many different combinations and interactions of multiple genes are implied. In addition, some genetic variants may be too rare to ever form a substantial subgroup within a study. Importantly, this genetic heterogeneity does not preclude the usefulness of clinical studies that evaluate the broad, unparcellated construct of ASD, as it is defined nowadays. While 'ASD', like many other disease spectra, is an umbrella term for a plethora of diseases, the label in itself is not redundant for neurobiological studies. The multitude of genetic backgrounds of ASD likely cluster to some extent in their targets in the brain. It is unlikely that neurobiological studies of ASD will ultimately be fully stratified on unique, homogeneous genetic background. From the field of genetics, it is evident that in large samples, even a less precise "proxy-phenotype" can lead to identification of relevant variants. This is likely the case for neuroimaging studies of the broad ASD construct as well.

Despite the pressure to identify novel neurobiological correlates, future studies should strive to identify more robust neurobiological findings, by seeking replication in an independent sample using similar methodology (Bernhardt, Di Martino, Valk and Wallace 2016). Further, developmental psychopathology should be studied more often in the context of normal development (Di Martino, Fair, Kelly, Satterthwaite, Castellanos et al. 2014), with careful consideration of representative control groups. Large-scale longitudinal studies, that cross broad age ranges, will ultimately provide more insight in the underlying trajectories of psychopathological neurobiology than cross-sectional "snapshots" of development.

Despite the strong genetic influence on ASD, specific attention is warranted for the study of preventable causes of ASD. Although partly explained by more lenient diagnostic criteria and more readily recognized symptoms, there is some consensus that the prevalence of ASD is rising. Environmental factors likely play a role in this (Hallmayer, Cleveland, Torres, Phillips, Cohen et al. 2011). There is a lot to be gained from researching these causes and trying to understand the mechanisms through which they act. This is perfectly illustrated by the

association of maternal vitamin D deficiency during pregnancy and later development of ASD (Vinkhuyzen, Eyles, Burne, Blanken, Kruithof et al. 2016). Although replication of this finding is warranted, it would be interesting to evaluate whether prenatal vitamin D supplementation could decrease the number of babies born with ASD.

Importantly, it remains essential to better identify young children at risk for developing symptoms of ASD as early as possible. There is evidence that early rigorous intervention, consisting of intensive in-home therapy in language and social reciprocity, leads to better outcomes in childhood (Estes, Munson, Rogers, Greenson, Winter et al. 2015).

CONCLUSION

In this thesis, we assessed multiple aspects of the neurobiology of ASD along a continuum of traits. We discussed the use of continuous phenotypes of ASD in the search for the underlying neurobiology and considered heterogeneity in symptoms and etiology as potential complicating factors in this type of research. So, to conclude, we can ask the question that was proposed by Happé and colleagues: is it time to give up on a single explanation of ASD (Happe, Ronald and Plomin 2006)? The answer is probably yes; it is time to give up on a *single* neurobiological explanation. It is probably best not to speak of the search for a "neural signature" of ASD, which implies a specific pattern of brain differences with nearly perfect sensitivity and specificity for the disorder (Lilienfeld et al. 2015). It is more likely that the inconsistency that we observe in studies of the neurobiology of ASD reflects actual heterogeneity across the autism spectrum itself. It, therefore, is unlikely that a single consistent neurobiological phenotype will be found that adequately describes the entire autism spectrum (Bernhardt et al. 2016). The neurobiology of ASD is likely highly idiosyncratic, in the sense that every patient with ASD may show their own deviation from typical brain development (Hahamy, Behrmann and Malach 2015). However, even if ASD is a broad diagnosis, large-scale neuroimaging studies of patients with the diagnosis can lead to identification of relevant and common brain correlates. Ultimately, on the individual level, each person with ASD may exhibit their own profile on each of these parameters, and perhaps such profiles could ultimately guide treatment decisions. Therefore, it would be too early to quit the search for the underlying neurobiology of the broader spectrum of ASD altogether, or to completely abandon the case-control design.

Importantly, behavior originates in the brain and can ultimately only be understood from the brain, even if differences are not obvious or even visible. As analysis technology improves, neuroimaging research remains an obvious and promising way to tap into this complex system. Continuous and categorical phenotypic approaches should complement each other in the search of neurobiological markers of psychopathology. At the same time, the search

for the underlying neurobiology should never occupy all available resources in psychiatric research. There should always be a simultaneous effort in developing interventions. Ideally, neurobiological research leads to a better understanding of the disorder so these interventions can become more targeted.

REFERENCES

Abrahams, B. S. and D. H. Geschwind (2008). "Advances in autism genetics: on the threshold of a new neurobiology." Nat Rev Genet **9**(5): 341-355.

Andersen, S. L. (2003). "Trajectories of brain development: point of vulnerability or window of opportunity?" Neurosci Biobehav Rev **27**(1-2): 3-18.

Aoki, Y., O. Abe, Y. Nippashi and H. Yamasue (2013). "Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies." Molecular Autism **4**.

Baron-Cohen, S., S. Wheelwright, R. Skinner, J. Martin and E. Clubley (2001). "The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians." J Autism Dev Disord **31**(1): 5-17.

Bernhardt, B. C., A. Di Martino, S. L. Valk and G. L. Wallace (2016). "Neuroimaging-Based Phenotyping of the Autism Spectrum." Curr Top Behav Neurosci.

Betancur, C. (2011). "Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting." Brain Res **1380**: 42-77.

Birnbaum, R., A. E. Jaffe, T. M. Hyde, J. E. Kleinman and D. R. Weinberger (2014). "Prenatal expression patterns of genes associated with neuropsychiatric disorders." Am J Psychiatry **171**(7): 758-767.

Bolte, S., F. Poustka and J. N. Constantino (2008). "Assessing autistic traits: cross-cultural validation of the social responsiveness scale (SRS)." Autism Res **1**(6): 354-363.

Bos, D. J., J. Merchan-Naranjo, K. Martinez, L. Pina-Camacho, I. Balsa, L. Boada, H. Schnack, B. Oranje, M. Desco, C. Arango, M. Parellada, S. Durston and J. Janssen (2015). "Reduced Gyrification Is Related to Reduced Interhemispheric Connectivity in Autism Spectrum Disorders." J Am Acad Child Adolesc Psychiatry **54**(8): 668-676.

Brown, A. S. (2012). "Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism." Dev Neurobiol **72**(10): 1272-1276.

Bullmore, E., P. Fletcher and P. B. Jones (2009). "Why psychiatry can't afford to be neurophobic." Br J Psychiatry **194**(4): 293-295.

Chaste, P., L. Klei, S. J. Sanders, V. Hus, M. T. Murtha, J. K. Lowe, A. J. Willsey, D. Moreno-De-Luca, T. W. Yu, E. Fombonne, D. Geschwind, D. E. Grice, D. H. Ledbetter, S. M. Mane, D. M. Martin, E. M. Morrow, C. A. Walsh, J. S. Sutcliffe, C. Lese Martin, A. L. Beaudet, C. Lord, M. W. State, E. H. Cook, Jr. and B. Devlin (2015). "A genome-wide association study of autism using the Simons Simplex Collection: Does reducing phenotypic heterogeneity in autism increase genetic homogeneity?" Biol Psychiatry **77**(9): 775-784.

Coghill, D. and E. J. Sonuga-Barke (2012). "Annual research review: categories versus dimensions in the classification and conceptualisation of child and adolescent mental disorders--implications of recent empirical study." J Child Psychol Psychiatry **53**(5): 469-489.

Colvert, E., B. Tick, F. McEwen, C. Stewart, S. R. Curran, E. Woodhouse, N. Gillan, V. Hallett, S. Lietz, T. Garnett, A. Ronald, R. Plomin, F. Rijsdijk, F. Happe and P. Bolton (2015). "Heritability of Autism Spectrum Disorder in a UK Population-Based Twin Sample." JAMA Psychiatry **72**(5): 415-423.

Connell, A., B. M. Bullock, T. J. Dishion, D. Shaw, M. Wilson and F. Gardner (2008). "Family intervention effects on co-occurring early childhood behavioral and emotional problems: a latent transition analysis approach." J Abnorm Child Psychol **36**(8): 1211-1225.

Constantino, J. N. (2002). Social Responsiveness Scale (SRS), Manual. Los Angeles, Western Psychological services.

Constantino, J. N. (2011). "The quantitative nature of autistic social impairment." Pediatr Res **69**(5 Pt 2): 55R-62R.

Constantino, J. N. and T. W. Frazier (2013). "Commentary: The observed association between autistic severity measured by the social responsiveness scale (SRS) and general psychopathology--a response to Hus et al.(2013)." J Child Psychol Psychiatry **54**(6): 695-697.

Constantino, J. N., C. Lajonchere, M. Lutz, T. Gray, A. Abbacchi, K. McKenna, D. Singh and R. D. Todd (2006). "Autistic social impairment in the siblings of children with pervasive developmental disorders." Am J Psychiatry **163**(2): 294-296.

Constantino, J. N., P. D. Lavesser, Y. Zhang, A. M. Abbacchi, T. Gray and R. D. Todd (2007). "Rapid quantitative assessment of autistic social impairment by classroom teachers." J Am Acad Child Adolesc Psychiatry **46**(12): 1668-1676.

Cooke, R. W., A. Lucas, P. L. Yudkin and J. Pryse-Davies (1977). "Head circumference as an index of brain weight in the fetus and newborn." Early Hum Dev **1**(2): 145-149.

Davis, J. M., J. G. Keeney, J. M. Sikela and S. Hepburn (2013). "Mode of genetic inheritance modifies the association of head circumference and autism-related symptoms: a cross-sectional study." PLoS One **8**(9): e74940.

de la Marche, W., I. Noens and J. Steyaert (2015). "[Dimensional measures in autism spectrum disorders: do we know what we measure?]

Dimensionele maten bij autisme-spectrumstoornissen: weten we wat we meten?" Tijdschr Psychiatr **57**(12): 897-901.

Di Martino, A., D. A. Fair, C. Kelly, T. D. Satterthwaite, F. X. Castellanos, M. E. Thomason, R. C. Craddock, B. Luna, B. L. Leventhal, X. N. Zuo and M. P. Milham (2014). "Unraveling the miswired connectome: a developmental perspective." Neuron **83**(6): 1335-1353.

Ducharme, S., M. D. Albaugh, T. V. Nguyen, J. J. Hudziak, J. M. Mateos-Perez, A. Labbe, A. C. Evans, S. Karama and G. Brain Development Cooperative (2016). "Trajectories of cortical thickness maturation in normal brain development--The importance of quality control procedures." Neuroimage **125**: 267-279.

Duvekot, J., J. van der Ende, F. C. Verhulst and K. Greaves-Lord (2015). "The Screening Accuracy of the Parent and Teacher-Reported Social Responsiveness Scale (SRS): Comparison with the 3Di and ADOS." J Autism Dev Disord **45**(6): 1658-1672.

El Marroun, H., T. J. White, N. J. van der Knaap, J. R. Homberg, G. Fernandez, N. K. Schoemaker, V. W. Jaddoe, A. Hofman, F. C. Verhulst, J. J. Hudziak, B. H. Stricker and H. Tiemeier (2014). "Prenatal exposure to selective serotonin reuptake inhibitors and social responsiveness symptoms of autism: population-based study of young children." Br J Psychiatry **205**(2): 95-102.

Estes, A., J. Munson, S. J. Rogers, J. Greenson, J. Winter and G. Dawson (2015). "Long-Term Outcomes of Early Intervention in 6-Year-Old Children With Autism Spectrum Disorder." J Am Acad Child Adolesc Psychiatry **54**(7): 580-587.

Fischbach, G. D. and C. Lord (2010). "The Simons Simplex Collection: a resource for identification of autism genetic risk factors." Neuron **68**(2): 192-195.

Fischl, B. and A. M. Dale (2000). "Measuring the thickness of the human cerebral cortex from magnetic resonance images." Proceedings of the National Academy of Sciences of the United States of America **97**(20): 11050-11055.

Folstein, S. E. and B. Rosen-Sheidley (2001). "Genetics of autism: complex aetiology for a heterogeneous disorder." Nat Rev Genet **2**(12): 943-955.

Fombonne, E., B. Roge, J. Claverie, S. Courty and J. Fremolle (1999). "Microcephaly and macrocephaly in autism." J Autism Dev Disord **29**(2): 113-119.

Gaugler, T., L. Klei, S. J. Sanders, C. A. Bodea, A. P. Goldberg, A. B. Lee, M. Mahajan, D. Manaa, Y. Pawitan, J. Reichert, S. Ripke, S. Sandin, P. Sklar, O. Svantesson, A. Reichenberg, C. M. Hultman, B. Devlin, K. Roeder and J. D. Buxbaum (2014). "Most genetic risk for autism resides with common variation." Nat Genet **46**(8): 881-885.

Geschwind, D. H. and P. Levitt (2007). "Autism spectrum disorders: developmental disconnection syndromes." Curr Opin Neurobiol **17**(1): 103-111.

Giedd, J. N., J. Blumenthal, N. O. Jeffries, F. X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A. C. Evans and J. L. Rapoport (1999). "Brain development during childhood and adolescence: a longitudinal MRI study." Nature Neuroscience **2**(10): 861-863.

Goddard, M. N., S. van Rijn, S. A. Rombouts and H. Swaab (2015). "White matter microstructure in a genetically defined group at increased risk of autism symptoms, and a comparison with idiopathic autism: an exploratory study." Brain Imaging Behav.

Gottesman, II and T. D. Gould (2003). "The endophenotype concept in psychiatry: etymology and strategic intentions." Am J Psychiatry **160**(4): 636-645.

Green, C., D. Z. Loesch and C. Dissanayake (2015). "A review of physical growth in children and adolescents with Autism Spectrum Disorder." Developmental Review **36**.

Grove, R., A. Baillie, C. Allison, S. Baron-Cohen and R. A. Hoekstra (2013). "Empathizing, systemizing, and autistic traits: latent structure in individuals with autism, their parents, and general population controls." J Abnorm Psychol **122**(2): 600-609.

Habas, P. A., J. A. Scott, A. Roosta, V. Rajagopalan, K. Kim, F. Rousseau, A. J. Barkovich, O. A. Glenn and C. Studholme (2012). "Early Folding Patterns and Asymmetries of the Normal Human Brain Detected from in Utero MRI." Cerebral Cortex **22**(1): 13-25.

Hahamy, A., M. Behrmann and R. Malach (2015). "The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder." Nat Neurosci **18**(2): 302-309.

Hallmayer, J., S. Cleveland, A. Torres, J. Phillips, B. Cohen, T. Torigoe, J. Miller, A. Fedele, J. Collins, K. Smith, L. Lotspeich, L. A. Croen, S. Ozonoff, C. Lajonchere, J. K. Grether and N. Risch (2011). "Genetic heritability and shared environmental factors among twin pairs with autism." Arch Gen Psychiatry **68**(11): 1095-1102.

Hanson, E., R. Bernier, K. Porche, F. I. Jackson, R. P. Goin-Kochel, L. G. Snyder, A. V. Snow, A. S. Wallace, K. L. Campe, Y. Zhang, Q. Chen, D. D'Angelo, A. Moreno-De-Luca, P. T. Orr, K. B. Boomer, D. W. Evans, S. Kanne, L. Berry, F. K. Miller, J. Olson, E. Sherr, C. L. Martin, D. H. Ledbetter, J. E. Spiro, W. K. Chung and C. Simons Variation in Individuals Project (2015). "The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population." Biol Psychiatry **77**(9): 785-793.

Happe, F., A. Ronald and R. Plomin (2006). "Time to give up on a single explanation for autism." Nat Neurosci **9**(10): 1218-1220.

Herculano-Houzel, S., C. Watson and G. Paxinos (2013). "Distribution of neurons in functional areas of the mouse cerebral cortex reveals quantitatively different cortical zones." Front Neuroanat **7**: 35.

Hus, V., S. Bishop, K. Gotham, M. Huerta and C. Lord (2013). "Factors influencing scores on the social responsiveness scale." J Child Psychol Psychiatry **54**(2): 216-224.

Insel, T., B. Cuthbert, M. Garvey, R. Heinssen, D. S. Pine, K. Quinn, C. Sanislow and P. Wang (2010). "Research Domain Criteria (RDoC): Toward a New Classification Framework for Research on Mental Disorders." American Journal of Psychiatry **167**(7): 748-751.

Kanner, L. (1968). "Autistic disturbances of affective contact." Acta Paedopsychiatr **35**(4): 100-136.

Kates, W. R., I. Ikuta and C. P. Burnette (2009). "Gyrification Patterns in Monozygotic Twin Pairs Varying in Discordance for Autism." Autism Research **2**(5): 267-278.

Kendell, R. and A. Jablensky (2003). "Distinguishing between the validity and utility of psychiatric diagnoses." Am J Psychiatry **160**(1): 4-12.

Kendler, K. S. (2013). "What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn." Mol Psychiatry **18**(10): 1058-1066.

Lai, M. C., M. V. Lombardo, J. Suckling, A. N. V. Ruigrok, B. Chakrabarti, C. Ecker, S. C. L. Deoni, M. C. Craig, D. G. M. Murphy, E. T. Bullmore, S. Baron-Cohen and M. A. Consortium (2013). "Biological sex affects the neurobiology of autism." Brain **136**: 2799-2815.

Lilienfeld, S. O., K. C. Sauvigne, S. J. Lynn, R. L. Cautin, R. D. Latzman and I. D. Waldman (2015). "Fifty psychological and psychiatric terms to avoid: a list of inaccurate, misleading, misused, ambiguous, and logically confused words and phrases." Front Psychol **6**: 1100.

Lundstrom, S., Z. Chang, M. Rastam, C. Gillberg, H. Larsson, H. Anckarsater and P. Lichtenstein (2012). "Autism spectrum disorders and autistic like traits: similar etiology in the extreme end and the normal variation." Arch Gen Psychiatry **69**(1): 46-52.

Lyall, K., J. N. Constantino, M. G. Weisskopf, A. L. Roberts, A. Ascherio and S. L. Santangelo (2014). "Parental social responsiveness and risk of autism spectrum disorder in offspring." JAMA Psychiatry **71**(8): 936-942.

Mandy, W. and M. C. Lai (2016). "Annual Research Review: The role of the environment in the developmental psychopathology of autism spectrum condition." J Child Psychol Psychiatry **57**(3): 271-292.

Martino, D. J., S. A. Strejilevich, E. Marengo, A. Ibanez, M. Scapola and A. Igoa (2014). "Toward the identification of neurocognitive subtypes in euthymic patients with bipolar disorder." J Affect Disord **167**: 118-124.

Moreno-De-Luca, A., D. W. Evans, K. B. Boomer, E. Hanson, R. Bernier, R. P. Goin-Kochel, S. M. Myers, T. D. Challman, D. Moreno-De-Luca, M. M. Slane, A. E. Hare, W. K. Chung, J. E. Spiro, W. A. Faucett, C. L. Martin and D. H. Ledbetter (2015). "The role of parental cognitive, behavioral, and motor profiles in clinical variability in individuals with chromosome 16p11.2 deletions." JAMA Psychiatry **72**(2): 119-126.

Nishihara, R., T. J. VanderWeele, K. Shibuya, M. A. Mittleman, M. Wang, A. E. Field, E. Giovannucci, P. Lochhead and S. Ogino (2015). "Molecular pathological epidemiology gives clues to paradoxical findings." Eur J Epidemiol **30**(10): 1129-1135.

Ogino, S., P. Lochhead, A. T. Chan, R. Nishihara, E. Cho, B. M. Wolpin, J. A. Meyerhardt, A. Meissner, E. S. Schernhammer, C. S. Fuchs and E. Giovannucci (2013). "Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease." Mod Pathol **26**(4): 465-484.

Ozonoff, S., K. Heung, R. Byrd, R. Hansen and I. Hertz-Picciotto (2008). "The onset of autism: patterns of symptom emergence in the first years of life." Autism Res **1**(6): 320-328.

Panizzon, M. S., C. Fennema-Notestine, L. T. Eyler, T. L. Jernigan, E. Prom-Wormley, M. Neale, K. Jacobson, M. J. Lyons, M. D. Grant, C. E. Franz, H. Xian, M. Tsuang, B. Fischl, L. Seidman, A. Dale and W. S. Kremen (2009). "Distinct Genetic Influences on Cortical Surface Area and Cortical Thickness." Cerebral Cortex **19**(11): 2728-2735.

Plomin, R., C. M. Haworth and O. S. Davis (2009). "Common disorders are quantitative traits." Nat Rev Genet **10**(12): 872-878.

Raznahan, A., P. Shaw, F. Lalonde, M. Stockman, G. L. Wallace, D. Greenstein, L. Clasen, N. Gogtay and J. N. Giedd (2011). "How Does Your Cortex Grow?" Journal of Neuroscience **31**(19): 7174-7177.

Robinson, E. B., K. C. Koenen, M. C. McCormick, K. Munir, V. Hallett, F. Happe, R. Plomin and A. Ronald (2011). "Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%)." Arch Gen Psychiatry **68**(11): 1113-1121.

Robinson, E. B., B. St Pourcain, V. Anttila, J. A. Kosmicki, B. Bulik-Sullivan, J. Grove, J. Maller, K. E. Samocha, S. J. Sanders, S. Ripke, J. Martin, M. V. Hollegaard, T. Werge, D. M. Hougaard, P.-S. S. I. B. A. G. i, B. M. Neale, D. M. Evans, D. Skuse, P. B. Mortensen, A. D. Borglum, A. Ronald, G. D. Smith and M. J. Daly (2016). "Genetic risk for autism spectrum disorders and neuropsychiatric variation in the general population." Nat Genet **48**(5): 552-555.

Ronald, A., F. Happe, P. Bolton, L. M. Butcher, T. S. Price, S. Wheelwright, S. Baron-Cohen and R. Plomin (2006). "Genetic heterogeneity between the three components of the autism spectrum: a twin study." J Am Acad Child Adolesc Psychiatry **45**(6): 691-699.

Ronald, A., F. Happe, T. S. Price, S. Baron-Cohen and R. Plomin (2006). "Phenotypic and genetic overlap between autistic traits at the extremes of the general population." J Am Acad Child Adolesc Psychiatry **45**(10): 1206-1214.

Rosenberg, R. (2000). "The case of dementia: psychiatry or neurology?" Acta Psychiatr Scand **102**(5): 319-320.

Sanders, S. J., M. T. Murtha, A. R. Gupta, J. D. Murdoch, M. J. Raubeson, A. J. Willsey, A. G. Ercan-Sencicek, N. M. DiLullo, N. N. Parikshak, J. L. Stein, M. F. Walker, G. T. Ober, N. A. Teran, Y. Song, P. El-Fishawy, R. C. Murtha, M. Choi, J. D. Overton, R. D. Bjornson, N. J. Carriero, K. A. Meyer, K. Bilguvar, S. M. Mane, N. Sestan, R. P. Lifton, M. Gunel, K. Roeder, D. H. Geschwind, B. Devlin and M. W. State (2012). "De novo mutations revealed by whole-exome sequencing are strongly associated with autism." Nature **485**(7397): 237-241.

Schaer, M., M. C. Ottet, E. Scariati, D. Dukes, M. Franchini, S. Eliez and B. Glaser (2013). "Decreased frontal gyrification correlates with altered connectivity in children with autism." Frontiers in Human Neuroscience **7**.

Sebat, J., B. Lakshmi, D. Malhotra, J. Troge, C. Lese-Martin, T. Walsh, B. Yamrom, S. Yoon, A. Krasnitz, J. Kendall, A. Leotta, D. Pai, R. Zhang, Y. H. Lee, J. Hicks, S. J. Spence, A. T. Lee, K. Puura, T. Lehtimaki, D. Ledbetter, P. K. Gregersen, J. Bregman, J. S. Sutcliffe, V. Jobanputra, W. Chung, D. Warburton, M. C. King, D. Skuse, D. H. Geschwind, T. C. Gilliam, K. Ye and M. Wigler (2007). "Strong association of de novo copy number mutations with autism." Science **316**(5823): 445-449.

Segovia, F., R. Holt, M. Spencer, J. M. Gorriz, J. Ramirez, C. G. Puntonet, C. Phillips, L. Chura, S. Baron-Cohen and J. Suckling (2014). "Identifying endophenotypes of autism: a multivariate approach." Front Comput Neurosci **8**: 60.

Shaw, P., N. J. Kabani, J. P. Lerch, K. Eckstrand, R. Lenroot, N. Gogtay, D. Greenstein, L. Clasen, A. Evans, J. L. Rapoport, J. N. Giedd and S. P. Wise (2008). "Neurodevelopmental trajectories of the human cerebral cortex." Journal of Neuroscience **28**(14): 3586-3594.

Shaw, P., M. Malek, B. Watson, W. Sharp, A. Evans and D. Greenstein (2012). "Development of Cortical Surface Area and Gyrification in Attention-Deficit/Hyperactivity Disorder." Biological Psychiatry **72**(3): 191-197.

Siegel, M., K. A. Smith, C. Mazefsky, R. L. Gabriels, C. Erickson, D. Kaplan, E. M. Morrow, L. Wink, S. L. Santangelo, Autism and C. Developmental Disorders Inpatient Research (2015). "The autism inpatient collection: methods and preliminary sample description." Mol Autism **6**: 61.

St Pourcain, B., K. Wang, J. T. Glessner, J. Golding, C. Steer, S. M. Ring, D. H. Skuse, S. F. Grant, H. Hakonarson and G. Davey Smith (2010). "Association between a high-risk autism locus on 5p14 and social communication spectrum phenotypes in the general population." Am J Psychiatry **167**(11): 1364-1372.

Stoner, R., M. L. Chow, M. P. Boyle, S. M. Sunkin, P. R. Mouton, S. Roy, A. Wynshaw-Boris, S. A. Colamarino, E. S. Lein and E. Courchesne (2014). "Patches of Disorganization in the Neocortex of Children with Autism." New England Journal of Medicine **370**(13): 1209-1219.

Sucksmith, E., I. Roth and R. A. Hoekstra (2011). "Autistic traits below the clinical threshold: re-examining the broader autism phenotype in the 21st century." Neuropsychol Rev **21**(4): 360-389.

Van Essen, D. C. (1997). "A tension-based theory of morphogenesis and compact wiring in the central nervous system." Nature **385**(6614): 313-318.

Van Hecke, A. V., S. Stevens, A. M. Carson, J. S. Karst, B. Dolan, K. Schohl, R. J. McKindles, R. Remmel and S. Brockman (2015). "Measuring the plasticity of social approach: a randomized controlled trial of the effects of the PEERS intervention on EEG asymmetry in adolescents with autism spectrum disorders." J Autism Dev Disord **45**(2): 316-335.

Vinkhuyzen, A. A. E., D. W. Eyles, T. H. J. Burne, L. Blanken, C. J. Kruithof, F. C. Verhulst, V. W. Jaddoe, H. Tiemeier and J. J. McGrath (2016). "Gestational Vitamin D deficiency and autism-related outcomes: The Generation R Study " In Revision.

Wallace, G. L., N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2010). "Age-related temporal and parietal cortical thinning in autism spectrum disorders." Brain **133**: 3745-3754.

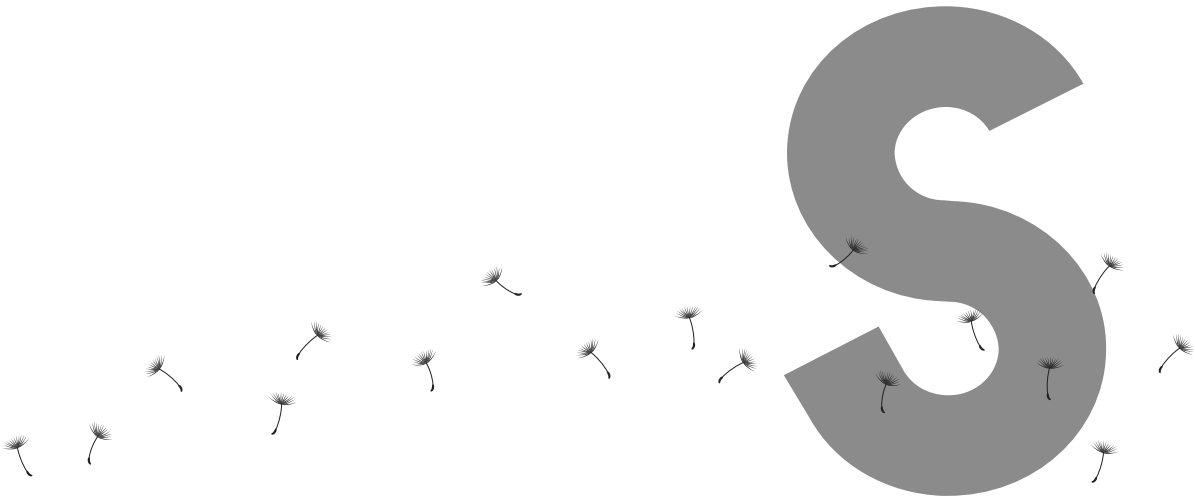
Wallace, G. L., B. Robustelli, N. Dankner, L. Kenworthy, J. N. Giedd and A. Martin (2013). "Increased gyrification, but comparable surface area in adolescents with autism spectrum disorders." Brain **136**: 1956-1967.

White, T., S. Su, M. Schmidt, C. Y. Kao and G. Sapiro (2010). "The development of gyrification in childhood and adolescence." Brain and Cognition **72**(1): 36-45.

Willsey, A. J., S. J. Sanders, M. Li, S. Dong, A. T. Tebbenkamp, R. A. Muhle, S. K. Reilly, L. Lin, S. Fertuzinhos, J. A. Miller, M. T. Murtha, C. Bichsel, W. Niu, J. Cotney, A. G. Ercan-Sencicek, J. Gockley, A. R. Gupta, W. Han, X. He, E. J. Hoffman, L. Klei, J. Lei, W. Liu, L. Liu, C. Lu, X. Xu, Y. Zhu, S. M. Mane, E. S. Lein, L. Wei, J. P. Noonan, K. Roeder, B. Devlin, N. Sestan and M. W. State (2013). "Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism." Cell **155**(5): 997-1007.

Witwer, A. N. and L. Lecavalier (2008). "Examining the validity of autism spectrum disorder subtypes." J Autism Dev Disord **38**(9): 1611-1624.

Yang, D. Y., D. Beam, K. A. Pelphrey, S. Abdullahi and R. J. Jou (2016). "Cortical morphological markers in children with autism: a structural magnetic resonance imaging study of thickness, area, volume, and gyrification." Mol Autism **7**: 11.



Summary
Samenvatting

SUMMARY

Despite evidence from genetic and phenotypic studies that points to a dimensional structure of child psychiatry, where traits of disorders extend to the general population, most neuroimaging studies are still designed as clinical case-control studies. In this thesis, we explored the neurobiology of various dimensions of child psychopathology, primarily focusing on autistic traits. In addition, we studied internalizing and externalizing symptoms in children. Instead of using the traditional case-control framework, we applied alternative approaches to define the phenotypes of interest. Two approaches of quantifying psychiatric symptoms were applied. In the context of Autism Spectrum Disorder (ASD), we utilized the fact that the social impairment in autism can be conceptualized along a continuum, at which ASD represents the severe end. Second, using a latent class approach of dimensional data, we evaluated categorical constructs of internalizing and externalizing problems in the general population. The studies in this thesis were performed in the Generation R cohort, a large prospective population-based cohort study in Rotterdam, the Netherlands. In this cohort, children are followed from prenatal life onwards. A large subgroup of children participated in a neuroimaging component of the study, which included structural and functional MRI scans, as well as a thorough neuropsychological assessment. The first aim of this thesis was to study various characteristics of the underlying neurobiology of ASD, using the continuum of traits in the general population. The second aim of this thesis was to study cognitive deficits and neurobiological correlates of internalizing and externalizing problems. Part I of this thesis addresses the first aim and focuses on various aspects of the neurobiology of autism, utilizing continuous trait measures.

In **chapter 2**, we studied the association between autistic traits and several characteristics of cortical morphology, including gyrification; the pattern of folding of the cerebral cortex. Children with more autistic traits showed widespread areas of decreased gyrification of the cortex. We found that this association was present along a continuum of autistic traits: after excluding children with the highest levels of autistic traits and confirmed ASD, the association remained in cortical areas involving the left temporal lobe and left precuneus. In addition, we found similar effects when comparing a small sample of children with confirmed ASD to age and gender-matched controls. Taken together, our findings in cortical morphology lend support to an extension of the neurobiology of autistic traits to the general population.

In **chapter 3**, we focused on prenatal brain growth using ultrasound measurements. Altered postnatal trajectories of brain growth are often reported in ASD, particularly in the first year of life, but much less is known about prenatal head growth trajectories. Evaluating prenatal growth trajectories of head circumference measured with ultrasound, we showed an inverse relationship between prenatal head growth and autistic traits later in life; a lower rate of growth in prenatal head circumference was associated with more autistic traits. However,

no differences in prenatal head growth were found between children with confirmed ASD and controls. This could be due to a lack of power, heterogeneity in pathways leading to ASD, or limited specificity of the finding for ASD. Our results may indicate that prenatal head growth is involved in the development of autistic traits, and future research should clarify the mechanisms involved.

According to the developmental disconnection hypothesis, ASD may arise from aberrant development of long- and short-range connections in the brain. Connectivity in the brain is partly facilitated by white matter tracts. In **chapter 4**, we studied the relation of autistic traits and microstructural integrity of white matter tracts in the brain. While we found no widespread associations between autistic traits and white matter integrity, we did observe a negative association between autistic traits and white matter integrity in the left superior longitudinal fasciculus, a long-range white matter tract that has been implicated in children with ASD. This association remained when excluding children with a confirmed diagnosis of ASD, and a trend to lower white matter integrity in this area was observed when comparing a small subset of children with ASD to controls. Thus, in the superior longitudinal fasciculus, there is evidence of a continuum of white matter integrity along the spectrum of autistic symptoms. Since we only found a very localized association, some other white matter abnormalities that are commonly reported in white matter studies in children with ASD may not relate to continuously measured social problems in the general population, and may instead be restricted to clinically affected children.

In **chapter 5**, we investigated dynamic characteristics of resting-state connectivity in children, and subsequently evaluated those characteristics in relation to autistic traits. We used a novel analysis method that relaxed the assumption that resting-state connectivity remains static over the course of several minutes. We hypothesized that relaxing this stationarity assumption would reveal novel features of both autism and typical brain development. When using this 'chronnectomic' (recurring, time-varying patterns of connectivity) approach to resting-state data of young children, we showed that dynamic patterns could be captured in four transient states that differed in the patterns and the degree of connectivity. Furthermore, we demonstrated that children with higher levels of autistic traits spent more time in a globally disconnected state. This pattern was consistent with that of young, typically developing children, which may suggest that children with autistic traits exhibit delayed characteristics of functional brain maturation.

The second part of this thesis addresses the second aim and focuses on specific cognitive and neurobiological characteristics of children with internalizing and externalizing symptoms. In **chapter 6**, we studied specific cognitive problems in children with internalizing, externalizing and comorbid problems. Internalizing and externalizing symptoms were studied both continuously and categorically, using both variable-centered and person-centered analyses. In the first approach, broadband scores of internalizing and externalizing symptoms

along a continuum were used. However, children with high levels of internalizing symptoms often also have high levels of externalizing symptoms, potentially obscuring specific relations between cognition and psychopathology. In the second approach, to remedy this issue, latent classes of children with relatively homogeneous symptom patterns were identified from several continuous dimensions of psychopathology, including a class of children with mostly internalizing symptoms, and a class of children with predominantly externalizing symptoms. Consistently, these two different modelling approaches demonstrated that children with internalizing and externalizing symptoms show distinct cognitive profiles. Children with more externalizing symptoms performed lower in the attention/executive functioning domain, while children with more internalizing symptoms showed impairment in verbal fluency and memory. In the most severely affected class of children with internalizing and externalizing symptoms, we found specific impairment in the sensorimotor domain. This study illustrates the specific interrelation of internalizing and externalizing symptoms and cognition in young children and the potential utility of the latent class approach to separate their unique contributions to cognition.

In **chapter 7**, we studied specific brain morphological correlates related to internalizing behavior in young children, accounting for externalizing comorbidity, by using two approaches similar to what was used in chapter 6. In the first approach, continuous internalizing scores were adjusted for externalizing scores, however, no association between and brain morphological measures was found. In the second approach, using the empirically defined classes of behavior, we showed that internalizing problems in children were related to thinner cortex mostly in temporal, but also in frontal regions. These findings suggest that cortical morphological differences observed in young children with internalizing problems might be similar to those implicated in clinical depression or anxiety in older populations.

Finally, in **chapter 8**, we discuss the main findings of these studies in the context of recent literature, and we discuss methodological considerations, as well as implications of these studies for further research and clinical practice.

SAMENVATTING

Genetische en fenotypische studies wijzen erop dat kinderpsychiatrie binnen een dimensionele structuur beschreven kan worden, waarbij bepaalde trekken ook voorkomen in de algemene bevolking. Desondanks hebben de meeste neuroimaging studies nog altijd een case-control ontwerp. In dit proefschrift hebben wij de neurobiologie onderzocht van verschillende dimensies van kinderpsychiatrie, waarbij wij ons met name richtten op autistische trekken. Daarnaast hebben wij ook internaliserende en externaliserende symptomen bij kinderen onderzocht. In tegenstelling tot het traditionele case-control ontwerp hebben wij gebruik gemaakt van andere methoden om het fenotype van interesse te definiëren. We hebben twee methoden toegepast. In de context van Autisme Spectrum Stoornissen (ASS) hebben wij gebruik gemaakt van het feit dat de sociale problemen in autisme gezien kunnen worden als deel van een continuüm, waarbij kinderen met een ASS-diagnose zich aan het ernstige eind van het spectrum bevinden. Ten tweede hebben wij dimensionele informatie middels een latente klasse-analyse tot categorische internaliserende en externaliserende constructen teruggebracht. De studies in dit proefschrift zijn uitgevoerd binnen de Generation R studie, een grote populatie-gebaseerde cohortstudie in Rotterdam. Binnen dit cohort worden kinderen vanaf de prenatale periode gevolgd. Een grote subgroep van deze kinderen nam deel aan een neuroimaging sub studie, waarbij enerzijds structurele en functionele MRI-scans werden gemaakt, en anderzijds een uitgebreid neuropsychologisch onderzoek werd afgenomen. Het eerste doel van dit proefschrift was om verschillende aspecten van de onderliggende neurobiologie van ASS te onderzoeken, gebruikmakend van het continuüm van autistische trekken in de algemene bevolking. Het tweede doel was om de cognitieve problemen en hersencorrelaten behorende bij internaliserende en externaliserende problemen te onderzoeken. Deel I van dit proefschrift gaat in op het eerste doel, en richt zich daarbij op verschillende aspecten van de neurobiologie van autisme, gebruikmakend van continue maten om autistische trekken te meten.

In **hoofdstuk 2** bestudeerden wij de associatie van autistische trekken met verschillende aspecten van corticale morfologie, waaronder gyrificatie, het patroon van vouwing van de cortex. Kinderen met autistische trekken lieten een globale vermindering van gyrificatie zien. Wij vonden dat deze associatie te beschrijven was aan de hand van een continuüm: de relatie bleef bestaan in een deel van de gebieden na het excluderen van kinderen met de meeste autistische trekken en kinderen met een klinische diagnose van ASS. Daarnaast vonden we een vergelijkbare relatie in een kleine groep kinderen met een bevestigde ASS-diagnose, wanneer wij hen vergeleken met een controlegroep. Concluderend steunen onze bevindingen betreffende corticale morfologie het idee dat kenmerken van de neurobiologie van autisme te generaliseren zijn naar de algemene bevolking.

In **hoofdstuk 3** richtten wij ons op prenatale hersengroei, gemeten met echoscopie. Bij ASS wordt vaak gesproken van veranderde trajecten van hersengroei, vooral in het eerste levensjaar, maar er is veel minder bekend over prenatale groeipatronen. Met behulp van echo's om groeipatronen van prenatale hoofdomtrek te meten, vonden wij een omgekeerde relatie tussen hoofdomtrek en de ontwikkeling van latere autistische trekken: een verminderde prenatale hoofdgroei was geassocieerd met autistische trekken later in het leven. Daarentegen, we vonden geen verschillen in prenatale hoofdgroei tussen kinderen met een bevestigde diagnose van ASS en controles. Dit zou kunnen komen door een gebrek aan statische power, heterogeniteit in de biologische paden die tot ASS kunnen leiden, of het feit dat de bevinding niet erg specifiek is voor ASS. Onze resultaten wijzen erop dat hoofdgroei mogelijk betrokken is bij de ontwikkeling van ASS en vervolgonderzoek moet verricht worden om de onderliggende mechanismen op te helderen.

Volgens de "developmental disconnection" hypothese zou ASS kunnen ontstaan als gevolg van verstoringen in de ontwikkeling van lange- en korte afstandsverbindingen in de hersenen. Connectiviteit in de hersenen wordt ten dele mogelijk gemaakt door witte stof banen. In **hoofdstuk 4** hebben wij de relatie tussen autistische trekken en microstructurele integriteit van deze banen onderzocht. We vonden geen wijdverbreide relatie tussen autistische trekken en microstructurele integriteit, maar wel een negatieve associatie tussen autistische trekken en witte stof integriteit in de linker superieure longitudinale fasciculus, een lange afstandsbaan die vaak in verband wordt gebracht met ASS. Deze associatie bleef bestaan wanneer we kinderen met een bevestigde ASS-diagnose excludeerden en we zagen een vergelijkbaar effect in dit gebied wanneer we een kleine subgroep kinderen met ASS vergeleken met een controlegroep. Dus, in de superieure longitudinale fasciculus is er bewijs voor het bestaan van een continuüm in witte stof integriteit, gerelateerd aan het spectrum van autistische trekken. Omdat we deze relatie louter in een klein gebied vonden, is het goed mogelijk dat bepaalde andere witte stof afwijkingen die vaak worden gerapporteerd bij kinderen met ASS alleen bij de meest ernstig aangedane kinderen voorkomen.

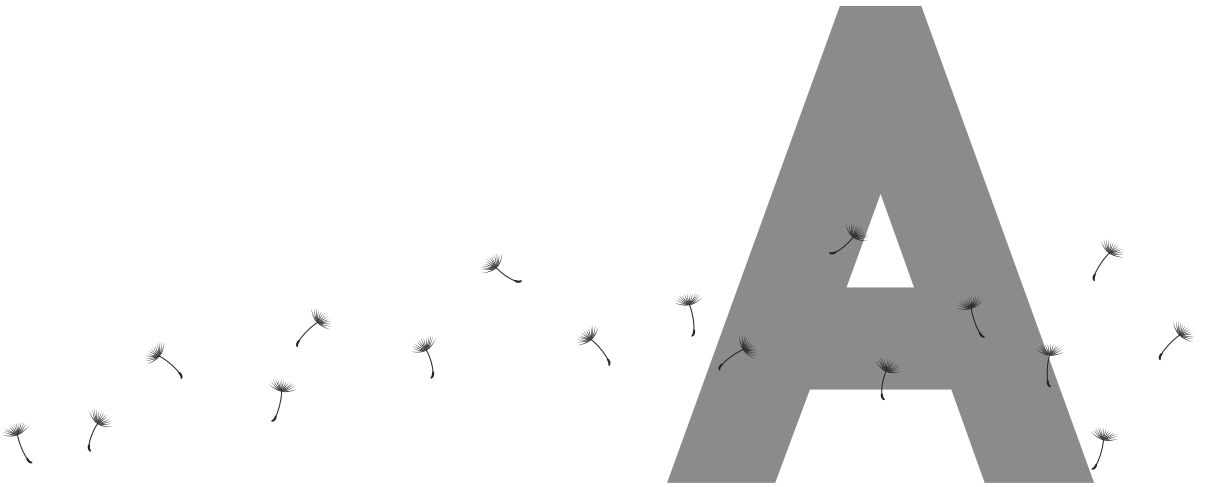
In **hoofdstuk 5** hebben wij dynamische kenmerken van connectiviteit van het brein in rust onderzocht, ook wel resting-state connectiviteit, en deze kenmerken vervolgens gerelateerd aan autistische trekken. We gebruikten daarbij een nieuwe methode, die niet meer uitgaat van de aanname dat connectiviteit stabiel is over een periode van enkele minuten. Wij veronderstelden dat het loslaten van deze aanname zou kunnen leiden tot het aantonen van nieuwe kenmerken van autisme en normale hersenontwikkeling. Wanneer we deze methode toepasten op resting-state data van jonge kinderen lieten we zien dat dynamische patronen van connectiviteit samengevat kunnen worden in vier dynamische staten, die van elkaar verschilden in patronen van connectiviteit en mate van verbondenheid. Verder lieten wij zien dat kinderen met meer autistische trekken meer tijd doorbrengen in een staat waarin verschillende delen van de hersenen niet erg verbonden zijn. Dat patroon zagen we ook bij

jonge kinderen die zich normaal ontwikkelen. Dit zou kunnen betekenen dat kinderen met autistische trekken kenmerken van vertraagde hersenontwikkeling laten zien.

In het tweede deel van dit proefschrift gaan we in op het tweede doel en richten we ons op specifieke cognitieve en neurobiologische kenmerken van kinderen met internaliserende en externaliserende symptomen. In **hoofdstuk 6** bestudeerden we specifieke cognitieve problemen van kinderen met internaliserende, externaliserende en comorbide symptomen. Internaliserende en externaliserende symptomen hebben we zowel continu als categorisch gedefinieerd. In de eerste aanpak keken we naar totaalscores van internaliserende of externaliserende symptomen aan de hand van een spectrum. Echter, kinderen met veel internaliserende symptomen hebben ook vaak veel externaliserende symptomen, hetgeen mogelijk specifieke relaties tussen cognitie en psychopathologie kan verhullen. In de tweede aanpak hebben wij dit probleem getracht te vermijden, door gebruik te maken van latente klassen van kinderen die een relatief homogeen patroon van symptomen lieten zien. Daaronder vielen een klasse met kinderen die vooral internaliserende symptomen lieten zien en een klasse met kinderen die vooral externaliserende symptomen vertoonden. Beide aanpakken wezen erop dat kinderen met internaliserende en externaliserende symptomen verschillende cognitieve patronen vertonen. Kinderen met meer externaliserende symptomen scoorden lager op het gebied van aandacht/executief functioneren, terwijl kinderen met vooral internaliserende symptomen slechter scoorden op verbale en geheugentesten. In de klasse met de meest ernstig aangedane kinderen vonden wij specifieke problemen in het sensomotorische domein. Deze studie laat zien dat internaliserende en externaliserende symptomen een specifieke relatie met cognitie hebben in jonge kinderen en illustreert het nut van een latente klasse-aanpak bij het ontrafelen van hun unieke bijdrage aan deze relatie.

In **hoofdstuk 7** bestudeerden we specifieke morfologische kenmerken gerelateerd aan internaliserend gedrag bij jonge kinderen. Wij hielden daarbij rekening met externaliserende comorbiditeit op twee verschillende manieren, lijkend op de aanpak van hoofdstuk 6. In de eerste aanpak corrigeerden we internaliserende scores voor externaliserende scores, maar daarbij vonden we geen relatie met morfologie van het brein. In de tweede aanpak gebruikten we empirisch tot stand gekomen klassen van gedrag, waarbij we lieten zien dat internaliserende problemen in kinderen gerelateerd waren aan dunnere cortex, vooral in temporale, maar ook in frontale gebieden. Deze bevindingen wijzen erop dat de verschillen in corticale morfologie die we zien bij jonge kinderen met internaliserende problemen wellicht lijken op de verschillen in corticale morfologie die studies bij volwassenen met angst of depressie hebben laten zien.

Tenslotte bespreken we in **hoofdstuk 8** de belangrijkste bevindingen uit deze studies in de context van recente literatuur, en bespreken we methodologische overwegingen, alsmede implicaties van deze studies voor verder onderzoek en de klinische praktijk.



A

Publications and manuscripts

PhD portfolio

Authors and affiliations

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Dankwoord

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PUBLICATIONS AND MANUSCRIPTS

Blanken LME, Mous SE, Ghassabian A, Muetzel RL, Schoemaker NK, El Marroun H, van der Lugt A, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H, White T. Cortical morphology in 6-to-10-year-old children with autistic traits – A population-based neuroimaging study. American Journal of Psychiatry, 2015, 172(5): 479-483.

Blanken LME, White T, Mous SE, Basten MMGJ, Muetzel RL, Jaddoe VW, Wals M, van der Ende J, Verhulst FC, Tiemeier H. Cognitive functioning in children with internalising, externalising and dysregulation problems: a population-based study. European Child & Adolescent Psychiatry, accepted for publication.

Blanken, LME*, Muetzel RL*, Rashid B*, Miller R, Damaraju E, Arbabshirani MR, Erhardt EB, Verhulst FC, van der Lugt A, Jaddoe VW, Tiemeier H, White T*, Calhoun V*. From Chronnectivity to Chronnectopathy: connectivity dynamics of typical development and autistic traits. Submitted for publication.

Blanken LME, Muetzel RL, Jaddoe VW, Verhulst FC, van der Lugt A, Tiemeier H, White T. White matter microstructure in children with autistic traits. Manuscript in preparation.

Blanken LME*, Dass A*, Alvares G, Van der Ende J, Schoemaker NK, El Marroun H, Hickey M, Pennell C, Maybery M, Dissanayake C, Jaddoe VW, Verhulst FC, Tiemeier H, White T, Whitehouse A. A prospective study of fetal head growth in children, autistic traits and autism spectrum disorder. Manuscript in preparation.

Blanken LME*, Ghassabian A*, Muetzel RL; Basten MMGJ, Verhulst FC, El Marroun H, Yeung E. Jaddoe VW, Tonya White T, Tiemeier H. Brain morphology and internalizing problems in young children: a population-based study. Manuscript in preparation.

Meffert H, **Blanken LME**, Blair KS, White SF, Blair JR. The influence of valence and decision difficulty on self-referential processing. Front Hum Neurosci, 2013. (7): 46.

Miliku K, Vinkhuyzen AA, **Blanken LME**, McGrath JJ, Eyles DW, Burne TH, Hofman A, Tiemeier H, Steegers EA, Gaillard R, Jaddoe VW. Maternal vitamin D concentrations during pregnancy, fetal growth patterns, and risks of adverse birth outcomes. American Journal of Clinical Nutrition, 2016;103(6):1514-22.

Mous SE, Schoemaker NK, **Blanken LME**, Thijssen S, van der Ende J, Polderman TJ, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H, White T. The association of gender, age, and intelligence with neuropsychological functioning in young typically developing children: The Generation R study. Appl Neuropsychol Child, 2016, epub ahead of print.

Vinkhuyzen AA, Eyles DW, Burne TH, **Blanken LME**, Kruithof CJ, Verhulst F, Jaddoe VW, Tiemeier H, McGrath JJ. Prevalence and predictors of vitamin D deficiency based on maternal mid-gestation and neonatal cord bloods: The Generation R Study. J Steroid Biochem Mol Biol, 2015, epub ahead of print.

Muetzel RL, Mous SE, van der Ende J, **Blanken LME**, van der Lugt A, Jaddoe VW, Verhulst FC, Tiemeier H, White T. White matter integrity and cognitive performance in school-age children: A population-based neuroimaging study. Neuroimage, 2015, (119):119-28.

Muetzel RL, **Blanken LME**, Thijssen S, Van der Lugt A, Jaddoe VW, Verhulst FC, Tiemeier H, White T. Resting-state networks in 6-to-10 year-old children. Human Brain Mapping, in press.

Serdarevic F, Ghassabian A, van Batenburg-Eddes T, White T, **Blanken LME**, Jaddoe VW, Verhulst FC, Tiemeier H. Infant muscle tone and childhood autistic traits. A longitudinal study in the general population. Autism Research, in revision.

Vinkhuyzen AA, Eyles DW, Burne THJ, **Blanken LME**, Kruithof CJ, Verhulst FC, Jaddoe VW, Tiemeier H, McGrath JJ. Gestational vitamin D deficiency and autism-related traits: The Generation R Study. Molecular Psychiatry, under review.

Adams HHH, Hibar DP, Chouraki V, Stein JL, …**Blanken LME**, …Debette S, Medland SE, Ikram MA, Thompson PM. Common genetic variation underlying human intracranial volume highlights developmental influences and continued relevance during late life. Nature Neuroscience, accepted for publication.

Muetzel RL, **Blanken LME**, van der Ende J, El Marroun H, van der Lugt A, Jaddoe VW, Verhulst FC, Tiemeier H, White T. Childhood psychiatric symptoms and white matter development: a longitudinal population-based neuroimaging study. American Journal of Psychiatry, in revision.

Langen C, Muetzel RL, **Blanken LME**, Tiemeier H, van der Lugt A, Jaddoe VW, Verhulst FV, Niessen WJ, White T. Differential patterns of age-related cortical and subcortical functional connectivity in school-age children: A connectome-wide association study. Manuscript in preparation.

Bouhuis RH, **Blanken LME**, Muetzel RL, van der Lugt A, El Marroun H, Jaddoe VW, Verhulst FC, Tiemeier H, White T. Cavum septum pellucidum in the general population and its relation to surrounding brain structures, cognitive function and emotional or behavioral problems. Manuscript in preparation.

White T, Muetzel RL, El Marroun H, **Blanken LME**, Jansen P, Bolhuis K, Kocevska D, Jaddoe VW, van der Lugt A, Verhulst FC, Tiemeier H. Pediatric population neuroimaging and the Generation R Study: the second wave. Manuscript in preparation.

Di Martino M, O'Connor D, Chen B, Alaerts K, … **Blanken LME**, …Cameron Craddock C, Lord C, Leventhal B, Milham M. The Autism Brain Imaging Data Exchange II for enhancing studies of the intrinsic brain connectome in autism. Manuscript in preparation.

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1. PhD Training		
General courses		
Masters degree Health Sciences, specialisation Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands		
Erasmus Summer Programme:		
Principles of Research in Medicine and Epidemiology	2012	0.7
Clinical Decision Analysis	2012	0.7
Methods of Public Health Research	2012	0.7
Markers and Prognostic Research	2012	0.7
The Practice of Epidemiologic Analysis	2012	0.7
Pharmaco-epidemiology	2012	0.7
Conceptual Foundation of Epidemiologic Study Design	2013	0.7
Principles of Genetic Epidemiology	2013	0.7
From problem to solution in public health	2012	0.7
Psychiatric Epidemiology	2013	0.7
Women's Health	2013	0.7
History of Epidemiologic Ideas	2013	0.7
Social Epidemiology	2013	0.7
Core Curriculum:		
Study Design	2013	4.3
Clinical Epidemiology	2013	5.7
Methodologic Topics in Epidemiologic Research	2013	1.4
Biostatistical Methods I: Classical Methods for Data-analysis	2012	5.7
Biostatistical Methods II: Classical Regression Models	2012	4.3
Advanced courses:		
Missing Values in Clinical Research	2013	0.7
Courses for the Quantitative Researcher	2012	1.4
Principles of Epidemiologic Data-analysis	2013	0.7
Specific Courses		
MRI Safety course, Erasmus MC, Rotterdam, the Netherlands	2010	0.3
Endnote course, Erasmus MC, Rotterdam, the Netherlands	2012	0.3
Zoeken in Pubmed, Erasmus MC, Rotterdam, the Netherlands	2012	0.3

Zoeken in andere databases, Erasmus MC, Rotterdam, the Netherlands	2012	0.3
Basiscursus Regelgeving en Organisatie Klinische trials, Erasmus MC, Rotterdam, the Netherlands	2012	1.0
Tools to parcellate the brain and its relation to function, Honolulu, USA	2013	0.3
Reproducible Neuroimaging Honolulu, USA	2015	0.3
FSL & FreeSurfer course, Oxford, UK	2014	2.0
International Conferences		
Human Brain Mapping, Hamburg, Germany (poster presentation)	2014	1.2
Human Brain Mapping, Honolulu, USA (poster presentation)	2015	1.2
International Meeting for Autism Research, Baltimore, USA (poster presentation)	2016	0.9
Workshops, Meetings and Symposia		
Symposium 'Brain Development and Developmental Disorders', Utrecht, the Netherlands	2012	0.3
Sophia Research Days, Rotterdam, the Netherlands (oral presentation)	2014	0.6
Child and Adolescent Psychiatry Colloquia, Erasmus MC, Rotterdam, the Netherlands (1 oral presentation)	2010-2014	1.0
Generation R research meetings, Erasmus MC, Rotterdam, the Netherlands (oral presentation)	2010-2014	1.0
Workshop "Media contacts for researchers", Erasmus MC, Rotterdam, the Netherlands	2015	0.1
Workshop "How to present your poster", Erasmus MC, Rotterdam, the Netherlands	2015	0.3
Various workshops of "VENA", Network for academic women in the Erasmus MC	2013-2015	1.0
2. Teaching Activities		
Supervising Master's Theses		
Angelica Uitterlinden (Clinical Neuropsychology, Leiden University)	2012	3.0
Attention in Children with the CBCL Dysregulation Profile		
Roos Bouhuis (Medicine, Erasmus MC)	2013	3.0
Cavum septum pellucidum in the general population and its relation to surrounding brain structures, cognitive function and emotional or behavioral problems.		
Nienke de Bles (Clinical Neuropsychology, Leiden University)	2015	3.0
The relation between autistic traits and neuropsychological functioning in school-aged children from the general population		
Other Teaching Activities		
Supervision of and giving lectures for 2nd year medical students, Erasmus University, Rotterdam, the Netherlands	2014-2015	1.0
Supervising various workgroups for 3rd year medical students, Erasmus University, Rotterdam, the Netherlands	2014-2015	1.0
Lectures for Landelijke Onderwijsdag Kinder- en Jeugdpsychiatrie (two lectures)	2014-2015	1.0
Supervision of and giving lectures for 3rd year medical students, Erasmus University, Rotterdam, the Netherlands	2014-2015	1.0
3. Other Activities		
Coordination of MRI brain incidental findings	2013-2014	3.0
Autism case ascertainment through general practitioners	2015-2016	6.0
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Laura Mary Elisabeth Blanken was born on the 29th of April 1986 in Amsterdam, the Netherlands. She grew up in Naarden and in 2004, she graduated cum laude from the Willem de Zwijgercollege in Bussum. She went on to study Medicine at Leiden University. Laura always had a special interest in psychiatry and during her studies she took part in an honors program called 'Psychiatric Disorders across the Life-span: A Biopsychosocial Interplay'. Her interest in neuroimaging was triggered by elective courses, such as "Imaging Technology" and "Cognitive Neuroscience". As part of her scientific internship, she explored the field of stress research and studied the influence of the mineralocorticoid receptor activation on learning strategies and memory in mice. During her clinical rotations, her long-standing interest in psychiatry was further confirmed at an internship at Curium, Academic center for Child and Adolescent Psychiatry and she chose Acute Psychiatry (Parnassia, Den Haag) and Child and Adolescent Psychiatry (Bascule, Duivendrecht) as elective and senior internships. In 2010, she graduated cum laude from Medical school. She went on to do clinical work at an outpatient clinic for children with disruptive behavior disorders at the Bascule, Academic center for Child and Adolescent Psychiatry in Duivendrecht. From January to April 2012, she visited the National Institutes of Mental Health as a special volunteer to perform a neuroimaging study under the supervision of Dr. James Blair, at the Unit of Affective and Cognitive Neuroscience. In April 2012, she started the work described in this thesis at the Department of Child and Adolescent Psychiatry and the Generation R Study Group at Erasmus MC-Sophia in Rotterdam. In 2013, Laura obtained a Master of Science Degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences. From April to September 2016, Laura was a visiting fellow at professor Baron-Cohen's Autism Research Centre in Cambridge.
In 2017, Laura will start her residency in Psychiatry at the Amsterdam Medical Center. She hopes to go on to combine clinical practice and research in psychiatry.

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