From THE DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

AUTISM SPECTRUM DISORDER IN ADULTS – BIOLOGICAL DIMENSIONS

Irina Manouilenko

Karolinska Institutet

Stockholm 2013

Published by Karolinska Institutet. Printed by Larserics Digital Print AB

Bookcover: " The brain is the universe" by Camilla Manouilenko Rosales

ISBN 978-91-7549-226-1

To Camilla, Valentina, Anatoly, Alexey

ABSTRACT

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental conditions characterized by difficulties in social interaction, communication and the presence of repetitive or stereotyped behaviors. Previous studies have demonstrated structural and functional abnormalities in different brain regions in ASD. Motor difficulties, unusual perception and minor physical anomalies have been reported but not systematically investigated in the adult population with ASD and normal intelligence.

In **Study I**, the internal consistency as well as diagnostic and concurrent validity of the Swedish version of the Ritvo Autism Asperger Diagnostic Scale - Revised (RAADS-R) were evaluated. The results imply that the Swedish version of the RAADS-R has good psychometric properties and is strongly correlated with the Autism-Spectrum Quotient (AQ). The RAADS-R captures ASD symptoms and can be used for screening of ASD as well as in the assessment of ASD in adults with normal intelligence.

In **Studies II and III**, regional cerebral blood flow (rCBF) was assessed by positron emission tomography (PET) in thirteen adults with ASD and ten neurotypical controls after psychiatric and neurological assessments. In comparison with the neurotypical controls, individuals with ASD showed significantly increased cerebral blood flow bilaterally in large parts of cerebellum, occipital associative cortex and posterior parietal cortex. In Study III, principal components corresponding to "Autistic/ADHD symptoms", "Sensori-motor integration" and "Intelligence/motor sequencing" were identified by factor analysis based on the normalized scores of 13 neuropsychological measures. The positive correlation between ''Autistic/ADHD traits'' and rCBF in the caudate indicates a possible association of CBF changes with the executive impairments and ritualistic or stereotyped behaviors typical for ASD. Furthermore, "Sensorimotor integration" was correlated with rCBF in the occipital visual cortex, reflecting an atypical visual perception often reported in ASD. Cerebral blood flow in the left thalamus was negatively correlated with all three factors which supports the implication of this brain region in the pathophysiology of ASD. Autistic traits and ADHD symptoms were associated with shared neural substrates whereas sensory-motor deficits were grouped in another independent factor and correlated with rCBF in other regions.

In **Study IV**, minor physical anomalies (MPAs) were investigated in 53 individuals with ASD and 50 age- and gender matched controls. The ASD group showed significantly more MPAs in comparison to the control group. Moreover, MPAs were correlated with severity of symptoms and overall functioning according to the Global Assessment of Functioning (GAF).

On the whole, various behavioral, cognitive, neurological and morphological signs are suggested to converge into the ASD phenotype. Thus, in order to understand the complexity of ASD it seems meaningful to include assessment of ADHD symptoms, subtle neurological abnormalities and minor physical anomalies in the clinical evaluation of adults with ASD.

LIST OF PUBLICATIONS

I. Andersen LM, Näswall K, Manouilenko I, Nylander L, Edgar J, Ritvo RA, Ritvo E, Bejerot S.
The Swedish Version of the Ritvo Autism and Asperger Diagnostic Scale: Revised (RAADS-R). A Validation Study of a Rating Scale for Adults.
Journal of Autism and Developmental Disorders, 2011, 41: 1635-1645

II. Pagani M, Manouilenko I, Stone-Elander S, Odh R, Salmaso D, Hatherly R, Brolin F, Jacobsson H, Larsson SA, Bejerot S.
Brief Report: Alterations in Cerebral Blood Flow as Assessed by PET/CT in Adults with Autism Spectrum Disorder with Normal IQ.
Journal of Autism and Developmental Disorders, 2012, 42: 313-318

III. Manouilenko I, Pagani M, Stone-Elander S, Odh R, Salmaso D, Hatherly R, Brolin F, Jacobsson H, Larsson SA, Bejerot S.
Autistic traits, ADHD symptoms, neurological soft signs and regional cerebral blood flow in adults with autism spectrum disorders.
Research in Autism Spectrum Disorders, 2013, 7:566-578

IV. Manouilenko I, Eriksson Jonna M., Humble Mats B., Bejerot S.
Minor Physical Anomalies in Adults with Autism Spectrum Disorders and Healthy Controls.
(submitted)

CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview Revised
ADOS	Autism Diagnostic Observation Schedule
ADOS-4	Autism Diagnostic Observation Schedule, module 4
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ASRS	Adult ADHD Self-Report Scale
ASD	Autism Spectrum Disorder
AQ	Autism Spectrum Quotient
APA	American Psychiatric Association
AUC	Area under ROC curve
BA	Brodmann's area
CBF	Cerebral blood flow
CNV	Copy numbers variations
CMRgl	Cerebral metabolic ratio of glucose
CT	Computed Tomography
DISCO	Diagnostic Interview for Social and Communication disorders
DNA	Deoxyribonucleic acid
DMN	Default mode network
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th edition
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th edition
GAF	Global Assessment of Functioning
fMRI	Functional magnetic resonance imaging
IQ	Intelligence quotient
M.I.N.I.	Mini International Neuropsychiatric Interview
MM	Minor malformation
MPA	Minor physical anomalies
MRI	Magnetic resonance imaging
NES	Neurological Evaluation Scale
OCD	Obsessive compulsive disorder
PDD-NOS	Pervasive developmental disorder- Not Otherwise Specified
PET	Positron emission tomography
PV	Phenogenetic variant
RAADS-R	Ritvo Autism and Asperger Diagnostic Scale-Revised
rCBF	Regional cerebral blood flow
ROC	Receiver operating characteristic
SCID	Structured Clinical Interview for DSM-IV
SD	Standard deviation
SNPs	Single-nucleotide polymorphisms
SPECT	Single photon emission computed tomography
SPM	Statistical parametric mapping
WURS	Wender Utah Rating Scale

1 INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition with persistent impairments in social interaction and communication, and restricted, repetitive patterns of behavior, interests or activities as well as unusual sensory responses. ASD should be considered a syndrome with a high degree of variability (i.e. the autistic spectrum) with impact on the overall functioning in everyday life. Usually, the ASD symptoms are already present in the early childhood but in some cases they may not be obvious until social demands exceed an individual's limited abilities.

Although, historically ASD has always been viewed as a pediatric disorder, in the last 10-20 years research has demonstrated that autism spectrum related problems and symptoms persist into adulthood. In accordance with the recently published DSM-5 classification system, ASD includes previously separate diagnostic categories according to DSM-IV such as Autistic Disorder, Asperger's Disorder and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) (APA, 1994). This updated classification contributes to the view of autism as a spectrum of conditions which affect individuals on variety of different levels.

According to recent data from the UK, the lifetime cost for adults with high-functioning ASD is estimated to approximately 2.9 million pounds (around 32 million Swedish crowns).
In adults with ASD, implicit costs lead to loss of employment for the individuals and their parents, accompanied by a raised living cost in terms of supported accommodation or a care home (Knapp et al., 2007). However, those adults who have ASD and fall in the normal IQ range appear to become lost in services and they tend to go without accesses to community services or supporting program (Brugha et al., 2011).

1.1 ETIOLOGY

The etiology of ASD implies a complex interaction between genetic and environmental variables.
The primary risk factor for autism is a predetermined genetic component at 70-90%, and siblings of individuals with ASD are at increased risk in comparison to people in the general population (Bailey et al., 1995; Steffenburg et al., 1989). Since parents and siblings of people with autism frequently show subtle autistic traits as well as subtle deviations in cognitive, language and motor abilities, a so called Broader Autism Phenotype has been identified through twin and family studies (Sucksmith et al., 2011; Bolton et al., 1994; Sasson et al., 2013; Le Couteur et al., 1996).

Moreover, several prenatal risk factors were found to be associated with diagnosis of ASD, such as an advanced maternal and paternal age along with an advanced grandparental age (Gillberg, 1980; Frans et al., 2013). Finally an array of additional risk factors can include mothers born abroad, mothers with low pre-pregnancy weight, maternal viral infection during the first trimester and maternal bacterial infection in the second trimester, maternal exposure for valproic acid or thalidomide during the early

pregnancy, breech presentation of foetus, pre-pregnancy diabetes, gestational diabetes and pre-eclampsia (Atladottir et al., 2010; Stromland et al., 1994; Burstyn et al., 2010; Gardener et al., 2009; Barnevik-Olsson et al., 2010) as well as urbanization (Lauritsen et al., 2005).

ASD is highly heritable, but the route of inheritance is unclear. Although extensive research has been carried out in identifying the carrier-promoting genes, no single gene has been linked to ASD yet. Furthermore, latest analysis of *de novo* mutations and copy numbers variations (CNV) suggest multiple genes contribution to the disorder rather than a single protagonist gene (O'Roak et al., 2012). However, two types of autism, sporadic (or "simplex") and familial (or "multiplex"), have been identified in genetic studies and it was suggested that these two types are genetically different. The rate of spontaneous mutations (be it deletions or duplications) such as CNV in sporadic cases was significantly higher than in the familial cases (Sebat et al., 2007).

Recent evidence suggests that epigenetic changes, presumably of environmental origin contribute to the development of ASD. Epigenetic changes regulate gene expression through DNA methylation without changing the actual DNA sequences. This mechanism of interaction between genes and environment was explored by studying pattern and number of DNA methylation in relation to severity of autistic traits (Wong et al., 2013). Indeed, there are several medical conditions that are etiologically linked to autism including tuberous sclerosis, neurofibromatosis, phenylketonuria, fragile X syndrome and 15q11-13 deletions.

1.2 PREVALENCE

ASD has a prevalence rate of 1-2 % in the child population (Wingate et al., 2012) and there is no evidence for lower rates of ASD among adults (Brugha et al., 2011). The male: female ratio is about 4:1 (Fombonne, 2009). The lower sex ratio 3:1 was observed in those individuals with higher rates of minor physical anomalies accompanied by a greater frequency in cognitive impairment (Miles et al., 2005). Almost all the prevalence data comes from studies performed in high-income countries, little evidence is available from middle-low income countries. The prevalence rates in Europe and America are at the same level for autistic disorder (median 19/10 000 in Europe; 22/10 000 in America) and PDD-NOS (62/10000 in Europe and 65/10000 in America) (Elsabbagh et al., 2012), however studies from Asian regions report much more inconsistent results (16.1- 181/10 000) (Kim et al., 2011; Wong and Hui, 2008).

1.3 PHENOTYPES

The manifestation of ASD symptoms (i.e. the phenotypical expressions) varies across the spectrum. A behavioral description of ASD can be made on the basis of the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), clinical observations or using self-report scales. Lorna Wing suggested four subgroups of social style are useful in clinical setting: Aloof group, Passive group, Active-but-odd group and Loners (Wing, 1997) (Table 1).

Table 1. Lorna Wings` suggestions on subtypes of ASD

Aloof group	Passive group	Active-but-odd group	Loners
Aloof and indifferent to others. Fit most closely into the popular picture of autism. They do not use non-verbal body language to accompany or substitute spoken speech. Gestures tend to be confined to pulling people along to use them as tools to obtain some desired object. Eye contact is inappropriate during social situations. Resistance to change in their elaborate repetitive routines. Their repetitive behavior usually consists of odd movements of limbs and body, known as motor stereotypies, and fascination with simple sensory stimuli, such as bright lights.	Social impairment presents as not taking social initiatives but responds to them. They have the impairments in communication and imagination and additional features similar to the aloof group, but usually in less florid form. They tend to be more amenable in behavior and less obviously upset at interference with their repetitive routines. Some have intellectual ability in the average or high range and manage to complete mainstream school, at least in the primary years.	Active social approaches that are naive, odd, inappropriate, and one-sided. They tend to fit Asperger's clinical descriptions of his syndrome. Their speech is often fluent with good grammar and vocabulary but repetitive and not used for reciprocal conversation. Play is concerned only with one or a few themes and usually not shared with other children. The repetitive routines take the form of fascination with and talking about particular topics. Any topic can become a special interest and the focus may change from time to time.	The most subtle form. Found in people of average, high, or outstanding intellectual ability, including fluent speech, who tend to prefer to be alone, lack empathy, and are concerned with their own interests regardless of peer-group pressures. As adults, may follow successful careers, sometimes of high academic distinction. Some learn the rules of social interaction by rote, while others remain solitary by choice. Some marry, but partners may report a lack of emotional rapport. Describe their experiences of the world as a confusing and frightening place.

In addition to the behavioral phenotypes, a wide range of the so-called intermediate phenotypes (endophenotypes) have been explored in ASD. Endophenotypes are heritable traits that link genes and observable symptoms or behaviors. They may be represented through neurophysiological, biochemical, endocrinological, neuroanatomical or neuropsychological features (Gottesman and Gould, 2003).

1.4 ATYPICAL BRAIN DEVELOPMENT

The autism phenotype is a consequence of an atypical brain development (Kaplan et al., 2001; Kaplan et al., 2006) during pre- and postnatal periods of life (Acosta and Pearl, 2003). Histopathological studies in ASD have shown increased neurogenesis, abnormal distribution, number and size of neurons, changes in the elaboration of axons, dendrites and synapses, and dysfunction of neuronal pathways (Bailey et al., 1998; Bauman and Kemper, 1985).

According to a growth dysregulation hypothesis in autism, abnormal differentiation in affected brain areas might result in atypical neuronal organization and functional activity (Akshoomoff et al., 2002). All these processes can impact on neuronal synchronization between and within neurofunctionally segregated regions and pathways and result in disconnectivity and dissynchrony in the autistic brain (Gepner and Feron, 2009). However, similar phenomena were also found in Attention Deficit Hyperactivity Disorder (ADHD) (Fair et al., 2010). From the clinical point of view, these anatomo-functional similarities in neurodevelopmental conditions may be associated to a high degree, not only due to the co-existence of different neurodevelopmental dysfunctions, but also to impairments in various neuropsychological dimensions, such as communication and language, social interaction, motor coordination, attention, activity, mood, and/or sleep, as shown in children and summarized in the acronym ESSENCE Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations (Gillberg, 2010).

1.5 DIAGNOSTIC PROCEDURE IN ADULTS

To diagnose ASD in adults is a complicated clinical task since DSM-IV criteria were not developed for adults, and parents are not always available for describing childhood symptoms that are a requisite for an ASD diagnosis. However, due to the clinical awareness of this disorder, an increasing group of psychiatric patients are diagnosed with ASD today (Murphy et al., 2011). In addition, high prevalence of co-morbid psychiatric disorders (Hofvander et al., 2009; Joshi et al., 2010; Rydèn and Bejerot, 2008) and a wide range of co-occurring symptoms (Reiersen et al., 2007; Reiersen and Todd, 2008) are often present in adults with ASD .

The ASD diagnosis in adults is based on the observation of behaviors, history of childhood symptoms, results from self-rating scales, structured and semi-structured interviews with patients and an interview with parents when this is possible. However, there are several difficulties in diagnosing ASD in adults. Not everyone has a next of kin who can provide information about childhood symptoms, which is necessary for several diagnostic interviews, and not everyone wants to involve their parents in the diagnostic procedure. Sometimes, only self-reports and clinical observations are the available tools.

Presently there are only two validated widely used self- administered scales that purport to measure autistic symptoms in adults. One is the Autism-Spectrum Quotient (AQ) (Baron-Cohen et al., 2001), the other one is the Ritvo Autism and Asperger Diagnostic

Scale-Revised (RAADS-R)(Ritvo et al., 2008). Both are useful tools for screening autistic traits, measure of severity and for providing a symptom profile. However, the Gold standard for diagnosis of ASD is Autism Diagnostic Observation Schedule (ADOS) and the Diagnostic Interview for Social and Communication disorders (DISCO) or Autism Diagnostic Interview Revised (ADI-R), in addition to patient records and a clinical interview. Notably, no single instrument is sufficient for diagnostic purposes due to the complexity of the clinical presentation of ASD. For instance, a previous study in children has found that the combination of an informant's self-reports of childhood symptoms and clinical observations are more stable over time than results from a single assessment scale (Kim and Lord, 2012; Lord et al., 2006).

1.5.1 Self-rating scales in diagnostic procedure

In general, adults with ASD have a well recorded history of problems related to their impairments in terms of social interaction and communication, accompanied by patterns of restricted, repetitive behaviors and/or activities. Consequently, people with ASD have low rates of employment and social relationships (i.e. friendships or intimate relationships) and rarely live independently (Howlin and Moss, 2012). Adults with ASD often suffer from comorbid depression, anxiety disorder or obsessive-compulsive disorder (Tantam and Girgis, 2009) which might lead to referrals to a psychiatric consultant.

In the diagnostic procedure of adults with ASD, self-rating scales may be used for screening purposes as well as to support the clinician´s evaluation. At times the healthcare practitioners lack the opportunity to include information from parents or significant others since some adults do not want to involve them in assessment procedures, or parents may be deceased. In these cases self-ratings can provide important information about symptoms in childhood, current symptoms and severity. In addition, for some people with ASD it may be easier to respond to paper-and-pen questions to allow extra time to think through the response alternatives without experiencing the stress of an interview.

Autistic symptoms in adults can be measured by two of the validated self-rating scales: the Autism-Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) that assesses cognitive aspects of ASD, and the Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R) which covers both sensory-motor functions and cognitive traits (Ritvo et al., 2008).

Despite the advantages of those self-reports scales we need to be aware of their limitations. Individuals with ASD generally have poor insight into their symptoms (Woodbury-Smith et al., 2005), deeming any self-ratings of their ASD symptoms unreliable. Thus high self-rating scores require consideration as they might be high either because symptoms are more severe, or due to an overestimation of subjective ASD symptoms. In addition, it needs to be recognized that a person with ASD may self-score highly but nevertheless be less affected than the scores suggest. Also, attention or mentalizing deficits may lead to misinterpretations of the meaning of the questions. Therefore, some people with ASD might require assistance when filling out self-rating scales.

1.5.2 Differences between DSM-IV and DSM-5 criteria

The DSM-IV criteria for autism and Asperger syndrome were developed for diagnosis in childhood, specifically from the ages 5-8 years. Therefore, it has been difficult to apply the DSM-IV criteria when diagnosing adolescents and adults. Moreover, females are much less often diagnosed than males with ASD (Shattuck et al., 2009). The aim with introducing the new DSM-5 criteria for ASD was to establish more useful diagnostic criteria as well as to improve the sensitivity and specificity (Swedo et al., 2012).

The most important change is the term Autism Spectrum Disorder that will replace earlier diagnostic subcategories, i.e. Autistic Disorder, Asperger's Disorder and PDD-NOS. This change underscores that former subcategories were actually variants of the same underlying condition with variations in severity, intelligence and language level.
Secondly, assessment of functional impairment in everyday life will be made according to the level of severity. The defined severity level is dependent on how much support the individual needs due to impairments in social communication, restricted interests and repetitive behaviors. Thirdly, persistent deficits in social communication and social interaction will include two domains from DSM-IV (Communication and Social Interaction) in addition to abnormal reactions to sensory input. This item is included in the criteria within the domain "restricted, repetitive patterns of behaviors".
The item "delay in language development" is no longer a diagnostic requirement. The age of onset of functioning delays have been extended until "social demands exceed limited capacities" along with presence of symptoms in early childhood.
Finally, a new diagnostic category "Social Communication Disorder" has been added to the DSM-5. This diagnosis will be given to individuals with difficulties in "social communication and social interaction", but without "repetitive/restricted behavior and interests".

1.5.3 Co-occurrence of autistic traits, ADHD-symptoms and sensory-motor impairments

The co-morbidity of psychiatric disorders has been discussed over the years. Bonnie Kaplan introduced the term Atypical Brain Development as an alternative to previously used Minimal Brain Dysfunction to underscore both weaknesses and strengths that may be seen as consequence of deviant brain development (Kaplan et al., 2001). In this conceptual framework a term 'co-occurrence' was suggested as more appropriate when discussing co-existence of psychiatric disorders (Kaplan et al., 2006). It was found that a substantial proportion of the genetic variance for ASD was shared with other neurodevelopmental disorders such as ADHD and developmental coordination disorder (Lichtenstein et al., 2010). Until recently, the diagnostic criteria for ADHD in the DSM-IV have not allowed for patients with ASD to be given an ADHD diagnosis (APA, 1994; Kooij et al., 2010). However, investigations indicate that ADHD symptoms are present in about 20-80% of children (Sturm et al., 2004) and reported in almost half of the clinical adult ASD population (Rydèn and Bejerot, 2008; Hofvander et al., 2009). Moreover, the severity of the ASD seems to correlate with the co-occurrence of ADHD symptoms (Holtmann et al., 2007).

Impairment of motor control, including neurological soft signs (i.e. subtle impairments of sensory integration, motor coordination and difficulties in sequencing complex motor tasks) is common in ASD (Mahone et al., 2006; Mayoral et al., 2010; Sahlander et al., 2008; Tani et al., 2006; Jansiewicz et al., 2006). It is often assumed that there is a relationship between motor dysfunction and the core impairments in ASD (Leary and Hill, 1996a) leading to the conclusion that motor dysfunction precedes the symptoms of linguistic and social problems (Teitelbaum et al., 2004).

The so-called psychiatric comorbidity is frequent in the ASD-population (Hofvander et al., 2009; Joshi et al., 2010; Rydén and Bejerot, 2008). Co-existing disorders alongside ASD can be described as distinct categories, but presumably they share common neural substrates. Anxiety, depressive symptoms and OCD-features are not included in the ASD diagnostic criteria but they are often expressed by people with ASD and arguably should be viewed as part of ASD phenotype. This is supported by the recognition that certain brain regions that are engaged in ASD are also affected by other psychiatric disorders (Ravindran et al., 2009; Kleinhans et al., 2010; Menzies et al., 2008; Seminowicz et al., 2004).

Recently, specific single-nucleotide polymorphisms (SNPs) in calcium-channel activity genes were found to be correlated with a range of psychiatric disorders with onset in childhood (ASD, ADHD) as well as some in adulthood (schizophrenia, bipolar disorder and major depression) (Smoller et al., 2013). The findings suggest that various psychiatric disorders may share genetic substrates.

1.6 BIOMARKERS AND ENDOPHENOTYPES

A biomarker (biological marker) is an indicator of biological processes (normal or pathogenic) or responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001). A biomarker can be objectively measured, it is associated with particular condition or disorder and it is stable across and within individuals. From clinical point of view, the biomarker represents a characteristic or a variable that related to how patients feel or the level of functioning. Furthermore, biomarkers can be applied to identify possible risk factors or to support a diagnosis.

1.6.1 Biomarkers in autism

In the past two decades a number of studies have sought to determine biomarkers for ASD. However, a major problem with such type of study is the complexity of the ASD phenotype as symptoms overlap with other psychiatric disorders and also individuals can change their position on the ASD spectrum over time. Therefore, only few clinically useful biomarkers have been identified (Walsh et al., 2011), such as biomarkers for susceptibility (e.g. common and rare genetic variants, epigenetic and environmental factors) (Bolton et al., 2012; Sebat et al., 2007), diagnostic biomarkers (e.g. brain morphology and function, head size, serum or urinary metabolites) (Waiter et al., 2004; Ecker et al., 2010; Herbert, 2005; Lainhart et al., 2006; Yap et al., 2010; Napolioni et al., 2013) and predictive biomarkers (e.g. minor physical anomalies in children) (Ozgen et al., 2012). However, much uncertainty still exists about the relationship between proposed biomarkers and clinical impairments. Thus, exploring

endophenotypes (i.e. intermediate phenotypes) has been suggested as one possible strategy in integrating neurobiological finding with phenotypical expression of the disorder (Sacco et al., 2010; Aldridge et al., 2011; Losh et al., 2008).

1.6.2 Neuroimaging in ASD

ASD is viewed as a distributed neural system disorder that disproportionately impairs many higher order abilities. Earlier studies suggest broad involvement of cortical and subcortical regions such as orbitofrontal cortex, inferior frontal gyrus, superior temporal sulcus and gyrus, middle temporal gyrus, right parahippocampal gyrus, amygdala, fusiform gyrus, anterior and posterior cingulate gyrus, caudate, thalamus and cerebellum (Amaral et al., 2008). These regions are considered to be the neuroanatomical substrates for language, speech, mentalizing processes and empathy; functions highly affected in individuals with ASD (Frith and Frith, 2003). Also, changes in basal ganglia, orbitofrontal cortex, anterior cingulate and thalamus are related to repetitive behaviors typically seen in ASD (Langen et al., 2011).

The human brain is anatomically and functionally organized into complex networks, which allows both segregation and integration of information. Functional MRI (fMRI) and Positron Emission Tomography (PET) studies have postulated that ASD is a disorder of disconnectivity and dissyncrony among different brain regions (Gepner and Feron, 2009). Underconnectivity at the long-range level and overconnectivity at the low-range level were found in ASD (Minshew and Keller, 2010).

The default mode network (DMN) has been found to be active mainly at rest when individuals are not engaged in any cognitive tasks and focused on self-generated mental processes, including autobiographical memory retrieval, future visualization and conceiving the perspectives of others (Gusnard et al., 2001; Raichle et al., 2001). Therefore, DMN is of particular interest in ASD since it represents the active organized baseline mode of brain function (Papo, 2013). Activation of the core regions of DMN such as medial frontal regions (the medial prefrontal cortex and anterior cingulate), medial parietal areas (the precuneus and posterior cingulate), lateral parietal areas (left and right angular gyri), and medial temporal areas (hippocampus and parahippocampal gyrus) were investigated during resting states of individuals with ASD (Kennedy et al., 2006; Cherkassky et al., 2006; Kennedy and Courchesne, 2008). In order to understand the relationship between brain and behavior, behavioral characteristics have been included in studies in which brain perfusion has been investigated. For instance, regional activity in the DMN in ASD has been correlated with the severity of social dysfunction, communication deficits and restricted and repetitive behaviors (Monk et al., 2009; Weng et al., 2010).

1.6.3 Neurological Soft Signs

The neurological soft signs are neurological impairments that do not indicate focal neurological symptoms and therefore are assumed to be non-specific signs of cerebral dysfunction. Neurological soft signs include subtle neurological abnormalities of sensory integration, motor coordination and sequencing of complex motor tasks (Buchanan and Heinrichs, 1989). A high prevalence of neurological soft signs compared to healthy controls was found in patients with schizophrenia, bipolar disorder

(Negash et al., 2004), obsessive–compulsive disorder (Bolton et al., 1998; Tumkaya et al., 2012) and ASD (Tani et al., 2006). Previous studies, mainly performed in patients with schizophrenia, have reported significant correlations between neurological soft signs, severity of symptoms and cognitive functions (Peralta et al., 2010; Arango et al., 1999; Sewell et al., 2010). More recently, neurological soft signs were suggested as potential endophenotypes in schizophrenia (Smit et al., 2012; Chan and Gottesman, 2008).

Adults with ASD have deficits in both gross and fine motor skills (Mayoral et al., 2010; Sahlander et al., 2008). Weak central coherence, perceptual hypersensitivity, defective automatization of movements and clumsiness are also often described. It is reasonable to argue that the neurological soft signs can be one of the potential endophenotypes for ASD and indicate an increased vulnerability for ASD.

1.6.4 Minor Physical Anomalies

Minor Physical Anomalies (MPAs) are defined as small anomalies in the formation of the craniofacial region and limbs, they occur without any significant cosmetic or functional impact to the individual. They are assumed to represent markers of deviant morphogenesis during the first or early second trimester of pregnancy (Waldrop et al., 1968; Spranger et al., 1982) and have ectodermal embryonic origins in common with the developing brain. MPAs include minor malformations and phenogenetic variants. *Minor malformations* are qualitative defects of embryogenesis arising during organogenesis and are true deviations from normal. *Phenogenetic variants* are quantitative defects arising after organogenesis and represent the exact equivalents of normal anthropometric variants (Spranger et al., 1982; Opitz, 2000) (Table 2). MPAs are stable over time and can be easily assessed in children as well as in adults (McNeil and Cantor-Graae, 2000). Previous studies suggest that genetic factors as well as prenatal events, such as maternal bleeding following foetal hypoxia, gestational diabetes, medication use or toxaemia contribute to MPAs (Campbell et al., 1978; Stromland et al., 1994; Gardener et al., 2009; O'Roak et al., 2012).

Recent evidence suggests a significant association between MPAs and autism (Ozgen et al., 2010). Moreover, it has been postulated that MPAs may be used as indicators of severity of the disorder (McGrath et al., 1995). Since high frequencies of MPAs have been found in patients with schizophrenia, affective disorder, ADHD and Tourette syndrome in comparison with neurotypical controls, they have been suggested to be markers of risk for certain psychiatric disorders (Akabaliev et al., 2011; Trixler et al., 2001; Waldrop et al., 1978; Csabi et al., 2008; Ismail et al., 1998). Specifically in studies of children with ASD excessive MPAs have been found when compared to typically developing children (Ozgen et al., 2011; Rodier et al., 1997; Tripi et al., 2008; Walker, 1977). However, far too little attention has been paid to investigation of MPAs in adults with ASD and little is known about association between MPAs and ASD symptoms in relation to the level of everyday functioning. Only one study has examined MPAs in adults with ASD and normal-intelligence using an MRI scan (Hardan et al., 2006a). Therefore, it is of essence to assess whether MPAs can be reliably applied within a clinical practice.

Table 2. Minor physical anomalies classified as Minor malformations (MM) or Phenogenetic variants (PV) according to the Waldrop scale

Minor physical anomalies	Minor Malformation or Phenogenetic Variant
Head	
Head circumference (glabella–opisthocranion circumference):	PV
1.5-2 SD	
2 SD	
Fine electric hair:	MM
Hair soon awry	
Hair unmanageable	
Eyes	
Epicanthus (the point of union where upper and lower lids join the nose):	PV
Partly covered	
Deeply covered	
Intercanthal distance/hypertelorism (approximate distance between tear ducts):	PV
Moderately increased	
Extensively increased	
Ears	
Seating ears - bottom of ears in line with:	PV
Area between mouth and nose	
Mouth (or lower)	
Adherent ear lobes Lower edges of ears extend:	MM
Moderate/Straight back toward rear of neck	
Extensive/Upward and back toward crown of head	
Asymmetrical ears (normal morphology but difference in the size and protrusion from head. If one ear is low seated this is assessed as a asymmetry on this item)	PV
Malformed ears (incomplete or absence development of helix, scapha, antihelix, tragus or anotia)	MM
Mouth	
High/steepled palate (the roof has an acute angle rather than an arch or a narrow flat area across the top) Roof of mouth:	PV
Flat and narrow at the top	
Definitely steepled	
Furrowed tongue (one with deep ridges not along the center line of the tongue)	MM
Hands	
Curved fifth finger (clinodactyly):	MM
Moderately curved	
Extensively curved	
Single transverse palmar crease (a single uninterrupted palmar crease from the radial to the ulnar border of the hand)	MM
Fifth-finger stubbing	MM
Feet	
Third toe longer than second toe:	
Equal in length to second	PV
Definitely longer than second	
Partial syndactylia of second and third toes (partial or total fusion of toes)	MM
Big gap between first and second toes (the distance between the first and the second toes is the same or exceeds the width of the second toe)	PV

1.6.5 Ethical challenges

In recent years, there has been a debate on whether the autism is a disability or a ”cognitive difference” (Kapp et al., 2013; Jaarsma and Welin, 2012; Baker, 2006). On one hand, autism has been seen as a lifelong condition related to atypical brain development and higher occurrence rates of certain disabilities as a consequence. These disabilities may require various types of support and treatment. On the other hand, association of autistic traits with scientific skills, music talents or presence of savant abilities in people with ASD has highlighted the positive aspects of the condition (Baron-Cohen et al., 2001). Moreover, severity of the impairments can change over time and follow-up studies of adults have postulated an overall decrease in the severity of symptoms (Howlin et al., 2004; Farley et al., 2009). Therefore, individuals with ASD can change their position within the broad spectrum from lower to higher functioning over time. Also, on the individual level people with ASD may exhibit traits that range from the almost normal and less impaired end of the spectrum to the other more pathological end. This reflects the neurobiological heterogeneity of ASD and might be taken into account when we try to identify biological markers or endophenotypes for ASD. In addition, exploring the neurobiological bases of ASD may increase our understanding of different dimensions of ASD and contribute to early and hopefully correct diagnosis.

2 AIMS

The general aim of this thesis is to study neurobiological dimensions of ASD and investigate whether organic deviations could be found in adults with ASD in comparison with neurotypical controls.

Specifically the aims of the studies were:

Study I: To evaluate the Swedish version of the Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R) with regards to internal consistency, temporal stability, diagnostic accuracy and concurrent validity.

Study II: To examine the regional cerebral blood flow in adults with ASD as compared to a group of neurotypical controls.

Study III: To investigate the neural correlates of autistic traits, the symptoms of inattention, hyperactivity/impulsivity and neurological soft signs in adults with ASD and neurotypical controls.

Study IV: To investigate the prevalence, as well as topographical pattern of minor physical anomalies (MPAs) in adults with ASD in comparison with neurotypical controls and to investigate whether the presence of MPAs can be used as a marker of impairment.

3 METHODS

Several different methods were used in the studies included in this thesis, i.e. the evaluation of the psychometrical properties of Swedish version of the RAADS-R scale, neuroimaging and anthropometric assessment of MPAs.

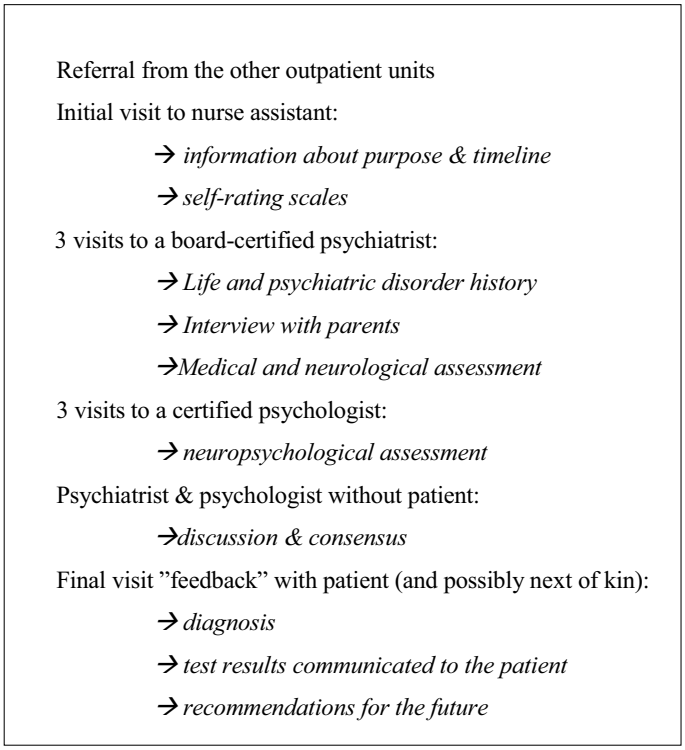
Table 3. Summary of aims, participants and methods in studies I-IV

Study	Aim	Participants	Methods
I	To assess internal consistency, temporal stability, diagnostic accuracy and concurrent validity of the Swedish version of the RAADS-R	Adults with ASD, n=75 Neurotypical adults, n=190 Psychiatric patients, n=7	RAADS-R AQ
II	To compare the regional cerebral blood flow distribution in adults with ASD to a group of neurotypical controls	Adults with ASD, n=13 Neurotypical adults, n=10	RAADS-R AQ ADOS-4 M.I.N.I. SCID-I & SCID-II PET-scan
III	To explore the neural correlates of autistic traits, symptoms of inattention, hyperactivity/impulsivity and neurological soft signs in adults with ASD and neurotypical controls		RAADS-R AQ NES ASRS ADOS-4 M.I.N.I. SCID-I & SCID-II PET-scan
IV	To investigate the prevalence, as well as topographical pattern of minor physical anomalies (MPAs) in adults with ASD in comparison to neurotypical controls, and to investigate whether the presence of MPAs can be used as a marker of impairment	Adults with ASD, n=53 Neurotypical adults, n=50	ADOS-4 AQ Waldrop scale GAF-symptoms GAF-functioning

3.1 PARTICIPANTS AND PROCEDURE

Most of the ASD patients who participated in the studies included in the present thesis underwent their ASD diagnostic assessment at the Neuropsychiatric unit at Northern Stockholm psychiatric clinic according to the model developed by Susanne Bejerot for clinical setting (see figure 1).

Figure 1. The pathway for neuropsychiatric assessment at the Neuropsychiatric unit, Northern Stockholm psychiatric clinic in 2008.



Patients were referred from outpatient units. During their first visit, patients met with an assistant nurse and received an information sheet regarding the purpose and schedule for the examination. The patients also completed self-rating scales and an assistant nurse was present for support if needed. Thereafter, the patients were scheduled for three visits with a board-certified psychiatrist, specialized in the diagnosis and treatment of adults with ASD and ADHD. The psychiatrist conducted interviews with the patients and their parents regarding early as well as present symptoms. In addition, an assessment of impairment level was performed. In order to screen for comorbidity a structured interview screening for Axis-I disorders, Mini International Neuropsychiatric Interview (M.I.N.I., 5.00 version) (Sheehan et al., 1998) and a semi-structured interview for past psychiatric disorders were used. General clinical impairment of symptoms and function were assessed with the Global Assessment of Functioning checklist (GAF) (APA, 1994). A number of different instruments were used for

assessment of ASD and ADHD symptoms. All patients underwent a neurological and a general medical examination. Finally, patients also underwent a set of neuropsychological assessments during their three visits with a certified psychologist.

The psychiatrist and psychologist discussed each patient's symptomatology in order to reach a consensus about the diagnosis. During the last visit the patients were informed about their diagnosis and test results. Support and information was also offered, regarding access to community facilities for people with ASD.

3.1.1 Study I

3.1.1.1 Participants

Seventy-five adults with ASD (Asperger's disorder, n=73; PPD-NOS or atypical autism, n=2) and 197 controls (neurotypical adults, n=184; adults with other psychiatric disorders, n=13) participated in the study (Table 4).

Table 4. Sex ratio and age by group in Study I

Group	N	Male : female	Mean age (SD), range
ASD	75	36 : 35*	34 (13), 19-75
Control	197	80 : 116*	31 (9), 20-62
Total sample	272	120 : 152	33 (12) 19-75

*Information on sex was missing for one subject in the control group and four subjects in the ASD group

3.1.1.2 Procedure and data collection

23 individuals with ASD were recruited from the Neuropsychiatric unit, Northern Stockholm psychiatric clinic (n=17) and a specialized unit in Lund (n=6). Additional, 52 individuals with ASD who participated in various research projects at Northern Stockholm psychiatric clinic were also included. All ASD individuals were diagnosed with ASD in adolescence or adulthood by an experienced clinician and diagnosis was supported by assessments with either ADOS or DISCO. None of the ASD participants had mental retardation.
The control group consisted of doctors and students from three campuses in Sweden and 60 subjects who comprised control groups from previous research studies.

RAADS-R was administered to all participants. Also, a subset of 39 individual with ASD and 49 controls completed the AQ. If the participants did not understand a question, they had the opportunity to ask the investigator for clarification.
The response rate was set at a minimum of 80 percent for inclusion in the study. This led to the exclusion of two subjects in the ASD group.

3.1.2 Study II and III

3.1.2.1 Participants

Thirteen adults with normal intelligence, diagnosed with ASD in adolescence or in adulthood, and ten age-, sex- and IQ-matched neurotypical controls were included in studies (II and III). Exclusion criteria for all subjects were learning disabilities, a

history of brain damage, current or past medical or neurological disorders, epilepsy, alcohol abuse or dependence, past or present substance abuse and psychosis. In addition, neurotypical controls were excluded if they had any past or current psychiatric or personality disorder, psychotropic medication and psychiatric disorders in first-degree family members.

Demographic characteristics and descriptive statistics are presented in table 5.

Table 5. Demographic and clinical characteristics of individuals with ASD compared with neurotypical controls in Study II and III

Variables		ASD n=13	Control n=10
Age, years		31.8 (8.6)	28.5 (7.5)
Male : female		7:6	5:5
Full scale IQ		104.2 (17.1)	115.7 (10.8)
	Verbal IQ	105.3 (16.4)	114.6 (13.2)
	Performance IQ	101.5 (17.6)	114.2 (9.9)
Handedness, right : left		12:1	9:1
Education	< 9 years, n	4	0
	9-12 years, n	4	3
	> 12 years, n	5	5
	university degree, n	0	2
Civil status, single : cohabit		12:1	6:4
Have children, yes : no		0:13	3:7
Independent living, yes : no		11:2	10:0
In full time work/studies, yes : no		3:10	10:0
Nicotine use, yes : no		3:9*	2:8
Global Assessment of functioning, total		54 (7.5)	86 (7.4)
	Symptom-GAF	54.7 (6.8)	87.8 (7.2)
	Function-GAF	56.3 (8.2)	90.7 (5.3)
Ritvo Autism Asperger Diagnostic Scale-Revised, total		109.7 (28.8)	19.6 (14.9)
Adult ADHD Self-Report Scale, total		32.2 (10.4)	19.6 (7.2)
	Inattention	18.4 (6.4)	11.1 (5.4)
	Hyperactivity/Impulsivity	14.7 (6.2)	8.5 (3.4)
Wender Utah Rating Scale, total		57,9 (40.5)	11.5 (7.1)
Neurological Evaluation Scale, total		16 (6.7)	5 (3.0)

*missing data in one subject

3.1.2.2 Procedure and data collection

Twelve neurotypical controls were recruited from the community in the Stockholm region. One participant was excluded since a first-degree family member had ASD and another participant withdrew leaving ten controls for the PET analysis.

Recruitment of individuals with ASD was performed by a letter of request sent to 357 individuals registered at a community based unit for adults with ASD and to patients with ASD at the Neuropsychiatric unit, Northern Stockholm psychiatric clinic. Fifty-five individuals with ASD were willing to participate in the study. Two individuals were excluded on the basis of epilepsy and a history of alcohol and drug dependence (see Figure 2). The final selection was based on the desired distribution of sex. All but one, of Asian descent, were Caucasians.

Previous neuropsychiatric assessments which included extensive interviews, rating scales, neuropsychological assessments and interviews with parents of the individuals were requested for all ASD individuals. According to these, 11 individuals met the DSM-IV criteria for Asperger disorder and two were diagnosed with highly-functioning autism. The clinical diagnosis of ASD was confirmed in all individuals with the ADOS module 4 (ADOS-4). Thereafter, two board-certified psychiatrists specialized in diagnosing ASD in adults, agreed on the diagnosis and type of social style, according to Wing's definition (Table 6).

Psychiatric and psychological assessments

The structured interview for Axis-I disorders Mini International Neuropsychiatric Interview (M.I.N.I., version 5.0.0.) (Sheehan et al., 1998) was administered to the ASD individuals whereas the Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders 4th edition (DSM-IV), Axis I Disorders (SCID-I) and Structured Clinical Interview for DSM-IV-R Personality Disorders (SCID-II) were administered to the neurotypical controls in order to rule out psychiatric disorders. General clinical impairment and function were assessed according the DSM-IV.
Past and current medical disorders, family history of mental disorders, education level, marital status and employment status were also covered in the semi-structured interview.
Global Assessment of Functioning (GAF) (APA, 1994) was estimated and the intelligence quotient was assessed using the full Wechsler Adult Intelligence Scale-Revised (WAIS-III-R) for all participants.

Eight individuals with ASD met diagnostic criteria for additional psychiatric disorders and six subjects were at the time being treated with psychotropic medication (Table 7).

Figure 2. Flow chart: Recruitment of individuals with ASD in Study II and III

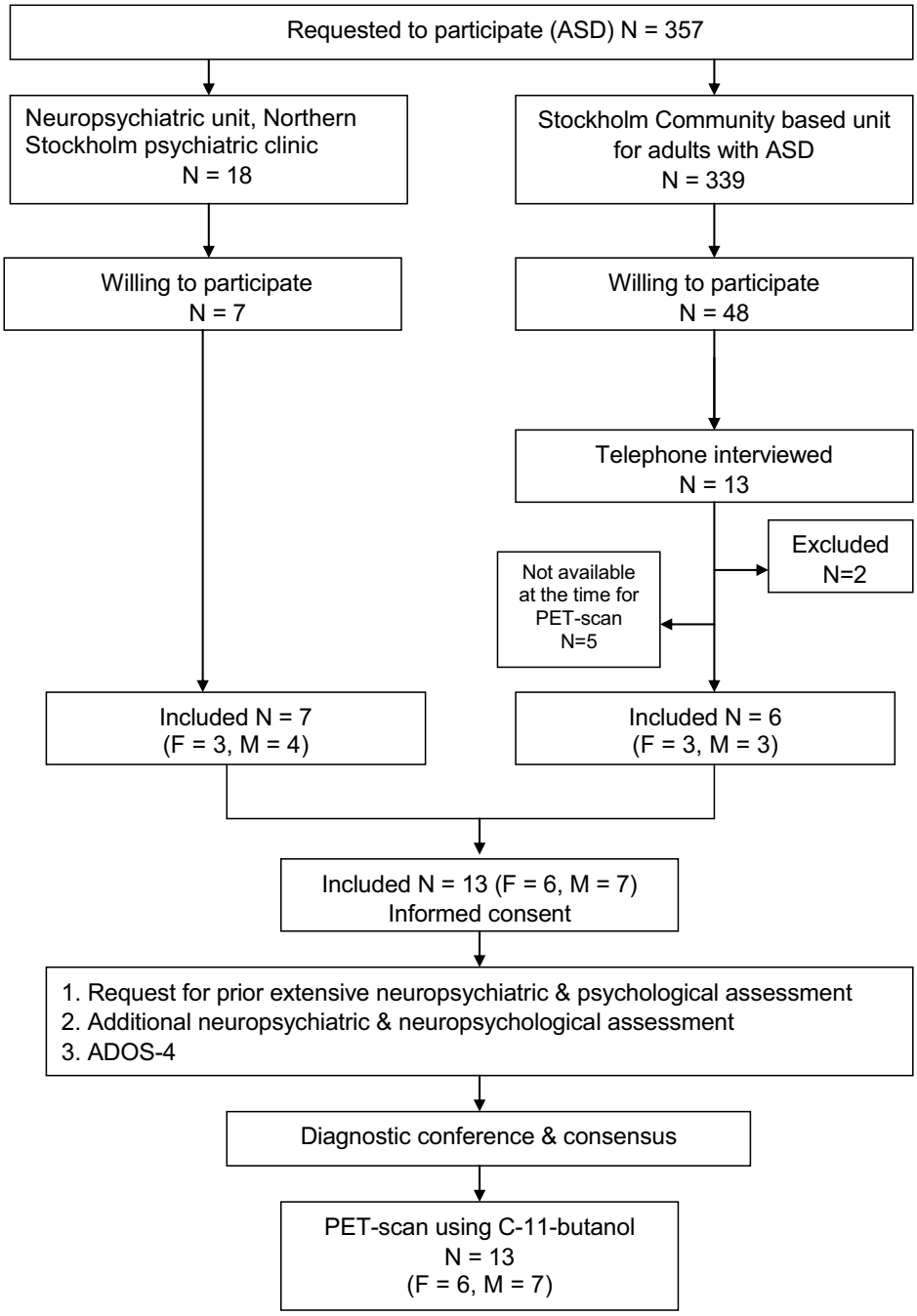


Table 6. Individual clinical characteristics for individuals with ASD in Study II and III

Sex	Autistic symptoms									ADHD symptoms				NSS
	ADOS					AQ	RAADS-R	Wings` subtype*		ASRS			WURS	NES
	Communi-cation	Recipro-cal Social Interac-tion	Communi-cation and Social Interaction	Imagination/ creativity	Stereotyped behaviors and restricted interests			Loners	Active-but-odd	Inatten-tion	Hyper-activity and impulsivity	Total ASRS		
M	2	5	7	0	1	34	93		Yes	18	13	31	56	7
M	4	7	11	1	0	36	73	yes		12	6	18	22	11
M	5	10	15	2	1	30	115		Yes	13	4	17		15
M	2	6	8	0	2	23	87		Yes	8	7	15	45	26
M	4	7	11	1	1	29	129	yes		27	12	39	51	28
M	5	11	16	2	1	40	153	Yes		22	22	44	53	18
M	3	5	8	1	0	39	163		Yes	26	18	44	56	25
F	2	7	9	0	1	22	82		Yes	24	20	32		12
F	5	10	15	2	0	25	136	Yes		14	11	25	21	16
F	3	7	10	2	0	24	114		Yes	23	19	42	32	15
F	4	11	15	1	3	27	107		Yes	25	17	42	38	13
F	3	5	8	0	1	18	76		Yes	13	21	34	21	12
F	2	5	7	0	2	29	99		Yes	14	21	35	74	8

*No ASD subject was classified as "passive" or "aloof" type;
ADOS: Autism Diagnostic Observation Schedule; ASRS: Adult ADHD Self-Report Scale; AQ: Autism Spectrum Quotient; NES: Neurological Evaluation Scale; NSS: Neurological Soft Signs; RAADS-R: Ritvo Autism and Asperger Diagnostic Scale-Revised ; WURS: Wender Utah Rating Scale

Table 7. IQ, handedness, current medication, comorbidity, general functioning in the ASD group in Study II and III

Sex	IQ: Fu/V/P	Handedness	Medication	Comorbidity	GAF (Tot/S/Fn)
M	116/120/107	R	None	GAD	51/51/50
M	135/130/134	R	None	None	51/51/60
M	130/122/134	R/L	Cit*	None	65/65/65
M	89/91/88	R	None	None	55/55/55
M	97/107/83	R	None	GAD, MD, Dys, PD, SAD	50/50/50
M	87/84/92	R	Con, Pro, Rem, Rit, Ste,Ther	BN, SAD	51/51/61
M	98/103/90	R	Efe, Xan, Met, Stra	GAD, OCD	52/52/55
F	87/83/94	R	Bus	Agor, GAD, PD	51/51/50
F	90/98/82	R	None	Agor, Dys, GAD, OCD	50/45/50
F	91/96/87	R	Par, Rem	Dys, SAD	40/50/40
F	110/107/112	R	Zol*	None	61/61/61
F	125/133/111	R	None	None	65/65/70
F	100/96/106	R	None	Agor	65/65/65

F, Female; M, Male; IQ, Intelligence Quotient, Fu, Full scale IQ; V, Verbal IQ; P, Performance IQ; R, Right; L, Left
GAF, Global Assessment of Function; S, Symptom; Fn, Function
Bu, Buspar; Cit, Citalopram; Con, Concerta; Efe, Efexor; Met, Metamina; Par, Paroxetin; Pro, Propavan; Rem, Remeron; Rit, Ritalin; Ste, Stesolid; Strat, Strattera; Ther, Theralen; Zol, Zoloft; Xan, Xanor;
Agor, Agorophobia; BN, Bulimia Nervosa; MD, Major Depression; Dys, Dystymic disorder; GAD, Generalized Anxiety Disorder; OCD, Obsessive Compulsive Disorders; PD, Panic Disorder; SAD, Social Anxiety Disorder.
*Successfully treated depression

Neuropsychiatric assessments

The Swedish version of the RAADS-R (Andersen et al., 2011; Ritvo et al., 2010) was used to assess cognitive aspects of ASD along with deficits in perception and sensory-motor integration.
Although all patients included in the study were diagnosed with ASD, many had additional symptoms of inattention and hyperactivity. The self-rating scale Adult ADHD Self-Report Scale (ASRS) was used for assessment of ADHD symptoms (Kessler et al., 2005).
Childhood ADHD symptoms were assessed using the Wender Utah Rating Scale (WURS) (Ward et al., 1993), which is also self-administered.

Neurological Assessment

Neurological examination was carried out with the Neurological Evaluation Scale (NES), which is a 26-item clinically administered instrument developed for the systematic evaluation of neurological soft signs (Buchanan and Heinrichs, 1989).

After the extended psychiatric assessment, all subjects underwent PET scans using [1-^{11}C]butanol to evaluate regional cerebral blood flow (rCBF).

3.1.3 Study IV

3.1.3.1 Participants

Fifty Swedish adults with ASD (24 females, 26 males; mean age 30 years) and 53 neurotypical controls (25 females, 28 males; mean age 30.4 years) participated in this study (Table 8). These subjects were included in a previous study on gender coherence (Bejerot et al., 2012).

Inclusion criteria were age range between 18 and 50 years and Caucasian descent. Exclusion criteria were any co-morbid neurological or genetic syndromes, diagnosed malformations, schizophrenia spectrum disorders or mental retardation, or having attended special education in primary or secondary school. Normal intelligence was assumed by mainstream schooling. Additional exclusion criteria for the control group were the presence of ASD in a first-degree family member, current psychiatric or personality disorder and use of any psychotropic medication.

Table 8. Sample characteristics of the ASD group and the neurotypical controls in Study IV

		ASD n=50	**Controls** n=53
Age, years, mean (SD)		30.0 (7.3)	30.4 (7.5)
Sex, n (%)	males	26 (52)	28 (53)
	females	24 (48)	25 (47)
Education, n	≤ 9 years	8	1
	≤ 12 years	18	7
	University level	24	45
Civil status, cohabit, n (%)		9 (18)	26 (49)
Have children, n (%)		8 (16)	11 (21)
The Autism-Spectrum Quotient, mean (SD)		29.4 (9.8)	11.2 (4.9)
GAF, past month, mean (SD)	Symptoms	55.6 (6.5)	97.5 (4.4)
	Functioning	55.0 (9.2)	97.6 (4.2)

3.1.3.2 Procedure and data collection

Individuals with ASD were recruited through an outpatient tertiary psychiatric unit, a community-based centre for adults with ASD and also through a website for people with ASD. All participants with ASD had been previously diagnosed according to the extensive standard procedure for diagnosing ASD in Sweden at the time of the study (Rydén and Bejerot, 2008). Furthermore, the diagnosis was confirmed with ADOS-4, patient records and a clinical interview performed by a psychiatrist experienced with ASD.

Neurotypical controls were recruited through advertisements on flyers and websites of various organisations and through word-of-mouth. Neurotypical controls were matched for sex and age to the ASD group.

All participants completed the AQ questionnaire (Baron-Cohen et al., 2001). Overall impairment in psychological, social, and occupational functioning was assessed by the DSM-IV Global Assessment of Functioning (GAF) (APA, 1994; Moos et al., 2002). Severity of symptoms (GAF-symptoms) and social, occupational, or school functioning (GAF-functioning) were assessed separately (Goldman et al., 1992).

All participants were assessed for minor physical anomalies and photographed in a standardized manner. The digital photos of face were close-up photos (front and in profile). Feet were photographed from beneath and from above.

MPAs were evaluated according to the modified Waldrop scale (Waldrop and Halverson, 1971) (Table 9). Head circumference was measured with a measuring tape and then categorized by reference to the control mean based on established scale norms (as 1.5 or 2 standard deviations greater than control mean). Two un-blinded assessors examined MPAs in all participants. In addition, two psychiatrists blinded to the participants´ diagnosis independently assessed eight of the total 16 items (epicanthus; hypertelorism; low-settled ears; adherent ear lobes; malformed ears; relative toe lengths; partial syndactylia; sandal gap between first and second toe) by using photographs of face (front and profile) and feet. If necessary, a consensus between observers was reached after discussion. Thus MPAs of mouth, hands and hair were assessed un-blinded.

Table 9. Measurements and frequencies of MPAs in the ASD group and the neurotypical control group (NC) according to the Waldrop scale, Study IV

Minor physical anomalies	Score	MM/ PV	ASD n (%)	NC n (%)
Head				
Head circumference:		PV		
1.5-2 SD	1		23(46)	27(50.9)
2 SD	2		8(16)	2(3.8)
Fine electric hair:		MM		
Hair soon awry	1		12(24)	4(7.5)
Hair unmanageable	2		1(2)	-
Eyes				
Epicanthus:		PV		
Partly covered	1		8(16)	7(13.2)
Deeply covered	2		1(2)	2(3.8)
Intercanthal distance/hypertelorism (approximate distance between tear ducts):		PV		
Moderately increased	1		22(44)	19(35.8)
Extensively increased	2		1(2)	1(1.9)
Ears				
Seating ears - bottom of ears in line with:		PV		
Area between mouth and nose	1		8(16)	9(17)
Mouth (or lower)	2		-	-
Adherent ear lobes Lower edges of ears extend:		MM		
Moderate/Straight back toward rear of neck	1		16(32)	14(26.4)
Extensive/Upward and back toward crown of head	2		18(36)	14(26.4)
Asymmetrical ears	1	PV	10(20)	3(5.7)
Malformed ears	1	MM	5(10)	4(7.5)
Mouth				
High/steepled palate Roof of mouth:		PV		
Flat and narrow at the top	1		12(24)	13(24.5)
Definitely steepled	2		-	3(5.7)
Furrowed tongue	1	MM	7(14)	4(7.5)
Hands				
Curved fifth finger (clinodactyly):		MM		
Moderately curved	1		2(4)	10(18.9)
Extensively curved	2		-	-
Single transverse palmar crease	1	MM	7(14)	-
Fifth-finger stubbing	1	MM	1(2)	
Feet				
Third toe:				
Equal in length to second	1	PV	2(4)	-
Definitely longer than second	2		-	-
Partial syndactylia of second and third toes	1	MM	-	3(5.7)
Big gap between first and second toes	1	PV	36(72)	40(75.5)

MM: Minor Malformation; PV: Phenogenetic variants;
Items are assessed according to descriptive anchor points (scored 0-2) depending on severity and "0" is defined as "no deviation"

3.2 CLINICAL ASSESSMENTS

3.2.1 M.I.N.I.; SCID-I; SCID-II interviews (Study II and III)

The Mini-International Neuropsychiatric Interview (M.I.N.I. version 5.0.0.) is a validated structured diagnostic interview for assessment of psychiatric disorders according to DSM-IV and ICD-10. It has been applied in clinical settings as well as in clinical trials and epidemiology studies (Sheehan et al., 1998). The M.I.N.I. consists of modules corresponding to diagnostic categories according to the DSM-IV. It takes about 15-45 minutes to complete and requires only "yes" or "no" answers. If necessary, the clinician can ask the patient for examples and clarifications.

Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders 4th edition (DSM-IV) Axis I Disorders (SCID-I) is a semi-structured standardized face-to-face diagnostic interview. The interview manual includes standardized interview questions and diagnostic algorithms for each module. The SCID-I interview comprises of modules for assessment of a number of DSM-IV disorders (First, 1998a).

Structured Clinical Interview for DSM-IV-TR Personality Disorders (SCID-II) covers eleven personality disorders (including Personality Disorder NOS) and the appendix categories Depressive Personality Disorder and Passive-Aggressive Personality Disorder (First, 1998b).

In Studies II and III, the SCID-I and SCID-II were implemented to rule out Axis-I and Axis-II disorders in neurotypical controls.

3.2.2 RAADS-R (Study I, II and III)

The Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R) is a self-report questionnaire based on the DSM-IV-TR and the ICD-10 criteria for autism and Asperger's disorder (Ritvo et al., 2008). It is an 80-item scale designed to assist in the diagnosis of autism and Asperger's disorder in adults. It consists of four subscales: Social Interaction, Language, Circumscribed Interests and Sensory Motor. Hence, the RAADS-R assesses deficits in perception and sensory-motor integration along with cognitive aspects of ASD. Each item is formulated as a statement from the patient's point of view (e.g. *"I often don't know how to act in social situations"*). Seventeen items are reversed in order to avoid response bias and to elicit information about skills or preferences acquired throughout the life span (e.g. *"I like to have close friends"*). The statements are answered on a four point Likert scale with the qualitative alternatives "never true", "true only when I was young (before the age of 16)", "true only now" and "true now and when I was young". The 63 "positively worded" statements are scored from 0-3, so that the longer a symptom has been present the more points it yields, and the 17 reversed statements are scored in the reverse order. A score of 65 or greater was suggested to be consistent with a clinical diagnosis of ASD and the scale has satisfying internal consistency (Ritvo et al., 2008; Ritvo et al., 2011).

3.2.3 AQ (Study I, II and IV)

The Autism-Spectrum Quotient (AQ) is a self-report questionnaire that measures autistic traits in individuals with normal intelligence. The AQ consists of 50 items that cover various abilities and is divided into five subscales: Social skills, Communication, Attention switching, Attention to detail and Imagination. Each item is scored on a four point Likert scale. The responses "definitely disagree" and "slightly disagree" are scored as 0 and "slightly agree" and "definitely agree" are scored as 1. Fifty percent of the items are reversely worded and scored. The discriminant validity and screening properties are suggested to be satisfactory in discriminating ASD from neurotypical controls (Baron-Cohen et al., 2001; Woodbury-Smith et al., 2005), however, in other psychiatric conditions the AQ differentiates the patient groups to a lesser degree (Ketelaars et al., 2008; Naito et al., 2010).

3.2.4 ADOS (Study II, III and IV)

The Autism Diagnostic Observation Schedule, module 4 (ADOS-4) is a semi-structured, standardized assessment of social interaction, communication, and imaginative use of materials for adolescents or adults with fluent speech suspected of having autism spectrum disorders (Lord et al., 2000). ADOS-4 has been widely used to support the clinical diagnosis of ASD. Recently, it was demonstrated that the ADOS-4 successfully distinguishes ASD from typical development, psychopathy and schizophrenia. Therefore, it is suggested to be helpful in differential diagnostics of psychiatric patients (Bastiaansen et al., 2011).

The ADOS-4 includes four subscales: Communication, Reciprocal Social Interaction, Imagination/Creativity and Stereotyped Behaviors-Restricted Interests. The ADOS-4 algorithms include only the combined score from the two subscales "Communication" and "Reciprocal Social Interaction", since the ADOS-4 does not offer an opportunity to measure restricted and repetitive behaviors reliably. The ADOS-4 postulates a threshold score of 7 for non-specific pervasive developmental disorder and a score of 10 or above for autism.

3.2.5 ASD social subtypes (Study II and III)

The four subtypes of social functioning in ASD, as proposed by Lorna Wing (Aloof group, Passive group, Active-but-odd group and Loners; (Wing, 1997) (for details see table 1), are useful because they offer a behavioral description of the social impairment. Interestingly, motor stereotypies and sensory abnormalities have been found in excess in the Aloof group in comparison with the Active-but-odd group whereas rituals and repetitive behaviors were more characteristic for the Active-but-odd group (Waterhouse et al., 1996). Therefore, motor abnormalities have been suggested to be a general marker for severity in children with ASD. On the whole, Wing`s subtypes of social functioning provide support for existence of a continuum of developmental severity. In addition, Wing`s subtypes may characterize behavioral subgroups for studies of neurobiological markers in ASD.

3.2.6 ASRS (Study II and III)

Adult ADHD Self-Report Scale (ASRS) is the WHO's self-rating scale for assessment of current inattention and hyperactivity/impulsivity symptoms in adults (Kessler et al.,

2005). It consists of eighteen questions based on DSM-IV criteria for ADHD (APA, 1994). ASRS consists of two subscales: Inattention and Hyperactivity/Impulsivity with scores ranging from 0 (no symptoms) to 72.

3.2.7 Neurological Evaluation Scale (Study II and III)

The Neurological Evaluation Scale (NES) is a 26-item clinically administered instrument developed for the systematic evaluation of the presence and severity of neurological soft signs (Buchanan and Heinrichs, 1989). It consists of four subscales for assessment of Sensory integration (stereognosis, graphaesthesia, extinction, right/left confusion), Motor coordination (tandem walk, rapid alternating movements, finger-thumbs opposition, finger-nose test), Sequencing of complex motor tasks (fist-ring test, fist-edge-palm test, Ozeretski test of rapid alternating movements, rhythm tapping) and other neurological signs such as Romberg sign, tremor, mirror movements, synkinesis, convergence, gaze impersistence, primary reflexes and short-term memory. The items are rated from 0 to 2 where 0 represent "no abnormality", 1 is "mild but definitive impairment" and 2 correspond to "marked impairment". NES has been widely used in psychiatric populations with schizophrenia (Chan et al., 2010; Peralta et al., 2010; Sewell et al., 2010; Heinrichs and Buchanan, 1988), Asperger syndrome (Tani et al., 2006), bipolar disorder (Negash et al., 2004) and obsessive-compulsive disorder (Mergl and Hegerl, 2005; Poyurovsky et al., 2007).

In Studies II and III, the NES subscales scores were used as a measure of sensory-motor function deficits, in addition to self-reported difficulties in the Sensory Motor subscale of the RAADS-R.

3.2.8 The Waldrop scale (Study IV)

The modified Waldrop scale (Waldrop and Halverson, 1971) is an instrument for assessment of minor physical anomalies (MPAs). It consists of 16 items that evaluate occurrence of MPAs in six anatomic body areas: head, eyes, ears, mouth, hands and feet (Table 9). The most of MPAs are assessed according to descriptive anchor points (scored 0-2) depending on severity. Head circumference measure is categorized by reference to the control mean based on established scale norms (as 1.5 or 2 standard deviations greater than control mean). The variables "asymmetrical ears", "malformed ears", "furrowed tongue", "single transverse palmar crease", "fifth-finger stubbing", "partial syndactylia of second and third toes", "big gap between first and second toes" are scored as absent (score 0) or present (score 1).

The Waldrop scale and its later modifications were able to discriminate patients with schizophrenia, affective disorder and autism from neurotypical controls (Akabaliev et al., 2011; Campbell et al., 1978; Compton et al., 2011; Walker, 1977). Assessment of MPAs according to the Waldrop scale takes about ten minutes and does not require any advanced equipment. Thus it is easily performed in the clinician`s office. However, there are several limitations regarding the low sensitivity and inter-rater reliability, the subjectivity in assessment of certain items (hair quality, hypertelorism, curved fifth finger) and the effects of ethnic origin (Trixler et al., 2001; Krouse and Kauffman, 1982).

3.2.9 GAF (Study II, III and IV)

The DSM-IV Global Assessment of Functioning (GAF) assesses the overall impairment in psychological, social, and occupational functioning. The GAF score is a combined measure of symptom severity and level of functioning, it ranges from 1 to 100 (APA, 1994; Moos et al., 2002). Low scores indicate more severe symptoms and low functioning. In order to capture these two different dimensions, severity of symptoms (GAF-symptoms) and social, occupational, or school functioning (GAF-functioning) can be assessed separately (Goldman et al., 1992).

3.3 NEUROIMAGING

Alterations in the function of the brain and underlying pathophysiology of disorders can be investigated by functional neuroimaging. During the last 30 years, functional brain imaging has been increasingly applied to psychiatric disorders. In the field of neurodegenerative disorders, Single Photon Emission Computed Tomography (SPECT) and PET allow for identification of neurodegenerative disorders with a sensitivity and specificity approaching 80-90% (Shaffer et al., 2013; Owen et al., 2011). However, lower rates of accuracy are reached for schizophrenia, post-traumatic stress disorder, major depression and childhood onset neurodevelopmental disorders such as ASD and ADHD. The links between the findings in functional brain imaging studies and the neural substrates of ASD and ADHD have not been clearly established yet.

A dynamic coupling between cerebral blood flow (CBF) and brain metabolism as assessed by regional cerebral metabolic rates of glucose (rCMRGlu) has generally been assumed.

A reliable diagnosis is achieved by identifying those structures, in which the modification of CBF deviates from normality. The diagnostic value of a functional brain image is increased if the patient's scan can be compared to an average scan obtained from a group of controls.

In the recent past a three-dimensional (3-D) semi-automatic approach has been implemented in the identification of brain regions and CBF changes.

3.3.1 Brief introduction to PET instrumentation

PET produces images of the body by detecting the radiation emitted following radioactive substances interactions with tissues and enables to measure important functional processes such as blood flow (^{15}O-water; ^{11}C-butanol) and glucose metabolism (^{18}F-FDG). In a PET scan, the patient is injected with a radioactive substance and placed on a flat table that moves through a "donut" shaped housing. This housing contains the circular gamma ray detector array, which has a series of scintillation crystals.

PET radioactive substances contain a radioactive atom emitting positron. The positron collides with electron resulting annihilation, i.e. the emission of two 511 kV photons. These two photons are emitted at opposite angles and detected by the circular gamma ray detector array in the PET camera. PET camera records only those 511 kV gamma rays that appear within a certain narrow time frame at a 180 degree angle (i.e. coincidence detection), all other background radiation will be effectively filtered out resulting in improved spatial resolution. Modern PET cameras are able to achieve a spatial resolution in the range of 4-8 mm. The scintillation crystals convert the gamma rays, emitted from the patient, to electrical signals. These electrical signals are then processed by the computer to generate images using the voxel-by-voxel method. Initially, the images of relative tracer distribution are spatially normalized to a predefined PET template (i.e. Montreal Neurological Institute, MNI, space) and

smoothed to account for individual gyral differences and brain anatomy. Thereafter, a comparison of scans is made on a voxel-by-voxel basis in which every voxel reflects the same anatomical location.

In studies II and III, the PET radiotracer, [1-^{11}C]butanol, was chosen for its advantage in measuring CBF more accurately than ^{15}O water, due to the latter's diffusion limitations at higher blood flows (Raichle et al., 1976). Butanol, as a blood flow tracer labeled with either ^{11}C or ^{15}O, has been shown to have a high degree of reliability for human studies and proven to be directly proportional to CBF (Saha et al., 1994; Herscovitch et al., 1987; Quarles et al., 1993). Additionally, the longer half-life of ^{11}C (20 min vs 2 min for ^{15}O) facilitated transportation of multi-patient doses to the clinical PET-CT, which is instrumental in enabling the performance of this clinical group analysis. The logistic feasibility suggests, where possible, a more widespread use of [1-^{11}C]butanol as a radiotracer in PET CBF studies.

The CBF is assumed to be related to neuronal activity and CBF alterations can reflect deviant function of various brain regions which are involved in the pathophysiology of disorders.

Through the years, different approaches in assessing neuronal activity have been used. One is to investigate task-related brain activity (i.e. when a person is involved in task solving during the scan) and the other one to assess the level of resting state activity. The latter is targeted by internally-directed thoughts and autobiographical reminiscences processing emotions, perception of social interactions, experience of learning (Lewis et al., 2009) and is proposed to be functionally important. Changes in resting state activity were found for populations with Alzheimer's disease, bipolar disorder, major depression, schizophrenia, autism and ADHD (Buckner et al., 2008; Liu et al., 2013; Sheline and Raichle, 2013; Monk et al., 2009; Schweitzer et al., 2003).

The resting state condition during the experiment is defined by a constant condition without stimuli or a cognitive task performance. Individuals are asked to close their eyes and rest quietly for 5–10 minutes immediately before, during and after radiopharmaceutical injection. This then allows for data collection of endogenously generated neural activity.

In Studies II and III, resting rCBF was investigated in thirteen individuals with ASD and normal IQ (>70) in comparison with ten neurotypical (i.e. healthy) controls.

3.3.2 PET- Scanning protocol

Siemens Biograph 64 Positron Emission Tomography/Computed Tomography (PET/CT) scanner with a spatial resolution of 5 mm was used for data collection. The system combines a high-speed ultra 32-detector-row (672 detectors per row) CT unit, a PET scanner with 32448 LSO crystals in 52 rings and an axial field of view 21,6 cm.

The examination started with a CT-scan of the head. This would allow for a later correction of attenuation and photon scatter. Then a bolus of [1-^{11}C]butanol (300 MBq) was injected simultaneously as PET acquisition started. Data were acquired during the

list mode for 5 minutes and were reconstructed into transverse images for rCBF evaluations. The interval between 40 and 100 sec after injection was identified by the analysis of the uptake curve as the optimal window from which to extract the raw data and reconstruct the images. During this interval the [1-^{11}C]butanol uptake reached a plateau before starting to decrease over time.

The protocol implemented was chosen in order to keep the time spent in the scanner to an absolute minimum. Subjects were resting in the waiting room approximately 30 minutes before injection and all procedures in the PET room lasted no longer than 10 minutes in order to minimise any impact on measured CBF. This is of utmost importance in psychiatric patients, since they are often unable to comply with the demands of functional neuroimaging. Having to wait for the examination and their discomfort and unease in being restricted in a narrow space during the scan may easily impact on the results. None of the patients studied here showed any anxiety spikes or panic and none of the images contained artefacts from motion in the gantry. A psychiatrist attended all the scanning sessions as a reassurance for the participants with ASD.

3.3.3 Image preprocessing

Data were analyzed with SPM2 (Wellcome Department of Cognitive Neurology, London, UK) as implemented in Matlab 6.5.1. Raw data were subjected to affine and non-linear spatial normalization to a predefined PET template based on the MNI (Montreal Neurological Institute) reference brain through a bilinear interpolation method into a common anatomical space. The images were spatially normalized and smoothed with a 10 mm (FWHM) isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. In addition, images were globally normalized using proportional scaling (0.5) to remove confounding effects in global CBF changes, with a grey matter masking threshold of 0.8.

3.4 STATISTICS

The statistical analyses used in this thesis are presented in table 10.

Table 10. The summary of statistics used in studies I-IV

Statistical analysis	Study I	Study II	Study III	Study IV
Descriptive statistics	x	x	x	x
Correlations (Pearsons *r*)	x			
Spearman`s rank correlation test	x			x
One-way analysis of variance (ANOVA)	x			
Analysis of covariance (ANCOVA)		x		
Mann-Whitney *U*-test			x	
Principal Component Analysis			x	
Analysis of functional PET imaging data SPM2		x	x	

3.4.1 Study I: Statistical analysis

Internal consistency of the Swedish version of RAADS-R was estimated with Chronbach's alpha (Chronbach's alpha is a function of intercorrelation among items and scale length).

The test-retest reliability and concurrent validity of RAADS-R and AQ in the ASD group was evaluated by correlation analyses (Pearsons *r*). For estimations of concurrent validity of RAADS-R and AQ in the control group the Spearmans rank order coefficient was computed, as the variables were not normally distributed in the control sample.

A ROC (receiver operating characteristics)-graph was generated in order to examine the diagnostic validity (i.e. ability of RAADS-R to discriminate between the two groups).

To explore group differences, a between-group ANOVA was performed comparing RAADS-R total and subscale scores by Group (ASD/control) and gender (male/female).
Analyses were made in SPSS, version 17. Missing scores were replaced with the individual means.

3.4.2 Study II and III: Statistical analysis

Proportions of categorical variables at baseline were compared using the chi-square test and frequency data were computed through the Fisher's exact test. Values of continuous measures were compared by either the *t*-test, or for non-normally distributed variables, the non-parametric Mann-Whitney *U*-test. The *p*-level was set as <0.05.

In Study II, the CBF group differences were assessed by voxel-based analysis in SPM2. Group analyses between ASD and neurotypical controls as well as between patients with and without medication were performed by the ''compare populations: one scan per subject (ANCOVA)'' model. The ''single subject: covariates only'' model was used to correlate the CBF in all participants and their IQs adjusting for age, sex and

educational level. Significant differences between the groups were thresholded at $p_{corrected} < 0.001$ at cluster level and $p_{uncorrected} < 0.01$ at voxel level.

In Study III, a principal component analysis was conducted, based on 13 original variables: the ADOS and GAF scores, in addition to subscale scores of RAADS-R, ASRS, NES and verbal and performance IQ. These original variables were truncated into three common factors (i.e. principal components). In order to correct for the large variability in the neuropsychiatric scales scores, data were normalized into a standard score (z-score). The obtained z-scores for the 13 chosen variables were then submitted to OpenStat (Rummel, 1970) for statistical analysis.
Minimal root to rotate was set to 1.0 and the maximal number of iterations to 25. Factors with eigenvalues larger than 1 were initially extracted. Variables, with an absolute factor loading greater than 0.5, were regarded as representative of the factor. This value is purely arbitrary, but it is a commonly used reference since it explains a moderate part of the variance of the factor (Pagani et al., 2009). Kaiser-Meyer-Olkin MSA values higher than 0.7 were regarded as significant.

Positive and negative correlations between rCBF distribution and each of the three factors were carried out separately in subjects with ASD and neurotypical controls. The "single-subjects covariates only" design model of SMP2 was implemented. All analyses were adjusted for age and gender. Due to the explorative nature of the study and the number of subjects, statistical thresholds of $p = 0.05$ at voxel height, $p_{corrected} < 0.05$ at cluster level and $p_{uncorrected} < 0.001$ at voxel level were applied.

3.4.3 Study IV: Statistical analysis

Comparisons between the ASD group and controls were made using Student's *t*-test for continuous variables and Pearson Chi-Square-distribution for categorical variables.

In addition to the total MPA score, the Craniofacial-MPA index was calculated from scores of the Head, Eyes, Ears and Mouth subscales and the Periphery-MPA index was calculated from scores of Hands and Feet subscales. Differences between means of the MPA total scores, the sub scores from the six body areas (Head, Eyes, Ears, Mouth, Hands and Feet), the Craniofacial-MPA and the Periphery-MPA indexes were analysed.

The participants were split into a Low-MPA group, defined as having a total Waldrop score below 5, and a High-MPA group, defined as having a total Waldrop score of 5 or greater. Bivariate correlations were performed to examine the relationships between MPAs, autistic traits (according to AQ), severity of symptoms (GAF-s) and overall functioning (GAF-f) in the Low-MPA as well as in the High-MPA groups. Due to skewness of AQ and MPA distributions, Spearman's rank correlation test was used.
The analyses were conducted using in SPSS software, version 17.

3.5 ETHICS AND PERMISSIONS

The studies were approved by the Regional Ethical Review Board in Stockholm (study I-IV) and the Radiation Safety Committee of Karolinska University Hospital (Study II and III). Written informed consent was obtained from each participant.

4 RESULTS

Table 11. Summary of results in studies I-IV

	Study I	Study II	Study III	Study IV
Research hypothesis	The Swedish version of the RAADS-R would produce good psychometric properties such as diagnostic accuracy, internal consistency, temporal stability and concurrent validity	Adults with ASD would show alterations in regional cerebral blood flow during resting state in comparison with a group of neurotypical controls	Autistic traits, ADHD symptoms and neurological soft signs would share neural substrates and correlate with regional cerebral blood flow in the temporo-parieto-occipital brain regions	Adults with ASD would show higher rates of MPAs than neurotypical controls; The pattern of topographical distribution of MPAs would differentiate individuals with ASD from neurotypical controls. Rates of MPAs would correlate with severity of symptoms and overall functioning.
Results	Mean RAADS-R is higher in ASD group. Sensitivity 0.91. Specificity 0.93. Strong correlation between RAADS-R and AQ. Satisfying internal consistency.	Increased cerebral blood flow was found in the ASD group in parahippocampal area and posterior cingulate, as well as in visual and temporal cortex, putamen, caudatus, substantia nigra and cerebellum.	Factors corresponding to ‘‘Autistic/ADHD traits’’, ‘‘Sensory-motor integration’’ and ‘‘Intelligence/Motor sequencing’’ were identified. In the ASD group, positive correlations with cerebral blood flow were found for ‘‘Autistic/ADHD traits’’ in caudate bilaterally and the inferior parietal lobule, for ‘‘Sensory-motor integration’’ in parieto-occipital cortex and for ‘‘Intelligence/Motor sequencing’’ in the right temporal cortex. Notably, CBF in the left thalamus correlated negatively with all three factors.	In the ASD group, the means of Craniofacial-MPA index, Head subscale score and MPA- total score were significantly higher in comparison to the control group. Significantly more ASD participants showed a high rate (i.e.>5) of MPAs than the controls. Association between MPA scores and severity of impairments according to the GAF scale was also found.
Conclusion	The Swedish RAADS-R is a reliable and valid instrument.	Adults with ASD and normal IQ have alterations in cerebral blood flow in the right hemisphere, the limbic-striatal system, the parieto-temporal lobes, the hippocampus and the primary visual cortex. These regions are involved in the phenotypic expression of the disorder.	Autistic traits and ADHD symptoms were associated with shared neural substrates. Sensory-motor deficits were grouped in another independent factor and associated with cerebral blood flow in different regions.	MPAs are suggested to serve as potential risk markers for ASD. Examination of morphological features can support diagnosis of ASD and may be helpful in estimation of psychological, social, and occupational functioning.

4.1 STUDY I

Study I investigated psychometric properties such as diagnostic accuracy, internal consistency, temporal stability and concurrent validity of RAAD-R. As mentioned in the introduction, there are only two validated and widely used self-report scales for assessment of autistic traits in adults with normal IQ. The RAADS-R is one of them, and the Swedish version needed to be validated.

As expected the ASD group had a significantly higher mean score than the controls on the RAADS-R: mean RAADS-R total score was 118.7 in the ASD group and 33.8 in the control sample.

Sensitivity reached 0.91 and specificity 0.93 at an optimal cut-off (i.e.72, that is somewhat higher than 65 suggested for the English version (Ritvo et al., 2011). The area under the ROC curve (AUC) was estimated at 0.96 (Std. err. 0.012, 95 % CI 0.94-0.98), indicating high overall accuracy. This implies that the probability of a randomly selected subject with ASD scoring higher than a randomly selected subject without ASD was approximately 96 % in this sample. Notably, specificity could be increased to 0.93 without losing sensitivity if the cut-off was set at 72.

The correlation between RAADS-R and AQ was strong in the ASD group ($r=0.84$, $p<0.001$) as well as in controls ($\rho=0.70$, $p<0.0001$) supporting good concurrent validity.

The Swedish version of RAADS-R showed satisfying internal consistency: Cronbach`s coefficient alpha for the total scale was 0.92 for the ASD group and 0.96 in the controls. Internal consistencies for Social Interaction, Circumscribed Interests and Sensory Motor subscales were also satisfactory ($\alpha=0.73$-0.87 in the ASD group and $\alpha=$ 0.73- 0.89 in the controls), however that was not the case for the Language subscale ($\alpha=0$ .58 in ASD group and 0.22 in the controls).

In conclusion, the study illustrates that the Swedish version of the RAADS-R is a reliable and valid instrument for assessment of autistic traits in adults with ASD and of normal intelligence.

4.2 STUDY II AND III

Study II investigated differences in CBF between adults with ASD and neurotypical controls whereas study III studied the two groups at a deeper functional level examining the neurobiological correlates of the autistic traits, ADHD symptoms and neurological soft signs as clinically assessed by appropriate scales.

The correlational analysis between CBF and IQ, as performed on all participants revealed no significant differences.

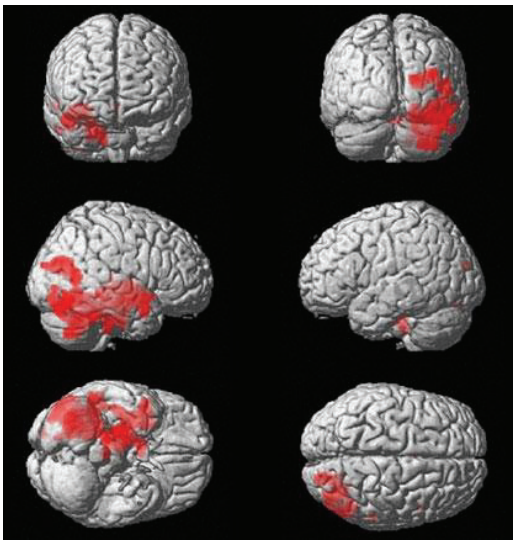
In Study II, CBF in the ASD group was increased in the right limbic structures including the parahippocampal (Brodmann Areas, BA 28) and posterior cingulate (BA

30) cortex, as well as the visual (BAs 17) and temporal cortex (BAs 37, 38, 39), putamen, caudatus, substantia nigra and cerebellum (Figure 3).

When controlling for drug intake, no CBF difference between medicated versus non-medicated patients was found.

The conclusion drawn from this preliminary study was that adults with ASD have a different pattern of resting brain activity in comparison with neurotypical controls, and possibly utilize alternative brain networks in comparison with neurotypical controls.

Figure 3. 3D rendering showing those regions (in red) in which CBF was significantly higher in ASD in comparison to neurotypical controls.



Top left: frontal view, top right: posterior view, middle left: right-side view, middle right: left-side view, left down view: from below, right down view: from above

In Study III, a principal component analysis of symptom scores identified three factors: "Autistic/ADHD traits"; "Sensory-motor integration" and "Intelligence/Motor sequencing". These factors were independent of age and educational level. Each of the three factors was in both groups correlated to significant increases (i.e. positive correlations) and decreases (i.e. negative correlations) in rCBF (Figure 4 and 5).

Positive correlations between the "Autistic/ADHD traits" factor and CBF were found in the occipital and temporal cortex in neurotypical controls. In ASD, positive correlations were found in the caudate bilaterally and in the right inferior parietal lobule, posterior cingulate and motor cortex. Also, CBF in right putamen and prefrontal cortex in ASD was negatively correlated to "Autistic/ADHD traits" factor.

In ASD, the “Sensory-motor integration” factor was positively correlated with CBF in the occipital association cortex while negative correlations were found in neurotypical controls in the same region. However, the opposite correlations were found for putamen, where the correlation of “Sensory-motor integration” factor with CBF was positive in neurotypical controls but negative in ASD participants.

The factor “Intelligence/Motor sequencing” was correlated with CBF only in the ASD group, positively in the right temporal cortex and negatively in the left insula and right uncus (Table 12).

The left thalamus correlated negatively with all three factors in the ASD group.

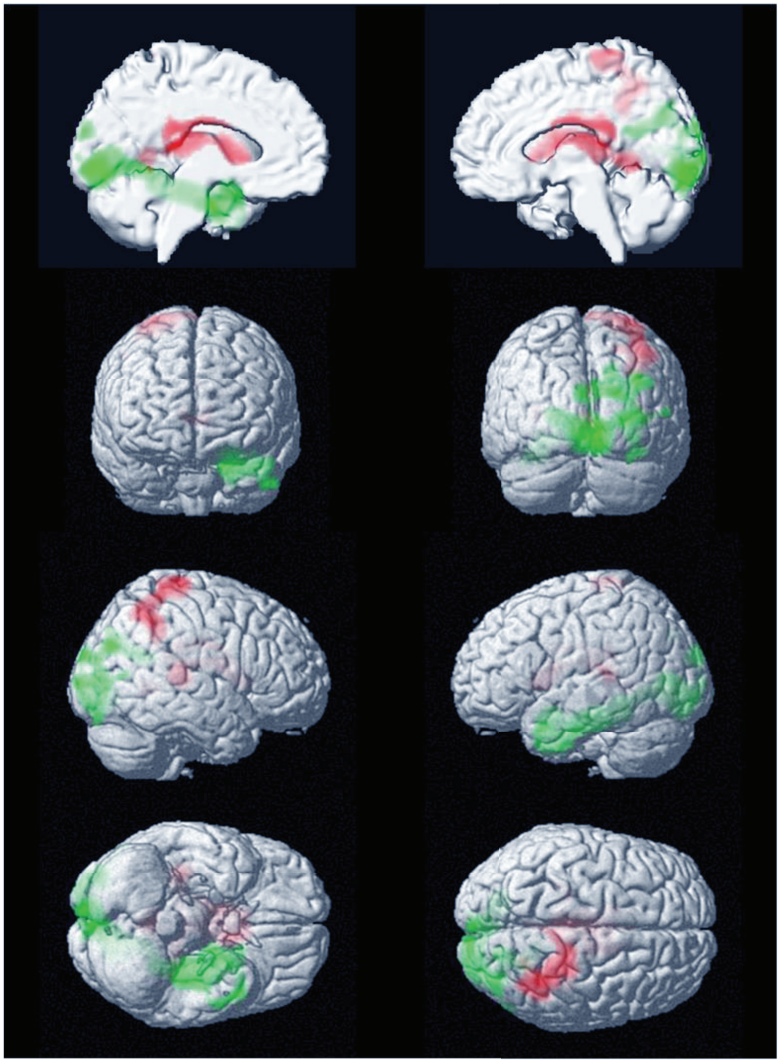
In conclusion, this study demonstrated that autistic traits and ADHD symptoms share common neural substrates. The correlation between ‘‘Autistic/ADHD traits’’ and rCBF in the caudate is possibly associated with the executive impairments and ritualistic or stereotyped behaviors apparent in ASD. Furthermore, sensory-motor deficits were correlated with rCBF in the occipital visual cortex, involved in atypical visual perception in ASD. On the whole, various behavioral and neurological symptoms are suggested to converge into the ASD phenotype.

Table 12. Regions with positive and negative correlations between the z-scores of the three factors and CBF

Structures	Brodmann area	F1. Autistic/ADHD traits				F2. Sensory-motor integration				F3. Intelligence/Motor sequencing			
		ASD, n=13		Control, n=10		ASD, n=13,		Control, n=10		ASD, n=13		Control, n=10	
		pos	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	Neg
Ventral Lateral Nucleus	Thalamus		R L				L				L		
Lentiform nucleus	Putamen		R				R L	L					
	Caudate	R L											
Sensory-motor cortex	3	R											
Precuneus	7					R							
Insula	13									R	L		
Cuneus	17			R L		L							
Cuneus	18			R		R L			R				
Fusiform Gyrus	19					R L			R L			R	
Middle Temporal Gyrus	21							L		R			
Superior Temporal Gyrus	22				R			L		R			
Superior Temporal Gyrus	38			L									
Temporo-parietal Junction	39/40	R		R									
Parahippocampal Gyrus	Hippocampus		R										
Posterior Cingulate	23	L											
Parahippocampal Gyrus	35			L									
Parahippocampal Gyrus	36			L									
Parahippocampal Gyrus	37			L		R							
Uncus	20										R		
Inferior Frontal Gyrus	45									R			
Superior Frontal Gyrus	10, 11		R										
Anterior Cingulate	32		R										
Inferior Frontal Gyrus	34						R L						
Inferior Frontal Gyrus	47						R						

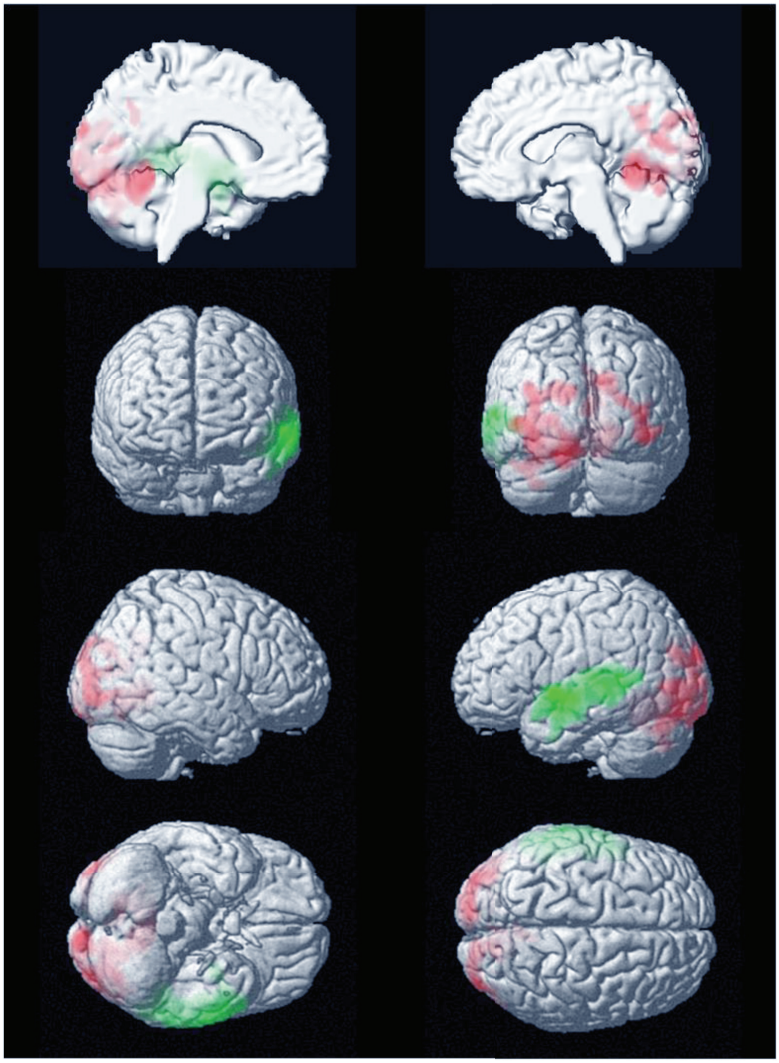
R: right; L: left

Figure 4. Positive correlation between rCBF and "Autistic/ADHD traits" in ASD (in red) and controls (in green).



Regions showing a positive correlation between rCBF and factor "Autistic/ADHD traits" in ASD (in red) and controls (in green). The first row represents the medial aspect of left (on the left) and right (on the right) hemispheres; the second row represents the anterior (on the left) and posterior (on the right) aspect on the brain; the third row represents the lateral aspect of the right (on the left) and of the left (on the right) hemispheres; the fourth row represents the inferior (on the left) and the superior (on the right) aspects of the brain

Figure 5. Positive correlation between rCBF and "Sensory-motor integration" in ASD (in red) and controls (in green).



Regions showing a positive correlation between rCBF and factor "Sensory-motor integration" in ASD (in red) and controls (in green). The first row represents the medial aspect of left (on the left) and right (on the right) hemispheres; the second row represents the anterior (on the left) and posterior (on the right) aspect on the brain; the third row represents the lateral aspect of the right (on the left) and of the left (on the right) hemispheres; the fourth row represents the inferior (on the left) and the superior (on the right) aspects of the brain.

4.3 STUDY IV

Study IV investigated the prevalence, as well as topographical pattern of MPAs in adults with ASD in comparison to neurotypical controls and examined whether MPAs can be correlated with severity of symptoms and overall functioning.

The main finding of the study was a negative correlation of both GAF-symptoms and GAF-functioning with the Craniofacial-MPA index, indicating that more MPAs in the craniofacial region were associated with lower overall GAF. The positive correlation of the Ears subscale with the AQ, and negative correlation with GAF-functioning was found in the subgroup of participants with high MPA frequencies (i.e. >5 in total MPA score). Thus, a higher frequency of MPAs in this region was associated with a higher frequency of autistic traits and a lower overall functioning (i.e. low GAF-f). In addition, a higher Head subscale score was associated with more severe psychiatric symptoms (i.e. low GAF-s) (Table 13).

Table 13. Correlations between MPA-total, MPA subscales, AQ and GAF for the Low-MPA and High-MPA groups in Study IV

	Low-MPA (total MPA<5)			**High-MPA (total MPA>5)**		
Individuals with ASD, n (%)	23 (46)			27 (54)		
Neurotypical controls, n (%)	37 (70)			16 (30)		
	AQ	**GAF-S**	**GAF-F**	**AQ**	**GAF-S**	**GAF-F**
Craniofacial-MPA index	0.20	-0.07	-0.06	0.12	**-0.37***	**-0-34***
Head	0.14	-0.01	-0.05	0.01	**-0.35***	-0.25
Eyes	0.08	0.06	0.11	-0.13	0.12	0.16
Ears	-0.06	0.10	0.12	**0.31***	-0.29	**-0.32***
Mouth	0.15	**-0.28***	**-0.28***	-0.15	0.14	0.03
Periphery-MPA index	0.06	-0.02	-0.03	-0.12	0.18	0.2
Hands	0.03	-0.11	-0.09	-0.16	0.08	0.18
Feet	0.06	0.02	0.01	-0.03	0.19	0.14
TOTAL MPA	0.24	-010	-0.10	0.03	-0.26	-0.23

Significances of correlations (Spearman's rank correlation test) are denoted by *p < 0.05

Furthermore, the means of Craniofacial-MPA index, Head subscale score and MPA-total score were significantly higher in the ASD group, compared with the control group (Table 14).

Table 14. Mean MPA scores for the ASD and neurotypical control groups in Study IV

	ASD (n=50)	**Neurotypical controls (n=53)**		
	Mean (SD)	Mean (SD)	*t*	*p*
Craniofacial-MPA index	3.64 (1.97)	2.69 (1.51)	-2.44	0.017
Head	1.06 (0.89)	0.66 (0.67)	-2.57	0.012
Eyes	0.68 (0.77)	0.61 (0.82)	-0.41	n.s.
Ears	1.52 (1.27)	1.09 (1.07)	-1.84	n.s.
Mouth	0.38 (0.60)	0.43 (0.60)	0.45	n.s.
Periphery-MPA index	0.95 (0.69)	1.0 (0.65)	0.38	n.s.
Hands	0.19 (0.43)	0.19 (0.40)	-0.16	n.s.
Feet	0.76 (0.52)	0.81 (0.48)	0.52	n.s.
TOTAL MPA	4.59 (1.91)	3.79 (1.67)	-2.26	0.026

5 DISCUSSION AND FUTURE DIRECTIONS

5.1 USE OF SELF-RATING SCALES

The RAADS-R was developed on the basis of DSM-IV criteria for ASD to support the clinical assessment of ASD in adults with normal IQ (Ritvo et al., 2008). In addition to evaluation of social impairments and repetitive and stereotyped pattern of behaviors, the RAADS-R covers symptoms of sensory-motor deficits that were recently introduced into the DSM-5 criteria for ASD (APA, 2013). The scale assesses symptoms in the perspective of life-time course (i.e. current and childhood symptoms).

Study I found support for the Swedish version of RAADS-R in that it meets requirements for being a reliable and valid instrument, useful for identifying autistic traits in adults with ASD. The important finding was that the Swedish version of RAADS-R differentiates ASD individuals from controls at a higher cut-off level (i.e. 72) in comparison to the international validation study of the English version (i.e. 65). The Social Interaction, Circumscribed Interests and Sensory Motor subscale scores showed good internal consistency and produced large group differences. Interestingly, the results imply that the Sensory Motor subscale could differentiate between groups as good as the Social Interaction and Circumscribed Interests subscales. This finding corroborates the ideas of Ssucharewa, Asperger and Kanner, who suggested that sensory-motor impairments are essential characteristics of ASD (Ssucharewa, 1926; Kanner, 1943; Asperger, 1944). Moreover, motor dysfunction is suggested to be related to the core dysfunctions in ASD (Leary and Hill, 1996b). As motor symptoms precede the social and linguistic problems (Teitelbaum et al, 2004) they also constitute an early visible biological marker for atypical brain development. In line with this, impaired integration of sensory input with motor commands was also reported, supporting a postulation of cerebellar dysfunction in Asperger syndrome (Gowen and Miall, 2005).

On the whole, ASD individuals scored significantly higher than neurotypical controls, and 60 percent of the variance was explained by diagnostic group membership. This indicates that the RAADS-R captures relevant features for differentiating ASD from neurotypical controls. Notably, some patients with other psychiatric disorders scored very high on the RAADS-R, although they were few in numbers. In contrast, psychiatric patients with non-ASD diagnoses scored relatively low in an English-American study testing RAADS-R in clinical populations (Ritvo et al., 2011). Therefore, it is important to further examine whether RAADS-R can differentiate between ASD and other psychiatric conditions.

As already mentioned in the introduction it is important to use self-rating scales together with clinical judgment and observations by informants. The current study supports the use of RAADS-R as an initial clinical assessment tool, used for screening of individuals with possible ASD, but the results should be interpreted in a wider context of a clinical evaluation.

5.2 CEREBRAL BLOOD FLOW DIFFERENCES IN ASD

Cerebral blood flow in adults with ASD was compared to a group of neurotypical controls in Study II. The results show an increased rCBF in a large portion of the posterior right cerebral and cerebellar hemispheres, particularly in the brain regions which have been previously implicated in ASD.

These results are in agreement with previous PET studies in which a global increase in CMRgl and metabolic abnormalities were found in networks between the frontal cortex, parietal cortex and subcortical regions in fourteen highly functioning young ASD males at rest (Rumsey et al., 1985). It was speculated that the reduced neuronal connectivity was due to a reciprocal inhibition between the frontal and parietal structures that causes deficits in arousal, motivation and sensory-motor integration. Our finding of a CBF increase in the primary visual cortex is consistent with decreased occipital alfa waves in EEGs (Cantor et al., 1986) and increased glucose metabolism in the calcarine cortex (Siegel et al., 1992) in ASD. Increased CBF in the primary visual cortex may be due to a heightened emotional status and increased processing of visual information (Belmonte and Yurgelun-Todd, 2003).

During the years, the cerebellum has been implicated in the pathophysiology of ASD. However, the report of lower Purkinje cell numbers and density observed in post-mortem studies mainly in mentally retarded or epileptic patients with ASD is in contrast with the finding of enlarged cerebellum in most MRI studies in which high-functioning individuals with ASD were recruited (Amaral et al., 2008). Hence, even if controversy remains concerning the anatomical consistency of cerebellar alterations, our finding of increased CBF in the anterior and posterior lobe suggests a role of the cerebellum in high-functioning individuals with ASD. The cerebellum is known to be involved in regulation of neural activity in networks involved in emotions and higher cognitive functions (D'Angelo and Casali, 2012), in other words, it may affect a person's ability to process and organize information and react to non-verbal signals and emotional clues (Timmann and Daum, 2007). It has been suggested that impairment of executive skills, flattening of affect, and abnormal social behavior can arise from dysfunction of the pathways connecting the cerebellum to the prefrontal, limbic and striatal regions of the brain (Schmahmann and Sherman, 1998; Critchley et al., 2000).

CBF increases were also found in the parieto-temporal lobes and in the hippocampus. The hippocampus, like the amygdala, is involved in social learning, cognitive functions and emotional processing (DeLong, 1992). Possibly, it plays a role in the deficits in social behavior and social cognition typically seen in individuals with ASD. The CBF increases found in the parieto-temporal region may reflect the involvement of the mentalizing networks in the pathophysiology of ASD (Frith and Frith, 2003).

Previous studies have reported positive correlations between repetitive behavior and caudate enlargement in young adults and adolescents with ASD, resulting in disturbances of the striato-thalamic circuitry (Hollander et al., 2005). This network is important in resource-demanding tasks that support working-memories demands and its relative rCBF increase might be related to repetitive thoughts during the examination in individuals with ASD. Neuroanatomical differences between groups may also

contribute to the observed CBF changes. Thicker grey matter has been reported in the temporal lobe (Hardan et al., 2006b) and in the cerebellum of individuals with ASD. Moreover, grey matter volume reductions have been found in the prefrontal lobe and the parietal networks (McAlonan et al., 2005). Structural studies in ASD have demonstrated increased neurogenesis, abnormal size, number and distribution of neurons, changes in the elaboration of axons, dendrites and synapses, and dysfunction of neuronal pathways (Bailey et al., 1998). All these factors can pervasively impair interregional connectivity and result in regionally distributed differences in brain anatomy, possibly causing functional loops following focal activation.

5.3 CORRELATIONS OF CEREBRAL BLOOD FLOW WITH AUTISTIC TRAITS, ADHD-SYMPTOMS AND NEUROLOGICAL SOFT SIGNS

Study II explored CBF differences between two comparison groups whereas Study III investigated specific contribution of various brain regions to different symptomatic dimensions in ASD phenotype. The neurobiological substrates underlying autistic traits, ADHD symptoms and neurological soft signs were examined in the same sample.

The findings in Study III seem to be consistent with other research which found that the ASD phenotype involves not only autistic traits, but also symptoms of inattention, hyperactivity and motor problems (Brieber et al., 2007; Reiersen and Todd, 2008; Rydèn and Bejerot, 2008; Plenty et al., 2013). Moreover, co-occurrence of autistic traits, ADHD symptoms and neurological soft signs is associated with specific patterns of changes in resting rCBF in adults with ASD. This supports previously reported results that indicate a common neural substrate for symptoms of inattention, hyperactivity and autistic symptoms in children with ASD (Brieber et al., 2007; Yamasaki et al., 2010). In addition, changes in white matter volume in the left primary motor and premotor cortex could predict poorer motor skills in children with autism in comparison to groups of ADHD and healthy controls (Mostofsky et al., 2007).

Since principal component analysis was suggested to be appropriate for exploring underlying etiological and pathophysiological causes (Dworzynski et al., 2009; Steer et al., 2010), this approach was applied in Study III to investigate whether resting rCBF correlates to three principal components or factors: “Autistic/ADHD traits”, “Sensory-motor integration” and “Intelligence/Motor sequencing”.

The most interesting finding was that autistic traits and ADHD symptoms got grouped into one factor and therefore imply an association with common neural substrates in certain brain regions such as the temporo-parietal junction, sensory-motor cortex, caudate as well as middle and superior temporal gyrus. All these areas have previously been reported to be implicated in both ASD and ADHD (Ecker et al., 2012; Fassbender and Schweitzer, 2006; Lombardo et al., 2011).

Another important finding was that clinically assessed scores in the sensory-motor integration when grouped in one independent factor, positively correlated with rCBF in the cuneus and fusiform gyrus in the ASD group. Similar findings were previously

reported in ASD with increased right-lateralized activation in occipital regions (Belmonte and Yurgelun-Todd, 2003; Damarla et al., 2010; Manjaly et al., 2007), excess pattern of cortical thickness in secondary visual area (BA 18) (Ecker et al., 2010) and abnormal gamma band activity in the visual cortex related to lack of inhibitory processing at the low-level perception (Brown et al., 2005). Atypical visual perception and visuospatial abilities in individuals with ASD can be related to impairments in social communication (Dakin and Frith, 2005; Sutherland and Crewther, 2010), Theory of Mind and empathy processing (Völlm et al., 2006). However, atypical visual processing can also be linked to the savant capacities and exceptional attention to details.

Previously, face identification, face processing and object perception were associated with activation in fusiform gyrus (Bly and Kosslyn, 1997). We found a positive correlation between the "Sensory-motor integration" factor and the rCBF in fusiform gyrus area, in which increased grey matter volume was previously demonstrated in individuals with ASD (Waiter et al., 2004). Notably, the impairment in face recognition has been suggested to be one of several factors that influence development of social skills (Barton et al., 2004).

In study III, all three factors showed negative correlations with rCBF in the left thalamus.
This is in line with the previously reported metabolic changes in the thalamus bilaterally (Haznedar et al., 2006) and abnormal neurotransmission in individuals with ASD (Bernardi et al., 2011). In addition, presence of neurological soft signs was related to thalamus abnormalities in earlier studies (Dazzan et al., 2006; Thomann et al., 2009).

Impaired integration of sensory inputs with motor commands has been reported, supporting a cerebellar and thalamus dysfunction in ASD as well as in ADHD (Gowen and Miall, 2005; Hardan et al., 2008). Thalamo-cortical functional connectivity was investigated by Zhang et al. and correlations between the parieto-occipital cortex and the lateral portion of the thalamus were found in neurotypical controls (Zhang et al., 2008). It was proposed that sensory integration deficits in autism may arise from alterations in such connections based on deviant synaptic modulation (Hardan et al., 2008; Horwitz et al., 1988). Moreover, enhanced subcortico-cortical connectivity in autism has been suggested to compensate for a reduced cortico-cortical long-range connectivity (Mizuno et al., 2006; Paakki et al., 2010). Involvement of the thalamus in the networks subserving alerting of attention may be linked to cognitive deficits in ASD (Fan et al., 2005).

On the whole, ADHD symptoms and neurological soft signs in our ASD sample are shown to be parts of the ASD phenotype. Moreover, autistic traits and ADHD symptoms are suggested to share neural substrates. Thus, this study endorses the appropriateness of assessing neurological soft signs in adults with ASD. However, more research on this topic needs to be undertaken as the association between neurophysiological alterations and clinical impairments is not clearly understood yet. Since the sample size was small in these two studies more research is needed with larger populations. It would be of interest to investigate how CBF is related to other

biological markers, such as signs of neuroinflammation and abnormalities of the auditory brainstem response.

In the future, connectivity studies might allow more detailed analysis of the cross-regional neuronal relationships underlying ASD. Cortical as well as sub-cortical pathological networks can be uncovered by applying principal or independent component analyses to functionally (Pick Atlas) or anatomically (Automated Anatomical Labeling) pre-segmented brain areas. This will be possible when a larger number of patients and healthy controls are recruited resulting in reliable statistics. Moreover, the use of electroencephalography has to be also considered since it will allow on-line monitoring for long periods during both activation and resting-state of the neurobiological changes occurring in psychiatric disorders (Pagani et al., 2012). In this respect, recent technical and software advancements have improved statistical and cortical representation of EEG activity differences between groups.

5.4 MINOR PHYSICAL ANOMALIES, AUTISTIC TRAITS AND FUNCTIONING

Study IV investigated the presence of MPAs in adults with ASD and behavioral correlates such as autism severity and functioning. The prevalence and patterns of MPAs in adults with ASD were compared to those of neurotypical controls. The results of this study suggest a link between MPAs, presence of autistic traits and level of functioning. In comparison to the neurotypical controls adults with ASD showed higher rates of MPA resulting in a higher total Waldrop score, as well as higher scores in the craniofacial region. In addition, two out of 16 MPAs (i.e. “fine electric hair” and “single palmar crease”) showed capacity to differentiate ASD from neurotypical controls, but neither of them are specific for ASD, they are also often found in patient with e.g. schizophrenia (Akabaliev et al., 2001). Possibly, this reflects shared genetic susceptibility, overlapping neural substrates and overlap in clinical impairments (Braff et al., 2007).

The findings of the current study are consistent with previous results in studies of children with ASD that reported higher rates of MPAs in comparison to typically developing children (Ozgen et al., 2011; Rodier et al., 1997; Tripi et al., 2008; Walker, 1977).

Our study identified two subgroups with different frequencies of MPAs: “Low MPA” and “High MPA”. Individuals with ASD were overrepresented in the “High MPA” group and they possibly represent a complex (i.e. sporadic) type of autism (Miles et al., 2005). It was proposed that single environmental insults during early foetal life and non-transmitted genetic events contribute to the aetiology of complex autism. Hence, individuals with high rates of MPAs represent a specific subgroup of ASD with higher risk for poorer outcomes in comparison with the so-called “familial” type.

All MPAs were divided into subscales by body region. Differences in the mean of the Head subscale (but not in the other body regions) were observed between the ASD group and the neurotypical controls. Previous research has shown that increased head

circumference correlates with more severe social impairment in children with ASD (Lainhart et al., 2006). More recently it was suggested that specific genes are associated with two reciprocal subtypes (i.e. CHD8-gene with macrocephaly and DYRK1A-gene with microcephaly) (O'Roak et al., 2012), although these genes are suggested to only contribute to about one percent of the observed sporadic ASD cases.

In the present study, the associations of Head score and Craniofacial-MPA index with severity of symptoms, indicate that deviations in this region could, at least to some extent predict severity of ASD symptoms. In addition, earlier studies have shown that minor anomalies of ears could discriminate children with autism (mostly with mental retardation) from typically developing children (Campbell et al., 1978; Rodier et al., 1997; Walker, 1977). We could not replicate these findings, nevertheless self-rated autistic traits correlated positively to the Ears subscale score in the High-MPA group and excess MPAs in this specific region were associated with lower functioning in the High-MPA group.

Notably, a substantial subgroup of neurotypical controls exhibited MPAs, mostly in the lower range, whereas the participants in the ASD group were almost equally distributed between having high and low total MPA scores. These rates were comparable to scores found in neurotypical controls in previous studies (Green et al., 1994; Ozgen et al., 2011; Trixler et al., 2001; Tripi et al., 2008; Sivkov and Akabaliev, 2003) suggesting that quantitative measures of MPAs are appropriate in the clinical assessment.

On the whole, MPAs are suggested to be external markers for atypical brain development. Research questions that future studies need to address are the clinical value of MPAs including influences of gender, IQ, genotype and family history of psychiatric and neurological disorders. In addition, it would be of interest to make direct comparisons of the topographical patterns of MPAs between ASD, schizophrenia, ADHD and other conditions, allowing for between-group analyses in search of various diagnostic patterns.

5.5 METHODOLOGICAL CONSIDERATIONS

Selection of participants

One important limitation of Study I was the lack of matching between groups with respect to gender. Although age and normal intelligence level (i.e. IQ>70) were equally distributed in both groups, females were overrepresented in the control group with risk of overestimation of the specificity of the RAADS-R.

There are several limitations in Studies II and III. First, we included six ASD subjects treated with psychotropic drugs, which may have affected rCBF. However, un-medicated patients are seldom found in clinical ASD population. When the subjects with co-occurring symptoms and medication were removed from the analysis, the remaining group showed a similar pattern of rCBF increases suggested to be specific for ASD. The findings of increased rCBF associated with ASD would be in fact

supported by the use of psychotropic drugs, known to decrease CBF such as SSRIs and anxiolytics.

In Study IV, only Caucasians were included because hair, shape of eyes and ears often vary between different ethnic groups. Thus, the present findings cannot be generalized to other ethnic groups.

Clinical evaluation and assessments

In Study I, a substantial proportion of neurotypical controls (i.e. n=90) were not seen in person by the investigators since they completed RAADS-R anonymously.

In Studies II, III and partly in Study IV, the participants were assessed un-blinded which may contribute to observer bias and overestimation of pathological features in patients. However, individuals with ASD have obvious social impairments and since neuropsychiatric and physical examination requires personal interaction, the patients' behaviors in such clinical settings may un-blind a clinician or researcher. Also, patients with ASD often express reluctance at having to be examined by an unfamiliar clinician.

Statistical limitations

In Study I, the scores of RAADS-R in the control group were non-normally distributed; therefore it was not possible to use parametric statistics. It is known that ANOVA is robust against a deviation from normality, if it is not caused by outliers. Therefore, we also ran the analyses with the outliers removed. The results continued to be robust with the exception of overall larger effect sizes. Nevertheless, it is possible that the non-normality of the distribution could affect the results in some way.

In Studies II and III, the analyses were performed accepting significances of $p<0.05$ uncorrected at peak level. Due to the small sample size (not unusual in neuroimaging studies) this liberal choice was adopted to avoid type II errors attributable to over-conservative thresholds (Oishi et al., 2005). Given the exploratory nature of this analysis and considering the relatively low sensitivity of PET without repeated measures, higher thresholds could lead to false-negative results.

In Study IV, the sample was also relatively small which increases the risk for type II errors and limits the power. On the other hand, we did not correct p values for multiple comparisons, which increases the risk for type I error. Thus, the statistical significance of the results should be interpreted with caution and future studies should preferably include larger samples.

6 CONCLUSIONS AND CLINICAL IMPLICATIONS

This thesis has investigated neurobiological aspects of ASD and possible organic deviations in adults with ASD. On the whole, a neurobiological model for ASD is supported by the current findings. The results of the neuroimaging studies enhance the understanding of the neurobiological basis of different symptomatic dimensions in adult ASD. Co-occurrence of inattention, hyperactivity and motor problems in ASD has frequently been observed by clinicians. Studies II and III contribute to the evidence suggesting that autistic traits, ADHD-symptoms and deficits in motor and sensory-integration are associated with both specific and common neural substrate. Accordingly, it seems appropriate to assess symptoms of ADHD and sensory-motor abnormalities in adults with ASD.

Since the Swedish version of RAASD-R has been successfully validated it can be used by clinicians for screening as well as for support of an ASD diagnosis in adults. In addition, MPAs may serve as risk markers and indicate need for additional medical and social support.

7 SUMMARY IN SWEDISH

Autismspektrumtillstånd utgörs av en grupp utvecklingsrelaterade neuropsykiatriska tillstånd som kännetecknas av svårigheter avseende socialt samspel och ömsesidig kommunikation samt av förekomst av repetitiva eller stereotypa beteenden. Tidigare studier har påvisat strukturella och funktionella avvikelser i olika hjärnstrukturer vid autismspektrumtillstånd. Motoriska svårigheter, små morfologiska avvikelser och en annorlunda perception är välkända men inte alltid systematiskt undersökta i den vuxna populationen med autismspektrumtillstånd och med normal begåvning.

I **delarbete I** har intern konsistens, diagnostisk och samtidig validitet av den svenska versionen av Ritvo Autism Asperger Diagnostic Scale - Revised (RAADS-R) utvärderats. Resultaten visade att den svenska versionen har goda psykometriska egenskaper samt korrelerar starkt med skattningsskalan Autism-Spectrum Quotient (AQ). RAADS-R fångar upp autistiska symptom och kan användas för screening och vidare utredning av autismspektrumtillstånd hos vuxna med normal begåvning.

I **delarbetena II och III** ingick tretton personer med autismspektrumtillstånd och tio kontroller. Samtliga undersöktes med positronemissionstomografi (PET) efter att ha genomgått en psykiatrisk intervju och en neurologisk undersökning. I jämförelse med kontrollgruppen hade personerna med autismspektrumtillstånd signifikant ökat cerebralt blodflöde bilateralt i stora delar av lillhjärnan, i occipitala associativa och i posteriora parietala hjärnbarken. I delarbete III identifierades tre kluster av faktorer som motsvarar ”autistiska drag och ADHD-symtom”, ”sensomotorisk integration” och ”intelligens och sekvens av motoriska rörelser”. I autismspektrumgruppen visade en korrelationsanalys mellan blodflödesindex och faktorn ”autistiska drag och ADHD-symtom” positiva korrelationer i nucleus caudatus bilateralt och i inferiora parietalloben vilket kan relateras till brister i exekutiva funktioner såväl som till rigida, stereotypa eller tvångsmässiga beteenden. Faktorn ”sensomotorisk integration” korrelerade positivt med cerebralt blodflöde i parieto-occipitala hjärnbarken vilket möjligen återspeglar en atypisk visuell perception som ofta ses hos personer med autismspektrumtillstånd. Det cerebrala blodflödet i vänster talamus korrelerade negativt med alla tre faktorer vilket poängterar betydelsen av detta hjärnområde för patogenesen vid autismspektrumtillstånd. Sammanfattningsvis visar delarbete II och III att den neurobiologiska basen för autistiska drag, ADHD-symtom och subtila neurologiska avvikelser delvis är gemensam.

I **delarbete IV** undersöktes små morfologiska avvikelser hos 53 personer med autismspektrumtillstånd och 50 köns- och åldersmatchade kontroller. Autismspektrumgruppen hade signifikant fler små morfologiska avvikelser i jämförelse med kontrollgruppen och dessa avvikelser var associerade med svårighetsgrad av symtom och funktionsnedsättning skattad med GAF-skalan.

Fenotypen för autismspektrumtillstånd innefattar flera beteendemässiga, kognitiva och neurobiologiska avvikelser. För att uppskatta komplexiteten vid autismspektrumtillstånd är det meningsfullt att ta hänsyn till förekomst av ADHD-symtom samt subtila neurologiska och morfologiska avvikelser.

8 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to many wonderful people who have contributed to making this thesis possible and supported me in my work.

First of all, I would like to thank all study participants who sacrificed their time and made these research projects possible.

I would like to acknowledge a debt of gratitude to my main supervisor Associate Professor **Susanne Bejerot** for giving me the opportunity to become a PhD-student and for encouraging me in my research. You always believed in me during this long journey.

I deeply want to thank my co-supervisor and former clinic head at the Northern Stockholm psychiatric clinic, Professor **Anna Åberg Wistedt** for the opportunity to become a resident physician in psychiatry, for your enthusiasm, energy, and all the support I have received during these years.

Also, a deep thank to my co-supervisor **Marco Pagani** for your humor and for guiding me through the neuroimaging jungle.

I would especially like to thank:

My mentor Professor Emeritus **Lennart Wetterberg** for all of the worthwhile discussions we have had during the years.

My co-authors on these projects and especially Professor **Sharon Stone-Elander**, Professor **Hans Jacobson**, **Mats Humble** and **Jonna Eriksson**. Without you this work would not have been possible! All co-workers at the Department of Nuclear Medicine, Karolinska University Hospital for friendly collaboration. Also, Associate Professor **Håkan Källmén** for assistance with statistical topics.

My former clinic head at the Northern Stockholm psychiatric clinic **Anna Stenseth** for support in the beginning of my doctoral studies.

My present head of clinic **Kersti Gabrielsson** and head of department **Helena Persson** at the Järvapsykiatrin for your kind support and giving me the opportunity to combine work and research. Also, all colleagues and co-workers at the Järvapsykiatrin, in particular at the psychosis department, for good collaboration and great support.

My dear friends and colleagues **Maia Alvariza**, **Elizabet Haking** and **Jeanette Georgieva** for your optimism, believing in me and moral support during many years of work and research. Thank you for numerous lunches and discussions about all aspects of life and tips on how to survive! Jeanette, I want to thank you for all your help editing my thesis and all the great comments, I really appreciate it!

My research school fellow **Emma** for all those hours we studied together and never gave up as well as shared a lot of fun. Many thanks to **Selma** who gave us support!

My dear friends **Lena**, **Natalia** and **Alexandra** for being the best friends I ever could have and yours kids **Nina**, **Catalina**, **Niklas**, **Alexander** and **Vera** for lots of funny moments throughout the years. Natalia and Lena, you have taught me to never give up! Nina, thanks for believing in my skiing abilities! Many thanks to my friends **Marina**, **Jury** and **Mattias** for interrupting my writing with energizing peeks at Domarudden.

My dearest dearest friends **Lena** and **Urban** for your love and support!

От всего сердца хочу поблагодарить **Инну Абрамовну Косаревскую** за веру в меня, любовь, вдохновение и поддержку! Без Вас я не достигла бы этой высоты.

Я бесконечно благодарна **Любовь Григорьевне Дисенбаевой** за теплоту, любовь и такие нужные слова поддержки на этом сложном и увлекательном пути!

My dear parents **Valentina** and **Anatoly**, my brother **Alexey,** for your love and for encouraging me in my studies, work and research. I am who I am now thanks to you. Without your help and support I could never have done this dissertation. I love you!
Я вас безумно люблю!

Last and most important, I would like to thank my lovely daughter **Camilla**, мое солнышко и счастье, for your love, patience, strength, optimism and managing our life during these last months. You are the most important person in my life and
I love you so much!

These studies were supported by grants from the Praktikertjänst AB; Psykiatrifonden, Sweden and through the regional agreement on medical training and clinical research (ALF/Pick-Up) between Stockholm County Council and Karolinska Institutet.

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J Autism Dev Disord (2011) 41:1635–1645
DOI 10.1007/s10803-011-1191-3

ORIGINAL PAPER

The Swedish Version of the Ritvo Autism and Asperger Diagnostic Scale: Revised (RAADS-R). A Validation Study of a Rating Scale for Adults

Lisa M. J. Andersen · Katharina Näswall · Irina Manouilenko · Lena Nylander · Johan Edgar · Riva Ariella Ritvo · Edward Ritvo · Susanne Bejerot

Published online: 16 February 2011

Abstract There is a paucity of diagnostic instruments for adults with autism spectrum disorder (ASD). This study evaluates the psychometric properties of the Swedish version of the Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R), an 80-item self-rating scale designed to assist clinicians diagnosing ASD in adults. It was administered to 75 adults with ASD and 197 comparison cases. Also, a subset completed the Autism Spectrum Quotient (AQ). Three out of four subscales had high internal consistency. Sensitivity was 91% and specificity was 93%. The ASD subjects had significantly higher mean scores on all subscales. ASD females had higher scores than ASD males on the sensory motor subscale, a dimension not included in the AQ. RAADS-R showed promising test re-test reliability.

Keywords Autistic disorder · Asperger syndrome · Psychiatric status rating scales · Self assessment (Psychology) · Diagnostic techniques and procedures · Adult

L. M. J. Andersen · K. Näswall
Department of Psychology, Stockholm University, Stockholm, Sweden

K. Näswall
Department of Psychology, University of Canterbury, Christchurch, New Zealand

I. Manouilenko · S. Bejerot
Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

L. Nylander
Department of Clinical Sciences, Psychiatry, Lund University, Lund, Sweden

J. Edgar
Faculty of Health Sciences, Linköping University, Linköping, Sweden

R. A. Ritvo
Yale University School of Medicine, Yale Child Study Center, New Haven, CT 06520, USA

E. Ritvo
UCLA School of Medicine, The Neuropsychiatric Institute, Los Angeles, CA 90024, USA

S. Bejerot (✉)
Northern Stockholm Psychiatry, St Göran Hospital, 112 81 Stockholm, Sweden
e-mail: susanne.bejerot@ki.se

Autism spectrum disorder (ASD) is characterized by impairments in social relatedness, communication and restricted patterns of behavior and interests (APA 2000). The variability of symptom expression in ASD is striking, a fact which has contributed to the continuous revisions and broadening of definitions and diagnostic criteria since Kanner's (1943) original descriptions. Symptoms vary depending on level of cognitive functioning, verbal ability and age, amongst other things (Volkmar et al. 1997). The current consensus is that these differences are due to variations in severity rather than distinct subtypes (Gilchrist et al. 2001; Howlin 2003). This is reflected in the proposed revisions for DSM-V, where the diagnosis Asperger's disorder is subsumed under the diagnosis Autism Spectrum Disorder.

In Britain, the prevalence of undiagnosed autistic adults is found to be 1% (Brugha et al. 2009). This indicates undiagnosed ASD is a major public health issue in England, and most likely in other countries as well. Since most cases of ASD are diagnosed in childhood much effort has been put into developing strategies for early detection.

Thus, many ASD individuals with milder impairments go through childhood and adolescence without receiving a diagnosis. In a large British study, Barnard et al. (2001) found that 29% of individuals with high functioning autism and 46% of those with Asperger's disorder had not received this diagnosis until late adolescence or adulthood, which is in line with experiences in Sweden (Rydén and Bejerot 2008). One reason for this is probably the relatively recent inclusion of Asperger's disorder in the diagnostic manuals DSM-IV and ICD-10. Other reasons may be that high intelligence and verbal ability can compensate for, and camouflage, other impairments, or that existing difficulties are mistaken for expressions of other psychiatric or psychosocial problems (Gillberg 2002). Also, other psychiatric disorders and symptoms often coexist with ASD (Bejerot and Wetterberg 2008). Impairments may become more pronounced in the transition to adulthood, when demands on self reliance and ability to structure one's life increase, while social skill becomes even more important for academic as well as occupational achievements (Hendricks and Wehman 2009; Tantam 1991). Thus it is important to be able to make a diagnosis of ASD in adults.

The symptoms and impairments of ASD manifest themselves differently with increasing age. Several cross sectional and longitudinal studies suggest a trend towards general symptom abatement in adolescence and adulthood (Billstedt et al. 2007; Esbensen et al. 2009; Fecteau et al. 2003; Seltzer et al. 2003; Seltzer et al. 2004; Shattuck et al. 2007). There is some evidence that there is a risk of excluding older, high functioning individuals when standard diagnostic instruments and algorithms are utilized (Boelte and Puostka 2000; Fecteau et al. 2003; Lord et al. 1994).

There are several specific difficulties in diagnosing autism spectrum disorders in adults. Significant others who can provide information about childhood symptoms may be absent, which is necessary both for ADI-R (Lord et al. 1994) and DISCO (Leekam et al. 2002). Also, many subjects may not want to involve their parents in the diagnostic procedure. Presently there are only two validated, self-administered scales that purport to measure autistic symptoms in adults. One is the Autism Spectrum Quotient (AQ, Baron-Cohen et al. 2001) a research and screening instrument described in detail below. The other is the Ritvo Autism and Asperger Diagnostic Scale (RAADS, Ritvo et al. 2008).

The RAADS was developed (Ritvo et al. 2008) to accommodate the need for diagnostic tools specifically tailored for adults with ASD. The objective of the present study is to evaluate the Swedish version of the RAADS-R (a modified version of the RAADS) with respect to internal consistency, test re-test reliability, diagnostic accuracy and concurrent validity.

Methods

Participants

The total sample comprised 272 adult subjects ages 19–75. Two groups of participants were recruited: 75 with ASD (the ASD group) and 197 without ASD (comparison cases). See Table 1 for their sex ratio and age distribution. Subjects with ASD were recruited among patients diagnosed at the Neuropsychiatric unit, Northern Stockholm psychiatry ($n = 17$) or at a specialized unit in Lund ($n = 6$). In addition, 52 subjects with ASD who were participants in various research projects at Northern Stockholm Psychiatry were included. All subjects with ASD were examined by an experienced clinician and their diagnosis was confirmed by either the Autism Diagnostic Observation Schedule-Generic (ADOS-G, Lord et al. 2000) (in Stockholm) or the Diagnostic Interview for Social and Communication Disorders (DISCO) (in Lund). Administration of these instruments requires extensive training. Seventy-three subjects were diagnosed with Asperger's disorder, and 2 with PPD-NOS (atypical autism). The standardized assessment of ASD in the Neuropsychiatric units in Stockholm and Lund include intelligence testing with WAIS. All included subjects had an IQ above 70, in other words no one fulfilled the diagnostic criteria for intellectual disability. All subjects had received their initial ASD diagnosis in adolescence or adulthood.

The comparison cases consisted of 61 doctors and medical students, 69 university students from three campuses in Sweden, and 60 subjects who comprised comparison cases in the research studies mentioned above. In addition, 7 psychiatric patients who were assessed for ASD but did not meet criteria were included. Out of these subjects, 6 met criteria for other psychiatric disorders (schizotypal personality disorder, ADHD, social anxiety disorder, depression, bipolar disorder, and delusional disorder). In the comparison cases another 6 subjects reported that they had a psychiatric diagnosis (depression, social anxiety disorder, generalized anxiety disorder, personality disorder NOS, and obsessive compulsive disorder). The study was approved by the Regional Ethic committee in Stockholm, and informed consent was obtained from all participants.

Table 1 Sex ratio and age by group

Subject group	N	Male: Female	Mean age (SD), min–max
ASD	75	36:35	31 (9), 26–62
Comparison cases	197	80:116	34 (13), 19–75
Total sample	272	120:152	33 (12), 19–75

Information on sex was missing for 1 subject in the comparison cases and 4 subjects in the ASD group

Measures

Two measures were used in the study: RAADS-R (Ritvo et al. 2010) and the AQ (Baron-Cohen et al. 2001). The RAADS-R is a revised version of the Ritvo Autism and Asperger Diagnostic Scale (RAADS), a self rating scale developed by Ritvo et al. (2008) to serve as an aid in the diagnosis of ASD in adults of normal intelligence. Items were formulated from DSM-IV and ICD-10 criteria for autism, which were operationalized to match the symptom expression in adults based on the authors' clinical experience. The original RAADS encompassed 78 items which were divided into three subscales to assess functioning in the domains of social interaction, language/communication and sensory motor/stereotypies (Ritvo et al. 2008). Following the 2008 pilot study, some alterations were made to the scale. Revisions included elimination of three items to improve internal consistency, adding more items on circumscribed interests, and some modifications to the subscales, like splitting up the sensory motor/stereotypies subscale into two separate scales.

Presently, the RAADS-R encompasses 80 items which are divided into four domains to assess functioning in: (a) social interaction, (b) language, (c) circumscribed interests and (d) sensory motor symptoms. Each item is formulated as a statement from the patient's point of view (e.g. *"I often don't know how to act in social situations"*). 17 items are reversed in order to avoid response bias and to elicit information about skills or preferences acquired throughout the life span (e.g. "*I like to have close friends*") (Ritvo et al. 2008). See "Appendix" for the full content of the various subscales. The statements are answered on a four point Likert scale with the qualitative alternatives "never true", "true only when I was young (before the age of 16)", "true only now" and "true now and when I was young". The 63 "positively worded" statements are scored from 0 to 3, so that the longer a symptom has been present the more points it yields, and the 17 reversed statements are scored in the reverse order (marked with an * in the "Appendix"). Higher scores are indicative of ASD in all subscales. The original RAADS pilot study (Ritvo et al. 2008) yielded promising results. In a sample comprising 37 subjects with autistic disorder or Asperger's disorder, 41 subjects with no psychiatric condition and 16 subjects with various psychiatric disorders outside the autism spectrum, RAADS demonstrated perfect sensitivity and specificity, as all subjects with an ASD obtained scores of 77 or higher whereas all subjects without an ASD scored at or below 64. Internal consistencies for the three subscales ranged from poor ($\alpha = 0.60$) to good ($\alpha = 0.84$). In a recent multicenter study the RAADS-R also demonstrated excellent diagnostic accuracy as well as improved internal consistency (Ritvo et al. 2010).

The RAADS-R was translated into Swedish by Susanne Bejerot, M.D., with assistance of Dr Lena Nylander. It was back translated by a bilingual translator, after which it was compared to the original and no modifications were deemed necessary.

The AQ (Baron-Cohen et al. 2001) was designed as a brief, self-administered questionnaire purporting to measure the degree to which any adult with normal intelligence has "autistic traits". The rationale underlying the scale is the assumption that autism lies at the upper end of a spectrum of traits which are normally distributed in the population (Baron-Cohen et al. 2001). The AQ comprises 50 items, divided into five domains: (a) social skill, (b) communication, (c) attention switching, (d) attention to detail, and (e) imagination. The questions are answered on a 4-point Likert scale, where "definitely disagree" and "slightly disagree" are scored as 0, and "slightly agree" and "definitely agree" is scored as 1 for half the questions, while the rest are reversely worded and scored.

The AQ has been evaluated both as a research instrument and as a screening instrument. In a pilot study, Baron-Cohen et al. (2001) found that AQ scores produced the hypothesized group differences between subjects with and without ASD, between students of science versus humanities, and between men and women in the general population. AQ has also been found to have screening properties (Hoekstra et al. 2008; Woodbury-Smith et al. 2005), however, in one study it did not differentiate between patients with mild ASD and patients with other psychiatric conditions (Ketelaars et al. 2007). The internal consistencies of the subscales have ranged from poor to fair in different studies (Austin 2005; Baron-Cohen et al. 2001; Hoekstra et al. 2008; Hurst et al. 2007). Several studies examining the factor structure of the AQ have found that a two- or three-factor solution fitted the data better compared to the five originally proposed domains (Austin 2005; Hoekstra et al. 2008; Hurst et al. 2007). Swedish participants were administered a translated version of the AQ which has not been validated.

Procedure

All participants completed RAADS-R. A subset of 39 ASD patients and 49 comparison cases completed AQ as well. If the subject did not understand a question an investigator was available to offer clarification. All personal data was coded and all data analyses were made in SPSS, version 17. The response rate was set at a minimum of 80% of the questions for inclusion in the study. This led to the exclusion of two subjects, both female ASD patients. For the remaining data, isolated missing scores were replaced with the individual mean.

Results

Internal Consistency and Test–Retest Reliability

The internal consistency was assessed separately in the ASD group and in the comparison cases. Cronbach's coefficient alpha for the total scale was estimated at 0.92 in the ASD group and at 0.94 in the comparison cases. Internal consistencies for the four subscales were: social interaction $\alpha = 0.87/0.89$ (ASD group/comparison cases), language, $\alpha = 0.58/0.22$ (ASD group/comparison cases), circumscribed interests $\alpha = 0.73/0.73$ (ASD group/comparison cases), and sensory motor $\alpha = 0.81/0.77$(ASD group/comparison cases). Item 2 in the language subscale (*I often use words and phrases from movies and television in conversations*) had a negative corrected item-total correlation, and by removing it alpha for this subscale could be increased to 0.70/0.40 (ASD group/comparison cases).

Test -retest reliability was assessed in a subset of subjects comprising 12 with ASD who had completed RAADS-R on two separate occasions with 3–6 months interval. The total scores on the two occasions were strongly and positively correlated ($r = 0.80$, $p = 0.002$). Strong and significant correlations were also obtained for three of the subscale scores: social interaction ($r = 0.76$, $p = 0.004$), circumscribed interests ($r = 0.73$, $p = 0.002$) and sensory motor ($r = 0.84$, $p = 0.001$). For the language subscale the correlation was not statistically significant ($r = 0.43$, $p = 0.161$).

Correlation with the Autism-Spectrum Quotient

The degree of agreement between RAADS-R and AQ was assessed by comparing 35 subjects with ASD and 49 comparison cases. Correlation analyses between RAADS-R and AQ total and subscale scores were performed separately in the comparison cases and in the ASD subjects. In the ASD group there was a strong, positive correlation between RAADS-R and AQ (see Table 2).

In the comparison cases, a Spearman's rank order coefficient was computed as the variables were not normally distributed. The correlation between AQ and RAADS-R total scores was strong ($\rho = 0.70$, $p < 0.0001$). RAADS-R subscale; social interaction, circumscribed interests, and sensory motor all had moderate to strong correlations with AQ Total score (ρ = 0.51–0.72, all $p < 0.0001$). The language subscale however was not significantly correlated with AQ total or any of the subscale scores (ρ = 0.06–0.17, all $p > 0.05$).

Distribution of Scores

The distribution of scores is shown in Fig. 1a–e. As evident from the histograms, the scores of the comparison cases had strong positive skewness (2.17) and were markedly peaked (kurtosis = 6.78). The scores of the ASD group did not depart significantly from normality (skewness = 0.02, kurtosis = −0.33). The median, minimum, and maximum scores of the two groups are shown in Table 3.

Group and Sex Differences

To explore group differences ANOVAs were performed comparing RAADS-R total and subscale scores by Diagnosis (ASD versus comparison cases) and Sex. Five subjects were excluded from the analysis due to missing information on sex. Mean total and subscale RAADS-R scores for the ASD and comparison cases are shown in Table 4, together with the results of the ANOVAs. As indicated, main effects of Diagnosis were found across all tests, the ASD subjects scoring higher than the comparison cases on the full scale as well as all four subscales. There was no main effect of Sex on the Total score, but there was

Table 2 Correlations (Pearsons *r*) between RAADS-R and AQ total and domain scores in 35 subjects with ASD

AQ total and subscales	RAADS-R total and subscales				
	RAADS-R total score	Social interaction	Language	Circumscribed interests	Sensory motor
AQ total score	0.84***	0.75***	0.63***	0.79***	0.60***
Social skill	0.79***	0.73***	0.65***	0.65***	0.56***
Communication	0.82***	0.79***	0.52***	0.72***	0.62***
Attention switching	0.68***	0.62***	0.53***	0.71***	0.44**
Attention to detail	0.30	0.12	0.22	0.40*	0.41*
Imagination	0.55***	0.54***	0.47**	0.52***	0.27

* Significant at the 0.05 level

** Significant at the 0.01 level

*** Significant at the 0.001 level

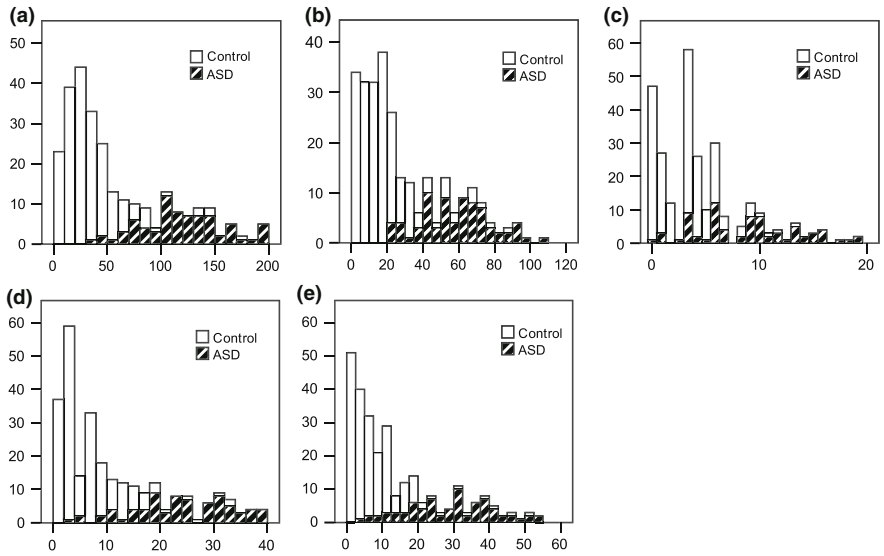

Fig. 1 Total RAADS-R and domain scores in the comparison cases group and ASD group. **a** Total RAADS-R. **b** Subscale: Social interaction. **c** Subscale: Language. **d** Subscale: Circumscribed interests. **e** Subscale: Sensory motor

Table 3 Median, minimum and maximum RAADS-R total and domain scores in the ASD group (N = 75) and the Comparison cases (N = 197)

Domain (no of items)	ASD			Comparison cases		
	Median	Min	Max	Median	Min	Max
Total RAADS-R (80)	114	34	198	28	0	175
Social interaction (39)	59	20	105	15	0	90
Language (7)	9	0	19	3	0	13
Circumscribed interests (14)	23	3	40	4.5	0	33
Sensory motor (20)	30	3	54	5.6	0	50

a significant two-way interaction between Diagnosis and Sex. In the comparison cases the males obtained higher total scores than females, whereas in the ASD group females obtained higher total scores than males. *T*-tests revealed that these sex differences were not significant when assessed either in the comparison cases ($t = 1.193$, $df = 137$, $p = 0.166$) or in the ASD subjects ($t = -1.847$, $df = 69$, $p = 0.069$).

On the social interaction and circumscribed interests subscales there were no significant main effects of Sex, nor any Diagnosis × Sex interaction. On the language subscale there was no main effect of Sex, but a significant Diagnosis × Sex interaction, comparison case males scored higher than comparison case females ($t = 2.370$, $df = 194$, $p = 0.019$). Females in the ASD group scored somewhat higher than males, though this difference did not reach significance ($t = -1.801$, $df = 69$, $p = 0.076$). Lastly, on the sensory motor subscale women generally obtained higher scores than men. There was also a significant Diagnosis × Sex interaction. *T* tests showed that women in the ASD group, but not in the comparison cases, obtained significantly higher scores than males ($t = -3{,}769, df = 69, p < 0.0001$).

Eight comparison cases and 3 subjects in the ASD group had scores that deviated markedly from the mean (at least 2 standard deviations). If these outliers were excluded from the analysis, the patterns of which Diagnosis and Sex differences were significant did not change.

Ability to Differentiate Between ASD and Comparison Subjects

In order to further examine the ability of RAADS-R to distinguish between the two groups, a ROC-graph was generated. The area under the ROC curve (AUC) was estimated at 0.96 (Std. err. 0.012, 95% CI 0.94–0.98), indicating high overall accuracy. This means that the probability of a randomly selected subject with ASD scoring higher than a randomly selected subject with no ASD was approximately 96% in this sample. Table 5 shows the sensitivity and specificity of RAADS-R total score at various cut-offs between 50 and 100. If sensitivity

Table 4 Mean RAADS-R total and domain scores and standard deviations for males and females in the comparison cases versus ASD group

Domain	Comparison cases *M* (SD)			ASD *M* (SD)			Diagnosis			Sex			Group × Sex		
	Males	Females	Tot	Males	Females	Tot	*F*	*p*	η^2	*F*	*p*	η^2	*F*	*p*	η^2
Total score	37.2 (32.1)	31.4 (23.9)	33.8 (27.6)	110.5 (36.5)	127.2 (39.8)	118.7 (38.8)	391.61	0.000	0.598	1.61	0.206	0.006	7.00	0.009	0.026
Social interaction	20.1 (17.8)	15.5 (13.5)	17.4 (15.6)	56.7 (18.7)	58.2 (20.9)	57.4 (19.7)	291.11	0.000	0.525	0.43	0.515	0.002	1.73	0.189	0.007
Language	3.4 (2.9)	2.6 (2.3)	2.9 (2.6)	7.7 (4.2)	9.7 (5.1)	8.7 (4.8)	159.08	0.000	0.377	1.52	0.219	0.006	10.13	0.002	0.037
Circumscribed interests	6.5 (6.4)	5.9 (5.9)	6.1 (6.1)	22.5 (9.8)	24.9 (8.2)	23.7 (9.1)	320.75	0.000	0.549	0.84	0.360	0.003	2.29	0.132	0.009
Sensory motor	7.6 (9.3)	7.6 (7.2)	7.6 (8.1)	24.3 (11.5)	34.3 (10.1)	29.2 (12.3)	298.57	0.000	0.532	15.98	0.000	0.057	15.97	0.000	0.057

The table also shows the results of the ANOVAs: *F*, *p*-values and partial η^2 (N = 266*, *df* = 1)

* Information on sex was missing for 1 subject in the comparison cases and 4 subjects in the ASD group, which were excluded from the analysis

Table 5 Sensitivity and specificity of RAADS-R at various cut-off scores (N = 272)

Cut-off	Sensitivity	Specificity
50	0.960	0.817
51	0.960	0.827
53	0.960	0.838
54	0.960	0.848
55	0.960	0.853
56	0.947	0.863
57	0.947	0.868
58	0.947	0.873
59	0.947	0.878
60	0.933	0.883
61	0.920	0.883
62	0.907	0.883
63	0.907	0.888
64	0.907	0.904
65	0.907	0.909
66	0.907	0.914
69	0.907	0.919
71	0.907	0.924
72	0.907	0.929
73	0.893	0.929
73	0.880	0.929
74	0.867	0.929
75	0.867	0.934
76	0.867	0.939
78	0.840	0.939
80	0.827	0.939
81	0.813	0.939
82	0.813	0.949
83	0.800	0.949
84	0.800	0.954
85	0.787	0.954
87	0.787	0.959
89	0.787	0.964
90	0.773	0.964
91	0.773	0.970
93	0.760	0.970
97	0.747	0.970
100	0.733	0.970

and specificity are given equal priority, a cut-off of 72 achieved the best compromise, with sensitivity 0.907 and specificity 0.929.

Discussion

The present study evaluates the psychometric properties of the Swedish version of the RAADS-R. The results indicate

that RAADS-R is reliable, has good diagnostic validity and thus can be a useful aid in the diagnostic assessment of ASD in adults.

Internal Consistency and Test Re-test Reliability

The internal consistency was fair or good for three of the subscales: social interaction, circumscribed interests and sensory motor. The language subscale however demonstrated poor internal consistency as measured with Cronbach's alpha. Part of the explanation for this is likely attributable to the fact that this subscale only consists of 7 items, as Cronbach's alpha is a function of both intercorrelation among items and scale length (Nunnally 1978), but it could also be a result of cultural nuances between the English speaking world and Sweden. For example, one item (*I often use words and phrases from movies and television in conversations*) was reversely correlated with the scale, implying that this item does not work the way it was intended, and that modification or removal of this item should be considered in the Swedish version. Preliminary estimates of the test re-test reliability of the total score and three of the domain scores were very promising, again with exception of the language subscale. However, these results should be interpreted with caution as the sample size for the test–retest analysis was very small.

Diagnostic Validity

As expected, the ASD group obtained significantly higher scores than the comparison cases group on the total RAADS-R score as well as all four domain scores, indicating that the Swedish RAADS-R captures symptoms, characteristics and experiences that are relevant to the differentiation of patients with ASD from neurotypical subjects. Ritvo et al. (2010) suggests a cut-off of 66 for differentiating between patients with and without autism spectrum disorders in a study including nine-centers in four English-speaking countries. In the present study specificity could be somewhat increased, while maintaining the same level of sensitivity, if the cut-off was set somewhat higher, at 72. Although lower than in the Ritvo et al. (2008) study, the levels of sensitivity and specificity obtained in the present study must be considered good for a self rating instrument. Self rating thus seems to be a viable method of assessing impairments in adults of normal intelligence with ASD. This is supported by previous studies which have also found that these individuals generally have insight into, and are able to reliably report on, their own difficulties and way of functioning (Baron-Cohen et al. 2001; Cederlund 2007; Ritvo et al. 2008; Woodbury-Smith et al. 2005).

However, the overlapping by nine percent in each group serves as a reminder that self ratings are not exact or perfect. Moreover, some individuals with severe forms of ASD tend to lack sufficient insight, and for this reason give normal responses. This underscores the need for complementary basic instruments for systematic observations, such as the High functioning Autism Asperger Scale (HAGS) (Bejerot et al. 2001). It is also recommended that a clinician be present during the completion of RAADS-R in order to clarify any confusion and to assess the reliability of the patients' responses (Ritvo et al. 2008).

As previously noted, 8 comparison cases (4 males and 4 females) obtained very high RAADS-R scores. 4 of these had undergone neuropsychiatric assessments and 3 were diagnosed with social anxiety disorder, ADHD, bipolar disorder, and schizotypal personality disorder. Another individual demonstrated many characteristics of ASD, and was assessed as having fulfilled diagnostic criteria in childhood; however presently he had no significant impairments. The fact that 3 out of the 12 subjects with a psychiatric disorder other than ASD obtained very high scores could be considered a problematic result. This underscores the importance of examining whether RAADS-R can differentiate between ASD and other psychiatric conditions, which may have overlapping symptoms. In addition, self-rating instruments alone are not sufficient in ambiguous cases; here clinical interviews are crucial to obtaining an accurate diagnosis.

Concurrent Validity

The overall strong and positive correlations between RAADS-R and AQ support the concurrent validity of the two instruments, although correlations provide only a rough estimate of the similarities and differences between them. The correlations between the domain scores are difficult to interpret as no factor analysis was performed on either instrument in the present study and previous factor analytic studies have suggested that the internal structure of the AQ does not fit the suggested domains (Austin 2005; Hoekstra et al. 2008; Hurst et al. 2007). The language and sensory motor domains within the RAADS-R had relatively modest correlations with the total AQ score compared to the social interaction and circumscribed interests domain scores. For the language subscale this might be due to poor internal consistency. The sensory motor subscale however showed good internal consistency and produced large group differences, implying that this subscale measures something unique and which is not included in the AQ conceptualization of autism. If one examines the content of the two scales this makes sense, as the AQ does not include questions on abnormal responses to sensory stimuli but stresses cognitive factors.

Sex Differences

A trend for females with ASD to score slightly higher than males with ASD is indicated, and this pattern was either the reverse or not replicated in the comparison cases. The higher scores in females with ASD could have several explanations: females may have greater insight into their symptoms than males; they may exaggerate their symptoms more; they may in fact have more symptoms than the males; female ASD might be more difficult to detect, thus the ones that are detected may have more extreme symptoms; or it could simply be a Type I Error. The sex difference in the comparison cases supports the male brain theory for autism, i.e. that males in general have more "autistic traits" than females in the normal population. Females with ASD scored higher than males on the sensory motor subscale. This may point towards a true sex difference in the symptomatology. Perhaps, in the future, these traits could serve as markers in genetic studies. In the comparison cases men scored slightly higher than women on the language subscale, but due to various problems with this subscale, one should be cautious with interpretations at this stage.

Limitations and Suggestions for Further Research

Some methodological limitations should be noted. First, the participants of the two groups compared in the study were not matched with respect to gender, age or intelligence. The age distributions of the two groups were roughly similar and all participants were in the range of intelligence above intellectual disability (i.e. IQ $>$ 70). However, the ratio of females to males was proportionally greater in the comparison cases compared to the ASD group. The fact that women were in majority in the comparison cases might possibly have led to a slight overestimation of the specificity of the RAADS-R as comparison case females obtained slightly lower mean scores than comparison case males, although this difference was not statistically significant. Furthermore, not all of the subjects in the comparison case group were seen in person by the investigators or screened for psychiatric disorders. This is true for 29 of the students as well as for the 61 doctors and medical students. A few of these subjects did obtain remarkably high scores on the RAADS-R. As it was completed anonymously the investigators did not have the possibility to examine those who were high scorers.

A third limitation has to do with the fact that the scores in the comparison cases were essentially non-normally distributed (which is to be expected with this type of instrument), thus using parametric statistics would be somewhat dubious. ANOVAs have proven to be rather robust against a deviation from normality, as long as it is not caused by outliers (Tabachnick and Fidell 2007). In an attempt to compensate for this, the analyses were also performed with outliers removed, and results were identical with the exception of larger effect sizes overall. However it is possible that the non-normality of the distribution may have affected the results in some way; the sex effects are probably the most vulnerable as the differences in means between men and women are much smaller than the differences between the ASD group versus the comparison cases.

Finally, future studies are needed to assess the sensitivity and specificity of the RAADS-R for subjects with other specific DSM diagnoses such as OCD, Social Anxiety Disorder, severe personality disorder and schizophrenia. It should be noted that different cut-off limits may be optimal with other comparison populations.

Conclusion

The results of the present study indicate that the Swedish RAADS-R is a reliable and valid instrument that can be a useful tool for clinicians when diagnosing the possibility of ASD in adults. This self-administered rating-scale is easily administered and user-friendly, properties which both are valuable and cost-effective. Three of the subscales have adequate psychometric properties, with the language subscale being the weakest for reasons discussed.

Appendix

See Tables 6, 7, 8, 9.

Table 6 RAADS-R subscale—Social interaction items

Social interaction	
1.* I am a sympathetic person	20. I like to copy the way certain people speak and act. It helps me appear more normal
6.* I can "put myself in other people's shoes"	21. It can be very intimidating for me to talk to more than one person at the same time
8. I only like to talk to people who share my special interests	22. I have to "act normal" to please others and make them like me

Table 6 continued

Social interaction	
11. I miss my friends or family when we are apart for a long time	23.* Meeting new people is usually easy for me
14. I'd rather go out to eat in a restaurant by myself than with someone I know	26.* I like having a conversation with several people, for instance around a dinner table, at school or at work
17. Others consider me odd or different	31. I have never wanted or needed to have what other people call an "intimate relationship"
18.* I understand when friends need to be comforted	43.* I like to talk things over with my friends
25. It is difficult for me to understand how other people are feeling when we are talking	47.* I feel very comfortable with dating or being in social situations with others
37.* I am an understanding type of person	48.* I try to be as helpful as I can when other people tell me their personal problems
38. I do not connect with characters in movies and cannot feel what they feel	53.* I am considered a compassionate type of person
3. I am often surprised when others tell me I have been rude	54. I get along with other people by following a set of specific rules that help me look normal
5. I often don't know how to act in social situations	55. It is very difficult for me to work and function in groups
12. Sometimes I offend others by saying what I am thinking, even if I don't mean to	60. When talking to someone, I have a hard time telling when it is my turn to talk or to listen
28. It is very difficult for me to understand when someone is embarrassed or jealous	61. I am considered a loner by those who know me best
39. I cannot tell when someone is flirting with me	64. How to make friends and socialize is a mystery to me
44. I cannot tell if someone is interested or bored with what I am saying	68.* I can tell when someone says one thing but means something else
45. It can be very hard to read someone's face, hand and body movements when they are talking	69. I like to be by myself as much as I can
76. It is difficult to figure out what other people expect of me	72.* I enjoy spending time eating and talking with my family and friends
79. I am often told that I ask embarrassing questions	77.* I like to have close friends
80. I tend to point out other peoples mistakes	

* = reversed item

Table 7 RAADS-R subscale—Language items

Language	
2. I often use words and phrases from movies and television in conversations	35. The phrase "I've got you under my skin" makes me very uncomfortable
7. I have a hard time figuring out what some phrases mean, like "you are the apple of my eye"	58.* I can chat and make small talk with people
15. I cannot imagine what it would be like to be someone else	66. The phrase "he wears his heart on his sleeve" does not make sense to me
27. I take things too literally, so I often miss what people are trying to say	

* = reversed item

Table 8 RAADS-R subscale—Circumscribed interests items

Circumscribed interests	
9. I focus on details rather than the overall idea	50. Sometimes a thought or a subject gets stuck in my mind and I have to talk about it even if no one is interested
13. I only like to think and talk about a few things that interest me	52. I have never been interested in what most of the people I know consider interesting
24. I get highly confused when someone interrupts me when I am talking about something I am very interested in	56. When I am talking to someone, it is hard to change the subject. If the other person does so, I can get very upset and confused

Table 8 continued

Circumscribed interests	
30. I get extremely upset when the way I like to do things is suddenly changed	63. I like things to be exactly the same day after day and even small changes in my routines upset me
32. It is difficult for me to start and stop a conversation. I need to keep going until I am finished	70. I keep my thoughts stacked in my memory like they are on filing cards, and I pick up the ones I need by looking through the stack and finding the right one (or another unique way)
40. I can see in my mind in exact detail things that I am interested in	75. When I go somewhere, I have to follow a familiar route or I can get very confused and upset
41. I keep lists of things that interest me, even when they have no practical use (for example sports statistics, train schedules, calendar dates, historical facts and dates)	78. People tell me that I give too much detail

* = reversed item

Table 9 RAADS-R subscale—Sensory motor items

Sensory motor	
4. Sometimes I talk too loudly or too softly, and I am not aware of it	49. I have been told that I have an unusual voice (for example flat, monotone, childish or high-pitched)
10. I always notice how food feels in my mouth. This is more important to me than how it tastes	51. I do certain things with my hands over and over again (like flapping, twirling sticks or strings, waving things by my eyes)
16. I have been told that I am clumsy or uncoordinated	57. Sometimes I have to cover my ears to block out painful noises (like vacuum cleaners or people talking too much or too loudly)
19. I am very sensitive to the way my clothes feel when I touch them. How they feel is more important to me than how they look	59. Sometimes things that should feel painful are not (for instance when I hurt myself or burn my hand on a stove)
29. Some ordinary textures that do not bother others feel very offensive when they touch my skin	62.* I usually speak in a normal tone
33*. I speak with a normal rhythm	65. It calms me to spin around or to rock in a chair when I am feeling stressed
34. The same sound, color or texture can suddenly change from very sensitive to very dull	67. If I am in a place where there are many smells, textures to feel, noises or bright lights, I feel anxious or frightened
36. Sometimes the sound of a word or a high-pitched noise can be painful to my ears	71. The same sound sometimes seems very loud or very soft, even though I know it has not changed
42. When I feel overwhelmed by my senses, I have to isolate myself to shut them down	73. I can't tolerate things I dislike (like smells, textures, sounds or colors)
46. The same thing (like clothes or temperatures) can feel very different to me at different times	74. I don't like to be hugged or held

* = reversed item

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J Autism Dev Disord (2012) 42:313–318
DOI 10.1007/s10803-011-1240-y

BRIEF REPORT

Brief Report: Alterations in Cerebral Blood Flow as Assessed by PET/CT in Adults with Autism Spectrum Disorder with Normal IQ

Marco Pagani · Irina Manouilenko · Sharon Stone-Elander · Richard Odh · Dario Salmaso · Robert Hatherly · Fredrik Brolin · Hans Jacobsson · Stig A. Larsson · Susanne Bejerot

Published online: 13 April 2011

Abstract Specific biological markers for Autism Spectrum Disorder (ASD) have not yet been established. Functional studies have shown abnormalities in the anatomo-functional connectivity of the limbic-striatal "social" brain. This study aimed to investigate regional cerebral blood flow (rCBF) at rest. Thirteen patients with ASD of normal intelligence and ten IQ-, sex- and age-matched healthy controls (HC) underwent PET/CT using [1-^{11}C]butanol, a perfusion tracer. As compared to HC, ASD showed significant CBF increases in the right parahippocampal, posterior cingulate, primary visual and temporal cortex, putamen, caudatus, substantia nigra and cerebellum. No statistically significant correlation between CBF and IQ was found. The limbic, posterior associative and cerebellar cortices showed increased blood flow in ASD, confirming previous findings about the neurobiology of ASD.

Keywords High functioning autism · PET/CT · [1-^{11}C]butanol · Cerebral blood flow

M. Pagani · R. Odh · R. Hatherly · F. Brolin · H. Jacobsson · S. A. Larsson
Department of Nuclear Medicine, Karolinska University Hospital, Stockholm, Sweden

M. Pagani (✉) · D. Salmaso
Institute of Cognitive Sciences and Technologies, CNR, Via Palestro 32, 00185 Rome, Padua, Italy
e-mail: marco.pagani@istc.cnr.it

I. Manouilenko · S. Stone-Elander · S. Bejerot
Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Introduction

Autism spectrum disorder (ASD) is defined by impairments in social-communicative interaction, in understanding other people's complex emotions and in automatically processing social information and responding to it (Frith and Frith 2003), in addition to restricted patterns of behaviour and interests. However, most people with ASD demonstrate abnormalities in additional areas such as perception (Andersen et al. 2011) and motor functioning, which prevail into adulthood (Sahlander et al. 2008). Altered functional connectivity within/between functional territories and pathways has been suggested as possible explanation for ASD (Gepner and Féron 2009).

Functional and anatomical studies in ASD have shown localized metabolic alterations affecting various cortical and subcortical regions. However, no common regional abnormalities have been found across cerebral blood flow (CBF) or cerebral glucose metabolism (CMR_{gl}) studies even though a consensus judgement suggests focal hypoperfused areas in the thalamus, basal ganglia, parietal, temporal lobes, and cerebellum (Rumsey and Ernst 2000). The discrepant results are possibly due to the lack of homogeneity across studies (i.e. differences in age, IQ, verbal skills, handedness, socio-economical status, working capabilities, diagnostic issues and medication) and poorly matched control groups.

The aim of this study was to investigate the functional status at rest in highly-functioning subjects with ASD as compared to an IQ-, sex- and age-matched control group. The study was performed by assessing CBF using Positron Emission Tomography/Computerised Tomography (PET/CT) with in-house produced [1-^{11}C]butanol.

Methods

Participants

Thirteen adults with ASD and normal intelligence and ten matched healthy controls were included in the study. Demographic data are shown in Table 1. The study was approved by the local Ethics and Radiation Safety Committees. Written informed consent was obtained from all participants.

ASD Group

Six females and seven males were included in the study. Ten of the thirteen subjects had a social style defined as active odd, while three were characterized as loners or aloof (Wing 1997).

At the time of the study, eight of the subjects received a disability pension or were on sick leave, whereas the others were either studying or held a job. Ten had completed upper secondary school. Mini international neuropsychiatric interview (M.I.N.I.) was used to determine psychiatric comorbidity (Sheehan and Lecrubier 2006). Eight subjects had a psychiatric co-morbidity. Six were treated with psychotropic drugs (antidepressants SSRI/SNRI, n = 5; anxiolytics/hypnotics, n = 3; stimulants, n = 2).

Control Group

Ten IQ-, sex- and age-matched individuals without physical disorders or mental disabilities according to the Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders 4th edition (DSM-IV) Axis I Disorders (SCID-I) and SCID-II were the healthy controls (HC). The exclusion criteria for all participants were alcohol and substance-abuse, intellectual disability, epilepsy, psychosis, brain damage or neurological disorders.

Neuropsychiatric Assessment

The diagnoses of all ASD subjects were based on extensive interviews, rating scales and parental interviews at a specialized neuropsychiatric unit (Rydén and Bejerot 2008). They were further confirmed by the Autism Diagnostic Observation Schedule (ADOS-G) (Lord et al. 2000) and supported by the self-administered Ritvo Autism and Asperger Diagnostic Scale Revised (RAADS-R) (Andersen et al. 2011), and Autistic Quotient (AQ) (Baron-Cohen et al. 2001).

Functional levels were assessed with the DSM-IV Global Assessment of Functioning (GAF) (American Psychiatric Association 1994). Full scale IQ and all neuropsychiatric tests, with the exception of AQ, were administered to all participants.

Table 1 Demographics and neuropsychologic data for subjects with autism spectrum disorders (ASD) and healthy controls

Variable	ASD (n = 13)	Healthy controls (n = 10)	*p*
Sex (female:male)	6:7	5:5	0.85
Age, mean (range, SD)	31.8(20–48, 8.6)	28.5(20–42, 7.5)	0.35
Female, mean (range, SD)	27.7(20–38, 7.1)	31.8(20–42, 8.1)	0.38
Male, mean (range, SD)	35.3(25–48, 8.8)	25.2(20–34, 5.8)	0.05
WAIS-III-R, IQ			
Full scale IQ, mean (range, SD)	104.2(87–135, 17.1)	115.7(99–134, 10.8)	0.08
Verbal IQ, mean (range, SD)	105.3(83–133, 16.4)	114.6(94–135, 13.2)	0.18
Performance IQ, mean (range, SD)	101.5(82–134, 17.6)	114.2(102–130, 9.9)	0.07
Smoking (yes:no)	3:9*	2:8	0.96
Handedness (right:left)	12:1	9:1	0.85
Civil status (single:cohabit)	12:1	6:4	0.04
Have children (yes:no)	0:13	3:7	0.02
University education (yes:no)	5:8	8:2	0.02
In full time work/studies (yes:no)	3:10	10:0	<0.0001
GAF total mean (range, SD)	54(40–65, 7.5)	86(80–100, 7.4)	<0.0001
RAADS-R mean (range, SD)	110(73–163, 29)	20(1–46, 15)	<0.0001
AQ mean (range, SD)	29(18–40, 6.9)	Not assessed	

SD standard deviation, *WAIS-III-R* Wechsler Adult Intelligence Scale-III-Revised, *IQ* intelligence quotient, *GAF* General assessment of functioning, *RAADS-R* Ritvo autism and asperger diagnostic scale revised, *AQ* autistic quotient, * Missing data in one subject with ASD

Radiopharmaceutical

[1-^{11}C]Butanol was produced via the reaction of a Grignard reagent, with cyclotron-produced carbon-11 labelled carbon dioxide, followed by reduction with lithium aluminum hydride by an adaptation of the method in Brodack et al. (1988).

Scanning Protocol

The examinations were performed at rest in a lit room with eyes closed on a Siemens Biograph 64 Positron Emission Tomography/Computed Tomography (PET/CT) scanner, with a spatial resolution of about 4 mm. A bolus of [1-^{11}C]butanol (about 300 MBq) was injected manually in about 1–2 s simultaneously as the PET acquisition was started and data were acquired in the list mode for 5 min.

We identified in all patients the interval between 40 and 100 s after injection as the window with the maximal number of events from which raw data should be extracted and summed to reconstruct the images to be analysed. During this interval the [1-^{11}C]butanol uptake reached a plateau before starting to decrease with time.

The protocol implemented was chosen to keep the time in the scanner to the absolute maximum of 10 min enabling patients to comply with the demands of functional neuroimaging without any impact on the results. None of the subjects showed any anxiety spikes or panicked.

Statistical Analysis

Voxel-based analysis was performed using SPM2 (Wellcome Department of Cognitive Neurology, London, U.K.). Images of relative tracer distribution were spatially normalised into the stereotactic Montreal Neurological Institute (MNI) space to a predefined PET template. After global normalisation, images were smoothed with a Gaussian filter (8 mm FWHM) to account for individual gyral differences and brain anatomy. Correction of SPM coordinates to match the Talairach coordinates was achieved by the subroutine implemented by Matthew Brett (http://imaging.mrc-cbu.cam.ac.uk/imaging/CbuImaging). Brodmann areas were then identified, after importing the corrected coordinates, by Talairach Daemon Database (http://www.talairach.org/daemon.html) at a range of 0–2 mm from the isocenter.

Group analyses between ASD and HC and between patients with and without medication were performed by the "compare populations: one scan per subject (ANCOVA)" model. The "single subject: covariates only" model was used to correlate the CBF in all participants and their IQs adjusting for age, sex and educational level. Significant differences between the groups were thresholded at $p$corrected < 0.001 at cluster level and $p$uncorrected < 0.01 at voxel level. Only clusters containing more than 64 voxels ($8 \times 8 \times 8$ mm) were considered to be significant. Proportions of categorical variables at baseline were compared using the chi-square tests and frequency data were computed through the Fisher's exact test. Values of continuous measures were compared using the t test. The p-level was set to 0.05.

Results

As expected the scores of RAADS-R and GAF differed significantly between ASD subjects and HC (see Table 1).

Compared to HC, ASD showed CBF increases in the right limbic structures, namely the parahippocampal (Brodmann Areas, BA 28) and posterior cingulate (BA 30) cortex, as well as visual cortex (BAs 17) and parieto-temporal (BAs 37, 38, 39) cortex, putamen, caudatus, substantia nigra and cerebellum (see Table 2; Fig. 1). When ASD data were subtracted from those of controls, no statistically significant difference was found.

Analyses of the medicated versus non-medicated patients and the correlation analysis between CBF and IQ performed on all participants revealed no significant differences and did not impact on the CBF differences.

Discussion

The main finding of this preliminary study was an increased resting CBF in ASD in a relatively large portion of the right cerebral and cerebellar hemispheres including regions belonging to the so-called limbic-striatal system.

The CBF increases found in the right parieto-temporal lobes, in the parahippocampal gyrus and in the primary visual cortex may be due to a heightened emotional status, an increased processing of visual information and a more laborious engagement of cognitive functions of ASD subjects compared to HC. Though high-functioning individuals with ASD have social impairments, explicit intellectual skills processed mainly in the amygdalo-hippocampal junction are preserved (Critchley et al. 2000). Maybe our ASD subjects implemented explicit cognitive strategies to assist them during the PET examination.

These results are in agreement with previous PET studies that have shown a global increase in metabolism and abnormalities in networking across frontal cortex, parietal cortex and subcortical regions (Rumsey et al. 1985).

The increased cerebellar CBF in high-functioning ASD (Table 2) is suggestive of its role in firing neuronal activity in the brain stem and in systems involved in emotions and higher cortical functions and skills (Rumsey and Ernst

Table 2 Results for CBF increases in ASD as compared to HC

Cluster level		Voxel level		Talairach coordinates			Regions and Brodmann Areas	Range (mm)
p(cor)	K	Z-score	*p*(unc)	x	y	z		
0.001	6,408	3.12	0.001	18	−45	−12	R Cerebellum; Culmen	0
		2.84	0.002	29	−64	−16	R Cerebellum; Declive	0
		2.78	0.003	22	−14	−27	R Uncus; BA 28	1
		2.75	0.003	31	3	−4	R Putamen	1
		2.66	0.004	10	−18	−8	R Substantia Nigra	0
		2.58	0.005	46	7	−12	R Superior Temporal Gyrus; BA 38	1
		2.49	0.006	19	−76	14	R Cuneus; BA 17	0
		2.47	0.007	29	−58	−40	R Cerebellum; Cerebellar Tonsil	0
		2.46	0.007	45	−59	8	R Middle Temporal Gyrus; BA 39	2
		2.41	0.008	47	−62	−13	R Fusiform Gyrus; BA 37	1
		2.32	0.010	26	−64	9	R Posterior Cingulate; BA 30	1
		2.31	0.010	8	2	−1	R Nucleus Caudatus	1

p(cor) corrected *p*-value, *K* cluster size in voxels, *p(unc)* uncorrected *p*-value, *R* right, *BA* Brodmann area

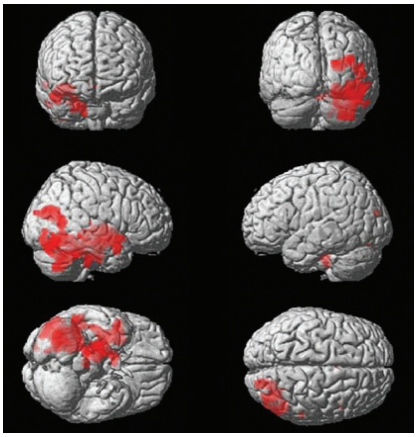

Fig. 1 3D rendering showing those regions in which CBF was significantly higher in ASD (n = 13) as compared with HC (n = 10). *Top left* frontal view, *top right* posterior view, *middle left* right-side view, *middle right* left-side view, *left down* view from below, *right down* view from above

2000). The CBF increase in the primary visual cortex is consistent with increased metabolism found in the calcarine cortex in ASD (Siegel et al. 1992).

Mean CMR_{gl} in highly functioning adult ASD was reported to be higher than in HC by Heh et al. (1989) and also in dispersed regions by Siegel et al. (1992). Other PET studies either failed to find differences between ASD and HC (Buchsbaum et al. 1992) or reported reduced metabolism in patients with intellectual disability and other comorbid conditions (DeLong and Heinz 1997).

The majority of the Single Photon Emission Computed Tomography (SPECT) studies in ASD, showing decreased or unchanged CBF distributions, have been performed on low-functioning individuals with various comorbidities and sedation (Zilbovicius et al. 1992; Chiron et al. 1995). This could explain the widely varying results of increased or normal CBF in high-functioning ASD compared to those with low IQ. This latter is suggested to play a key role in differentiating ASD subgroups (Witwer and Lecavalier 2008).

The CBF at rest reflects the baseline state of the brain, always active even during repose periods. In neuropsychiatric studies not employing specific brain activations during the scan, the subjects will process information and elaborate concepts and sensations. This resting activity might have contributed to the group differences found here.

Brain activation findings would suggest that subjects with ASD use different networks of activity and secondary strategies than HC and utilize alternative brain circuitry, possibly also at rest (Hazlett et al. 2004).

The experimental conditions during PET scanning may have caused a preoccupation and/or hypersensitivity to the external environment (Kennedy et al. 2006) in the ASD subjects, along with, consistent with the "central coherence theory" of autism (Frith and Happé 1994), a particularly high activation of visuospatial processes due to the new situation and to the amount of details perceived. This low-level enhanced perception is specifically processed in temporal-occipital regions (O'Connor and Kirk 2008) and might be absent in subjects with intellectual disability or in sedated subjects.

A CBF increase in the right hemisphere is compatible with proficiencies in visuo-spatial functions and with low scores in functions attributed to the left hemisphere. Using

quantitative ^{133}Xe-SPECT Chiron et al. (1995) found a higher CBF in the right hemisphere of ASD subjects at rest, with an inversion of hemispheric laterality compared to HC. Neuroanatomical differences between groups may also contribute to the CBF changes observed. Thicker grey matter has been reported in the temporal lobe (Hardan et al. 2006) and in the cerebellum. Just et al. (2004) hypothesized that in ASD the "under-connectivity" is due to processing centres not developing adequate connections, which causes potential association areas to develop independently and become hyper-specialized. Hence, the elevated CBF and/or CMR_{gl} found in some brain areas may derive from increased neuronal activity in redundant and poorly integrated circuits (Schwartz 1993), especially in domains most dependent on highly coordinated somato-sensory association processes (Herbert 2005).

Butanol as a blood flow tracer labelled with both ^{11}C and ^{15}O was validated two decades ago for human studies (Herscovitch et al. 1987; Quarles et al. 1993, for review see Saha et al. 1994) and was reported to more accurately measure rCBF than [^{15}O]water (Raichle et al. 1976). Additionally, the longer half-life of carbon-11 (20 min vs. 2 min for ^{15}O) facilitated transportation of multi-patient doses from the cyclotron lab to the clinical PET-CT, which was instrumental in enabling the performance of this study.

Findings in the previous ASD imaging literature have reported jeopardized cortical and subcortical abnormalities in flow and metabolism and have failed to identify any patterns specific for the disorder. This is possibly also due to inhomogeneity between patient and controls investigated across studies. Further inconsistencies might derive from the scanning and image analysis methodology used. Moreover, the high costs of functional neuroimaging often limit recruitment to an inadequate number of subjects, which increases the likelihood of Type II statistical errors.

In this investigation both the ASD and HC groups were specifically recruited for the study, and all underwent the same neuropsychological and neuropsychiatric tests. This might have reduced the contamination from confounding factors and of variables out of control. In order to investigate how our findings extend to other groups within the autistic spectrum, studies on larger and equally well-characterized cohorts of subjects will be needed.

Conclusion

In this preliminary study significant CBF differences were found between highly functioning ASD subjects and healthy controls in part of the posterior right hemisphere in limbic, posterior associative, visual and cerebellar cortices. This underscores the involvement of these regions in the phenotypic expression of the disorder and raises methodological and diagnostic issues in the evaluation of the heterogeneous findings in functional and neuroanatomical investigations on ASD.

Acknowledgments Authors wish to thank Psykiatrifonden, C.M. Lerici Foundation, Stockholm, Sweden, Praktikertjänst AB, Stockholm, Sweden; Stockholm County Council and Dipartimento per i Rapporti Internazionali, National Research Council (CNR), Italy for financial support.

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Research in Autism Spectrum Disorders 7 (2013) 566–578

Contents lists available at SciVerse ScienceDirect

Research in Autism Spectrum Disorders

Journal homepage: http://ees.elsevier.com/RASD/default.asp

Autistic traits, ADHD symptoms, neurological soft signs and regional cerebral blood flow in adults with autism spectrum disorders

Irina Manouilenko [a,b,*], Marco Pagani [c,d], Sharon Stone-Elander [a,e], Richard Odh [f], Fredrik Brolin [c], Robert Hatherly [c], Hans Jacobsson [c], Stig A. Larsson [g], Susanne Bejerot [a]

[a] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[b] Järva Psychiatric Out-patient Clinic, Praktikertjänst AB, Stockholm, Sweden
[c] Department of Nuclear Medicine, Karolinska University Hospital, Stockholm, Sweden
[d] Institute of Cognitive Sciences and Technologies, CNR, Rome, Italy
[e] Department of Neuroradiology, Karolinska University Hospital, Stockholm, Sweden
[f] Department of Radiation Protection, Swedish Radiation Safety Authority, Stockholm, Sweden
[g] Department of Diagnostic Physics, Karolinska University Hospital, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 20 December 2012
Accepted 24 December 2012

Keywords:
Autism spectrum disorder
ADHD
Neurological soft signs
Temporo-parietal junction
Thalamus
Caudate

ABSTRACT

The resting regional cerebral blood flow (rCBF) patterns related to co-occurring symptoms such as inattention, hyperactivity, neurological soft signs and motor problems have not yet been disclosed in autism spectrum disorders (ASD).

In this study thirteen adults with ASD and ten matched neurotypical controls underwent PET. The scores of rating scales for autistic traits, attention deficit hyperactivity disorder (ADHD) and neurological soft signs were included in a factorial analysis and correlated with rCBF. Factors corresponding to "autistic/ADHD traits", "sensory-motor integration" and "Intelligence/Motor sequencing" were identified. In the ASD group, positive correlations with CBF were found for "autistic/ADHD traits" in caudate bilaterally and the inferior parietal lobule, for "sensory-motor integration" in parieto-occipital cortex and for "Intelligence/Motor sequencing" in the right temporal cortex. Notably, CBF in the left thalamus correlated negatively with all three factors. Autistic traits and ADHD symptoms were associated with shared neural substrates. The correlation between "autistic/ADHD traits" and rCBF in the caudate is possibly associated with the executive impairments and ritualistic/stereotyped behaviors apparent in ASD. Furthermore, sensory-motor deficits were correlated with rCBF in the occipital visual cortex, involved in atypical visual perception in ASD. Various behavioral and neurological symptoms are suggested to converge into the ASD phenotype.

1. Introduction

Co-occurrence of symptoms consistent with attention deficit hyperactivity disorder (ADHD) and sensory-motor problems is considered to play an important role in the phenotype of autism spectrum disorders (ASD) (Reiersen & Todd, 2008; Sturm, Fernell, & Gillberg, 2004). Individuals with ASD have qualitative impairments in reciprocal social interactions

Abbreviations: CBF, cerebral blood flow; rCBF, regional cerebral blood flow; rCMRgl, regional cerebral metabolic rate of glucose; NES, Neurological Evaluation Scale; NSS, neurological soft signs.

* Corresponding author at: Järva Psychiatric Out-patient Clinic, Rinkebysvängen 70A, 4tr, 16374 Spånga, Stockholm, Sweden. Tel.: +46 8 12338515; fax: +46 8 7607950.
E-mail address: irina.manouilenko@ki.se (I. Manouilenko).

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http://dx.doi.org/10.1016/j.rasd.2012.12.008

and communication and have restricted repetitive and stereotyped patterns of behavior, interests and activities. ADHD is a broad diagnosis, characterized by inattention, hyperactivity and impulsivity. The diagnostic criteria for ADHD in the DSM-IV have not allowed for a patient with ASD to be given an ADHD diagnosis (American Psychiatric Association, 1994; Goldstein & Schwebach, 2004; Kooij et al., 2010). However, co-occurrence of ASD- and ADHD-related symptoms has been verified by population-based (Reiersen, Constantino, Volk, & Todd, 2007) and clinically based studies (Goldstein & Schwebach, 2004; Hofvander et al., 2009; Lee & Ousley, 2006; Mayes, Calhoun, Mayes, & Molitoris, 2012; Rydén & Bejerot, 2008). Investigations indicate that ADHD symptoms are present in about 20–80% of children with ASD (Sturm et al., 2004). Not only does the ADHD symptoms persist in adulthood (Hofvander et al., 2009), the severity of ASD seems to correlate with the co-occurrence of ADHD symptoms (Holtmann, Bolte, & Poustka, 2007). Moreover, a substantial proportion of clinically diagnosed children with ADHD meet the criteria for ASD (Rommelse, Franke, Geurts, Hartman, & Buitelaar, 2010; Sturm et al., 2004). For reliable diagnosis of ASD it is appropriate to investigate different dimensions of the disorder, since no single test or neurophysiological examination is sufficiently accurate (Steer, Golding, & Bolton, 2010).

Impairment of motor control, including neurological soft signs (i.e. subtle impairments of sensory integration, motor coordination and difficulties in sequencing complex motor tasks) is common in both ASD (Mahone et al., 2006; Mayoral et al., 2010; Price, Shiffrar, & Kerns, 2012; Sahlander, Mattsson, & Bejerot, 2008; Tani et al., 2006) and ADHD (Chan et al., 2010; Gustafsson, Thernlund, Ryding, Rosen, & Cederblad, 2000; Pasini & D'agati, 2009). Motor dysfunction might precede the symptoms of linguistic and social problems in ASD (Teitelbaum et al., 2004) and a combination of motor problems with ADHD predicted more autistic traits than ADHD alone (Reiersen, Constantino, Grimmer, Martin, & Todd, 2008). Impaired integration of sensory input with motor commands has also been reported, supporting the hypothesis of a cerebellar and thalamic dysfunction in ASD (Gowen & Miall, 2005; Hardan et al., 2006).

Neurological Evaluation Scale (NES), which is an instrument for the systematic evaluation of neurological soft signs (Buchanan & Heinrichs, 1989), has been widely used in psychiatric populations with schizophrenia (Heinrichs & Buchanan, 1988; Peralta et al., 2010; Sewell et al., 2010), bipolar disorder (Negash et al., 2004) and obsessive compulsive disorder (Mergl & Hegerl, 2005; Poyurovsky et al., 2007). Patient groups scored higher in NES than healthy controls in all these studies. Furthermore, structural correlates of neurological soft signs were established by their relationship with gray matter reductions in a group of healthy controls (Dazzan et al., 2006) and in patients with psychosis (Bersani et al., 2007; Janssen et al., 2009).

A small number of neuroimaging studies, mainly performed in children and adolescents, have investigated neural correlates in ASD in direct comparisons with individuals with ADHD and neurotypical controls (NC). Correlations were reported between regional gray matter volume in the medial temporal lobe and inferior parietal cortex and symptoms of inattention, hyperactivity and autistic symptoms (Brieber et al., 2007; Yamasaki et al., 2010). In addition, increased white matter volume in the left primary motor and premotor cortex predicted poorer motor skills in children with autism as compared to groups of ADHD and healthy controls (Mostofsky, Burgess, & Gidley Larson, 2007). All these studies suggest that anatomical changes in ASD are likely to impair functional connections and to affect motor and neuropsychological performances.

Several positron emission tomography (PET) studies in ASD have shown localized metabolic changes in various cortical and subcortical regions (Chiron et al., 1995; Heh et al., 1989; Siegel et al., 1992). However, no common regional abnormalities have been found in regional cerebral blood flow (rCBF) or cerebral glucose metabolism studies (rCMRgl), possibly due to inhomogeneity in the individuals with ASD and the different methodologies implemented in various studies (Buchsbaum et al., 1992; Rumsey & Ernst, 2000; Zilbovicius et al., 1992). Further investigations to better describe the neurobiological correlates of ASD are therefore needed. In a previous study we compared adults with ASD to a group of NC and found increased rCBF, which is an index of regional brain activity, in the posterior part of the right hemisphere, confirming the involvement of cortical and subcortical structures in the phenotypic expression of the disorder (Pagani et al., 2012).

The aim of this study was to investigate the neural correlates of autistic traits, the symptoms of inattention, hyperactivity/impulsivity and neurological soft signs in the same individuals. We hypothesized that autistic traits, ADHD symptoms and neurological soft signs would share neural substrates and would correlate to rCBF in the temporo-parieto-occipital brain regions previously implicated in ASD.

2. Methods

2.1. Participants

Thirteen normal-intelligence adults with ASD, diagnosed in adolescence or in adulthood, and ten age-, sex- and IQ-matched neurotypical controls were included in the study. Exclusion criteria for all subjects were mental retardation, a history of brain damage, current or past medical or neurological disorders, epilepsy, alcohol abuse or dependence, past or present substance abuse and psychosis. In addition, NC were excluded if they had any past or current psychiatric or personality disorder, psychotropic medication or psychiatric disorders in first-degree family members. Demographic characteristics and descriptive statistics are presented in Table 1.

2.1.1. Neurotypical controls

Twelve NC were recruited from the Stockholm region. One subject was excluded since a first-degree family member had ASD and another subject dropped out of the study.

Table 1
Demographic and clinical characteristics of individuals with ASD compared with neurotypical controls.

Variables	ASD $n = 13$	Control $n = 10$	p-Value
Age, years	31.8 (8.6)	28.5 (7.5)	0.35
Male: female	7:6	5:5	0.85
Full scale IQ	104.2 (17.1)	115.7 (10.8)	0.08
Verbal IQ	105.3 (16.4)	114.6 (13.2)	0.176
Performance IQ	101.5 (17.6)	114.2 (9.9)	0.066
Handedness, right: left	12:1	9:1	0.85
Education			
<9 years, *n*	4	0	
9–12 years, *n*	4	3	
>12 years, *n*	5	5	
University degree, *n*	0	2	
Civil status, single:cohabit	12:1	6:4	0.04
Have children, yes:no	0:13	3:7	0.02
Independent living, yes:no	11:2	10:0	
In full time work/studies, yes:no	3:10	10:0	<0.0001
Nicotine use, yes:no	3:9[a]	2:8	0.96
Global assessment of functioning, total	54 (7.5)	86 (7.4)	<0.0001
Symptom-GAF	54.7 (6.8)	87.8 (7.2)	<0.0001
Function-GAF	56.3 (8.2)	90.7 (5.3)	<0.0001
Ritvo Autism Asperger Diagnostic Scale-Revised, total	109.7 (28.8)	19.6 (14.9)	<0.0001
Adult ADHD Self-Report Scale, total	32.2 (10.4)	19.6 (7.2)	0.003
Inattention	18.4 (6.4)	11.1 (5.4)	0.008
Hyperactivity/impulsivity	14.7 (6.2)	8.5 (3.4)	0.010
Wender Utah Rating Scale, total	57.9 (40.5)	11.5 (7.1)	0.001
Neurological Evaluation Scale, total	16 (6.7)	5 (3.0)	<0.001

Mean values are presented with the standard deviation in parentheses. IQ, intelligence quotient; GAF, global assessment of functioning; ASRS, Adult ADHD Self-Report Scale.
[a] Missing data in one subject.

2.1.2. Individuals with ASD

Recruitment of subjects with ASD was performed by a letter of request sent to 357 individuals registered at the community based unit for adults with ASD and to patients with ASD at the Neuropsychiatric unit, Northern Stockholm psychiatric clinic. Of the total fifty-five subjects with ASD that were willing to participate, 20 were interviewed, two were excluded on the basis of epilepsy and a history of alcohol and drug dependence and five were not available at the time of the PET scan, resulting in seven recruitments from the clinic and six from the community. This final selection was based on the desired distribution of sex. All but one, of Asian descent, were Caucasian.

Previous neuropsychiatric assessments, which included extensive interviews, rating scales, neuropsychological assessments and interviews with parents of the subjects, were requested for all ASD subjects. According to these, 11 subjects met the DSM-IV criteria for Asperger's disorder and two were diagnosed with high-functioning autism (American Psychiatric Association, 1994). The clinical diagnosis of ASD was confirmed in all subjects with the Autism Diagnostic Observation Schedule (ADOS) by one of the authors (SB) (Lord et al., 2000). Thereafter, two of the authors (SB and IM), board-certified psychiatrists specialized in diagnosing ASD in adults, agreed on the diagnosis and type of social style, according to Wing's definition (Wing, 1997). Nine subjects were defined as ''active-odd'' and four as ''schizoid and loners''. No ASD subject was classified as ''passive'' or ''rigid formal'' type. Eight subjects fulfilled diagnostic criteria for additional 1–5 psychiatric disorders (Agorophobia; Bulimia Nervosa; Major Depression; Dystymic disorder; Generalized Anxiety Disorder; Obsessive Compulsive Disorder; Panic Disorder; Social Phobia), and six subjects were currently treated with psychotropic medication.

2.2. Psychiatric and psychological assessments

The structured interview for Axis-I disorders Mini International Neuropsychiatric Interview (M.I.N.I., version 5.00) (Sheehan et al., 1998) and a semi-structured interview for past psychiatric disorders were administered to the ASD subjects whereas the Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders 4th edition (DSM-IV), Axis I Disorders (SCID-I) and Structured Clinical Interview for DSM-IV-R Personality Disorders (SCID-II) were administered to the NC in order to rule out psychiatric disorders. General clinical impairment and function were assessed according the DSM-IV Global Assessment of Functioning (GAF) (American Psychiatric Association, 1994). The Wechsler Adult Intelligence Scale-Revised (WAIS-III-R) provided intelligence quotient (IQ) estimates. Past and current medical disorders, family history of mental disorders, educational level, marital status and employment status were also covered in the semi-structured interview.

2.2.1. Neuropsychiatric assessments

Standardized, semi-structured assessment, the ADOS, module 4, was used to confirm the diagnosis of ASD. The ADOS includes four subscales: Communication, Social Interaction, Imagination/creativity and Stereotyped behaviors and restricted interest. Because the ADOS is assessed within one hour, it does not offer an opportunity to measure restricted and repetitive behaviors satisfactorily. Thus, ADOS algorithms include only the combined score from the two subscales "Communication" and "Social Interaction".

The Swedish version of the Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R) is an 80-item self-report diagnostic questionnaire based on DSM-IV-TR and ICD-10 criteria (Ritvo et al., 2011). The RAADS-R is self-administered and consists of four subscales (Social Interaction, Language, Circumscribed Interests and Sensory Motor Symptoms). However, the language sub-scale was excluded in this study since it showed low internal consistency in a Swedish validation study (Andersen et al., 2011). RAADS-R assesses deficits in perception and sensory-motor integration along with cognitive aspects of ASD. Each question is scored 0–3 and a score of 65 or greater is consistent with a clinical diagnosis of ASD. The self-rating scale, Adult ADHD Self-Report Scale (ASRS), was used to measure ADHD symptoms (Kessler et al., 2005). It consists of eighteen questions based on the DSM-IV criteria for ADHD (American Psychiatric Association, 1994) and includes two subscales: inattention and hyperactivity/impulsivity. A sum of scores between 17 and 23 for each subscale indicates that the subject is likely to have ADHD, while scores above 23 indicate a high likelihood of ADHD. Childhood ADHD symptoms were assessed using the Wender Utah Rating Scale (WURS) (Ward, Wender, & Reimherr, 1993), which is also self-assessed.

2.2.2. Neurological assessment

Neurological examination was carried out with the Neurological Evaluation Scale (NES), which is a 26-item clinically administered instrument for the systematic evaluation of neurological soft signs (Buchanan & Heinrichs, 1989). It covers four functional domains: Sensory Integration Signs (audio-visual integration, stereognosis, graphaesthesia, extinction and right-left confusion), Motor Coordination Signs (tandem walk, rapid alternating movements, finger-thumb opposition and finger-to-nose test), Motor Sequencing Signs (fist-ring, fist-edge-palm, rhythm tapping production movements and Ozeretski test) and other, e.g. "hard" neurological signs such as Romberg sign, tremor, mirror movements, synkinesis, convergence, gaze impersistence, primitive reflexes and short-term memory. Each item is rated from 0 to 2 (0 = no abnormality, 1 = mild but definitive impairment, 2 = marked impairment).

2.3. PET-scanning protocol

After the extended psychiatric assessment, all subjects underwent PET scans using [1-^{11}C]butanol to evaluate rCBF. Butanol, as a blood flow tracer labeled with either ^{11}C or ^{15}O, has been shown to have a high degree of reliability for human studies (Raichle et al., 1976; Saha, MacIntyre, & Go, 1994). It was produced using an in-house cyclotron and radiochemistry lab and was rapidly transported to the PET camera site.

The examinations were performed using a Siemens Biograph 64 Positron Emission Tomography/Computed Tomography (PET/CT) scanner, with a spatial resolution of 5 mm. The system combines a high-speed ultra 32-detector-row (672 detectors per row) CT unit and a PET scanner with 32448 LSO crystals in 52 rings and an axial field of view 21.6 cm.

The head was first scanned by CT, so corrections for attenuation and photon scatter could be made. Thereafter a bolus of [1-^{11}C]butanol (300 MBq) was injected simultaneously as the PET acquisition was started and data were acquired in the list mode for 5 min. The dynamic data were reconstructed to transverse images for rCBF evaluations. The interval between 40 and 100 s after injection was identified as the optimal window from which the raw data were extracted to reconstruct the images to be analyzed. During this interval the [1-^{11}C]butanol uptake reached a plateau before starting to decrease over time.

2.3.1. Image preprocessing

Data were analyzed with SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK) as implemented in Matlab 6.5.1. Raw data were subjected to affine and non-linear spatial normalization to a predefined PET template based on the MNI (Montreal Neurological Institute) reference brain by a bilinear interpolation method into a common anatomical space. The spatially normalized set of images were then smoothed with a 10 mm (FWHM) isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. Images were globally normalized using proportional scaling (0.5) to remove confounding effects to global CBF changes, with a gray matter masking threshold of 0.8.

2.4. Statistical analysis

Proportions of categorical variables at baseline were compared using the chi-square tests. Due to the small sample size, frequency data were computed through the Fisher exact test. Values of continuous measures were compared using either the *t*-test or, for non-normally distributed variables, the non-parametric Mann–Whitney *U*-test. The *p*-level was set to 0.05.

A principal component analysis, based on 13 original variables (scores of ADOS and GAF total, RAADS-, ASRS- and NES-subscales, verbal and performance IQ), was performed in all subjects to reduce their number into common factors (i.e. principle components). Each factor will then explain a different part of the total variance of the data set. In order to correct for the large variability in neuropsychiatric scales scores, data were normalized to a standard score (*z*-score) subtracting the

population mean from an individual raw score and then dividing the difference by the population standard deviation. The obtained *z*-scores for the 13 chosen variables were then submitted to OpenStat (Rummel, 1970) for statistical analysis.

Principal Component Analysis was performed and varimax orthogonal rotation was applied to the normalized 13 native neuropsychological measures to identify those scores expressing a similar part of total variance. Minimal root to rotate was set to 1.0 and the maximal number of iterations to 25. Factors with eigenvalues larger than 1 were initially extracted. Variables, with an absolute factor loading greater than 0.5, were regarded as representative of each factor. This value is purely arbitrary, but it is commonly used since it explains a moderate part of the variance of the factor. Kaiser–Meyer–Olkin measure of sampling adequacy (MSA) values higher than 0.7 were regarded as significant.

Positive and negative correlations between rCBF and each of three factors were carried out separately in subjects with ASD and NC. The "single-subjects covariates only" design model of SMP2 was implemented. All analyses were adjusted for age and gender. Due to the explorative nature of the study and to the number of subjects, statistical thresholds of $p = 0.05$ at voxel height, $p_{\text{uncorrected}} < 0.05$ at cluster level and $p_{\text{uncorrected}} < 0.001$ at voxel level were applied. Only those clusters containing more than 125 ($5 \times 5 \times 5$ voxels, i.e. 11 mm $\times$ 11 mm $\times$ 11 mm) contiguous voxels were accepted as significant, based on the calculation of the partial volume effect resulting from the spatial resolution of the PET camera (about double the intrinsic spatial resolution). The resulting statistical parametric maps, SPM{t}, were transformed into normal distribution (SPM{z}) units. Because the SPM template does not completely match the Talairach brain, it is necessary to correct its coordinates. This was achieved by the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging). Brodmann areas (BAs) were then identified at a range of 1 mm from the corrected Talairach coordinates of the SPM output isocentres, after importing them into Talairach client (http://www.talairach.org/index.html).

2.5. Ethical considerations

The study was approved by the Regional Ethical Review Board in Stockholm and the Radiation Safety Committee of the Karolinska University Hospital. Written informed consents were obtained from all subjects.

3. Results

Comparisons of demographic variables between the ASD and NC revealed no significant differences in age, IQ, smoking, handedness or sex distribution.

Principal component analysis of symptom scores identified three factors independent of age and educational level referred to as "Autistic/ADHD traits" (F1); "Sensory-motor integration" (F2) and "Intelligence/Motor sequencing" (F3). The pattern of loadings on each of three factors is presented in Table 2.

Each of three factors was correlated in both groups to significant increases (i.e. positive correlations) and decreases (i.e. negative correlations) in rCBF (Table 3 and Figs. 1 and 2).

In NC, positive correlations between the "Autistic/ADHD traits" factor and CBF were found in the occipital and temporal cortex and, in ASD, in the caudate bilaterally and in the right inferior parietal lobule, posterior cingulate and motor cortex. The ASD group showed also negative correlations in the right putamen and prefrontal cortex.

For the "Sensory-motor integration" factor opposite relationships in the occipital association cortex were found in ASD and NC with positive and negative correlations, respectively. The same was found for the putamen, in which NC correlated positively and ASD negatively with CBF.

Table 2
Factor loadings of the neuropsychiatric scale scores.

Variables	Component			h^2
	F1. Autistic/ADHD traits	F2. Sensory-motor integration	F3. Intelligence/Motor sequencing	
Hyperactivity/impulsivity subscale in ASRS	**0.841**	−0.254	0.334	0.88
Circumscribed interests subscale in RAADS-R	**0.826**	0.376	0.039	0.93
Inattention subscale in ASRS	**0.804**	0.036	0.065	0.65
ADOS communication and Social interaction	**0.798**	0.341	0.250	0.81
Sensory Motor Symptoms subscale in RAADS-R	**0.766**	0.318	0.514	0.95
Global assessment of function, total	**−0.757**	−0.469	−0.277	0.87
Social Interaction subscale in RAADS-R	**0.751**	0.452	0.272	0.84
"Hard" signs NES	**0.563**	0.385	0.413	0.63
Motor Co-ordination Signs subscale in NES	0.032	**0.935**	0.058	0.87
Sensory Integration Signs subscale in NES	0.316	**0.618**	0.186	0.51
Verbal IQ	−0.244	0.030	**−0.866**	0.80
Performance IQ	−0.237	−0.206	**−0.890**	0.89
Motor Sequencing Signs subscale in NES	0.298	0.519	**0.727**	0.88
Cumulative variance explained	38.6	19.7	22.9	

Highest factor loadings are in bold. h^2 is the communality, i.e. the proportion of variance of a single item that is explained by the factor solution. ADOS, Autism Diagnostic Observation Schedule; ASRS, Adult ADHD Self-Report Scale; NES, Neurological Evaluation Scale; RAADS-R, Ritvo Autism and Asperger Diagnostic Scale-Revised; IQ, Intelligence quotient.

Table 3
Regions with positive and negative correlations between the z-scores of the three factors and CBF.

Structures	Brodmann area	F1. Autistic/ADHD traits				F2. Sensory-motor integration				F3. Intelligence/Motor sequencing			
		ASD, $n = 13$		Control, $n = 10$		ASD, $n = 13$,		Control, $n = 10$		ASD, $n = 13$		Control, $n = 10$	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Ventral lateral nucleus	Thalamus		R L				L				L		
Lentiform nucleus	Putamen		R				R L	L					
	Caudate	R L											
Sensory-motor cortex	3	R											
Precuneus	7					R							
Insula	13									R	L		
Cuneus	17			R L		L							
Cuneus	18			R		R L			R				
Fusiform gyrus	19					R L			R L			R	
Middle temporal gyrus	21							L		R			
Superior temporal gyrus	22				R			L		R			
Superior temporal gyrus	38			L									
Temporo-parietal junction	39/40	R		R									
Parahippocampal gyrus	Hippocampus		R										
Posterior cingulate	23	L											
Parahippocampal gyrus	35			L									
Parahippocampal gyrus	36			L									
Parahippocampal gyrus	37			L		R							
Uncus	20										R		
Inferior frontal gyrus	45									R			
Superior frontal gyrus	10, 11		R										
Anterior cingulate	32		R										
Inferior frontal gyrus	34						R L						
Inferior frontal gyrus	47						R						

ASD, individuals with ASD, R: right; L: left.

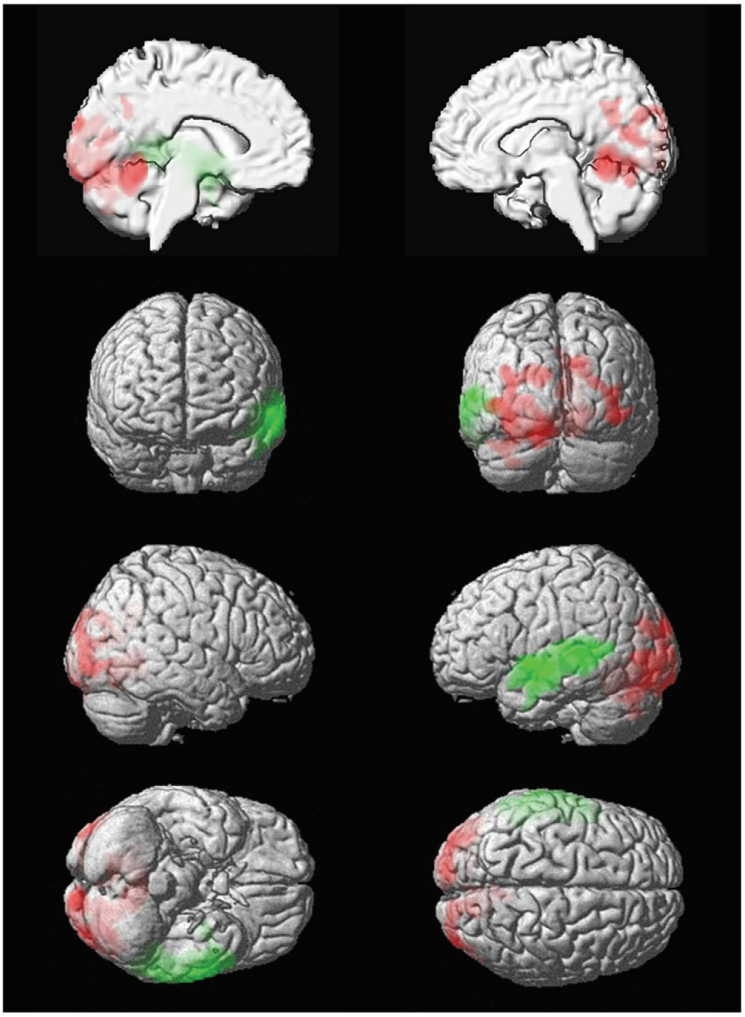

Fig. 1. Positive correlation between rCBF and "autistic/ADHD traits" in ASD (in red) and controls (in green). Regions showing a positive correlation between between rCBF and factor "autistic/ADHD traits" in ASD (in red) and NC (in green). The first row represents the medial aspect of left (on the left) and right (on the right) hemispheres; the second row represents the anterior (on the left) and posterior (on the right) aspect on the brain; the third row represents the lateral aspect of the right (on the left) and of the left (on the right) hemispheres; the fourth row represents the inferior (on the left) and the superior (on the right) aspects of the brain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The factor "Intelligence/Motor sequencing" was correlated with CBF only in the ASD group, positively in the right temporal cortex and negatively in the left insula and right uncus. The left thalamus correlated negatively with all three factors in the ASD group.

4. Discussion

In this study we could show that autistic traits and ADHD symptoms were grouped in the same factor ("Autistic/ADHD traits"). Thus, our data support the hypothesis that autistic traits and ADHD symptoms are correlated with resting rCBF in

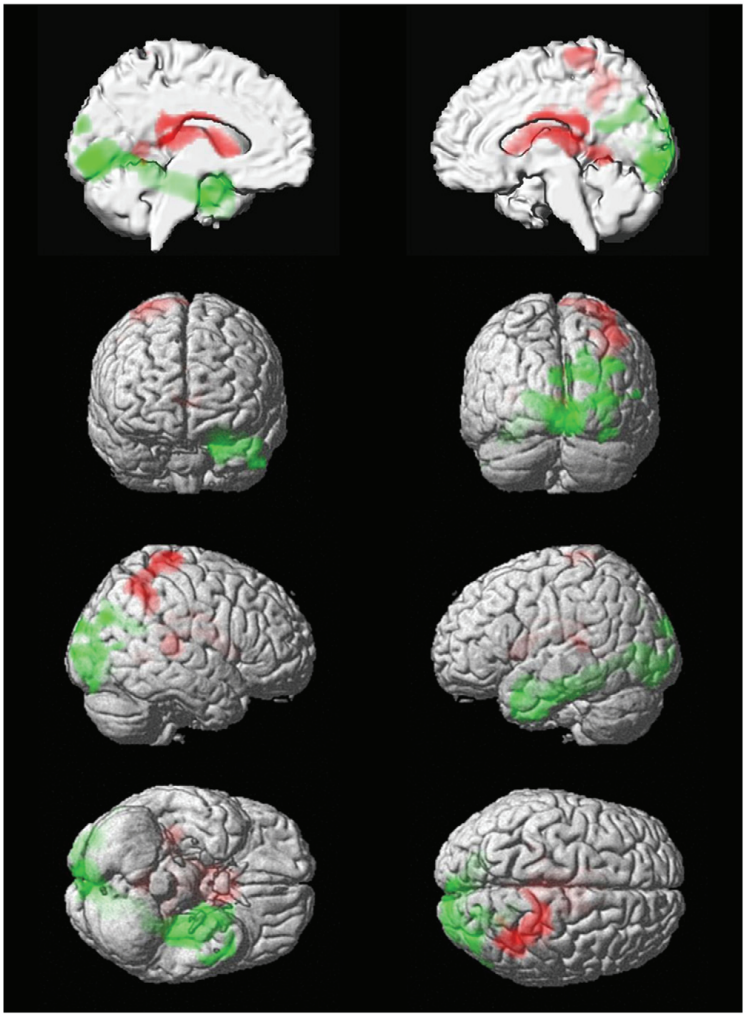

Fig. 2. Positive correlation between rCBF and "sensory-motor integration" in ASD (in red) and controls (in green). Regions showing a positive correlation between rCBF and factor "sensory-motor integration" in ASD (in red) and NC (in green). The first row represents the medial aspect of left (on the left) and right (on the right) hemispheres; the second row represents the anterior (on the left) and posterior (on the right) aspect on the brain; the third row represents the lateral aspect of the right (on the left) and of the left (on the right) hemispheres; the fourth row represents the inferior (on the left) and the superior (on the right) aspects of the brain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

superimposing brain regions. Neurological soft signs and intelligence/motor sequencing were correlated to rCBF independently from the ASD and ADHD dimensions.

4.1. "Autistic/ADHD traits" and rCBF

The positive relationship between CBF and the factor "Autistic/ADHD traits" in individuals with ASD reflects the neurobiological correlates of ASD- and ADHD symptoms in specific brain regions. These regions – the temporo-parietal junction, sensory-motor cortex, caudate and middle and superior temporal gyrus – have previously been reported to be

implicated in both ASD and ADHD (Ecker et al., 2012; Fassbender & Schweitzer, 2006; Lombardo, Chakrabarti, Bullmore, & Baron-Cohen, 2011).

The temporo-parietal junction (corresponding to BA 39/40) is implicated in complex behaviors, which require attention to biological motion (Allison, Puce, & McCarthy, 2000), spatial orientation of the eye gaze (Wicker et al., 2008) and integration of visual and auditory inputs with the purpose of providing meaningful social and emotional responses (Calvert, Campbell, & Brammer, 2000). It is also involved in moral judgment and in the ability to understand the intentions of other people and metaphors (Luria, 1970; Verhoeven, De Cock, Lagae, & Sunaert, 2010), further supporting the relevance of this area in ASD. Moreover, the tempo-parietal junction has been identified as a part of a right-lateralized ventral attention system related to distractibility (Fox, Corbetta, Snyder, Vincent, & Raichle, 2006) and inattention to salient social cues, implicated in both ASD and ADHD. In addition, higher activations in this region were also found in a PET study in subjects with autism compared to healthy controls during a verbal memory task (Hazlett et al., 2004).

We also found positive correlations between the factor "Autistic/ADHD traits" and CBF in the postcentral gyrus (BA 3), i.e. a part of the primary somatosensory cortex. Possibly, positive correlations in this region may be related to abnormal perceptions of somatic sensations emerging during the PET scanning.

The caudate has also been implicated in ASD by being associated with executive deficits and ritualistic behaviors (Haznedar et al., 2006; Hollander et al., 2005). Associative cortices project in a segregated manner, mainly to the nucleus caudatus in the striatum. Since the caudate is the crucial part of several neural circuits, the positive correlation between the factor "Autistic/ADHD traits" and CBF in the caudate could be explained by the strong anatomical and functional connections of this structure with temporal and parietal lobes. Because the human brain is anatomically and functionally organized into complex networks, it allows both segregation and integration of information. It is likely that connectivity networks are impaired in ASD at the long-range level and incremented at the low-range level (Minshew & Keller, 2010). This latter anatomo-functional looping might under certain circumstances cause excessive information processing, resulting in a local increase in metabolism and blood flow. Therefore, the negative correlation between the factor "Autistic/ADHD traits" and the prefrontal cortex is likely due to the long-range disconnection between temporo-parietal junction/posterior cingulate cortex and the rostral part of the brain and may be partly explained by presence of ADHD-symptoms (Solomon et al., 2009).

4.2. "Sensory-motor integration" and rCBF

This study also provided evidence that neurological soft signs are part of the ASD phenotype since the factor "Sensory-motor integration" included the scores of NES sub-scales for assessment of motor coordination and sensory-integration. Positive correlations with CBF were found in the cuneus and fusiform gyrus in ASD, whereas a negative correlation was found in NC, as shown in Table 3.

Impaired eye-to-eye gaze and facial expressions are included in the diagnostic criteria for ASD (American Psychiatric Association, 1994) and face recognition deficit was suggested to be one of several factors that could lead to poor social skills (Barton et al., 2004). In a neurotypical population, activation in the fusiform gyrus was associated with face identification, face processing and object perception (Bly & Kosslyn, 1997) and the dorsolateral occipital cortex was selectively activated by face matching tasks (Haxby et al., 1991). We found in patients a positive correlation between CBF and the factor "Sensory-motor integration" in fusiform gyrus, in which increased gray matter volume has been reported in ASD (Waiter et al., 2004). This positive correlation could be explained by the abnormally intense and generalized pattern of information flow in autistic perception and by the increased local neuronal activity in the visual cortex (Belmonte & Yurgelun-Todd, 2003).

Individuals with ASD tend to use a visually oriented, asocial processing style (Koshino et al., 2008) and visual strategies to solve cognitive problems, regardless of whether they are visual or verbal (Sahyoun, Belliveau, Soulières, Schwartz, & Mody, 2010). Atypical visual perception and visuospatial abilities in individuals with ASD may be linked to savant capacities and exceptional attention to details and possibly also to social interaction deficits (Dakin & Frith, 2005; Sutherland & Crewther, 2010; Völlm et al., 2006). Thus, the positive correlations shown in this study between the factor "Sensory-motor integration" and CBF in cuneus and fusiform gyrus in individuals with ASD, may reflect difficulties in the cross-modal integration of visual, auditive, proprioceptive and tactile information, typical for the disorder. Also, our findings confirm an involvement of the temporo-occipital regions, implicated in decoding intentions and emotions of other people.

4.3. "Intelligence/Motor sequencing" and rCBF

Positive correlation was found in the ASD group between the factor representing "Intelligence/Motor sequencing" and CBF in the right insula and middle and superior temporal gyrus. Insula (BA 13) is also involved in visuo-spatial (Damarla et al., 2010) and multisensory processing (Downar, Crawley, Mikulis, & Davis, 2001). Moreover, the insula was proposed to play a key role in switching attention between external stimuli and internal reflections (Sridharan, Levitin, & Menon, 2008) and in mediating attention to novel sensory stimuli. In addition, the insula is involved in temporal processing, phonological processing and visual–auditory integration (Bamiou, Musiek, & Luxon, 2003), functions that are affected in ASD. The positive correlation in this region between CBF and the factor "Intelligence/Motor sequencing" could possibly reflect compensatory mechanisms for the deficits in integrative functions (Ornitz, 1974). Also the insula, along with the superior temporal gyrus, contribute to neural networks for empathizing, consciousness and identity (Craig, 2002; Damasio et al., 2000; Singer et al., 2006). Interestingly, increased rCBF was found in the right insular cortex in individuals with gender identity disorder (GID)

(Nawata et al., 2010), a condition that is clearly overrepresented in ASD (de Vries, Noens, Cohen-Kettenis, van Berckelaer-Onnes, & Doreleijers, 2010). In addition, the increases of gray matter volumes in the temporo-parietal junction and in the insular cortex in individuals with GID was recently suggested to be related to the involvement of these regions in sensory-motor processing (Savic & Arver, 2011), which opens new directions for research on a "comorbidity" between ASD and GID.

The superior temporal gyrus (corresponding to BA 21, 22) is considered to be the neuroanatomical substrate for language, speech, mentalizing (Frith & Frith, 2003), empathy (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003; Völlm et al., 2006) and sensory integration (Dazzan et al., 2006). Thus, this region is considered to be the neural basis for social- and communication deficits in ASD and ADHD combined with substantial perceptual, motor and attention deficits (Gepner & Feron, 2009; Gillberg & Rasmussen, 1982; Herrington et al., 2007; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Leekam, Nieto, Libby, Wing, & Gould, 2007). In the present study the factor "Intelligence/Motor sequencing" was positively correlated with CBF in the superior temporal gyrus in the ASD group, but not in the controls.

4.4. Thalamus involvement

Notably, the left thalamus showed negative correlations with all three factors.

This is in agreement with the previously reported lower metabolic activity in the anterior thalamus bilaterally (Haznedar et al., 2006) and abnormal neurotransmission in individuals with ASD (Bernardi et al., 2011). Also, thalamus abnormalities have shown to be associated with an abundance of neurological soft signs in earlier studies (Dazzan et al., 2006; Thomann et al., 2009). Since the thalamus mediates both sensory perception and motor planning and serves as an active filter for the information flow to the cerebral cortex, it is suggested to be implicated in the inappropriate multisensory integration processing in ASD as well as in ADHD (Hardan et al., 2008; Zhu et al., 2008). In addition, the thalamus regulates cortical arousal through thalamo-cortical connections and can therefore elicit a hyperarousal condition, which is well known in ASD.

Taken together, a neurobiological-based model for ASD is supported by our findings and is consistent with previous reports about the co-occurrence of inattention, hyperactivity and motor control deficits in ASD. This is also in line with studies that showed phenotypic overlap between ASD and ADHD (Reiersen et al., 2008) and shared genetic variance (Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010). Moreover, our findings are in agreement with a previously reported underlying factor of a major effect contributing to both autistic traits and ADHD symptoms (Constantino et al., 2004). Therefore, co-occurring ADHD symptoms are suggested to be a representation of a broader ASD phenotype and not a separate co-morbid disorder, which is of relevance for diagnosis and treatment. Thus, the use of factor analysis of different signs and symptoms is suggested to be a valuable approach for exploring underlying ethiological and pathophysiological causes (Dworzynski, Happe, Bolton, & Ronald, 2009; Steer et al., 2010) and biological correlates in ASD (Sacco et al., 2010).

4.5. Measures of autistic traits

RAADS-R, a rating scale to assist in the assessment of the ASD diagnosis, was used in the present study to measure autistic traits. A wide selection of impairments in cognitive functions, the existence of repetitive and stereotyped behaviors and sensory-motor deficits are assessed by this instrument. Our results suggest that ASD is a clinical syndrome with various symptomatic dimensions composing the autism spectrum phenotype. Doubtlessly, the autism phenotype is a consequence of an atypical brain development (Kaplan, Crawford, Cantell, Kooistra, & Dewey, 2006; Kaplan, Dewey, Crawford, & Wilson, 2001), which affects brain connectivity and synchronicity (Gepner & Feron, 2009). Similar findings have been reported in ADHD (Fair et al., 2010). These anatomic and functional similarities may be associated to intertwined impairments in language, motor coordination, activity, mood, and sleep, as shown in children diagnosed with a neuropsychiatric disorder (Gillberg, 2010). In our adult ASD sample, co-occurrence of symptoms of ADHD and deficits in motor and sensory-integration, along with a number of other psychiatric and psychological problems are suggested to represent the clinical manifestation of this atypical brain development. Certain areas, engaged in ASD, are similarly affected in other psychiatric disorders (Kleinhans et al., 2010; Menzies et al., 2008; Ravindran et al., 2009; Seminowicz et al., 2004). In adults with ASD, coexisting sensory-motor abnormalities, assessed rather than self-reported, may represent a specific clinical subtype, separable from ASD alone, and supported by neuroimaging findings in this study. Inattention and hyperactivity seem to be integrated in the ASD diagnosis and share neural substrates.

4.6. Limitations

The analyses were performed accepting significances of $p < 0.05$ uncorrected at peak level. Due to the small sample size, not unusual in neuroimaging studies, this liberal choice was adopted to avoid type II errors attributable to over-conservative thresholds (Oishi et al., 2005). Effectively, given the exploratory nature of this analysis and considering the relatively low sensitivity of PET without repeated measures, higher thresholds could lead to false-negative results in PET studies. Another limitation was the lack of blinded assessors.

5. Conclusions

Although ASD and ADHD can be described as distinct categories, autistic traits and ADHD-symptoms were associated with common neural substrates, while sensory-motor deficits were grouped in another independent factor. These different

symptom dimensions may reflect underlying brain dysfunctions in atypical organized neuronal networks. Our findings contribute to the hypothesis that in ASD resting neural activity in local neuronal circuits is associated with metabolic and blood flow alterations.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgements

Financial support was provided for clinical research by Praktikertjänst AB, Stockholm, Sweden; Psykiatrifonden, Sweden; C.M. Lerici Foundation, Stockholm, Sweden, grants from the Swedish Research Council (No 523-2011-3646) and through the regional agreement on medical training and clinical research (ALF/Pick-Up) between Stockholm County Council and Karolinska Institutet.

The authors wish to thank Professor Emeritus Lennart Wetterberg for his review of the manuscript and Professor Anna Åberg Wistedt for support.

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IV

Minor physical anomalies in adults with autism spectrum disorder and healthy controls.

Irina Manouilenko

Karolinska Institutet, Sweden;

Praktikertjänst AB, Sweden

Jonna M Eriksson

Karolinska Institutet, Sweden

Mats B Humble

Örebro University, Sweden

Susanne Bejerot

Karolinska Institutet, Sweden

Corresponding author:
Irina Manouilenko, Järva psychiatric out-patient clinic, Rinkebysvängen 70A, 4tr,

163 74 Spånga, Stockholm, Sweden.

Email: irina.manouilenko@ki.se

Running title: Minor physical anomalies in ASD

Abstract

Minor Physical Anomalies (MPAs) are subtle abnormalities of the head, face and limbs, without any significant cosmetic or functional impact to the individual. They are assumed to represent external markers of developmental deviations during foetal life. MPAs are suggested to indicate severity in mental illness and constitute external markers for atypical brain development. Higher frequencies of MPAs can be found in children with autism. The aims of the present study were to compare the prevalence and patterns of MPAs in adults with autism spectrum disorder (ASD) with those in neurotypical controls, and to investigate whether MPAs are associated with symptom severity and overall functioning. Fifty adults with ASD and 53 healthy controls were examined with the Waldrop scale, an instrument for assessing MPAs. Significant differences between the ASD and the control group were found on the mean MPA-total scores, and also in the head and face region scores. Particularly, the shape and setting of the ears were associated with autistic traits. The findings suggest a link between MPAs, autistic traits and level of functioning. MPAs may potentially serve as risk markers for ASD, and careful examination of morphological features can give some estimation of functional level and severity of symptoms.

Keywords

Autistic disorder, minor physical anomalies, global assessment of functioning, phenotype; biomarker

Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders, characterized by atypical development and qualitative impairment in reciprocal social interaction and communication, and restricted repetitive and stereotyped patterns of behaviour, interests and activities. ASD includes autistic disorder, Asperger`s disorder, and pervasive developmental disorders not otherwise specified (American Psychiatric Association., 1994) and occurs in nearly 2% of the population (Wingate et al., 2012) . Males are more affected than females with a sex ratio at about 4:1 (Fombonne, 2009). ASD is remarkably heterogeneous and associated with impairments in various areas of a person's life. Since no specific biological markers for ASD have been identified, the diagnosis of ASD is based on expert evaluation of cognitive, language, social and emotional functioning along with developmental progress. In population studies a strong evidence of genetic aetiology of ASD has been shown (Ronald et al., 2006; Bailey et al., 1995; Lichtenstein et al., 2010). Several prenatal and postnatal risk factors have also been suggested (Gillberg, 1980; Burstyn et al., 2010; Atladottir et al., 2010). Furthermore, autistic traits can be measured at sub-threshold level in the normal population suggesting that the autism phenotype lies along a continuum of quantitative traits (Baron-Cohen et al., 2001).

Minor Physical Anomalies (MPAs) are subtle morphological abnormalities of the craniofacial region and limbs, without any significant cosmetic or functional impact to the individual. They are assumed to represent markers of deviant morphogenesis during the first or early second trimester of pregnancy and to have ectodermal embryonic origins in common with the central nervous system and particularly the

developing brain. Genetic factors and prenatal events, such as maternal bleeding with following foetal hypoxia, gestational diabetes, medication use or toxaemia, may contribute to MPAs (Campbell et al., 1978; Stromland et al., 1994; Gardener et al., 2009).

MPAs include minor malformations and phenogenetic variants that are stable over time (McNeil and Cantor-Graae, 2000). *Minor malformations* are qualitative defects of embryogenesis arising during organogenesis and are true deviations from normal. *Phenogenetic variants* are quantitative defects arising after organogenesis and represent the exact equivalents of normal anthropometric variants (Spranger et al., 1982; Opitz, 2000).

Higher frequencies of MPAs can be found in patients with schizophrenia, affective disorder, ADHD and Tourette syndrome (Akabaliev et al., 2011; Trixler et al., 2001; Waldrop et al., 1978; Csabi et al., 2008; Ismail et al., 1998). In addition, MPAs are suggested to be indicators of severity of the illness (McGrath et al., 1995). Taken together, MPAs are markers for aberrant development and may be used as markers of risk for certain psychiatric disorders (Compton and Walker, 2009; Mittal and Walker, 2011).

In studies of children with ASD MPAs are suggested to be the external markers for atypical brain development and excessive MPAs have been found when compared to neurotypically developing children (Ozgen et al., 2011; Rodier et al., 1997; Tripi et al., 2008; Walker, 1977). Hitherto, only one study has examined MPAs in adults with

ASD and normal-intelligence. The interorbital and interlens distances were measured using MRI scan and showed that hypotelorism (i.e. short interorbital length) is a MPA that may be present in a subgroup of individuals with autism and IQ within the normal lower range (Hardan et al., 2006). However, advanced methodology such as MRI is mostly for research purposes. Also, other MPAs than orbital would be of interest to investigate in adults. Thus it is important to investigate whether MPAs can be assessed in the clinical practice.

The aim of the present study was (1) to investigate the prevalence, as well as (2) topographical pattern of MPAs in adults with ASD in comparison to neurotypical controls and (3) to investigate whether MPAs can be used in clinical procedures to support diagnosis of ASD and as markers of impairment.

We hypothesized that (1) adults with ASD would show higher rates of MPAs than neurotypical controls; (2) that the pattern of topographical distribution of MPAs would differentiate individuals with ASD from neurotypical controls and (3) that rates of MPAs would correlate with severity of symptoms and overall functioning.

Methods

Participants

Fifty Swedish adults with ASD (24 females, 26 males; mean age 30 years) and 53 neurotypical controls (25 females, 28 males; mean age 30.4 years) participated in the

study (Table 1). These subjects were included in a previous study on gender coherence, for details see (Bejerot et al., 2012).

Inclusion criteria for all participants were age between 18 and 50 years and Caucasian descent. Exclusion criteria were any neurological or genetic syndrome, diagnosed malformations, schizophrenia spectrum disorders or intellectual disability, or having attended special education in primary or secondary school. Normal intelligence was assumed by mainstream schooling. Additional exclusion criteria for the control group were ASD in a first-degree family member, current psychiatric or personality disorder and use of psychotropic medication.

Table 1. Sample characteristics of the ASD group and the neurotypical controls.

		ASD (n=50)	**Controls** (n=53)
Age, years, mean (SD)		30.0 (7.3)	30.4 (7.5)
Sex, males, n (%)		26 (52)	28 (53)
Education, n	≤ 9 years	8	1
	≤ 12 years	18	7
	University level	24	45
Cohabiting with partner, n (%)		9 (18)	26 (49)
Having children, n (%)		8 (16)	11 (21)
The Autism-Spectrum Quotient, mean (SD)		29.4 (9.8)	11.2 (4.9)
GAF, past month, mean (SD)	Symptoms	55.6 (6.5)	97.5 (4.4)
	Functioning	55.0 (9.2)	97.6 (4.2)

ASD: Autism Spectrum Disorder; GAF: Global Assessment of Functioning

Procedures and Materials

Participants were recruited between November 2006 and October 2010. Individuals with ASD were recruited through an outpatient tertiary psychiatric unit, a community-based centre for adults with ASD and also through a website for people with ASD. All participants with ASD had been previously diagnosed according to the rigorous and extensive standard procedure for diagnosing ASD in Sweden at the time of the study (approximately 18 hours of assessments that include diagnostic interviews, rating scales, neuropsychological assessments and structured interviews with parents of the subjects) (Rydén & Bejerot, 2008). The diagnosis was further confirmed with Autism Diagnostic Observation Schedule (ADOS), patient records and a clinical interview performed by a psychiatrist experienced with ASD (SB).

Neurotypical controls were recruited through advertisements in flyers and websites of various organisations, including, university campuses, student residences, private companies, dentists and vaccination centres, employment agencies and through word-of-mouth. They were enrolled after the ASD group in order to be matched for sex and age.

All participants completed the Autism Spectrum Quotient (AQ), a self-report questionnaire that measures autistic traits in individuals with normal intelligence. The AQ consists of 50 items that cover abilities within social skills, communication, attention switching, attention to detail and imagination. The discriminant validity and screening properties are suggested to be satisfactory (Baron-Cohen et al., 2001).
Overall impairment in psychological, social, and occupational functioning was assessed with the DSM-IV Global Assessment of Functioning (GAF), shown to have

good psychometric properties (Jones et al., 1995). The GAF score is a combined measure of symptom severity and level of functioning and range from 1 to 100 (American Psychiatric Association., 1994; Moos et al., 2002). Low scores indicate severe symptoms and low functioning. In order to capture these two different dimensions, severity of symptoms (GAF-symptoms) and social, occupational, or school functioning (GAF-functioning) were assessed separately (Goldman et al., 1992).

Written informed consents were obtained from all subjects. The study was approved by the Regional Ethical Review Board in Stockholm.

Assessment of minor physical anomalies (MPAs)

All participants were assessed for minor physical anomalies and photographed in a standardised manner, standing against a white wall in an examination room, which was used throughout the study. The digital photos included face close-up photos, front and in profile. Feet were photographed from beneath and from above. The participants were requested to wear a shower-hat to hide the hair, but not the ears. Excessive makeup and jewellery were removed before photographing.

The modified Waldrop scale (Waldrop & Halverson, 1971) was used to assess MPAs. It consists of 16 items that evaluate occurrence of MPAs in six anatomic body areas: head, eyes, ears, mouth, hands and feet. These MPAs were further classified as either *minor malformation* or *phenogenetic variants* (Opitz, 2000) (Table 2). Head circumference was measured with a measuring tape and then categorized by reference to the control mean based on established scale norms (as 1.0

or 1.5 standard deviations greater than control mean). All other items were assessed according to descriptive anchor points (scored 0-2) depending on severity. Eight items (epicanthus; hypertelorism; low-settled ears; adherent ear lobe; malformed ears; relative toe lengths; partial syndactylia; sandal gap between first and second toe) were estimated from photographs of face (front and profile) and feet by two psychiatrists (IM, MH), independently and blinded to the diagnosis. If their ratings on any item differed, they reached a consensus after discussion. MPAs of mouth, hands and item "fine electric hair" were examined by two un-blinded assessors (SB, JE).

Six subscales of MPAs were used: Head, Eyes, Ears, Mouth, Hands and Feet. In addition to the total MPA score, the craniofacial index (CF-MPA) was calculated from scores of the Head, Eyes, Ears and Mouth subscales and the periphery index (P-MPA) was calculated from scores of Hands and Feet subscales.

Table 2. Comparison of MPA rates between the ASD and neurotypical control groups.

Minor physical anomalies		Score	MM/PV	ASD n (%)	NC n (%)	*p*-value
Head						
Head circumference:			PV			
	1.5-2 SD	1		23(46)	27(50.9)	
	2 SD	2		8(16)	2(3.8)	
Fine electric hair:			MM			0.037*
	Hair soon awry	1		12(24)	4(7.5)	
	Hair unmanageable	2		1(2)	-	
Eyes						
Epicanthus (the point of union where upper and lower lids join the nose):			PV			
	Partly covered	1		8(16)	7(13.2)	
	Deeply covered	2		1(2)	2(3.8)	
Intercanthal distance/hypertelorism (approximate distance between tear ducts):			PV			
	Moderate	1		22(44)	19(35.8)	
	Extensive	2		1(2)	1(1.9)	
Ears						
Seating ears - bottom of ears in line with:			PV			
	Area between mouth and nose	1		8(16)	9(17)	
	Mouth (or lower)	2		-	-	
Adherent ear lobes Lower edges of ears extend:			MM			
	Moderate/Straight back toward rear of neck	1		16(32)	14(26.4)	
	Extensive/Upward and back toward crown of head	2		18(36)	14(26.4)	
Asymmetrical ears		1	PV	10(20)	3(5.7)	
Malformed ears		1	MM	5(10)	4(7.5)	
Mouth						
High/steepled palate Roof of mouth:			PV			
	Flat and narrow at the top	1		12(24)	13(24.5)	
	Definitely steepled	2		-	3(5.7)	
Furrowed tongue (one with deep ridges)		1	MM	7(14)	4(7.5)	
Hands						
Curved fifth finger:			MM			0.021 [a]
	Moderately curved	1		2(4)	10(18.9)	
	Extensively curved	2		-	-	
Single transverse palmar crease		1	MM	7(14)	-	0.005 *
Fifth-finger stubbing		1	MM	1(2)		
Feet						
Third toe:						
	Equal in length to second	1	PV	2(4)	-	
	Definitely longer than second	2		-	-	
Partial syndactylia of second and third toes		1	MM	-	3(5.7)	
Big gap between first and second toes		1	PV	36(72)	40(75.5)	

MM: Minor Malformation; PV: Phenogenetic variants; a higher scores in controls; level of significance *p < 0.05. Items are assessed according to descriptive anchor points (scored 0-2) depending on severity and “0” is defined as “no deviation”

Statistics

Comparisons between the ASD group and controls were made using Student's *t*-test for continuous variables and Pearson Chi-Square-distribution for categorical variables. Differences between means on the MPA total scores, the sub scores from the six body areas and the CF-MPA and P-MPA were analysed. Missing data, assumed to be random, were present for five participants with ASD and one of the controls. Missing data were replaced with group means.

Since total Waldrop scores of 5 or greater have been associated with various psychiatric conditions (Tripi et al., 2008; Trixler et al., 2001; Guy et al., 1983), the participants were split into a Low-MPA group, defined as having a total Waldrop score below 5, and the High-MPA group as having a total Waldrop score of 5 or greater. For both groups bivariate correlations were performed to examine the relationships between MPAs, autistic traits (AQ) and severity of symptoms (GAF-s) and overall functioning (GAF-f). Due to skewness of AQ and MPA distributions, Spearman's rank correlation test was used.

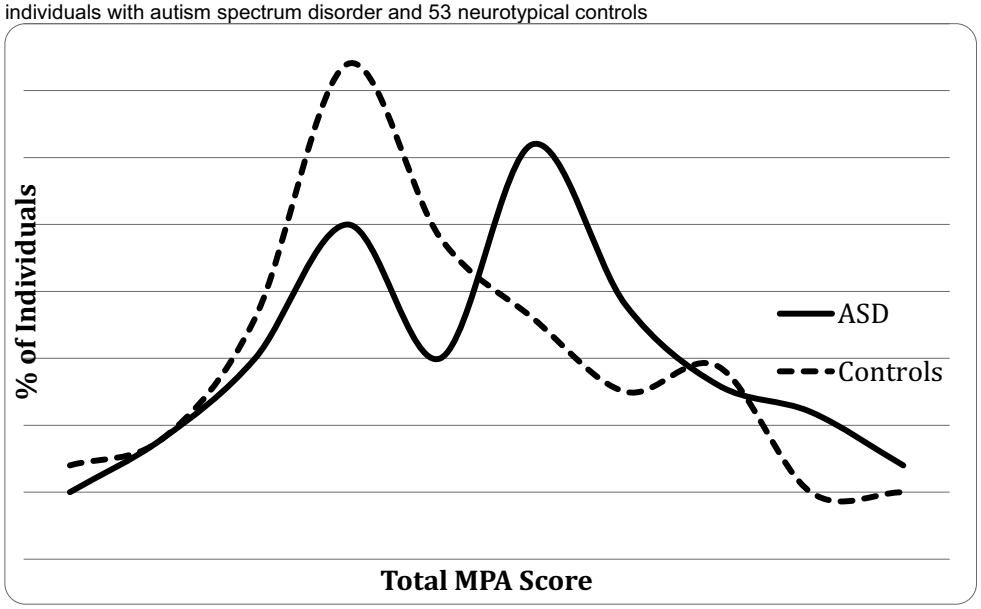
Results

Comparisons between the groups revealed no significant differences in age, but, as expected, large differences in all three symptom-related measures (Table 1).

The total MPA scores ranged from 1 to 9 in the ASD group and from 0 to 7 in the neurotypical control group, indicating that all participants in the ASD group

displayed at least one MPA. The distribution of the total MPA score in the ASD group showed two subgroups, one with high frequencies of MPAs and one with normal frequencies, see Figure 1.

Figure 1. Frequency distribution of total minor physical anomalies (MPA) scores in 50 individuals with autism spectrum disorder and 53 neurotypical controls



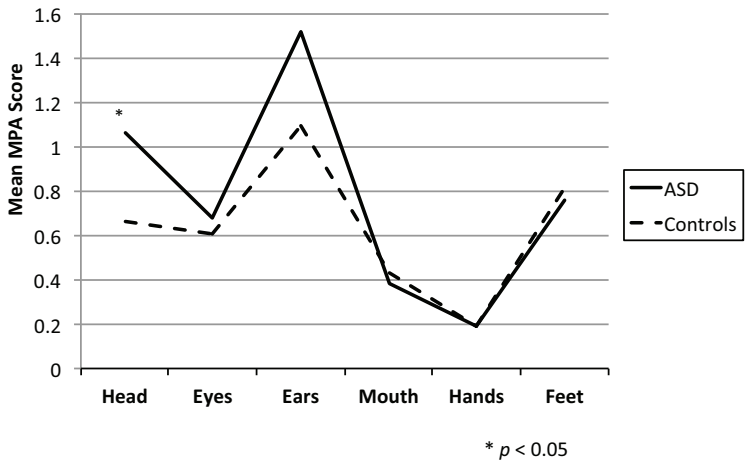
In the ASD group, the means of CF-MPA index, Head subscale score and MPA-total score were significantly higher in comparison to the control group, as shown in Table 3 and Figure 2.

Table 3. Mean MPA scores for the ASD and neurotypical control groups.

	ASD (n=50)	**Neurotypical controls (n=53)**		
	Mean (SD)	Mean (SD)	*t*	*p*
Craniofacial index (CF - MPA)	3.64 (1.97)	2.69 (1.51)	-2.44	0.017
Head	1.06 (0.89)	0.66 (0.67)	-2.57	0.012
Eyes	0.68 (0.77)	0.61 (0.82)	-0.41	n.s.
Ears	1.52 (1.27)	1.09 (1.07)	-1.84	n.s.
Mouth	0.38 (0.60)	0.43 (0.60)	0.45	n.s.
Periphery index (P - MPA)	0.95 (0.69)	1.0 (0.65)	0.38	n.s.
Hands	0.19 (0.43)	0.19 (0.40)	-0.16	n.s.
Feet	0.76 (0.52)	0.81 (0.48)	0.52	n.s.
TOTAL MPA	4.59 (1.91)	3.79 (1.67)	-2.26	0.026

ASD: Autism Spectrum Disorder; MPA: Minor physical anomalies

Figure 2. Mean of minor physical anomalies scores by body regions in ASD and neurotypical controls.



MPA = minor physical anomalies; ASD = autism spectrum disorder

When the sample was split into Low- and High-MPA groups, 60 individuals were allocated to the Low-MPA category and 43 individuals were categorized into the High-MPA group. Significantly more ASD participants showed a high rate of MPAs than the controls (χ^2=6.0, p = 0.014), see Table 4.

Table 4. Correlations between MPA-total, MPA subscales, AQ and GAF for the Low-MPA and High-MPA groups.

	Low-MPA (total MPA<5)			**High-MPA (total MPA>5)**		
Individuals with ASD, n (%)	23 (46)			27 (54)		
Neurotypical controls, n (%)	37 (70)			16 (30)		
	AQ	**GAF-S**	**GAF-F**	**AQ**	**GAF-S**	**GAF-F**
Craniofacial index (MPA-CF)	0.20	-0.07	-0.06	0.12	**-0.37***	**-0-34***
Head	0.14	-0.01	-0.05	0.01	**-0.35***	-0.25
Eyes	0.08	0.06	0.11	-0.13	0.12	0.16
Ears	-0.06	0.10	0.12	**0.31***	-0.29	**-0.32***
Mouth	0.15	**-0.28***	**-0.28***	-0.15	0.14	0.03
Periphery index (MPA-P)	0.06	-0.02	-0.03	-0.12	0.18	0.2
Hands	0.03	-0.11	-0.09	-0.16	0.08	0.18
Feet	0.06	0.02	0.01	-0.03	0.19	0.14
TOTAL MPA	0.24	-010	-0.10	0.03	-0.26	-0.23

ASD: Autism Spectrum Disorder; MPA: Minor Physical Anomalies; GAF-S: Global Assessment of Functioning-symptoms; GAF-F: Global Assessment of Functioning-functioning; AQ: The Autism-Spectrum Quotient. Significances of correlations (Spearman's rank correlation test) are denoted by *p < 0.05.

In the Low-MPA group a negative correlation was found between the Mouth subscale and both GAF-symptoms and GAF-functioning, in other words more MPAs

in this region were associated with lower overall functioning and more severe symptoms. All other correlations in the Low-MPA group were non-significant.

In the High-MPA group the Ears subscale was positively correlated with the AQ, and negatively correlated with GAF-functioning. Thus, more MPAs in this region were associated with more autistic traits and lower overall functioning (i.e. low GAF-f). A higher Head subscale score was related to more severe psychiatric symptoms (i.e. low GAF-s). The CF-MPA index was negatively correlated with both GAF-symptoms and GAF-functioning, indicating that more MPAs in the craniofacial region were associated with lower overall GAF (Table 4).

DISCUSSION

In support of the first hypothesis, higher total scores on the Waldrop scale were observed for the adults with ASD in comparison to neurotypical controls. Our findings are in accordance with previously reported higher rates of MPAs in children with ASD compared with typically developing children (Tripi et al., 2008; Ozgen et al., 2011). In addition, the second hypothesis was supported, since the topographical distribution of MPAs in the ASD group showed relatively higher stigmatisation in the craniofacial region. In contrast, the peripheral MPA-index did not differ between the two groups. Also, the third hypothesis was supported by the association between higher rates of MPAs in the craniofacial region and symptom severity and lower functioning according to the GAF. This finding is in line with previously shown correlations between facial phenotypes and clinical and behavioural characteristics in boys with ASD (Aldridge et al., 2011). Early in foetal morphogenesis, the

development of structures in the craniofacial region occurs simultaneously with central structures of the brain implicated in ASD (Haznedar et al., 2006; Kwakye et al., 2011; Frith and Frith, 2003). Therefore, presence of MPAs in craniofacial region may be related to atypical brain development resulting in lower level of functioning. Possibly, in analogy with schizophrenia, early cerebro-craniofacial dysmorphogenesis reflects an early stage in the development of ASD that occurs before behavioural manifestations (Waddington et al., 1999).

Self-report questionnaires in addition to clinical assessment of behavioural characteristics are widely used in diagnostic procedures. Additional examination of some objective morphological markers could further support the ASD diagnosis. In our study, self-rated autistic traits were correlated positively to the Ears subscale score in the High-MPA group. In addition, more MPAs in this specific region were associated with lower functioning in the High-MPA group. External ear malformations, such as low-settled ears, adherent ear lobes and posteriorly rotated ears have been associated with autism in several earlier studies (Rodier et al., 1997; Stromland et al., 1994; Christianson et al., 1994; Walker, 1977). Also, a possible link between external and inner ear anomalies has been proposed in children with autism (Konstantareas and Homatidis, 1987). Probably, the developmental anomalies of ears occur during neural tube formation in the first month of gestation – a time point when the developing brain is particularly sensitive to various teratogen factors (Kosling et al., 2009; Miller, 1991). In summary, assessment of Ears MPAs is suggested to be highly relevant for support of ASD diagnosis and in evaluation of

functional impairment in adult with ASD. However, in contrast to previous studies of children, in which minor anomalies of the ears could discriminate children with autism (mostly with intellectual disability) from typically developing children (Campbell et al., 1978; Rodier et al., 1997; Walker, 1977), this was not the case in the present study. Possibly, the difference is due to the normal intellectual functioning of the current participants.

In the Low-MPA group higher scores on the Mouth subscale were related to the lower functioning and greater severity of symptoms in line with earlier findings on children with ASD (Walker, 1977; Campbell et al., 1978; Tripi et al., 2008; Ozgen et al., 2011). On the other hand, MPAs of mouth may not be specific for ASD since higher rates of mouth MPAs have also been shown in bipolar I disorder (Akabaliev, et al., 2011). The Mouth subscale scores could not differentiate individuals with ASD from neurotypical controls in the present study.

We also found that a substantial subgroup of controls exhibited MPAs, but mostly in the lower range and comparable to scores shown in other neurotypical control groups (Green et al., 1994; Ozgen et al., 2011; Trixler et al., 2001; Tripi et al., 2008; Sivkov and Akabaliev, 2003). Moreover, whereas the ASD group was almost equally distributed between having high and low total MPA scores, most of the controls (70%) fell into the Low-MPA group.

MPAs as a method to differentiate ASD from neurotypical controls

Only two out of 16 MPAs measured with Waldrop scale, distinguished individuals with ASD from the controls; the minor malformations "fine electric hair" and "single palmar crease". Although the presence of a single palmar crease is a straightforward finding, the assessment of fine electrical hair tends to be subjective and should be interpreted with caution (McGrath et al., 1995; Krouse and Kauffman, 1982).

The palmar flexion creases develop during early foetal life, between week 8 and 13 of gestation, and arises due to interaction between genetic and environmental factors, as well as underlying movement of the developing hand in the foetus (Schaumann and Kimura, 1991). The palmar creases may be clearly seen at 13 weeks of gestation and they are the unique features of the hand. Since the flexion movements of the hands are closely associated with joint formation and muscular function, deviations in development of the palmar creases reflect either anatomical or functional alterations of the developing hand (Kimura, 1991). Therefore, the crease patterns may depend on the hand malformations or some developmental insult that occurs before the time of crease development. In addition, unusual palmar creases may be seen in children with some decreased foetal movement (Popich and Smith, 1970). In consequence, as the palmar creases are determined by the interplay of genetic and environmental factors, alterations may indicate intrauterine insults early in pregnancy and may be of predictive value for developmental disorders or genetic syndromes (Tay, 1979).

Another possibility to differentiate the groups was found when MPAs were divided into subscales by body region. Differences in the mean of Head subscale between ASD group and neurotypical controls were obtained, but not in the other five body regions (Figure 2, Table 3). In a study on children with ASD, increased head circumference was associated with a more severe social impairment (Lainhart et al., 2006). This relationship was partially supported by our findings showing a positive association between Head subscale score and severity of symptoms.

Limitations

This study has several important limitations. First, the sample was relatively small which increases risk for type II errors and limits the power. On the other hand, we did not correct *p* values for multiple comparisons, increasing the risk for type I error. Thus, the statistical significance of the results should be interpreted with taking these two factors into consideration. Future studies should preferably include larger samples. Second, we did not investigate all MPAs blinded. However, people with ASD have visible social impairments and since physical examination requires personal interaction, the patients' behaviors may reveal the diagnosis. Also, most patients with ASD would be reluctant to undress and be examined by an unfamiliar clinician. Moreover, several of the MPAs examined were in fact assessed blinded, as ears, eyes and feet were estimated from photographs. In this study only Caucasians were included because hair, shape of eyes and ears often vary between different ethnic groups. Thus, the present findings cannot be generalized to other ethnic groups.

Conclusions and clinical implication

In this study a link was shown between MPAs, autistic traits and level of functioning in adults with ASD. MPAs of head, ears, eyes, mouths and hands can easily and efficiently be assessed in the clinician's office. Information on MPAs may provide important information for the diagnostic process. In the current study, particularly the shape and setting of the ears are suggested to be associated with autistic traits. As anthropometric measures can be perceived as intrusive, methods that do not require the patient to undress is an advantage. A positive association between MPAs scores and severity of impairments according to the GAF scale was found in this study. Thus more MPAs may suggest need for more supportive interventions. MPAs are suggested to serve as potentially risk markers for psychiatric disorder and careful examination of morphological features can support diagnosis of ASD and be helpful in the estimation of psychological, social, and occupational functioning.

Acknowledgement

Financial support was provided for clinical research by Praktikertjänst AB, Stockholm, Sweden; and through the regional agreement on medical training and clinical research (ALF/Pick-Up) between Stockholm County Council and Karolinska Institutet.

The authors wish to thank Professor Emeritus Lennart Wetterberg and Professor Anna Åberg Wistedt for support.

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Institutionen för Klinisk Neurovetenskap

Autism spectrum disorder in adults – biological dimensions

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Föreläsningssalen Bryggarkungen, Westmanska Palatset, Holländargatan 17, Stockholm

Fredagen den 6 September, 2013, kl 13.00

av
Irina Manouilenko
Legitimerad läkare

Huvudhandledare:
Docent Susanne Bejerot
Karolinska Institutet
Institutionen för Klinisk Neurovetenskap

Bihandledare:
Professor Anna Åberg Wistedt
Karolinska Institutet
Institutionen för Klinisk Neurovetenskap

Marco Pagani, PhD
Institute of Cognitive Sciences and Technologies, CNR, Rome, Italy

Fakultetsopponent:
Professor emeritus Sten Levander
Malmö högskola
Institutionen för kriminologi

Betygsnämnd:
Professor Lisa Ekselius
Uppsala Universitet
Institutionen för Neurovetenskap

Professor Håkan Fischer
Stockholms Universitet
Psykologiska Institutionen

Professor Anne-Liis von Knorring
Uppsala Universitet
Institutionen för Neurovetenskap

Stockholm 2013

Abstract

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental conditions characterized by difficulties in social interaction, communication and the presence of repetitive or stereotyped behaviors. Previous studies have demonstrated structural and functional abnormalities in different brain regions in ASD. Motor difficulties, unusual perception and minor physical anomalies have been reported but not systematically investigated in the adult population with ASD and normal intelligence.

In **Study I**, the internal consistency as well as diagnostic and concurrent validity of the Swedish version of the Ritvo Autism Asperger Diagnostic Scale - Revised (RAADS-R) were evaluated. The results imply that the Swedish version of the RAADS-R has good psychometric properties and is strongly correlated with the Autism Spectrum Quotient (AQ). The RAADS-R captures ASD symptoms and can be used for screening of ASD as well as in the assessment of ASD in adults with normal intelligence.

In **Studies II and III**, regional cerebral blood flow (rCBF) was assessed by positron emission tomography (PET) in thirteen adults with ASD and ten neurotypical controls after psychiatric and neurological assessments. In comparison with the neurotypical controls, individuals with ASD showed significantly increased cerebral blood flow bilaterally in large parts of cerebellum, occipital associative cortex and posterior parietal cortex. In Study III, principal components corresponding to "Autistic/ADHD symptoms", "Sensori-motor integration" and "Intelligence/motor sequencing" were identified by factor analysis based on the normalized scores of 13 neuropsychological measures. The positive correlation between ''Autistic/ADHD traits'' and rCBF in the caudate indicates a possible association of CBF changes with the executive impairments and ritualistic or stereotyped behaviors typical for ASD. Furthermore, "Sensorimotor integration" was correlated with rCBF in the occipital visual cortex, reflecting an atypical visual perception often reported in ASD. Cerebral blood flow in the left thalamus was negatively correlated with all three factors which supports the implication of this brain region in the pathophysiology of ASD. Autistic traits and ADHD symptoms were associated with shared neural substrates whereas sensory-motor deficits were grouped in another independent factor and correlated with rCBF in other regions.

In **Study IV**, minor physical anomalies (MPAs) were investigated in 53 individuals with ASD and 50 age- and gender matched controls. The ASD group showed significantly more MPAs in comparison to the control group. Moreover, MPAs were correlated with severity of symptoms and overall functioning according to the Global Assessment of Functioning (GAF).

On the whole, various behavioral, cognitive, neurological and morphological signs are suggested to converge into the ASD phenotype. Thus, in order to understand the complexity of ASD it seems meaningful to include assessment of ADHD symptoms, subtle neurological abnormalities and minor physical anomalies in the clinical evaluation of adults with ASD.

ISBN 978-91-7549-226-1