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AUTISM SPECTRUM DISORDER BEYOND THE EXTREME MALE BRAIN

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ABSTRACT

Autism spectrum disorder (ASD) is an umbrella-term for neurodevelopmental conditions characterized by both impairments in communication and social interaction and unusually restricted and repetitive or stereotyped interests and behaviors. Although proven to have a strong genetic etiology, the full biological background of ASD remains unclear. The higher frequency of ASD among males implies that a sex-related perspective may shed light on both the etiology and behavioral differences between males and females with ASD.

The main aim of this thesis was to explore male and female ASD phenotypes and their possible relation to sex hormones, beyond the well-established high systemizing─low empathizing personality observed by the extreme male brain theory of autism.

Studies I and II examined sex-dimorphic parameters such as sex hormone levels, anthropometry, self-rated gender identity, gender role and sexuality in 26 men and 24 women with high-functioning ASD and 53 age- and gender-matched neurotypical controls. Photographs of faces and bodies as well as voice recordings were blindly assessed with respect to gender coherence. Women with ASD had higher testosterone levels and less feminine facial features, while men in the ASD group were assessed as having less masculine body characteristics and voice quality than respective controls. A weak gender identity and low libido was more common in adults with ASD than in the control group.

The hypothesis of prenatal testosterone as a risk factor for developing ASD and attention deficit/hyperactivity disorder (ADHD) was tested in study III in a cohort of dizygotic twins from the Child and Adolescent Twin Study in Sweden (CATSS). Parent-reported autistic and ADHD traits were compared between female index twins with either a co-twin brother or a co-twin sister. Contrary to what could be expected from a presumed testosterone transfer from a co-twin brother to his sister, girls with a co-twin brother had lower ASD and ADHD scores as compared to those who had a co-twin sister.

Study IV examined psychometric properties as well as sex/gender differences in RAADS-14 Screen, a short version of the RAADS-R self-assessment questionnaire for ASD. Responses from 135 adults with ASD, 508 adults with other psychiatric disorders and 590 non-psychiatric controls were analyzed, showing satisfactory psychometric properties for men and women alike. Women scored higher than men in the sensory reactivity domain across all groups.

In conclusion, aberrations in the expression of sex-dimorphic traits are present in ASD. Androgyny, weak gender identity and low libido seem overrepresented along with an extreme male cognitive style. These patterns are not easily explained solely by an effect of increased prenatal androgen blood levels. The observed sex-differences in ASD underscore the necessity of acknowledging possible diagnostic and biological sex-differences when conducting research on ASD.

LIST OF SCIENTIFIC PAPERS

I. Bejerot S, **Eriksson JM**, Bonde S, Carlström K, Humble MB, Eriksson E. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. Br J Psychiatry. 2012 Aug;201:116–23.

II. Bejerot S, **Eriksson JM**. Sexuality and Gender Role in Autism Spectrum Disorder: A Case Control Study. Schmitz C, editor. PLoS One. 2014 Jan 31;9(1):e87961.

III. **Eriksson JM**, Lundström S, Lichtenstein P, Bejerot S, Eriksson E. Effect of co-twin gender on neurodevelopmental symptoms: a twin register study. Mol Autism. 2016 Jan 12;7:8.

IV. **Eriksson JM**, Andersen LM, Bejerot S. RAADS-14 Screen: validity of a screening tool for autism spectrum disorder in an adult psychiatric population. Mol Autism. 2013 Dec 9;4(1):49.

CONTENTS

LIST OF ABBREVIATIONS

A	Androstendion
ADHD	Attention Deficit/Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule
ASD	Autism spectrum disorder
A-TAC	Autism–Tics, ADHD, and other Co-morbidities inventory
AQ	Autism Spectrum Quotient
AR	Androgen Receptor
AUC	Area under the curve
BMI	Body mass index
CAIS	Complete androgen insensitivity syndrome
CAH	Congenital adrenal hyperplasia
CATSS	Child and Adolescent Twin Study in Sweden
DHEAS	Dehydroepiandrosterone sulphate
DSM	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic
EMB	The extreme male brain theory of autism
EQ	Empathy Quotient
ER	Estrogen Receptor
ICD	International Classification of Diseases
MF	Masculinity – Femininity scale
PCOS	Polycystic ovary syndrome
RAADS-R	Ritvo Autism and Asperger Diagnostic Scale-Revised
ROC	Receiver operating characteristics
RORA	Retinoic acid-related orphan receptor alpha
SHBG	Sex hormone binding globulin
SQ	Systemizing Quotient
T	Serum Testosterone
TD	Typically developed

1 INTRODUCTION

Autism spectrum disorder (ASD) comprises a continuum of conditions with similar behavioral difficulties and aberrations in social interaction and communication as well as unusually restrictive and repetitive behavioral patterns observable from early childhood. ASD is a heterogeneous disorder with individual variation in both severity of impairment and level of intelligence. A distinctive feature in the whole range of ASD is male predominance. Most cases are diagnosed at an early age and a minority of children diagnosed with ASD present with a regression of previously developed abilities.[1] ASD commonly co-occurs with other neurological disorders such as epilepsy, intellectual disability, schizophrenia or Attention Deficit/Hyperactivity Disorder (ADHD) as well as other somatic manifestations, for example gastrointestinal problems, sleep disturbances and dysmorphic features.[2]

Diagnostics and research on ASD have traditionally focused on boys, however, interest in the clinical picture for adolescents and adults with ASD has increased in recent decades resulting in a rising number of adult diagnoses and an increased interest in comorbidities, sex-differences, and outcomes in adults with ASD. Theories associating ASD with extreme male intellect and an autistic spectrum fading into the normal population have opened the ASD research field for studies of traits and sex differences.

1.1 AUTISTIC TRAITS

The concept of autism and core autistic traits was first described in the first half of the twentieth century. In 1943, Leo Kanner described cases of "early infantile autism",[3] a low functioning ASD including mental retardation and little to no language. The typical traits of high functioning ASD were described already in 1926 by the Russian psychiatrist Grunya Efimovna Sukhareva[4] and in 1944 by Hans Asperger.[5] Each of these three pioneers thoroughly described the core autistic traits that are included in modern diagnostic criteria for ASD. Individuals with ASD show persistent impairments in social interaction and communication, including abnormal social-emotional reciprocity, difficulties in initiating and maintaining social interactions, deficits in nonverbal communication necessary for social engagement (for example, poor eye contact), and atypical speech. Further, individuals with ASD also present restricted patterns of behaviors and interests, including motor stereotypies, inflexibility to routines and insistence on sameness as well as circumscribed and fixated interests. Motor difficulties are also very common in this group.[6–8]

In 2013, with the new version of the Diagnostic and Statistical Manual of Mental Disorders, DSM-5, the previously separately defined disorders 'autistic disorder', 'Asperger disorder', 'childhood disintegrative disorder' and 'pervasive developmental disorder not otherwise specified' that described different levels of language development, intelligence and number of autistic traits were replaced by the collective term 'autism spectrum disorder' (ASD) to

acknowledge the difficulties in distinguishing distinct and clinically relevant autistic phenotypes based on those characteristics.

An abnormal response to sensory input is a common trait in ASD, with over 90% of those diagnosed with ASD displaying reduced or enhanced sensitivity of some kind.[9–11] Although mentioned in the original case reports, it was left out from the DSM-III and DSM-IV criteria, but was included as "hyper- or hypo-reactivity to sensory input or unusual interest in sensory aspects of the environment" in the DSM-5 criteria.

The core symptoms of autism are believed to be an effect of altered neuro-cognitive mechanisms. Two generalized cognitive mechanisms have been suggested to explain the core ASD symptoms: deficits in empathizing and an augmented tendency to systemize.[12] Within the empathizing concept lies the ability to identify mental states to self and others and to predict and understand other people's behavior accordingly, known as the *theory of mind,*[13,14] and also to react emotionally appropriately to another person's thoughts and feelings.[15] Systemizing is the drive to study, construct and maintain systems. The interplay between a strong need to systemize and a weak ability to empathize explains impaired and stereotyped social behavior and communication as well as narrow interests and resistance to change.[15]

1.2   THE EXTREME MALE BRAIN THEORY OF AUTISM

The capacities and inclinations to empathize and systemize varies among all individuals and have been suggested to be two different and important cognitive dimensions.[15] Two self-evaluation tests, the Systemizing Quotient (SQ)[16] and the Empathy Quotient (EQ)[17] have been developed to measure and describe cognitive style on these dimensions. Large studies of EQ and SQ in the general population show gender differences at a group level with more males reporting a strong drive to systemize and more females reporting a strong drive to empathize.[15,18,19] This female advantage in empathizing also results in an ability to decipher emotions from eye expressions in the Reading the Mind in the Eyes test[20] and pinpointing socially inappropriate behavior in short stories in the Faux Pas test.[21] Males have typically tested better on the Embedded Figures test,[22] being quicker to recognize a simple shape embedded in a complex shape.[23] Individuals with ASD, with their extreme drive to systemize and impairments in the empathy domain, can thus be regarded as having an extreme male intellect on these cognitive dimensions, a theory that has been named *The extreme male brain theory of autism* (EMB).[24] From a neurodevelopmental point-of-view, an extreme male intellect indicates that a masculinization of the brain has taken place during fetal brain development. The EMB has focused mainly on brain masculinization caused by elevated levels of prenatal androgens, especially testosterone, but does not exclude effects of other masculinizing factors such as genetic aberrances in the X- and Y-chromosomes.[25]

1.3 ETIOLOGY

ASD comprises a set of neurodevelopmental conditions with a broad etiological base that gives rise to the autistic core symptoms. However, a detailed etiology of ASD has not been established. Large studies of twin pairs, high-risk infant siblings and families have estimated the heritability of ASD to be around 50%,[26,27] with a concordance in ASD diagnosis of 76-95% between monozygotic twins.[28] This indicates a strong genetic influence with additional gene-environment interactions.

1.3.1 Genetic factors

Several known genetic disorders, such as fragile X syndrome, tuberous sclerosis, Smith–Magenis syndrome, Rett syndrome and Prader–Willi syndrome[29–32] often present with ASD. The disorder is also common in individuals born pre-term; however, most cases of ASD have no such straightforward etiology. Genetic studies have identified many genes associated with ASD. However, the identified genes only explain 10-20% of the total ASD population. These candidate genes are very diverse but, interestingly, a subset are related to synapse function, immune function, and sex hormone regulation[33–35] – all functions that have been related to ASD (e.g. altered synaptic pruning,[36] maternal infection associated with increased ASD risk[37] and elevated levels of prenatal testosterone associated with an increased number of autistic traits in children[38]).

1.3.2 Environmental factors

The environmental portion of the ASD etiology appears pre- or perinatal, caused mostly by events in first or second trimester that augment the effect of existing genetic aberrations. Prenatal environmental factors that have been suggested to increase risk for ASD are vitamin D insufficiency,[39] toxins, immunological factors such as maternal infection and autoimmunity,[37] and deficient testosterone regulation.[40]

1.3.2.1 Immunological factors

There are several indications that immunological factors affect the development of ASD subtypes. Several ASD candidate genes play important roles in immune function,[34] and maternal infection in the 1-2 trimesters is associated with higher rates of ASD in the child.[41,42] Additionally, both bacterial and viral maternal immune activation have yielded offspring with autism-like features in mice, rats and primates. Similar autism-like behaviors were also achieved by gestational flu exposure in mice.[43]

1.3.2.2 ASD and prenatal testosterone exposure

During fetal development, testosterone (T) is a key player in masculinization. In line with the EMB theory there is some evidence for an association between elevated levels of prenatal androgens, especially testosterone, and ASD. Amniotic testosterone levels have been found to positively correlate with poorer social skills at age four according to parent ratings.[44] One study has also indicated that steroidogenic activity is elevated in the amniotic fluid of fetuses

who will develop into children who receive ASD diagnoses.[45] An increased number of autistic traits[46] and reduced empathy[47] have been found in women with congenital adrenal hyperplasia (CAH), a genetic disease that results in high prenatal testosterone levels in both sexes.

The influence of androgens in women with ASD is further supported by the fact that androgen-related conditions are more common than expected in these patients[48] and by the observation that autistic traits in early childhood have been associated with late menarche, which is related to androgen status.[49] A recent study found that maternal polycystic ovary syndrome (PCOS) increased the odds of having a child with ASD by 59%,[50] further indicating a relation between elevated maternal testosterone levels and ASD.

1.4   IS THERE AN AUTISTIC BRAIN?

Although there are no biomarkers available that are specific enough to diagnose ASD, studies using brain imaging and pathological analyses of brain tissue have been used to identify differences in brain development as well as activation and connectivity in individuals with ASD. An altered growth trajectory of the whole brain has been suggested for ASD and considered a central neurodevelopmental feature of the disorder.[51] Leo Kanner[3] reported unusually large heads in 5 of 11 cases in his original description of autism and, after that, a higher frequency of large head circumference associated with macrocephaly has been reported in several studies.[52] Brain MRI studies have confirmed an increased frequency of enlarged cerebrum volume in ASD as compared with typically developed (TD) individuals throughout childhood.[51] However, this brain enlargement is only present in a subgroup of ASD and is mostly associated with regressive ASD.[53] Notably, microcephaly has also been reported in ASD children.[54]

Differences in hippocampal and cerebellar volume have also been indicated in individuals with ASD.[55,56] Furthermore, compared with age matched TD children, variation in amygdala volume is greater in 2-4 year-old children with ASD, and on average is bilaterally increased.[57,58] Bilateral activation of the amygdala has been related to the restricted repetitive interests in individuals with ASD as well as strong interests in TD people. Several aspects of social impairment related to autism, such as processing of facial emotions and joint attention ability, have been suggested to be linked to amygdala abnormalities.[58]

Several studies have also shown alterations in functional connectivity in ASD patient with decreased frontal-posterior connectivity and over-connectivity within the extrastriate cortex, frontal and temporal regions and amygdala.[59] Abnormal cortical growth trajectories together with alterations in functional connectivity and post-mortem findings of an excess of neurons in the prefrontal cortex in children with ASD[60] suggest an effect of abnormal synaptic pruning during ASD development.[61,62]

There is also some evidence for aberrant activation of brain regions related to theory of mind in individuals with ASD as compared with TD individuals.[63] Regions affected include the insula, posterior and anterior cingulate cortex, caudate nucleus, precuneus, lateral occipital cortex, and supramarginal gyrus.

1.4.1.1   Sex differences in ASD

Very few structural neuroimaging studies of ASD have focused on females; however there is some evidence of sex differences in early brain growth trajectories in ASD.[36,53] It has also been suggested with some evidence that preschool-aged girls with ASD show more neural abnormalities in the amygdala, temporal lobe, and cerebellum than boys of the same age with ASD.[64,65]

1.5   DIAGNOSING ASD

The diagnostic procedure for ASD is based on a clinical assessment of core autistic traits in conjunction with impairment of overall function in relation to defined diagnostic criteria.

The clinical assessment procedure for diagnosing ASD is an extensive process typically performed by a team that includes a certified psychologist and a fully trained psychiatrist specialized in diagnosing ASD. In adults, this multidisciplinary assessment includes observations of behaviors, history of childhood symptoms, results from structured and semi-structured interviews with the patient, assessment of intellectual ability and adaptive functioning and, when possible, an interview with a parent. A full assessment takes approximately 20 hours to complete.

Self-assessment questionnaires are often a part of the psychiatric diagnostic process in adults. Written questionnaires may relieve some patients from social stress and allow more time for introspection before responding. However, self-assessment is a subjective measure colored by the respondents' willingness to be honest counter-balanced by the drive to align with social norms, varying lack of self-insight and interpretation of the questions. It has been discussed whether the core symptoms of ASD might impair the individual's ability for personal introspection,[14] implying that self-rating questionnaires could be unsuitable in this group, however studies have shown that most adults with ASD and normal intelligence will validly self-assess both autistic and other personality traits.[66–68] The self-assessment questionnaires most widely used in the diagnostic procedure for ASD, and also included in the UK National Institute for Health and Care Excellence (NICE) guidelines, are the Ritvo Autism and Asperger Diagnostic scale – Revised (RAADS-R),[67] the Autism Spectrum Quotient (AQ)[69] and the EQ.[17]

Parental assessment is an important part of ASD diagnostics. Examples of parental interview instruments are the semi-structured interview Autism Diagnostic Interview-Revised (ADI-R),[70] the Diagnostic Interview for Social and Communication Disorders (DISCO),[71] the

Autism - Tics, AD/HD and other Comorbidities inventory (A-TAC),[72] a parental telephone interview, and parent report questionnaires, the Social Responsiveness Scale (SRS)[73] and AQ-Child.[74] In adults and adolescents, the parental interview is valuable to give a perspective of childhood symptoms. In child diagnostics parents contribute important information about behaviors and impairments in daily life that may not be visible during clinical sessions. Further, symptom debut and symptom perseverance are important factors where parents may also provide important information for adolescent and adult assessments. Difficulties with parental interviews include bias due to different expectations depending on the child's sex/gender or comparisons between siblings.

1.5.1 Diagnostic challenges

The current classification systems DSM-5 and International Classification of Diseases – 10th Ed. (ICD-10), aim to classify clinically meaningful groups of symptoms, that is, common clinical appearances, into different diagnoses. As psychiatric disorders currently lack biological diagnostic markers, the borders between different psychiatric disorders are not clear-cut increasing misdiagnosis risk in patients with an ambiguous phenotype. Specifically, it has been suggested that females with ASD present with a slightly different phenotype than males.

1.5.1.1 Differential diagnostics

The instruments used when diagnosing ASD are very good at recognizing ASD symptoms (high sensitivity) and weeding out non-psychiatric patients (high specificity). What complicates these diagnostics is that many patients have co-occurring psychiatric disorders with symptoms that may cause diagnostic confusion. An adult patient presenting with anxiety, difficulties with social interaction, dysphoria and difficulties concentrating may suffer from ASD, but the same symptoms are also common in other psychiatric diagnoses such as depression, ADHD and schizophrenia. Thus, the main difficulty in diagnosing such a patient is not to identify that she is suffering from a psychiatric disorder but to identify which particular diagnosis/es fits the best. As in-depth assessment is time consuming for psychiatric disorders in general and for neuropsychiatric disorders in particular, only those assessment procedures that are deemed necessary are initiated.

For many other psychiatric diagnoses, short questionnaires are available for quick screening to decide whether further evaluation is necessary. For ASD, short questionnaires have been lacking, with the AQ and RAADS-R being too long, unwieldy and time consuming for the psychiatric patient to complete and the short version of AQ, AQ-10, having insufficient evidence for screening in psychiatric settings.

1.5.1.2 Sex differences in the differential diagnostics

Diagnostic challenges differ in some ways between females and males. Male bias may increase the risk of a missed ASD diagnosis in females when symptoms are instead attributed to a comorbid, more female-biased diagnosis. This has been found for eating disorders, where

a large number of female patients present with signs of Asperger syndrome;[32,75] it has also been suggested that anorexia in some cases may be a sign of an underlying ASD rather than a comorbidity.[76]

1.5.1.3 Sex differences in autistic symptoms

Owing to the history of describing Asperger syndrome or high functioning ASD as a male disorder, the diagnostic criteria for ASD are still based on the typical characteristics of a male with ASD. It has been suggested that ASD in females often goes unrecognized because of a presentation that differs from male-typical ASD and also from a greater ability to camouflage symptoms.[25,77] Differences in presentation have been described in all three classical core symptom domains: the most well documented is the decreased number of restricted and repetitive patterns of behavior and interests,[78] but characteristics more often described in females with ASD include a greater awareness of the need for social interaction, an ability to mask difficulties by imitating other's behaviors, developmentally superior linguistic abilities, and superior imagination.[77,79]

Additionally, there are indications of male-biased diagnosis of boys and girls with the same symptom burden: 1) males are, on average, diagnosed at an earlier age,[80] even when boys and girls present with similar amounts of autistic traits,[81] 2) a study comparing children with equal levels of ASD traits that had either received an ASD diagnosis or not, showed that girls, but not boys, who meet diagnostic criteria for ASD show lower intellectual level and more behavioral problems than their non-diagnosed peers with similar levels of ASD traits.[82]

1.6   EXPLAINING THE MALE PREDOMINANCE

The male:female ratio of ASD has been estimated to 4-5:1, but varies from 2:1 in cohorts with low intellectual quotient (IQ) to 7:1 in high functioning cohorts.[83] Several non-mutually exclusive explanations for male predominance have been put forward, some focusing on an over-estimation of the male:female ratio and others suggesting biological models based on male-biased risk-factors and a female protective factors.

As previously mentioned, it has been suggested that the diagnostic process leads to underdiagnosis of females due to phenotypic differences for males and females with ASD[25,77] and a male biased diagnostic assessment discriminating females from ASD diagnoses even when criteria are met.[80–82]

The prenatal testosterone hypothesis of the EMB males supports the idea that males are more exposed to the risk factors for ASD. Furthermore, biological differences between the sexes may also contribute to a *female protective effect*, that is, biological factors specific for the female sex which counteract the effects of risk factors for ASD. This protective effect has recently been suggested to relate to sex-differences in neural plasticity at the synaptic and regional level.[84]

Several biological explanation models have been proposed and tested, and are more thoroughly reviewed by Lai *et al*.[85] Although there is no support for one single female protective genetic factor that could explain the sex bias,[86] there is evidence that females require a greater genetic burden to surpass the diagnostic threshold for an ASD diagnosis. *The multifactorial model of disease transmission* suggests that genetic liability for autism is normally distributed in the population and that there exists a minimum level of genetic liability causing ASD that is higher in females than in males.[87,88] A register study of siblings to probands with autistic impairments in the top 90th percentile found significantly more autistic impairments in siblings of female probands with autism than in siblings of male probands, indicating that a larger etiological load is required for females.[89]

A variant of this model explains differences in genetic liability between males and females with a qualitative difference in the etiological factors for ASD in males and females. One large genetic study found that *de novo* mutations more often lead to sporadic ASD in males than in females, but that the off-spring of the non-symptomatic females present with increased risk for familial ASD.[90] This suggests that *de novo* mutations have elevated penetrance in males compared with females, which is also supported by several genetic analyses of individuals with ASD showing a larger number of large de novo copy number variations (CNVs) in females than in males,[91,92] indicating different thresholds in males and females for developing symptoms.

A revision of this completely genetic view is *the multi-factorial sex/gender-differential liability model*. In this model, an etiological burden is added to the genetic load, suggesting a common genetic threshold; however, the liability distribution for the entire sex is shifted towards (in males) or away from (in females) this threshold by sex-differentiated genetic or environmental factors, e.g. an X chromosome protective effect or elevated risk by increased levels of prenatal androgens. Support for protective genes on the X-chromosome is indicated by the increased prevalence of autism in X-linked disorders like Turner syndrome (X0),[93,94] fragile-X syndrome (an absence or mutation of the FMR1 gene on the X chromosome)[95] and Rett syndrome (mutations of the MECP2-gene on the X chromosome).[96] On a slightly different note, an intricate gene by environment interaction has been proposed by Hu *et al*:[97] a deficiency in the expression of the retinoic acid-related orphan receptor alpha (*RORA*) gene in ASD causes dysregulated feedback loops with both sex hormones as well as the expression of several other genes related to ASD[35,98] and *RORA* dysfunction may have different impacts on males and females with ASD.[97]

1.7   SEX/GENDER DIFFERENCES IN BEHAVIOR

Although behavioral differences between human males and females are diffuse and by no means applicable on individual level, a range of differences have been measured on group level, and some already in infants. The largest behavioral differences are found in sexual preference (over 90% of adults report sexual attraction toward the opposite sex) and gender identity. Smaller differences have been found in empathy[99] and verbal fluency,[100,101] where females outperform males, and males generally express more physically aggressive

behavior[102] and proficiency in mental rotation than females.[103] Sexual desire is also generally higher in men.[104] The differences may be X-or Y-chromosome linked or dependent on prenatal as well as post-natal circulating hormone levels, but to some extent, they can also be socially determined. The term sex/gender is used to acknowledge the overlap between biological and social effects on behavior and, consequently, the difficulties to fully distinguish the effects.

1.7.1 Fetal sexual differentiation

The gonads develop in the sixth week of pregnancy. Testicles are developed if the sex determining gene on the Y chromosome (SRY) is present. When developed, the testicles start producing testosterone in a surge that lasts until gestational week 24. A sufficiently high level of testosterone is then essential for the formation of male genitals during the sexual organ development between gestational weeks 6 and 12. In the absence of sufficient testosterone exposure, female genitals form. Brain development is also affected by sex hormones. Both testosterone and estrogens are said to have organizational effects on the brain because they produce permanent changes to brain structures.

1.7.2 Prenatal testosterone and behavior

1.7.2.1 Animal studies

Studies on non-human mammals, from rodents to primates, have shown that prenatal testosterone levels influence sexual differentiation of the brain that will impact behavior.[105] Testosterone and its derivative estradiol have been shown to affect cell survival and neurite outgrowth. Early hormonal manipulation in animals has shown effects of testosterone on the development of neural regions resulting in altered patterns of apoptosis, connections between neural regions and neurochemical sensitivity.[106] Sex dimorphic behavior affected by the same hormonal manipulations, such as sexual behavior, aggressive behavior and juvenile rough-and-tumble play, are thought to be an effect of these neural changes.[106]

1.7.2.2 Human studies

The research on sex differences related to prenatal testosterone levels is complicated by difficulties in direct measurement of prenatal testosterone. The effects of high prenatal testosterone levels have therefore been studied in indirect ways. Congenital adrenal hyperplasia (CAH) is a rare genetic disease resulting in elevated androgen production in the adrenal glands. Comparisons between females with and without this condition show effects of elevated prenatal testosterone regarding increased male-typical toy, playmate and activity preferences.[107] Prenatal exposure to testosterone has been found to correlate positively with aggression[47,108,109] and negatively with empathy.[110,111]

1.8 ASSESSING PRENATAL TESTOSTERONE EXPOSURE

Several methods have been used to estimate prenatal testosterone exposure, the most common of which are measurements of testosterone levels in amniotic fluid (during pregnancy), umbilical cord blood (at birth) and using sex-dimorphic biomarkers as postnatal surrogate measures.[112] The effects of prenatal testosterone exposure on group level may also be studied in persons exposed to elevated levels of testosterone due to a known condition.

1.8.1 2D:4D

The ratio between the second and fourth digit lengths (2D:4D) is a sex-dimorphic measure widely used as a surrogate measure for fetal testosterone levels in the first trimester.[112] Sex differences in 2D:4D, showing a lower ratio in boys than in girls, have been detectable from 10 weeks and older,[113] with some variation of magnitude over time.[114,115] Females and males exposed to elevated levels of prenatal androgen due to CAH have lower 2D:4D[116,117] and males with complete androgen insensitivity syndrome (CAIS) have larger 2D:4D than same-sex controls indicating that this sex-dimorphism is in fact related to prenatal androgen exposure. Several studies have found a decreased 2D:4D in the ASD group.[118]

The usefulness of this measure as a surrogate for prenatal testosterone exposure has recently been questioned, noting that differences are small, with a large overlap between compared groups and sexes and thus do not reflect a linear or even a monotonically increasing relationship between 2D:4D and prenatal testosterone levels.[114]

1.8.2 Testosterone transfer

In animal studies, testosterone has been shown to transfer from male fetuses to adjacent fetuses via amniotic diffusion causing elevated levels of testosterone and masculinization in female fetuses.[119] It has been suggested that this phenomenon also occurs in human multiple pregnancies,[120–122] implying that comparisons of masculinization of sex-differentiated traits in female dizygotic (DZ) twins with either a male co-twin increasing prenatal testosterone exposure or a female co-twin not affecting testosterone levels, may shed light on the effects of elevated prenatal testosterone levels (Figure 1).
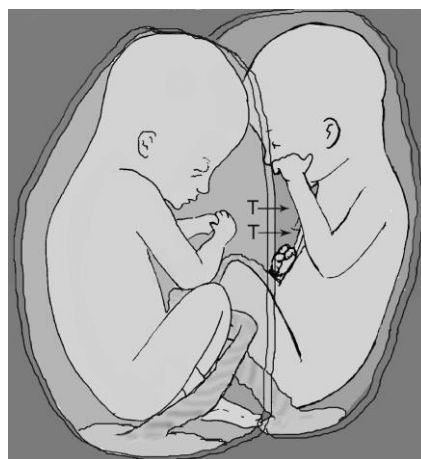
Figure 1 The testosterone transfer theory proposes that testosterone exposure through amniotic diffusion from a dizygotic male twin may cause masculinized characteristics in his female twin.

1.9 GENDER RELATED OBSERVATIONS IN AUTISM SPECTRUM DISORDER

A straightforward assumption along the prenatal testosterone hypothesis of the EMB would be that all patients with ASD have been exposed to additional prenatal testosterone during the entire sex-differentiation developmental process. If this is the case, all sex-dimorphic characteristics assumed to be differentiated in the fetus should be masculinized in people with ASD. Contrary to this conclusion, however, clinicians have observed that demasculinized appearance and behavior in males with ASD appear to be more frequent than in the general population.

1.9.1 Gender identity

Reports from clinics as well as studies indicate that the prevalence of ASD is increased among patients with gender identity disorders.[123] Preliminary data from the Swedish diagnosis register showed a 14% prevalence of ASD diagnoses among 18-24 year old individuals with a transsexualism (ICD-10 F64) diagnosis in 2010 (Kosidou Kyriaki, unpublished).

1.9.2 Gender appearance

Although individuals with ASD, as well as the general population, show great variability in physical appearance, clinical observations of ASD patients have suggested a larger prevalence of obesity[125,126] as well as an overrepresentation of physical features with an androgynous appearance, e.g. slenderness, delicate facial features and high pitched voices in male ASD patients.[4,5] Notably, this phenotype has not been described in female ASD patients.

1.10 SEXUALITY AND AUTISM SPECTRUM DISORDER

There is a paucity of studies concerning sexual orientation and sexual behavior in adolescents and adults with ASD. The core autistic traits indicate that sexual behavior and experience could be different in this group: difficulties with social relationships may complicate forming and maintaining romantic relationships, preoccupation with a special interest as well as the common resistance to touch, probably caused by a combination of hyper sensitivity and impairments concerning social relations, could potentially leave little space for interest in sex or romance and may be difficult to overcome. At the same time, presumed elevated levels of prenatal testosterone in ASD would potentially impact sexual behavior and orientation. Autobiographies and case studies of adults with ASD have indicated an existing romantic and sexual interest in many individuals with ASD,[127–129] but also higher frequencies of asexuality.[48,130] Higher rates of bisexuality have also been indicated.[129,130]

1.11 SEX HORMONES IN THE ADULT

Appearance and sexuality in adults is also affected by circulating sex hormones. Androgens, which bind to the androgen receptor (AR) are the sex hormones largely affecting male characteristics while estrogens, which bind to the estrogen receptor (ER), affect female characteristics. Both hormones are present in both sexes, together with a large amount of related hormones and precursors.

Sex hormone-binding globulin (SHBG) is important in the regulation of androgens and estrogens. It is synthesized in the liver and binds to androgens such as testosterone and estrogen in blood, regulating their accessibility to target cells. SHBG production is regulated by both hormonal and nutritional factors, and has been shown to increase during estrogen treatment and decrease after androgen administration.[131] Obesity is associated with decreased SHBG levels in both sexes, mainly due to increased liver fat.

Dehydroepiandrosterone sulphate (DHEAS) is an androgen mainly produced by the adrenal glands, but also in the gonads and brain. DHEAS must be converted to testosterone to affect AR. Its production varies with age: the fetal adrenal cortex secretes large amounts of DHEAS, however, after birth, DHEAS serum concentrations quickly decrease to very low levels. At the adrenarche at about age 7, DHEAS levels start increasing again and peak in the 20s-30s, followed by a steady decline with age.

Androstendion (A) is produced both by the gonads and the adrenal cortex. CAH leads to increased production of A due to 21-hydroxylase deficiency. In peripheral tissue, A is converted to testosterone, but can also be aromatized to estrogen.

Testosterone (T) is the most potent androgen. In men, it is mainly produced in the testes, but in women T is secreted by the adrenal cortex (25%) and the ovaries (25%), the remainder is produced peripherally from circulating DHEAS.[132] Circulating levels are in the range 0.6–2.5nmol/L. Testosterone concentrations vary during the menstrual cycle and reach a lowest point in the early follicular phase of the cycle, rising to a mid-cycle peak with luteal phase concentrations higher than those of the early follicular phase.[133]

Estradiol is the most potent and most abundant estrogen. In women, estradiol is mainly produced in the ovaries, and estradiol levels vary over the menstrual cycle. In men, T is converted to estradiol by the enzyme aromatase which, together with ER are abundant in brain regions related to sexual arousal, the penis and the testes. Estradiol has also been shown to modulate libido and erectile function in men.[134]

1.11.1 Sex hormones in autism spectrum disorder

Adolescent and adult levels of sex hormones in ASD have not been thoroughly studied; however, it has been indicated that increased serum levels of sex hormones and steroidopathy diagnoses are more common in women with ASD.[45,135,136] In men with ASD, studies are few and small and show no conclusive results.[135–137]

2 RATIONALE AND AIMS OF THIS THESIS

The extreme male brain theory of autism describes ASD as being characterized by an extreme male intellect and suggests that elevated levels of prenatal androgens may play an important role in the development of autism. There is support for a masculinization of the brain from structural and functional imaging studies as well as studies showing a correlation between elevated prenatal testosterone and increased number of autistic traits; however, some questions remain regarding to what extent prenatal testosterone or other lesser known sex-differentiating etiological factors are involved the development of ASD and through which mechanisms (e.g. fetal overproduction as in CAH, external testosterone exposure from mother with PCOS or a male twin; or gene-environment interactions such as dysregulated expression of the sex-dimorphic *RORA* gene).

Clinical observations have indicated a tendency for androgynous physical traits in males with ASD. Along the same vein, transgender clinics and self-biographical reports from individuals with ASD have indicated a weak gender identity and higher frequencies of asexuality and non-normative sexual orientation.[129] A better understanding of how traits beyond the extreme male cognition relates to typically developed males and females may add a new perspective to the sex/gender-related aspects of ASD, possibly providing evidence for a connection between androgens and ASD.

Studies I and II aim to study sex-differentiation in ASD beyond masculinization in systemizing and empathizing presented by the extreme male brain theory. Study III further attempts to test the prenatal testosterone hypothesis in ASD by examining whether female fetuses exposed to elevated levels of prenatal testosterone from a twin brother develop an increased number of autistic traits.

Study IV acknowledges the problem with identifying a previously unrecognized ASD in adult psychiatric patients. As underdiagnosis of ASD in females is believed to be a reason for the greater male:female ratio observed in high functioning ASD, additional instruments that aid the psychiatric differential diagnostics are necessary to counteract missed diagnoses. A short self-assessment instrument provides valuable, time-efficient screening for evaluation of the need for further investigation of a possible ASD. Recognizing a previously undiagnosed ASD in adult psychiatric patients may aid in treatment choices and allow adapted support, as well as increase self-acceptance.

2.1.1 Aims

Study I

To assess sex-dimorphic physical measures purportedly related to the prenatal androgen influence in men and women with and without ASD to shed further light on its possible biological underpinnings.

Study II

To explore psychological factors related to prenatal androgen influence, such as gender self-perception, gender role and sexuality in men and women with and without ASD.

Study III

To test the theory that prenatal testosterone increases autistic and ADHD traits by comparing the number of autistic and ADHD traits in DZ index twins with a female co-twin to those in DZ index-twins with a male co-twin.

Study IV

To examine the validity of the RAADS-14 Screen in a psychiatric population and to determine any sex-related differences.

3 MATERIALS AND METHODS

This thesis encompasses several different study designs: studies I and II are based on the same case-control study, analyzing sex/gender-dimorphic characteristics, study III is based on parent-reported data from a twin register, and study IV is a psychometric evaluation of a self-assessment questionnaire comprising anonymously collected questionnaire data.

Table 1 Summary of aims, participants and methods in studies I-IV.

Study	Aim	Participants	Methods
I	Explore differences in sex-dimorphic physical features between men and women with and without ASD.	*Case-control-study:* 50 adults with ASD (26 men and 24 women) 53 gender and age matched controls without ASD (28 men and 25 women).	Sex/gender separated comparisons of: anthropomorphic measures, serum hormone levels and blinded assessments of face, body and voice characteristics from photographs and recordings.
II	Explore differences in gender role, gender identity, gender typical childhood behavior and sexuality in men and women with and without ASD.		Sex/gender separated comparisons of: gender identity, gender role behavior and sexuality in ASD vs controls, using self-assessment questionnaires and interview.
III	Test effect of prenatal testosterone transfer on autistic and ADHD traits in DZ twins.	*Register study:* Dizygotic twins aged 9 or 12. 8477 males. 7835 females.	Using data from the Child and Adolescent Twin Study in Sweden (CATSS). Comparison of A-TAC scores between index-twins with either a female or a male co-twin.
IV	Psychometric validation of the RAADS-14 Screen, including analysis of differences between diagnosis groups and genders.	*Psychometric study:* 135 adults with ASD. 508 psychiatric controls. 590 non-psychiatric controls	Psychiatric and non-psychiatric participants completed the RAADS-14 Screen questionnaire. Analyses included construct validity, factor analysis, ROC-analyses, and group comparisons for diagnosis and gender.

3.1 PARTICIPANTS AND PROCEDURE

3.1.1 Studies I and II

Studies I and II were based on the same case control study material. The study started in November 2006 and selection of measures and procedures as well as assessment of participants in the ASD group was performed by Susanne Bejerot and Sabina Bonde. I joined the work in May 2009, and co-jointly assessed the three remaining participants in the ASD group together with Dr. Bejerot and subsequently recruited and assessed the control group.

3.1.1.1 Participants

Fifty adults, (24 women, 26 men) diagnosed with ASD and 53 controls (28 men, 26 women) matched for sex and age were included in this study.

Inclusion criteria for both groups were participants aged between 20 and 47 years who had parents of Swedish/White descent. The latter was to simplify masculinity/femininity assessment and to avoid possible variation due to cultural differences. Exclusion criteria were any known neurological or genetic syndrome, any disease or medication affecting androgen status, any congenital syndrome, diagnosed malformations, any psychotic episodes, intellectual disability or having attended special education in school up until ninth grade. Intelligence within the normal range was assumed by mainstream schooling. Exclusion criteria in the control group were a diagnosis of ASD or ASD in a first-degree family member, as well as large or multiple tattoos or piercings, in order to match the ASD group. All participants negated use of anabolic steroids or other androgen treatment. Hormonal contraceptive treatment was accepted.

Participants were recruited through advertisements via a number of channels. The ASD group was recruited through an outpatient tertiary psychiatric unit for adults with ASD (n=20), Asperger Center - a community-based center for adults with ASD (n=24), and through a website for adults with ASD (n=6). Figure 2 illustrates the recruitment process of the ASD group. All controls were recruited through flyers posted on bulletin boards at a non-profit keep-fit organization (Friskis & Svettis: n=23), Stockholm University (n=8), student accommodation houses (n=5), at dental clinics and vaccination centers (n=5), private companies (n=4), employment agencies (n=2) or by referral from friends (n=7).

3.1.1.2 Procedure

An initial telephone screening was conducted for each participant to assess inclusion and exclusion criteria. Information about the study and screening questionnaires were mailed to the participants before assessment sessions. In the ASD group, the assessment session started with an ADOS assessment to evaluate if the participant fulfilled ASD criteria. The remaining assessment procedure was identical for the ASD and control groups: 1) interview questions to collect background data, childhood gender behavior and psychiatric structural interviews e.g.

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MINI assessment; 2) physical examination and photography; and 3) completion of additional self-evaluation questionnaires. The participants were also given a referral for a blood test, and were instructed to take the test between 9:00AM and 13:00 PM.
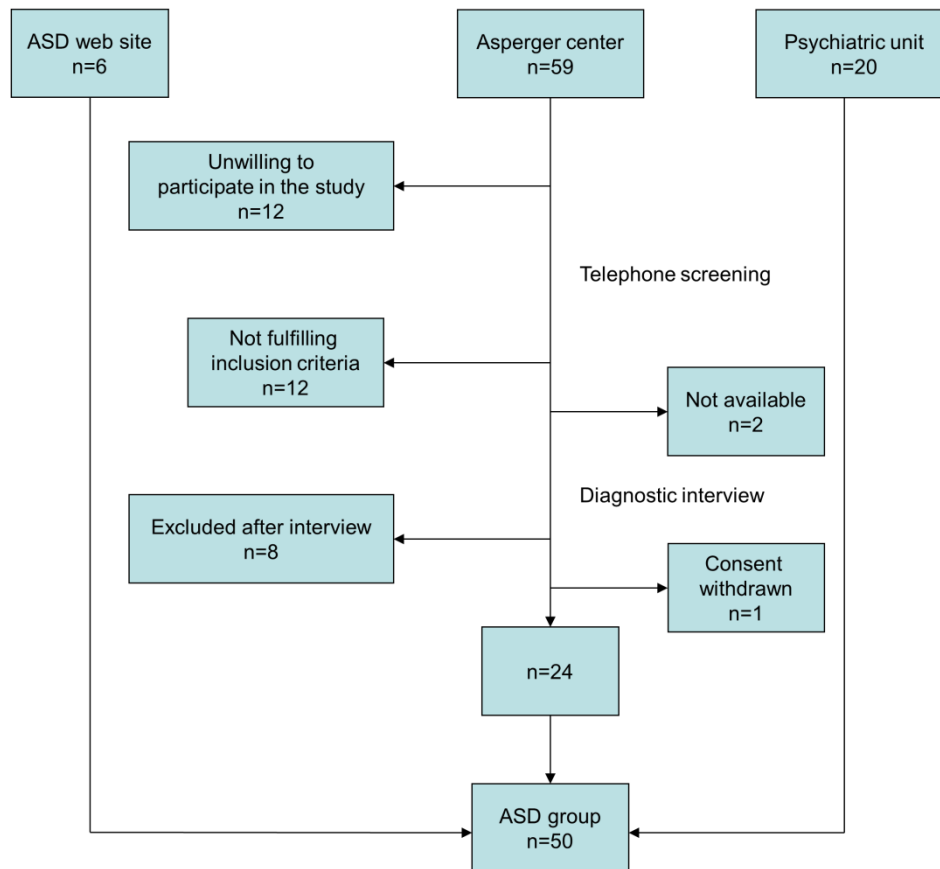
Figure 2 Recruitment flow chart: inclusion and exclusion of individuals with ASD in studies I and II.

3.1.1.3   Laboratory tests and adjustments

Blood samples for T, SHBG and DHEAS were collected between 9:00 AM and 13.20 PM. Due to the diurnal variability of male T,[138] T samples are most stable between 7:00 and 9:00 AM, after which stability declines. However, test times were fairly equally distributed in both groups and linear adjustment for the time of sampling in accordance with the results of Brambilla *et al*.[138] did not markedly affect the outcome of the analyses. Female testosterone has less diurnal variation, but there is some variation over the menstrual cycle, peaking at ovulation.[139]

The analyses of male and female T levels were conducted using both original data and data adjusted linearly for diurnal variation in men and days from ovulation in women. Additionally, as five women in the ASD group had body mass index (BMI) > 30 kg/m^2 (classified as obese) and obesity is associated with decreased levels of SHBG in serum as well as elevated levels of androgen,[140,141] we also analyzed the female groups excluding the obese women.

3.1.1.4 Anthropometrics

The circumference of the head, chest, waist, hips, ankles and wrists were measured with a regular millimeter tape-measure. A participant stated height was accepted. If the participant was unsure, the height was measured with millimeter precision stadiometer. Because of the large difference in BMI between the female ASD group and controls, that affected anthropometric measures (wrist, ankle and waist-hip ratio) as well as androgen levels, analyses were conducted after linear adjustment for BMI.

3.1.1.5 Digit ratio (2D:4D)

The second and fourth digits of both hands were measured, fingers fully extended, from the middle of the proximal crease to the fingertip using a Vernier caliper. The 2D:4D is the ratio between the second and the fourth digit measures of each hand.

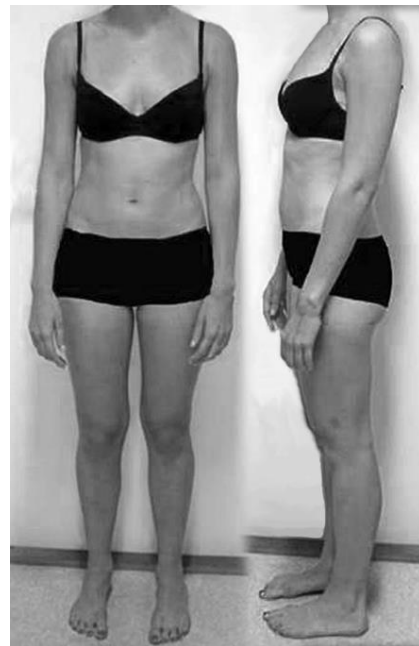### 3.1.1.6 Calibration of anthropomorphic measurements

As body measurements were conducted by three different assessors, a calibration assessment was conducted where eight people who were not included in the study were independently measured by all raters, yielding an inter-rater reliability of 0.96-1.0 (Cronbach's α).

3.1.1.7 Gender coherence assessment

Gender coherence in face, body and voice characteristics was blindly and independently assessed from photographs and voice recordings by a group of eight assessors.

Figure 3 Body photos for gender coherence assessment (published with the consent of the participant).

Voice samples were recorded with an analogue dictation machine while each participant read the same short story. The recordings were digitalized and normalized in volume before the assessment session.

Face and body photographs were taken both front-facing and in profile, full-body with the participants in undergarments, in a standardized manner against a white background in the same examination room (Figure 3). To avoid distraction from hair and hairstyle, participants' head hair was covered by a shower hat. Noticeable makeup and jewelry were removed before photographing.

To minimize judgement bias due to the undergarments worn, photos were digitally manipulated to show a similar neutral shape and color of underpants and, for women, bra.

Body and facial photos as well as voice recordings were assessed individually by eight independent assessors (four men and four women) aged 18-47 years who were selected to represent different ages and socioeconomic backgrounds.

The assessors were carefully instructed to estimate each participant's gender typical features without judging beauty. Face, body, and voice were assessed separately. Femininity in the females and masculinity in the males were rated in accordance with a five-point Likert scale of gender coherence (for women: 1 = *very feminine;* 2 = *more feminine than average;* 3 = *average;* 4 = *less feminine than average;* 5 = *a lot less feminine than average).*

Assessments were conducted on a computer for one gender at a time and in separate sessions for faces, bodies and voices. Within each session, and for each assessor, the order of the participants was randomized to minimize bias due to order or preconceptions from a previously assessed photo.

Inter-rater reliability was calculated using Cronbach's Alpha and was found satisfactory: face gender congruence $\alpha = 0.88$, body gender congruence $\alpha = 0.86$, and voice gender congruence $\alpha = 0.86$ (average inter-item correlations were between 0.46 and 0.49). These α-values did not increase when any evaluator's results were disregarded. A month later, a subset of the photos was re-evaluated by six persons in the assessor group, likewise showing satisfactory intra-rater reliability.

3.1.1.8   Sexuality questionnaire (Study II)

The questionnaire regarding sexuality was a compilation of questions about sexual orientation, sexual experience and libido, as well as romantic partnership and was constructed specifically for this study because existing questionnaires were lengthy and had ambiguously worded questions assumed to render problems for individuals with ASD.

Sexual orientation was examined with the question: "*Whom are you attracted to?*" The five response alternatives: *"Men", "Women", "Both men and women", "Neither",* and *"Other"* were dichotomized for heteronormativity, that is, opposite sex vs. any other response.

Sexual experience and libido were examined with the question: "*Age of sexual debut?*" and five questions with multiple response alternatives, as shown in Table 2. Multiple choice alternatives were dichotomized for low and high sexual interest in the analysis. Additional questions regarding partnership were: "*Do you have a partner?*" and "*Do you appreciate physical contact with a partner?*" Seven questions in the questionnaire were discarded. These questions and reasons for discarding them are presented in Table 3.

Table 2 Included questions regarding sexual experience and libido from the sexuality questionnaire in study II. The responses were dichotomized into lower and higher sexual interest.

Measure	Item	Response alternatives	
		Sexual interest	
		Lower	**Higher**
Intercourse	Have you had sexual intercourse?	"No"	"Yes"
Libido	Have you been sexually aroused the past month?	"No", "1–3 times"	"4–6 times", "7–15 times", "Every day"
Sexual initiative	Who takes initiative to have sex?	"No one" "Partner"	"Both", "I"
Sexual interest	Are you interested in sex?	"No, not at all", "Not very much"	"Yes, quite", "Very interested"
Orgasm frequency	Have you had an orgasm during the past month?	"No", "1-3 times"	"4-6 times", "7-15 times", "Every day"

Table 3 Discarded items from the sexuality questionnaire in study II.

Item	Reason for discarding
When did you enter puberty?	Participants could not respond with certainty.
How many sex partners have you had?	Free-text answer could not be analyzed.
How often do you think about sex?	Free-text answer could not be analyzed.
How many times have you had sexual intercourse during the past month?	Answers were biased by having a partner and possibly by time of year. Most controls were interviewed during summer while interviews of persons in the ASD group were conducted year-round.
How many times have you masturbated the past month?	Variation depended on whether the respondent had a partner.
Are you sexually aroused by something else than by humans, e.g. certain clothing, latex or something else?	Free text answers. Difficult to analyze with a masculinization perspective.
Sexual experience with same sex?	Ambiguous definition of sexual experience and may have reasons other than personal sexual attraction, e.g. sexual assault or coercion.

3.1.2 Study III

3.1.2.1 Participants

Study III used registry data of parent-rated neurodevelopmental traits from 16312 DZ twins, including 8477 males and 7835 females (Table 4), from the Child and Adolescent Twin Study in Sweden (CATSS), a Swedish twin registry.

The CATSS is an ongoing study on mental health problems that aims to track all twins born in Sweden since 1992. Parental assessment of the twins was conducted via telephone interview proximal to a child's ninth birthday. During the first three years of the CATSS, interviews were also conducted for parents of 12-year-old twins to increase the number of included birth cohorts. The CATSS response rate is 80% and the presence of neurodevelopmental disorders has little influence on the response rate.[142] Zygosity in all twins is determined by a DNA test.[143]

Table 4 Number of same-sex and opposite sex dizygotic twins and their age distribution in study III.

	No. of pairs	No. of 9-year old pairs	No. of 12-year old pairs
Same sex	3937	2799	1137
Male	2129[†]	1503	625
Female	1808	1296	512
Opposite sex	4219	3175	1044

[†]Assessment age data missing for one pair.

3.1.2.2 Procedure

This study examined the effects of elevated prenatal testosterone levels in girls on neurodevelopmental traits. The study is based on the assumption that testosterone diffuses from male to female fetuses sharing a womb, as postulated by the testosterone transfer theory. In the light of this, having a male co-twin should cause elevated testosterone levels in female twins. We tested whether female DZ twins with a male co-twin have more autistic and/or ADHD traits than a female DZ twin with a female co-twin.

Data on neurodevelopmental traits in DZ twins were acquired from the CATSS study. Within the CATSS, data on neurodevelopmental traits was collected through the parental interview The Autism–Tics, AD/HD, and other Comorbidities inventory (A-TAC).

Scores were compared between index-twins with a female co-twin and index-twins with a male co-twin and analyses were conducted separately for male and female index twins. The main analyses comprised the composite modules for ASD and ADHD. Additional sensitivity analyses were conducted for the five separate ASD and ADHD modules and, as comparison modules, for "perception", which is also related to ASD and therefore possibly affected by prenatal testosterone, and for "emotion" and "opposition", which are not assumed to be affected by prenatal testosterone levels (Table 5).

Table 5 A-TAC modules included in analyses in study III.

Tested constructs	A-TAC module
ASD	Language
	Flexibility
	Social Interaction
ADHD	Concentration/Attention
	Impulsiveness/Activity
Control modules	Perception
	Opposition[†]
	Emotion[†]

[†]Not assumed to be affected by prenatal testosterone levels.

3.1.3 Study IV

3.1.3.1 *Participants*

A total of 135 adults with ASD (64 men, 66 women), 508 adult psychiatric controls (191 men, 280 women) and 590 non-psychiatric controls (105 men, 400 women, and 85 persons of unknown sex) participated by anonymously responding to the abridged RAADS-R questionnaire. Additionally, data comprising 75 adults with ASD and 197 non-psychiatric adults from the validation study of the Swedish RAADS-R[144] was initially used to select items for the abridged questionnaire. Psychiatric participants were either patients recruited from 17 Swedish psychiatric outpatient clinics (n=541) or individuals responding to a web-based survey advertised in online communities and targeted to people diagnosed with ADHD, Asperger's disorder, social anxiety disorder and mood disorder (n=102). Only questionnaires endorsing that the respondent was diagnosed by a psychiatrist were included.

A total of 89 individuals were excluded from phases II and III due to: 1) entering the study as a psychiatric participant but failing to report a confirmed diagnosis (n=40); 2) entering the study as a non-psychiatric control but reporting a psychiatric diagnosis (n=11); 3) having checked all items identically, indicating not reading the statements (failing to notice the reversed item: n=35); 4) five or more missing items; or 5) having a diagnosis of intellectual disability (n=1).

3.1.3.2 *Procedure*

This study was conducted in three phases: the first phase was performed by Lisa Andersen, using data from the Swedish RAADS-R validation study. In this phase, the 18 RAADS-R items that best distinguished ASD from controls in each subscale were selected. The second phase, which was performed as my master's thesis project,[145] was a pilot study testing the 18 items of the abridged RAADS questionnaire in psychiatric outpatients and removing four items that failed to identify ASD in this population. Finally, the third phase, which is part of this thesis, validated the final RAADS-14 Screen in a psychiatric population comprising 77 individuals with ASD and 370 individuals without ASD but with another psychiatric disorder, including mood disorder, psychotic disorder, anxiety disorder, borderline personality disorder and ADHD. Sex/gender differences were also studied.

3.2 ASSESSMENT INSTRUMENTS

3.2.1 The Ritvo Autism and Asperger Diagnostic Scale-Revised (RAADS-R) (Studies I, II and IV)

RAADS-R is a self-assessment instrument developed as an aid in the ASD diagnostic procedure in adults. The 80 item questionnaire assesses autistic traits with 60 items that closely match diagnostic criteria in the DSM-IV in the domains of "social interaction", "language", and "circumscribed interests", and also including 20 items in the additional "sensory motor symptoms" domain added in the DSM-5. All items are statements formulated in the first person narrative. Examples of these items include: *"Sometimes I offend others by saying what I am thinking, even if I don't mean to"*, *"The phrase 'I've got you under my skin' makes me very uncomfortable"*, and *"Sometimes the sound of a word or a high pitched noise can be painful to my ears".* The four response alternatives *"true now and when I was young", "true only now", "true only when I was younger than 16"* and *"never true"* are evaluated on a four-point Likert scale. Sixteen statements are reversely formulated to identify skills acquired during the life-span and to limit the effect of response bias.[146] Good internal consistency has been shown for three of the subscales, while the internal consistency of the language subscale ranges from poor to acceptable.[67,144] With its 80 items the RAADS-R is comprehensive and can take up to an hour to complete for people with psychiatric problems. Sex differences were tested in the Swedish RAADS-R validation study.[144] Men and women diagnosed with ASD received equally high total scores but, in line with other research, women with ASD scored higher on the sensory motor subscale than men with ASD. The only subscale showing a significant sex-difference in the non-psychiatric comparison group was the language subscale where men reported more autistic traits.

3.2.2 The Autism Spectrum Quotient (AQ) (Studies I and II)

The Autism Spectrum Quotient (AQ) was used to examine the number of autistic traits in men and women in both the ASD and control groups.

AQ was developed as an instrument to measure autistic traits in the general population.[69] It consists of 50 items positively or negatively related to autistic traits designed to assess five different areas of functioning: social skills, communication, attention switching, attention to detail, and imagination, e.g. "*I enjoy social chit-chat*" and "*I notice patterns in things all the time*". Each item is evaluated on a four-point Likert scale ("*Definitely agree*", "*Slightly agree*", "*Slightly disagree*" and "*Definitely disagree*"). Scoring is often dichotomized to symptom agreement. Several validation studies have shown good psychometric properties, however with varying internal consistency of the five-factor model.[69,147,148] The instrument has good ASD screening properties in the normal population,[149] but it has been shown that certain groups, e.g. mathematicians[69] and individuals with schizophrenia[150] score high on the AQ without having ASD.

3.2.3 Autism Diagnostic Observation Schedule (ADOS) (Studies I and II)

ADOS module 4, is a semi-structured standardized observation instrument for adolescents or adults with fluent speech who are suspected of having ASD. It consists of several tasks and interview questions assessing autistic symptoms in terms of social interaction, communication, and imaginative use of materials.[151] ADOS module 4 is widely used as a tool to confirm an ASD diagnosis and was used as such in Studies I and II.

3.2.4 The MF-scale (Study II)

The MF scale[152] is a validated Swedish modification of the Bem Sex Role Inventory.[153] It measures stereotypical masculine and feminine behavioral traits with 43 items rated on a four-point Likert scale (1 = *"I totally disagree"*, 2 = *"I slightly disagree"*, 3 = *"I slightly agree"* and 4 = *"I fully agree"*). Male and female stereotypes (defined in the two subscales MF-M and MF-F) are assessed with 17 items each, and a further nine items are considered gender neutral. The MF-M scale contains statements regarding power, assertiveness, leadership abilities and competitiveness, while the MF-F mainly measures how tender, caring and dismissive a person rates him-/herself.

As Swedish gender norms have changed markedly since the development of the MF scale, we conducted a (yet unpublished) study to estimate how current masculine and feminine gender roles relate to the MF scale. A total of 637 Swedish-born adults (46% men and 54% women; age range 19–65 years) were recruited in conference halls and in public places in Stockholm. This study showed extant gender-differences with mean MF-M scores of 48.6 (6.3) for men and 45.3 (5.8) for women, and mean MF-F scores of 42.4 (4.8) for men and 46.4 (4.4) for women.

3.2.5 The Mini-International Neuropsychiatric Interview (MINI) (Studies I and II)

The Mini-International Neuropsychiatric Interview (MINI version 5.0.0.) is a validated structured interview to assess psychiatric disorders. It consists of modules that correspond to diagnostic categories according to the DSM-IV. It has been used in clinical settings as well as in research studies.[154]

3.2.6 The Autism–Tics, AD/HD, and other Comorbidities inventory (A-TAC) (Study III)

A-TAC is a telephone parental interview to assess neurodevelopmental traits in children. It consists of 20 modules assessing different clinically meaningful areas of neurodevelopmental problems with 96 questions that are largely based on the DSM-IV criteria for neuro-psychiatric disorders. AD/HD traits are assessed with the two modules "impulsiveness/activity" and "concentration/attention". The validated ASD assessment consists of the modules: "language", "social interaction" and "flexibility" (mirroring the DSM–IV subdomain of "restricted repetitive and stereotyped patterns"). Additional modules in A-TAC are: the "perception" module, which assesses hypo-/hyper- reactivity to sensory

input and corresponds to the "hyper- or hypo-reactivity to sensory input" ASD criterion in DSM-5; and fourteen modules not closely related to ASD or ADHD: "emotion", "opposition", "learning", "planning & organizing", "memory", "tics", "compulsions", "feeding", "separation", "conduct", "anxiety", "mood", "concept of reality", and "miscellaneous".

3.3 STATISTICAL ANALYSES

In all studies, comparisons of dichotomous variables were conducted using χ^2-test for significance analysis. Odds ratios and confidence intervals were included in study IV. Group means were compared using a Student's t-test, and Cohen's d for estimation of effect size. For heavily skewed data, a Mann–Whitney U test was used, with effect size calculated as $r = Z / \sqrt{N}$[155] (the effect size is considered large for $r \geq 0.5$, medium for $0.5 > r \geq 0.3$, and small for $0.3 > r \geq 0.1$). A two-tailed P value of < 0.05 was considered significant.

In studies I and II, adjustments were applied to some measures before analysis: missing data on the MF subscales and the AQ were substituted with mean values of all the items in the respective subscale; linear adjustments were conducted to compensate for strong influence of BMI on the variables of waist, hip, ankle and wrist measure and testosterone level. Additional adjustments for diurnal testosterone variation in men and for menstrual variation in women were conducted.

In study III, the A-TAC data was heavily skewed with over 50% of the participants scoring 0 on either ASD or ADHD traits. For this reason, all analyses were conducted with non-parametric methods. The effect of age was studied both with a Kolmogorov-Smirnov test comparing the distributions for the two age groups and with Quade's rank analysis of covariance.[156] The latter analysis was also used to study interaction of comorbid ASD or ADHD traits.

Study IV used a receiver operating characteristic (ROC) curve to evaluate the discriminatory power of the scale: for each score, the rate of true positives (sensitivity) was plotted against the rate of false positives (1-specificity). The area under the curve (AUC) is a measure of the power to discriminate between ASD and non-ASD. An AUC > 0.7 is considered acceptable. Aiming to achieve good screening properties with high sensitivity, the cut-off score was selected by choosing the lowest score corresponding to a true positive rate of 93% or greater in the combined phase II and III samples. An alternative cut-off was selected by finding the score yielding the maximum (specificity + sensitivity).

3.4 ETHICAL CONSIDERATIONS

All studies were approved by the Regional Ethical Review Board at Karolinska Institutet, Stockholm.

3.4.1 Studies I and II

There are several ethical aspects to these studies. First, exposing individuals with ASD to being photographed in underwear and responding to intimate questions about sexuality and sexual orientation is sensitive and requires a clear ability to consent. Second, pointing out that ASD is deviant in a sex/gender perspective could possibly marginalize this group even more. However, these aspects of the study were described in the advertisement for participation and the study was thoroughly discussed and approved by the local empowerment board within the Swedish Autism Society (Autism & Aspergerförbundet). All participants provided written informed consent prior to study and information was given that indicated participation could be ceased at any time during the assessment session. Participants received a reimbursement of approximately £95.

Participant ability to consent to the study was initially screened with a brief telephone interview ensuring that the individual had not been diagnosed with an intellectual disability and had attended mainstream schooling. None of the included participants had a caretaker or guardian. Further, in the ASD group, the ability to consent was also assessed by a psychiatrist during the initial interview. Three individuals that were deemed not to have the capacity to consent were subsequently excluded: two had a comorbid psychosis and one had epilepsy and brain damage.

3.4.2 Study III

All participation in the CATSS study is protected by the informed consent process – information is given about what data is being collected and participants can withdraw their consent and discontinue participation at any time.

3.4.3 Study IV

Information about the study was provided to all participants, who consented by completing the questionnaire. All questionnaires were collected without accompanying personal identification.

4 RESULTS

Table 6 Summary of results

Study	Research hypothesis	Results	Conclusion
Study I	Persons with ASD display androgynous physical features.	Elevated T levels and less feminine facial features were more common in women with ASD than in control women. Men with ASD showed less masculine body characteristics.	Androgynous physical features are more common in adult males and females with ASD than in TD adults.
Study II	Individuals with ASD display an androgynous gender identity and gender role as well as atypical sexuality.	Perceived gender typicality was shifted towards androgyny. Sexual interest was decreased in men and women with ASD as compared with TD men and women respectively.	Despite their extreme male cognitive style, individuals with ASD show androgynous gender perception and demasculinized gender role and sexuality.
Study III	Female DZ twins have more traits of ASD and ADHD if they have a co-twin brother than if they have a co-twin sister.	Parental reported scores of ASD and ADHD traits were lower in female DZ twins with a twin brother than in those with a twin sister.	In female DZ twins, having a male co-twin is **not** associated with elevated traits of ASD and ADHD.
Study IV	RAADS-14 Screen shows good psychometric properties in psychiatric outpatients.	The ASD group scored higher than all comparison groups. A cut-off score of 14 yielded a sensitivity of 97% and a specificity of 46% in the ADHD group and 64% in patients with other psychiatric disorders.	The RAADS-14 Screen is a promising measure for ASD screening in adult psychiatric outpatients.

4.1 STUDY I

4.1.1 Anthropometric scores

Women with ASD had significantly larger heads, higher BMIs and larger ankle circumferences and waist-hip ratios. However, differences in ankle measurement and waist-hip ratio were not significant after adjusting for BMI. The men with ASD had higher 2D:4D ratios than control men ($P = 0.04$).

4.1.2 Hormone levels

Women with ASD had higher serum levels of both total and bioactive T than control women. Differences remained significant after excluding participants taking estrogen contraceptives and after adjusting for BMI. No differences were detected in serum T levels between male groups. The expected negative correlation between DHEAS and age could not be detected in men or women with ASD, but was observed in the control group.

4.1.3 Gender coherence

ASD men were assessed to be less masculine regarding body constitution ($P < 0.001$) and voice characteristics ($P < 0.05$) than control men, while facial features were regarded less feminine ($P < 0.001$) in women with ASD than in control women.

4.2 STUDY II

4.2.1 Gender identity, gender role and gender typical childhood behavior

An atypical gender identity was more common in the ASD group than in the control group (χ^2 (N=103) = 10.1; $\phi = 0.31$; $P = 0.001$). When subdivided by sex, only the difference in women reached significance. More women with ASD than control women reported tomboy-behavior as a child, while there was no difference in reported gender typical childhood behavior between male groups.

Both men and women with ASD were less inclined to identify with masculine gender role traits and behavior than controls, as measured with the MF-M subscale. Within-group comparisons revealed similar scores for men and women with ASD on both masculine and feminine role identification whereas control men and women had similar scores for masculine role but not for feminine role identification.

4.2.2 Sexual preference

Sexual preference is presented in Table 7. Women with ASD reported higher non-heterosexual preference than control women. No significant difference was detected in sexual preference between male groups.

4.2.3 Sexuality

A total of 41% of the people in the ASD group reported having had sexual intercourse at least once in the previous month as compared with 79% in the control group. Notably, only two men (8%) and seven women (30%) in the ASD group reported having had sexual intercourse more than three times during that month – the number representing the median frequency in the control group.

Table 7 Reported sexual preference in men and women with ASD and controls.

	Men		Women	
	ASD (n=26)	Controls (n=28)	ASD (n=24)	Controls (n=25)
Attracted to		n (%)		
Men	2 (8)	2 (7)	9 (38)	21 (84)
women	22 (85)	24 (86)	1 (4)	0 (0)
both sexes	1 (3.5)	2 (7)	13 (54)**	4 (16)
neither sex	1 (3.5)	0 (0)	1 (4)	0 (0)

**P < .01

4.3 STUDY III

4.3.1 Main results

Female index-twins with a female co-twin were rated with a higher total ASD-score, ($P = 0.001$; $r = 0.04$) than female twins with a male co-twin. The same difference was also significant in two of the three ASD sub-modules: social interaction ($P = 0.01$; $r = 0.03$) and flexibility ($P = 0.005$; $r = 0.04$) and also in the equally ASD related module perception ($P = 0.004$; $r = 0.04$).

Similarly, female index-twins with a female co-twin were rated with more ADHD-traits ($P = 0.03$; $r = 0.03$) and specifically higher scores on concentration/attention ($P = 0.001$; $r = 0.04$) as compared with female twins with a male co-twin.

No differences between boys on total ASD or ADHD scores were visible; however, in sensitivity analyses, boys with a female co-twin had higher rank scores on concentration /attention ($P = 0.003$; $r = 0.04$) and flexibility ($P = 0.006$; $r = 0.03$) suggesting poorer concentration ability and lower grade of flexibility, than boys with a male co-twin.

4.4 STUDY IV

Study IV examined the psychometric properties of the RAADS-14 Screen self-evaluation instrument, in terms of reliability and discriminative power.

As the original RAADS-R was developed much in accordance with DSM-IV, two experienced clinicians were asked to independently map the RAADS-14 Screen items against

the DSM-5 criteria. The mapping was largely in unison and comprised all but one of the A and B criteria, excluding only the A4 stereotypy item that may be difficult for patients to self-evaluate.

4.4.1 Differences in total score

The median score of the ASD group was 32 (range $8 - 42$), compared with a score of 15 (range $0 - 42$; $r = 0.5$; $P < 0.001$), in the ADHD group and 11 (range $0 - 39$; $r = 0.7$; $P < 0.001$) in the Other psychiatric disorders group.

4.4.2 Gender differences in scoring

Table 8 shows median scores by gender. In the ASD group, there were only differences in the Sensory Reactivity domain where females scored higher. In the non-psychiatric group, males scored higher than females on both Mentalizing Deficits and Social Anxiety, but lower on Sensory Reactivity.

Table 8 Median scores of the RAADS-14 Screen and subscales in men and in women

Sample[†]	Sex	n	RAADS-14-Screen	Mentalizing Deficits	Social Anxiety	Sensory Reactivity
			Median [min-max]			
ASD	M	64	30 [0 - 42]	15 [0 - 21]	9.7 [0 - 12]	6 [0 - 9]
	F	66	34 [9 - 42]	18 [3 - 21]	9 [0 - 12]	8 [3 - 9][***]
ADHD	M	127	15 [0 - 36]	7 [0 - 21]	3 [0 - 12]	3 [0 - 9]
	F	185	15 [0 - 42]	8 [0 - 21]	3 [0 - 12]	3 [0 - 9][***]
Other psychiatric	M	64	11.5 [0 - 33]	4 [0 - 21]	4 [0 - 11]	2 [0 - 9]
Disorders	F	95	12 [0 - 39]	5 [0 - 21]	2 [0 - 12]	3 [0 - 9]
Non-psychiatric	M	105	3 [0 - 19]	1 [0 - 13][*]	1 [0 - 8][**]	0 [0 - 6]
	F	400	2.5 [0 - 28,7]	0 [0 - 18.7]	0 [0 - 10]	0 [0 - 9][*]

[†]Samples selected from the combined phases II and III.
[*]$P < .05$, [**]$P < .01$, [***]$P < .001$; differences are indicated in the sex with the higher score.

4.4.3 Symptom perseverance

Endorsement of symptom perseverance (childhood and adult symptoms) measured by a three-point item score is presented in Table 9. In the ASD group, 75% of the women and 57% of the men endorsed at least one persevering symptom from each domain (result not included in paper IV).

Table 9 Percentage of participants who scored at least one three-point item score* in a RAADS-14 domain and in all three domains.

Sample[†]	Sex	n	Mentalizing Deficits	Social Anxiety	Sensory Reactivity	All domains
					%	
ASD	M	64	82.1	89.6	65.7	56.7
	F	66	84.1	92.8	89.9	75.4
ADHD	M	127	44.4	53.5	42.4	19.4
	F	185	38.5	51.8	59.0	22.1
Other psychiatric disorders	M	64	29.9	53.7	31.3	9.0
	F	95	36.6	37.6	38.6	15.8
Non-psychiatric	M	105	7.6	18.1	12.4	0
	F	400	7.8	14.0	13.3	0.5

[†]Samples from the combined phase II and phase III populations.
*Three-point item score denotes endorsing a symptom both as adult and in childhood.

4.4.4 Reliability and discriminatory powers of RAADS-14 Screen

The RAADS-14 Screen showed excellent internal consistency between all 14 items (n = 1,233, α = 0.9). The discriminatory power of the RAADS-14 Screen was 91% in ASD vs other psychiatric disorders and 88% in ASD vs ADHD. No differences were detected in reliability measures or discriminatory powers when analyses were split by gender.
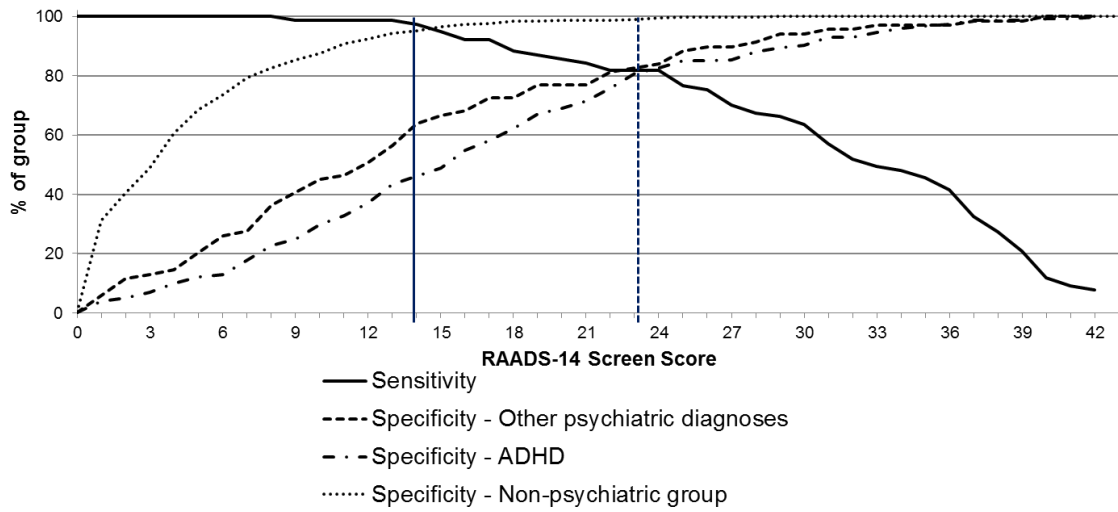
4.4.5 Cut-off scores

A high sensitivity demand of 97% was reached with a cut-off score of 14 or above. This yielded a specificity of 46% in the group with ADHD and 64% in the other psychiatric disorders group.

4.4.5.1 Additional results not presented in Paper IV

A more conservative measure would be a cut-off of 23 points yielding 81% sensitivity and 81% specificity out of the full psychiatric sample (Figure 4).

Cut-offs regarding number of items endorsed with three-point score gave similar results: a cut-off of at least three three-point scores yielded 95% sensitivity and 37- 65% specificity. A cut-off of at least six three-point scores yielded 80% specificity and 70% sensitivity in the ADHD group and 76% sensitivity in the other psychiatric disorders groups.

Figure 4 Sensitivity and specificity for the RAADS-14 Screen scores. A cut-off score ≥ 14 yielded a high sensitivity of 97% and a specificity of 46% in the ADHD group and 64% in the other psychiatric disorders group. A more conservative cut-off score ≥ 23 yielded a sensitivity of 81% and a specificity of 81% in both the ADHD and other psychiatric disorders groups.

5 DISCUSSION

This thesis takes on the subject of male preponderance for ASD using three different view-points: studies I and II explored sex/gender differences and similarities beyond the extreme male cognitive style of ASD in men and women with and without ASD; study III estimated the effect on the development of autistic traits in female fetuses by prenatal testosterone exposure from a twin brother; and study IV introduced a new screening instrument for ASD in adults with co-occurring psychiatric symptoms, thus aiming to reduce underdiagnosis, which is believed to particularly affect females with a high functioning ASD.

5.1 STUDIES I AND II

The extreme male brain theory of autism explains the core cognitive symptoms of ASD with the combination of increased systemizing and decreased empathizing ability and attributes this to a hyper-masculinization of the brain that is suggested to be associated with elevated prenatal testosterone levels. While the extreme male brain phenotype seems like an all-encompassing nature of ASD, masculinization in other known sex-dimorphic traits have appeared to be lacking in males but not in females with ASD.

Studies I and II aimed to study sex-differentiation in men and women with ASD with regard to physical and mental characteristics beyond the masculinized cognitive functions presented by the extreme male brain theory. In line with clinical observations but in opposition to a straightforward interpretation of the effects of elevated prenatal testosterone in males, we hypothesized that male typical behavior and perception would be weakened in both men and women with ASD and that gender identity would be less pronounced, as compared with TD men and women respectively.

5.1.1 Physical sex/gender differences

The study of anthropometrics and circulating sex hormone levels in ASD men and women, as compared with TD adults showed great variability in both groups. The anthropomorphic measures showed few significant differences when comparing ASD and control groups for each gender: a larger head circumference in women with ASD and a higher 2D:4D in men with ASD. However blinded assessment of gender-typicality in face, body and voice showed larger amount of androgyny in the rater-assessed parameters in both men and women with ASD. These findings lend support to the clinical observations that initially gave rise to this study that androgynous features are more common in people with ASD, but that androgyny is not an all-encompassing ASD phenotype.

In our study, we aimed to examine sex-differentiated characteristics affected by prenatal hormones, although the interpretation of the results also must account for pubertal hormones as well as hormonal effects of obesity. Our four group study design, comparing females and males separately, was unusual in ASD research at the time when the study was conducted and

the acknowledgement of the need for sex-differentiated studies describing the ASD group is an important strength of this study. This has later been underscored by Lai *et al.*[85]

A somewhat surprising finding was that ASD men had an elevated 2D:4D as compared with control men, indicating a lower prenatal testosterone level in ASD men. This result differs from most previous studies that have found decreased 2D:4D in ASD.[118] If 2D:4D were a reliable surrogate measure of prenatal testosterone exposure, this result would imply a very skewed recruitment of unusually masculinized control men or demasculinized ASD men. In a relatively small sample such as ours, this is possible; however, as the reliability of 2D:4D to predict prenatal testosterone has been found to be quite low[114] our result could also be unrelated to prenatal testosterone levels. The most likely explanation is that this is a difference by chance, since the significance level was high ($P < 0.05$), and several other tests were performed, increasing the risk for chance findings.

5.1.2 Gender identity and typicality

In line with the results on physical appearance, the ASD group showed more androgyny with a less pronounced gender identity and a lack of difference in gender perception ("*Do you perceive yourself as typical for your gender?*"). Women with ASD more often reported being a tomboy as a child than control women. One could argue that such gender identification could be an effect of a weaker interest in gender aspects combined with a general feeling of being different, but the existence of an inherent sense of gender is supported by the strong feeling of being born with the wrong sex expressed by transgendered people. In the same vein, the overrepresentation of ASD in both male-to-females and female-to-males with gender identity disorder supports our findings of a gender identity shift towards androgyny. It has been suggested that transsexuality could be explained, at least to some extent, by opposite organizational effects during the different periods involved in sex-differentiated development of the genitals and the brain;[157] for example, caused by a sudden change in prenatal hormone levels. If the sex-differentiation of the brain is continuously dependent on sex hormone levels, ASD could also be related to hormonal variability during fetal development.

5.1.3 Gender role

Identification with stereotypical masculine and feminine gender roles was measured with the two-dimensional MF-scale. Both ASD men and women showed a low identification with masculine role behavior (determination, strong leadership skills and competitiveness) as compared with control men and women respectively. With regard to female gender role behavior, no significant differences were detected between ASD and control groups in either sex. Interestingly, in the ASD group, no significant sex-difference was detected in the endorsement of masculine or feminine role behaviors. This gender role independence in ASD may be explained as a result of less organizational differences between men and women with ASD compared with TD men and women. In ASD the gender role is markedly demasculinized, as opposed to the masculinized pattern associated with empathizing-systemizing, in men and women alike. Still, the lack of a difference in gender role may also

be an effect of an inherent indifference to gender roles, perhaps in combination with generally impaired functioning in ASD. Further studies using different gender role measures and a psychiatric comparison group may shed light on this.

5.1.4 Sexuality

Similarly to gender role behavior, sexual drive and behavior appeared demasculinized in both men and women with ASD. It should be noted that a subgroup of both men and women with ASD reported normal to high sexual activity and sexual interest; however, as a whole group, ASD men and women reported a later sexual debut and lower sexual activity. Low sexual interest and less inclination to take sexual initiative were also reported more often in the ASD group.

Notably, eight individuals in the ASD group had not had their sexual debut, while this applied to none in the control group. There are several non-mutually exclusive explanations for this. First, a person with ASD has by definition impaired ability to from social relationships and many are socially withdrawn, creating obstacles to successfully approaching another person with romantic and/or sexual intent. Second, our findings of decreased libido and the experience of being asexual, also supported by self-biographical reports,[128,158,159] are also possible explanatory factors for a delayed or absent sexual debut. Third, as the two hit model[160] proposes adolescence as the second developmental hit for ASD causing limitations for the adolescent with ASD in the transition into an adult role and social demands on adaptive functioning, a delay in the adult transition may lead to a delayed sexual debut. Last, one can also speculate that, as non-normative sexual orientation and gender identity issues appear more common in the ASD group, this additional confusion may add to the difficulties mentioned above.

5.1.5 Limitations and considerations

There are several limitations to studies I and II that are worth considering. Recruitment of participants through advertisements will always increase the risk of selection bias. In this case, when the advertised theme was sex/gender differences and included being photographed in undergarments, blood-testing of sex-hormone levels and questions on sexuality, bias is unavoidable.

Some effects of bias can be estimated by comparing our data with those of other comparable study samples: in a Swedish age-equivalent clinical sample of 45 men and 39 women diagnosed with high functioning ASD,[161] cohabiting with a partner was equally as common as in this study. However, parenthood and university studies were only half as frequently reported in the clinical sample as in studies I and II, indicating that our sample was more socially adept and in some ways possibly less autistic than a random ASD sample. Thus, assuming that severity of symptoms is related to sex/gender characteristics also within the group diagnosed with ASD, our sample is expected to have less difference in characteristics than a random ASD sample. However, the relationship between severity of autistic traits within the ASD group and effect on sex/gender characteristics remains to be studied. At the

same time there are some indications that the control group was less gender typical than the general population: testosterone levels in the males were below the normal range and female controls scored higher on masculine behavior as measured by the MF scale than women in general. Together this could indicate that our data is actually an underestimation of differences.

Another problem with the selection of participants is the etiological heterogeneity of ASD. The great variability in masculinization/demasculinization of physical and gender identification characteristics indicate effects on subgroups of ASD. As these differences also depend on factors un-related to ASD, a larger samples size is required to fully assess differences in masculinization of features and subgroup accordingly.

There are also some limitations to the hormonal measures used in study I. There are uncertainties in male T measures due to diurnal variation, testosterone pulsatility and differences in physical activity before taking the blood test. Estrogen levels were unfortunately analyzed with insufficient sensitivity to detect differences in the low levels circulating in male blood. In females, sex hormone analyses have several uncertainties: sex hormonal variations over the menstrual cycle make subtle differences difficult to assess, and hormone levels are also affected by other factors such as obesity and hormonal contraceptives. However, excluding these groups was not an option as this would have increased selection bias.

Finally there is a limitation to the ascertainment, as ADOS-interview was conducted with the controls. Two of the male controls had RAADS-R scores of 82 and 84 respectively, which are well above the suggested cut-off score of 66. As they were both reporting normal function they were included in in the control group for analyses. However, analyses excluding these two controls did not change the significances of the results. Notably, both males were rated less masculine than average.

5.2 STUDY III

The main aim of study III was to explore whether female fetuses exposed to elevated levels of prenatal testosterone from a twin brother develop an increased number of autistic traits, as the extreme male brain theory would predict.

Since ASD and ADHD often co-occur, have male biased sex ratios and have been suggested to be connected with prenatal testosterone, our study comprised both disorders and similarly studied a set of other traits for comparison. Assuming that a dizygotic male twin fetus will expose it's co-twin to increased testosterone levels we compared the amount of autistic and ADHD traits between dizygotic twin girls with either a twin brother (testosterone exposure from amniotic diffusion) or a twin sister (no additional testosterone exposure).

Counter to our hypothesis, instead of finding increased autistic and ADHD traits in girls with a male co-twin as compared with girls with a female co-twin, the opposite result was found: traits of ASD and ADHD were more common in girls with a female co-twin than in girls with

a male co-twin. These differences were also present in all ASD-related modules except language. In the ADHD submodules, the difference was significant in the attention/concentration module but not in the impulsiveness/activity module. Interestingly, a higher rate of both boys and girls with a twin sister reached the lower cut-off for ADHD than those with a male co-twin.

There may be several explanations for the paradoxical result that twins with a female co-twin receive higher scores on ADHD and ASD symptoms. First, when a parent assesses twins, there is a risk of *contrast-effects* resulting from an unconscious comparison between the twins. Contrast-effect means that an existing difference between the twins seems even larger to the rater (e.g. a twin girl with a (female-typically) low amount of autistic traits who has a twin brother with a (male-typically) slightly larger amount of autistic traits might be rated as having even less traits, while two twin sisters with the same amount of traits will be unaffected by contrast effects; Figure 5). As ASD and ADHD traits are somewhat more common in the boys, any existing contrast effects would be more likely to induce a decrease in the scores of girls with a twin brother than in girls with a twin sister. However, there is no current evidence for contrast effects in parent ratings of these traits and none were detected in the ratings of impulsiveness/activity, language, emotion or opposition, or sex-dimorphic traits that would be expected to be similarly affected by contrast effect. As parent ratings of traits are common in twin studies, contrast effects in the assessment of neurodevelopmental traits need further study. Second, underestimation of ADHD symptoms may be the result of greater social responsiveness in girls.[162] Finally, the possibility exists that testosterone exposure from a male twin is characterized by a dosage or timing that has a protective sensitizing effect for other testosterone exposures or dysregulations.
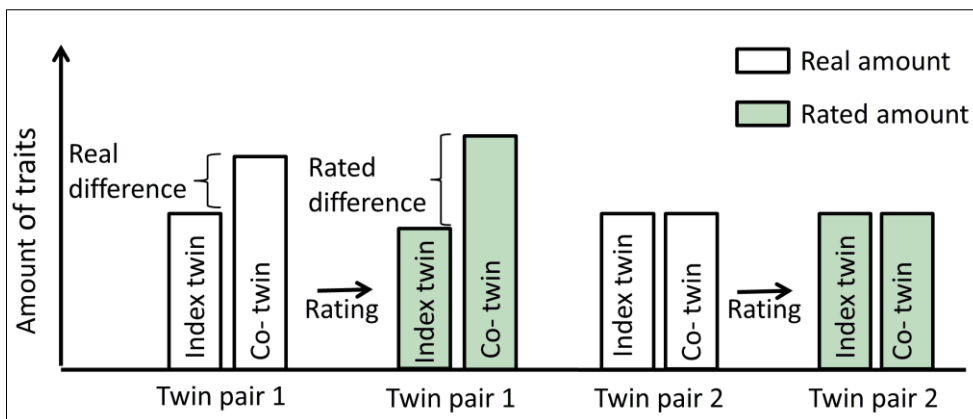
Figure 5 Contrast effect in twins: the observed difference in twin pair 1, results in affected parent ratings, amplifying the difference. Twin pair 2, lacking observable difference, will not be subject to contrast effects.

5.2.1 Limitations and considerations

Study III suffers from some limitations, first and foremost that the evidence for testosterone transfer in humans is not entirely clear, which makes it difficult to draw definitive conclusions regarding the lack of effect of the presumed elevated levels of prenatal testosterone.

There are also limitations is the assessment of neurodevelopmental symptoms using A-TAC. While parent assessment encompasses all types of situations and thus is based on more information than a clinician assessment, it is based on less knowledge of symptomatology and is more susceptible to contrast effects.[163] Furthermore, autistic traits are assumed to be normally distributed over the population but A-TAC shows a substantial floor effect. This means that any attempt to analyze the effect on neurodevelopmental traits in the whole population will be blunted by A-TAC's insensitivity to detect differences in subtle traits in half of the population.

5.3   STUDY IV

Study IV assessed the validity of the RAADS-14 Screen, a short version of the self-assessment questionnaire RAADS-R, in an adult psychiatric population without intellectual disability as well as in adults without a psychiatric diagnosis. The RAADS-14 Screen showed good psychometric properties and only takes a few minutes to complete, allowing for quick ASD screening in psychiatric outpatients.

Individuals diagnosed with ASD endorsed more items than the other groups, reaching a median score of 32 as compared with 15 in the ADHD group, 11 in the other psychiatric disorders group and 3 in the non-psychiatric group. Endorsement of the response alternative indicating symptoms both as an adult and in childhood was also more common in the ASD group.

As persevering symptoms with childhood onset is a necessary criterion for ASD, in contrast to most other psychiatric disorders with onsets in adulthood, another approach to evaluate the RAADS Screen questionnaire is to count the number of three-point scores, that is, the reports of life-long symptoms. The number of three-point scores proved an equally good screening criterion as total score. A combination of the two criteria, either two or more three-point scores or a total score > 12 provided an even higher sensitivity to the test; however, we assert that this adds too much complexity to the analysis for a clinical screening instrument.

The broad heterogeneity of ASD causes difficulties in diagnosing cases with an atypical presentation because of co-morbid conditions or unusual compensatory abilities to mask impairments. A more common atypical presentation in females has also been suggested to lead to an underdiagnosis of high-functioning ASD in females. A correct identification of ASD in both males and females is important both in the clinical understanding and treatment of patients and as a basis for future research. No sex/gender differences in discrimination power of RAADS-14 Screen could be detected in this study sample. However, it is important to note that the women in our ASD sample had received a diagnosis and may not represent the group that is most difficult to recognize.

Women scored higher than men in the Sensory Reactivity domain across all groups, supporting gender differences in sensitivity to noise and touch that have been previously

reported.[164,165] TD men scored higher than women in the Mentalising Deficits and Social Anxiety domains. As sensory symptoms were more common in women than in men in the ASD group as well, the inclusion of sensory symptom items in the RAADS-14 Screen is an important strength in its screening ability in women.

5.3.1 Limitations and considerations

The RAADS-14 Screen showed a very good ability to distinguish between ASD and each of the tested psychiatric diagnoses with the exception of ADHD for which the specificity was marginally lower. The reasons for this are several: ADHD and ASD share persevering symptoms, which makes differentiating between the disorders inherently difficult, co-existing ASD and ADHD has been found to be very common[166] and the ADHD sample included a number of patients who were later referred to a full ASD assessment, some of whom likely were diagnosed with ASD after our study was concluded.

There are some limitations with regard to the study design. All diagnoses were based on patient/clinician reports, and no confirmative diagnostics or cross reference controls in patient charts were conducted as participation was anonymous. Inclusion bias may also have affected the results, as patients were recruited from several different out-patient clinics, possibly by decreasing the numbers of participants with difficulties in completing questionnaires. However, as participation in research is optional, this is difficult to overcome. There is a risk that patients already diagnosed with ASD will more readily endorse autistic symptoms than yet undiagnosed patients who have not already identified themselves with the symptom descriptions. A clinical study examining final outcomes after ASD assessment and RAADS-14 Screen scores would provide a more accurate picture of the screening ability of the questionnaire.

6 CONCLUSIONS AND CLINICAL IMPLICATIONS

6.1 STUDIES I AND II

While the core aspects of ASD may be explained by an extreme male cognition pattern of high systemizing and low empathizing abilities, our studies show signs of a more androgynous physique in ASD, more ambiguous gender identity and gender typical behavior, a weaker sexual interest and a less specific heterosexual orientation encompassing both sexes. This indicates that at least a sub-category of ASD can be characterized as gender defiant.

Individuals with ASD often feel different and socially alienated from TD people. A broader understanding of common issues in ASD may be helpful in limiting their feelings of alienation and improve communication within support services and clinical settings. Awareness of the elevated rates of non-normative gender identity and sexuality among persons with ASD may give rise to efforts to attenuate the increased stress that adolescence poses on ASD.

6.2 STUDY III

Having a dizygotic twin brother is not associated with more autistic or ADHD traits. Instead, parents rate daughters with a twin sister as having slightly more ADHD and autistic traits than daughters with a twin brother. This study does not support the hypothesis that elevated prenatal testosterone levels result in increased frequency of autistic traits in dizygotic twins.

6.3 STUDY IV

Our study indicates that the RAADS-14 Screen has good psychometric properties and that both men and women diagnosed with ASD endorse significantly more items and more persevering symptoms than those with other psychiatric diagnoses, allowing for meaningful screening for ASD in psychiatric populations.

Self-assessment questionnaires do not provide enough information for diagnosing ASD. However, when time and resources are limited, the RAADS-14 Screen is a useful instrument to assess whether primary care patients and psychiatric out-patients need a referral for a full ASD assessment.

6.4 GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The data presented in this thesis suggest that sex differences in ASD do not follow clear patterns of masculinization. In addition to the core male cognitive style, we have observed increased androgyny in physical features and non-male typical patterns of gender roles,

sexual behavior, and reactivity to sensory input in individuals with ASD. This sex/gender defiant pattern, together with the lack of increased autistic traits in females prenatally exposed to testosterone from a twin brother, suggests a relationship between fetal masculinization and autistic traits that is, at least in subgroups of ASD, more complex than a straightforward relationship between fetal exposure to androgens and autistic traits. The recent findings of a dysregulated hormonal *RORA* feedback loop,[97] may provide a model for variability in androgen and estrogen sensitivity over areas and cell types.

ASD research is complicated by the heterogeneity of the disorder. Interrelated uncertainties regarding sex/gender differences in ASD range from possible flaws, both in the clinical definition and recognition of ASD, to the etiological heterogeneity.[85] Sex differences in etiology as well as presentation may result in misleading results when samples are small and no sex stratification is conducted. The broad range of sex differences in ASD indicated in this thesis underscores the necessity of including a sex/gender perspective in ASD studies.

7 SVENSK SAMMANFATTNING

Den neuropsykiatriska diagnosen autismspektrumtillstånd (AST) innefattar funktionsnedsättning och atypiskt beteende avseende social interaktion, kommunikation och stereotypa och repetitiva beteenden. AST beror till stor del på genetiska faktorer men även miljöfaktorer under fosterutvecklingen antas spela in. Hur dessa riskfaktorer samverkar är inte helt klarlagt men utmärkande för AST är att diagnosen är mer än fyra gånger vanligare hos män än hos kvinnor. Genom att beskriva och förstå hur könsskillnader är kopplade till AST kan vi få ökad förståelse för tillståndets ursprung och en individs risk att utveckla AST. Det är också av klinisk betydelse att förstå hur riskfaktorer som drabbar könen olika också kan påverka hur symptombilden kan skilja sig mellan kön och individer.

I den här avhandlingen har jag studerat AST ur ett könsperspektiv utgående från *The extreme male brain theory of autism* (EMB). EMB fastslår att autismens kärnsymptom kan förklaras av en hjärna som utvecklats med extremmanliga kognitiva drag vilket innebär en stark benägenhet till systematisering av omvärlden och en svagare förmåga till empati. Teorins hypotes är att förhöjda testosteronnivåer i fostrets hjärna påverkar dess utveckling, vilket resulterar i att individen får mer autistiska drag.

I studie I och II undersöktes fysiska och psykiska drag som kan tänkas ha påverkats av en maskulinisering till följd av förhöjda testosteronnivåer under fostertiden. Vi undersökte 50 män och kvinnor med AST och 53 köns- och åldersmatchade kontrollpersoner utan AST avseende könstypiskt utseende i kropp och ansikte, röstkvalitet, kroppsmått, könshormonnivåer, könsidentitet, sexualitet och sexuell preferens. Våra resultat indikerar att det inte föreligger någon generell maskulinisering hos vuxna med AST, utan att androgynt utseende är vanligare hos både män och kvinnor med AST än hos kontrollgruppen. Gruppen med AST uttryckte också en svagare könsidentitet och visade mindre skillnader i gendertypiskt beteende än kontrollerna.

I studie III undersöktes, med hjälp av registerdata, om flickor med en tvåäggstvilling som är pojke (och därmed utsatt för förhöjda halter testosteron under fostertiden) har fler autistiska eller ADHD-drag än flickor som har en flicktvilling. Överraskande nog visade våra data det motsatta resultatet – att flickor med en pojktvilling skattas med något lägre poäng för både AST och ADHD.

I studie IV har vi utvecklat och testat ett självevalueringsformulär för att screena för autism. Vi utgick från ett frågeformulär med 80 frågor, RAADS-R. Eftersom många psykiatriska symptom överlappar med symptom vid AST är det viktigt att skattningsinstrument testas på psykiatriska patienter, vilket gjordes i denna studie. De 14 frågor som bäst kunde särskilja AST från andra psykiatriska tillstånd resulterade i formuläret RAADS-14 Screen. Vår studie visar att formuläret har god potential som screeningformulär för AST i primärvård och psykiatrisk öppenvård och kan indikera om behov av en autismutredning föreligger.

8 ACKNOWLEDGEMENTS

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10 APPENDIX

RAADS-14-Screen

Please choose one of the following alternatives:

This is true or describes <u>me now and when I was young</u>.
This was true or describes me <u>only now</u> (refers to skills acquired).
This was true <u>only when I was young</u> (16 years or younger).
This was <u>never true and never described me</u>.
Please answer the questions according to what is true for *you*.
Check only one column per statement!

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Some life experiences and personality characteristics that may apply to you	True now and when I was young	True only now	True only when I was younger than 16	Never true
1. It is difficult for me to understand how other people are feeling when we are talking.	☐	☐	☐	☐
2. Some ordinary textures that do not bother others feel very offensive when they touch my skin.	☐	☐	☐	☐
3. It is very difficult for me to work and function in groups.	☐	☐	☐	☐
4. It is difficult to figure out what other people expect of me.	☐	☐	☐	☐
5. I often don't know how to act in social situations.	☐	☐	☐	☐
6. I can chat and make small talk with people.	☐	☐	☐	☐
7. When I feel overwhelmed by my senses, I have to isolate myself to shut them down.	☐	☐	☐	☐
8. How to make friends and socialize is a mystery to me.	☐	☐	☐	☐
9. When talking to someone, I have a hard time telling when it is my turn to talk or to listen.	☐	☐	☐	☐
10. Sometimes I have to cover my ears to block out painful noises (like vacuum cleaners or people talking too much or too loudly).	☐	☐	☐	☐
11. It can be very hard to read someone's face, hand, and body movements when we are talking.	☐	☐	☐	☐
12. I focus on details rather than the overall idea.	☐	☐	☐	☐
13. I take things too literally, so I often miss what people are trying to say.	☐	☐	☐	☐
14. I get extremely upset when the way I like to do things is suddenly changed	☐	☐	☐	☐