From Department of Women's and Children's health
Karolinska Institutet, Stockholm, Sweden

PSYCHOLOGICAL ASPECTS IN DIFFERENCES/DISORDERS OF SEX DEVELOPMENT

Anna Strandqvist

Stockholm 2018

All previously published papers were reproduced with permission from the publisher.
Cover: Original work of art, reproduced with permission from the artist, Kjell Strandqvist
Published by Karolinska Institutet.
Printed by EPRINT

ISBN 978-91-7831-145-3

Psychological aspects in differences/disorders of sex development

THESIS FOR DOCTORAL DEGREE (Ph.D.)

At Karolinska Institutet, to be defended in Leksell Lecture Hall, at Eugenia hemmet

Friday August 31, 2018 at 9 am

By

Anna Strandqvist

Principal Supervisor:
Professor Anna Nordenström
Karolinska Institutet
Department of Women's and Children's health
Division of Paediatric Endocrinology

Co-supervisor(s):
Professor Agneta Herlitz
Karolinska Institutet
Department of Clinical Neuroscience
Division of Psychology

Professor Agneta Nordenskjöld
Karolinska Institutet
Department of Women's and Children's health
Division of Paediatric Surgery

Associate Professor Louise Frisén
Karolinska Institutet
Department of Clinical Neuroscience
Centre for Psychiatry Research

Opponent:
Associate Professor Sven Mueller
Ghent University
Department of Experimental Clinical and Health Psychology

Examination Board:
Associate Professor Lisa Thorell
Karolinska Institutet
Department of Clinical Neuroscience
Division of Psychology

Associate Professor Fotios Papadopolous
Uppsala Universitet
Department of Neuroscience
Division of Psychiatry

Associate Professor Charlotte Höybye
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Experimental and Clinical Neuroendocrinology

Ring the bells that still can ring

Forget your perfect offering

There is a crack, a crack in everything

That is how the light gets in

Leonard Cohen, Anthem

ABSTRACT

Background Sex differentiation depends on a complex set of molecular events. These are at one point initiated by *SRY* gene expression and involve gene transcription, which in turn can be affected by the prescence or absence of different co-factors, availability of and exposure to sex hormones, presence and sensitivity of steroid receptors that directs growth and development. DSD conditions represent variations in typical sex differentiation with discordance between sex chromosomes, gonads, hormones or genital appearance. Both mechanisms underlying sex differentiation of body, brain, behavior and psychological outcome in many DSD conditions remain understudied.

Aim To increase knowledge of outcome in DSD patient groups regarding psychosocial and psychological parameters and contribute to the knowledge on biological factors with impact on sex differences in cognition and gender role behavior.

Study I

Methods Psychosocial outcome in men and women with congenital adrenal hyperplasia (CAH) (n=588) was assessed through registry studies with regards to education, income and participation in work life, dependency on social welfare, marriage and fertility. Patients were compared to controls from the general population matched for age, sex and place of living.

Results Individuals with CAH had less often completed primary education (OR=0.5), had more periods on sick leave (OR=1.7) and had children less often than controls (OR=0.3). Women with CAH were married less often (OR=0.2) and men were married more often than controls (OR=0.4). Women with SW had income in the top 20% more often (OR=2.0) and both men and women with SW had disability pension more often (OR=2.8).

Study II

Methods Psychiatric morbidity in women with complete androgen insensitivity (CAIS) (n=20), gonadal dysgenesis (GD) (n=13) and premature ovarian insufficiency (POI) (n=21) was assessed through a structured diagnostic interview (MINI-V) and self-report questions, and compared to age matched controls from the population.

Results Psychiatric morbidity was increased for women with CAIS and GD compared with controls from the general population (p=.003) but not in comparison to women with POI. Depression (p=.03) and anxiety disorder diagnoses (p=.003) were increased with an overrepresentation of obsessive compulsive disorder diagnoses (p<.001).

Study III and IV

Methods Cognitive abilities in women with CAIS (n=18), XYGD (n=6) and XXGD (n=7) and men with hypospadias (n=89) were assessed using a battery of cognitive tasks that typically yield sex differences and were compared to age matched controls.

Results The cognitive test battery revealed sex differences in the expected directions. Women with CAIS or XYGD performed more similar to female than male controls except on the test of emotion recognition and word fluency where they had lower performance. Women with XXGD outperformed all other groups on the emotion recognition task. There were no differences between men with hypospadias and male controls neither on the cognitive test battery nor the retrospective gender role behavior questions. Men with proximal hypospadias performed slightly lower overall than men with distal hypospadias.

Conclusions Psychosocial and psychiatric outcome is impaired in women with DSD and in some aspects for men with CAH. Results from the cognitive study in women with DSD support theories of androgen influence on cognitive abilities but factors related to karyotype may influence emotion recognition. Further studies are warranted particularly regarding mechanisms behind superior performance of women with XXGD on emotion recognition and general cognitive abilities in men with proximal hypospadias.

LIST OF SCIENTIFIC PAPERS

I. Strandqvist, A., Falhammar, H., Lichtenstein, P., Hirschberg, A. L., Wedell, A., Norrby, C., Nordenskjöld, A., Frisén, L., Nordenström, A. Suboptimal psychosocial outcomes in patients with congenital adrenal hyperplasia: epidemiological studies in a nonbiased national cohort in Sweden. *The Journal of Clinical Endocrinology & Metabolism*, 2014; *99* (4), 1425–1432.

II. Engberg, H., Strandqvist, A., Nordenström, A., Butwicka, A., Nordenskjöld, A., Hirschberg, A. L., & Frisén, L. Increased psychiatric morbidity in women with complete androgen insensitivity syndrome or complete gonadal dysgenesis. *Journal of Psychosomatic research* 2017;101, 122–127.

III. Strandqvist, A., Herlitz, A., Nordenskjöld, A., Örtqvist, L., Frisén, L., Hirschberg, A. L., & Nordenström, A. Cognitive abilities in women with complete androgen insensitivity syndrome and women with gonadal dysgenesis. *Psychoneuroendocrinology*, 2018;94

IV. Strandqvist, A., Örtqvist, L., Frisén, L., Nordenskjöld, A., Herlitz, A., Nordenström, A. No difference in cognitive performance or gender role behaviour between men with and without hypospadias. 2018 *Manuscript, submitted.*

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit hyperactivity disorder
AMH	Anti-müllerian hormone
AR	Androgen receptor
ASD	Autism spectrum disorder
CAIS	Complete androgen insensitivity syndrome
CAH	Congenital adrenal hyperplasia
FSH	Follicle stimulating hormone
fMRI	Functional magnetic resonance imaging
GD	Gonadal dysgenesis
GnRH	Gonadotropin releasing hormone
GT	Genital tubercle
HPA	Hypothalamic pituitary adrenal axis
HPG	Hypothalamic pituitary gonadal axis
KS	Klinefelter syndrome
LH	Luteinizing hormone
MGD	Mixed gonadal dysgenesis
MRKH	Mayer-Rokitansky-Küster-Hauser syndrome
NC	Non-classic
NPR	National patient register
PAIS	Partial androgen insensitivity syndrome
PAR	Pseudo autosomal region
POI	Premature ovarian insufficience
SDN-POA	Sexually dimorphic nucleus of the preoptic area
SRY	Sex determining region Y
SV	Simple virilising
SW	Salt wasting
21-OH	Steroid 21-Hydroxylase
TS	Turners syndrome
XCI	X chromosome inactivation

LIST OF DEFINITIONS

Aneuploidy - the presence of an abnormal number of chromosomes in a cell.

DSD - congenital conditions where chromosomal, gonadal, or anatomical sex is atypical.

Gender - the psychological experience of being male or female, in between, both or other.

Gender role - behaviors, preferences and traits that differ between males or females in a given culture and historical period.

Gender identity - the core sense of the self as male, female or other

Genotype - the genetic make up of an individual, for example a description of the gene and type of mutation

Haploinsufficiency - when only a single functional copy of a gene pair is present and the resulting phenotype is characterized by loss of function of this gene.

Heterozygous - the genomic state in which one of two copies of a gene is present.

Homozygous - the genomic state in which two copies of a gene is present.

Intersex - previously used term for DSD conditions in medical settings and literature. Presently used in settings other than medical to refer to DSD.

Karyotype - the number and appearance of chromosomes in the nucleus of a eukaryotic cell, is also used to refer to the test that detects this complement or measures the number.

Nuclear receptor - a class of proteins in cells that are sensitive to steroids and together with other proteins up regulate the expression of specific genes controlling the development, homeostasis and metabolism of the organism.

Phenotype - an individual's set of observable characteristics or traits.

QOL - Quality of life "an individuals perception of their position in life in context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns." - Quality of life group, WHO

Sex - the categories male, female or other based on physical or biological attributes such as sex chromosomes, gonads, internal or external reproductive structures.

Sexual orientation - the direction of romantic and sexual attractions to males, females both or other/neither.

1 INTRODUCTION

Sex and gender are important defining factors in society at large and on an individual level. The debate over the existence, magnitude and importance of sex differences and similarities is a somewhat eternal question that is discussed in many parts of academia and elsewhere. Despite this, there are still large knowledge gaps concerning the mechanisms, impact and significance of sex differences and debate over their origins and the relative contributions of nature and nurture. The knowledge field struggles with difficulties of teasing apart biological and social processes often acting in concert creating interactional effects, resulting in rich variation and a spectrum of outcomes.

Disorders or differences of sex development, DSD incorporates a vast number of conditions where somatic sex is atypical with regards to sex chromosomes, hormonal situation, gonads and/or genital anatomy. The spectrum includes a variety of conditions that can affect gender and identity development, growth, fertility and general health. Diagnoses in the spectrum range from benign variations to conditions requiring life long medication and medical interventions.

Much of what is known concerning biological sex differences and sex development in humans has been clarified through learning about underlying reasons for DSD conditions and the psychological and somatic development in patients with these conditons. Yet, the outcome and main mediators of psychosocial and psychosexual outcome in individuals with many of these rare conditions remains unknown and are understudied. Small patient groups and other methodological limitations hamper studies that have the potential to increase the understanding of biological contributions to sex differences. Lately, improved techniques in gene sequencing have made possible more precise genetic diagnostics and subdivision of diagnoses that were previously based on phenotype only. This, together with development of new research paradigms in experimental research has revealed an intricate interplay between specific genes and co-factors in interaction with endocrine systems that steer development along the molecular pathways towards sex differentiation with quite some variation within and between species.

1.1 SOMATIC SEX DEVELOPMENT AND DIFFERENTIATION.

Sex differentiation refers to the biological development steering the individual in either a male or a female direction. The nomenclature and theory concerning the mechanisms behind sex differentiation is developed through a vast amount of experimental animal research. Through translational research and clinical studies this theory is applied and constitute the basic framework for describing sexual differentiation in humans as well.

1.1.1 Sex determination, sex differentiation of genitals

The development of genitalia in a male or female direction is a fairly short but well directed growth period, steered by parallel activating and inhibitory factors, interacting in a precise temporal pattern. It begins early in life around the third week of gestation. The first phase is referred to as *sex determination*, a function of specific genes located on the sex chromosomes that will initiate and in most cases determine the direction of further development (1). Although sex chromosome aneuploidy occurs quite frequently, between 1/400 – 1/1000 births (2,3), sometimes resulting in sex chromosome complement of X only, men typically have XY chromosomes while the majority of women have XX chromosomes. In the first five weeks of fetal development, embryos have undifferentiated internal structures that later develop into internal and external genitals. The structures that will form the inner genitalia for male development are referred to as Wolffian ducts and the female structures as Müllerian ducts. Individuals of both sexes have bipotential gonads that can develop towards either ovaries or testes.

When the first determining events have occurred, subsequent sex typical development is referred to as *sex differentiation* (1). Many genes important for sex differentiation and as such, significant in the understanding of the etiology of DSD conditions, have been mapped out (4). Mutations in different genes may have different effect depending on karyotype and presence of other factors. For example a dysfunction in SF-1, a nuclear transcription factor important for gonadal formation, may cause premature ovarian insufficiency (POI) in 46,XX individuals, dysgenetic or absent testes in a 46,XY individual and may cause adrenal insufficiency in both (5).

Male

The development of male genitalia precedes that of female development and begins in the third week of gestation when testis development is initiated. The sex determining region of the Y chromosome, the *SRY* gene is at present described as the dividing agent that initiates male development by directing the bipotential gonad towards testes (6). It promotes expression of genes that activates and maintains the male gonadal differentiation pathway with differentiation of the cells into Sertoli and Leydig cells. The Leydig cells of the testes will secrete testosterone, which will drive further development and promote survival of the Wolffian ducts. The Sertoli cells will secrete anti-Müllerian hormone, which will induce regression of the Müllerian ducts (7).

In week five the cloacal folds form on the cloacal membrane and fuse to become a structure labeled the genital tubercle (GT) with the labioscrotal folds developing on either side of this structure. The distal part of the GT will subsequently elongate and differentiate into the penis while the labioscrotal folds fuse to form the scrotum. This is to a large part an androgen driven process. Testosterone (T) from the Leydig cells of the testes is converted in peripheral tissues into the more potent androgen; dihydrotestosterone (DHT) by the enzyme 5α-reductase. This enzyme is present in different concentrations in peripheral tissues, therefore

acting locally. The Wolffian ducts grow into vas deferens, the seminal vesicle and epididymis of the male inner genitalia. This development is typically finished by the 20th gestational week. Finally, the testicular hormone, insulin like factor-3 promotes the descent of the testes through the abdomen and testosterone facilitates their descent into the scrotum. This is generally completed by the 32th gestational week (1,8). Later in life, androgens (particularly testosterone) contribute to the development of secondary sex characteristic in puberty such as beard growth, lowered voice and increased bone and muscle mass. (9)

Female

In females the Y bound gene *SRY* is not present. As a consequence, ovary specific transcription factors and genes will initiate and maintain the development of the undifferentiated gonad into an ovary. No equivalent gene of *SRY* has been identified for ovary formation. The Müllerian ducts will persist and develop into oviducts, uterus, and the upper part of the vagina. The Wolffian ducts will degenerate and the genital tubercle will form a clitoris and the labioscrotal folds the labia. The lower part of the vagina is formed from the sinovagal bulb (1,4). Estrogens are the sex hormones produced by the ovaries. They are primarily responsible for the secondary sex differentiation of females particularly during pubertal development. Adrenal androgens induce secondary sex characteristics such as hair growth.

Several singular genes have been identified as important for the etiology of DSD conditions. Both genetic variant, expression of genes, and deficiencies of enzyme production or function can cause deviations from the path of typical sexual differentiation. Important aspects have been identified in terms of gene dosage effects, the temporal aspect, cofactor and transcription factor function. Variations in this development create the spectrum of DSD conditions (4,8,10).

1.1.2 Concepts in theory of sex development

Masculinization refers to development of typically male features as in the retention and development of the Wolffian structures or the changes that will promote the expression of typically male sexual behavior in adulthood. *Feminization* refers to the processes by which typically female development will take place. *Defeminization* is the term used for the development leading the individual towards development of a masculine phenotype. The degeneration of the Müllerian ducts is one such example. The logically derived term *demasculinization* has fewer applications. It can sometimes denote the disruption of a masculinizing process but since many masculinizing effects are more or less irreversible, the term is seldom used (11). In variations of genital development, *virilization* is sometimes used to describe growth in the male direction in females and *undermasculinization* is used to describe, “arrested development” in males.

1.1.3 Determinants and driving forces of sex differentiation

Sex chromosomes

Both men and women inherit an X chromosome from their mother. Women also inherit another X chromosome from their father while men inherit a Y chromosome from their father. The X chromosome carries around 900 genes, many of which are important for brain development (12). In individuals with more than one X chromosome gene dose equivalence is kept in balance by a mechanism referred to as X-chromosome inactivation (XCI) when a significant part of one of the X chromosomes is functionally silenced (13) Either the maternal or paternal X can be silenced, with variation in different tissue resulting in mosaicism across cell populations. Some genes, around 15%, located on the pseudo autosomal region (PAR) escape inactivation (14).

The Y chromosome carries as few as 80 genes, some of which are known to induce important sex differences. Mouse models where the gonadal and the chromosomal sex is decoupled (15) have been used to show hormone independent pathways of sex differentiation for differences in body size, metabolic disease and cellular tissue differences, independent of exposure to sex hormones (16,17).

Sex steroids

Sex steroids include hormones like estrogens, androgens and progesterone; signaling molecules that steer tissue development and regulate many of the bodys functions and growth processes. They are also important actors in somatic sex differentiation.

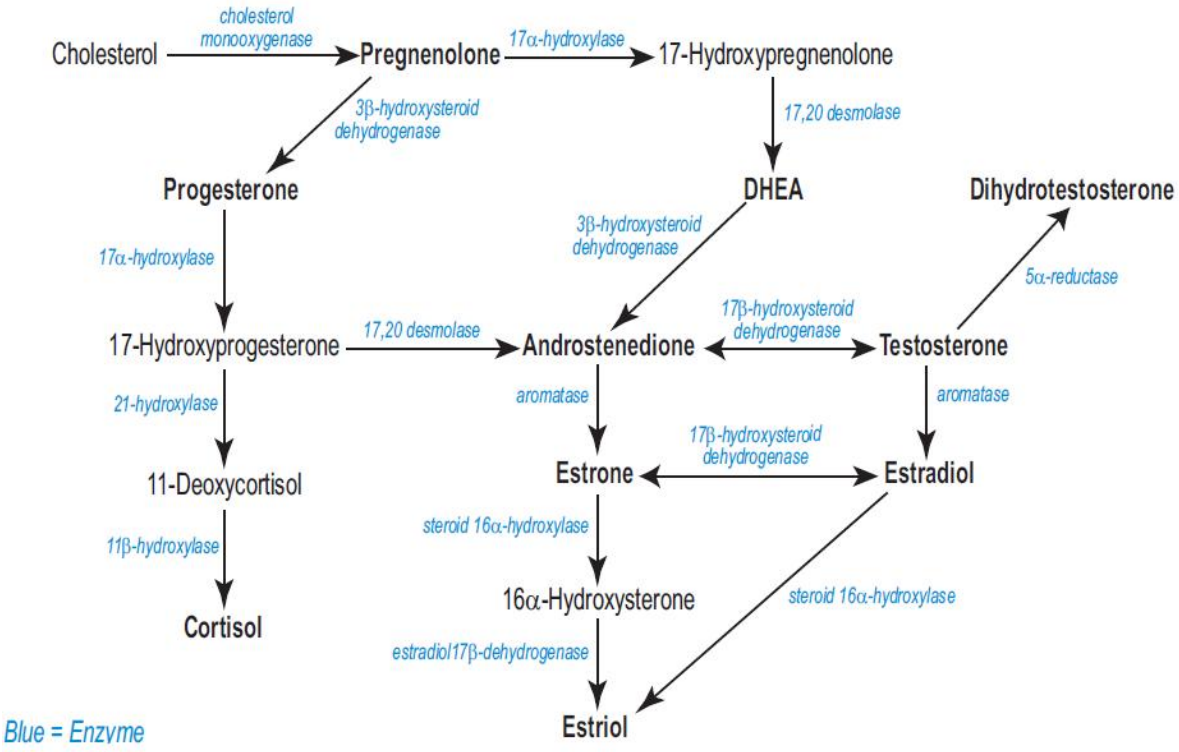

Fig 1. Overview of the human steroidogenesis.

Steroids are synthesized from cholesterol in the endocrine glands; the testes, ovaries and the adrenal glands. Steroidogenesis starts off with cholesterol, which is converted into pregnanes, such as progesterones and pregnenolone. From these metabolites, androgens, estrogens and the adrenal steroids, the mineralocorticoids and glucocorticoids are synthesized. The process of aromatization of androgens into estrogens, takes place throughout the body and in the brain. The action and effect of sex steroids are dependent on availability, which in turn depends on synthesis, transport, uptake, degradation, as well as prescece of locally converting enzymes and functional receptors.

Steroid receptors

Steroids signal by binding to a group of receptors that are called nuclear receptors. They interact with DNA together with co-factors and co-repressors with facilitating or inhibitory effects on gene expression. Steroid signaling can also occur on the cell membrane. These effects are more rapid and can occur directly in contrast to the more slow progression of changes in gene expression (11).

There is only one known androgen receptor (AR) and it is expressed in both sexes. The gene coding for the AR is located on the X-chromosome. The receptor is a protein that binds to DNA and induces masculine development (9). Several different androgens can bind to the same receptor with effect on different target genes. The sensitivity of the AR receptor can be tissue specific (9). For estrogen, at least three different estrogen receptors are described, localized in the cell nuclei, cytoplasm and at the cell membrane (18).

1.1.4 Neuroendocrine systems

The regulation of sex hormones takes place in endocrine systems that often depend on feedback mechanisms for homeostatic regulation. The regulation of endocrine processes involved in development, reproduction and ageing can be conceptualized and understood through a description of the HPG axis i.e., hypothalamic, pituitary, gonadal axis (19). The hypothalamus is a structure in the brain located below thalamus and is a part of the limbic system. This system links the pituitary gland with the nervous system in the brain by secretion of what is sometimes referred to as “neurohormones” (20). This implies that they mediate signal between other parts of the brain and the body, which is important for endocrine function. The hypothalamus releases GnRH to the pituitary gland, which in turn produce LH and FSH. In females these two hormones activate the ovaries that in turn produces estrogen and inhibin that regulates the menstrual cycle. Estrogen inhibits the production of GnRH by a negative feedback loop to the hypothalamus. In men, LH stimulates the testosterone production in the testes, which is crucial for spermatogenesis (20).

The HPA axis (hypothalamic-pituitary-adrenal axis) is another neuroendocrine system that regulates and is involved in many somatic processes, including stress response, immune reactions, digestion, mood and emotion, sexuality and energy storage. The release of vasopressin and corticotrophin releasing hormone from the hypothalamus stimulates secretion of adrenocorticotrophin (ACTH) from the pituitary gland. This in turn signals to the adrenal cortex to produce glucocorticoids, mostly cortisol, a hormone involved in varying functions such as glucose metabolism, immune system and stress regulation. Cortisol production results in negative feedback to the brain inhibiting both the hypothalamus and the pituitary gland (21).

Activity in these systems has three peaks in human development: early during gestation, post partum, also refered to as “minipuberty” and in puberty. Increases in sex typical hormones and subsequent decreases induced by feedback mechanisms, create a hormonal milieu that directs the individual’s general body growth, the development of genitalia in particular, sex characteristics, and also aspects of brain development (22). Further on, pregnancy and menopause also constitute periods of considerable systemic change in the hormonal situation.

1.1.5 Models of sex differentiation, organizing effects and critical periods

Gonadal and adrenal sex steroids exert permanent, sometimes irreversible, sometimes transient influences on sex related differences in the brain. Steroids have multiple ways of influencing brain development both in terms of structure and function. The underlying mechanisms are manifold including altering of cell survival and proliferation, synaptic connectivity (11) and cell migration (23) amongst others.

Organizational-activational effects

Phenomena that occur resulting from changes in levels of sex hormones that have priming or lasting effect are traditionally referred to as ”organizational” and assumed to occur during the early phases of prenatal or postnatal development but may also occur during puberty. Later, the hormonal influences that activate, modulate, or inhibit the effects are referred to as ”activating” (24,25). Examples of physical changes that are organizing include deepening of the voice and feminization such as breast development during puberty. An activational effect, i.e. one that can be reversed and depend upon continued stimulation to prevail include male body hair dispersion.

The organizing effects of sex steroids, particularly androgens on brain development have been mapped out through experimental studies in animals such as rodents, primates and marsupials. The behaviors investigated are mostly related to sexual behavior as this differs considerably between male and female rodents. In primates, other behaviors like play

behavior, vocal behaviors and interest in infants have been studied. While qualitative differences exist, most behaviors differ primarily in quantity between males and females (26).

Organizing effects have been shown to induce anatomical differences like the larger volume of the sexual dimorphic nucleus of the preoptic area (SDN-POA) and ventromedial hypothalamic nucleus (VMN), two regions associated with sexual behavior in male rodents (16). Activating effects of circulating androgens can also induce anatomical differences like the enlargement of amygdala in male rodents (16). The amygdala is a brain area involved in emotion, decision-making and is important in conditioning of responses. This has also been observed in humans where brain development during puberty gives rise to anatomical differences such as relative size difference before and after in amygdala and hippocampus (27). Sex hormone levels, particularly testosterone, has been found to be associated with anatomical differences in prefrontal cortex paralleling those of change in executive functions in males (28).

Critical periods

For some effects of sex steroids and their role in genetic events it has been shown that there are windows in time where the programmed event occur. If development takes a different path, this particular development will not spontaneously be induced later on. One such example in humans is male genital development which mainly takes place during the first trimester and if arrested or disturbed, will not continue completely after this critical period resulting in malformations such as bladder or cloacal extrophy, epispadias or hypospadias.

In experimental research different models exist to explain the mechanisms behind these phenomena. Periodical sensitivity and increasing resistance to sex hormones is one such mechanism, (25) epigenetic changes another, (22) to understand periods where development accelerates or occur in response to exposure and modulating effects of sex hormones.

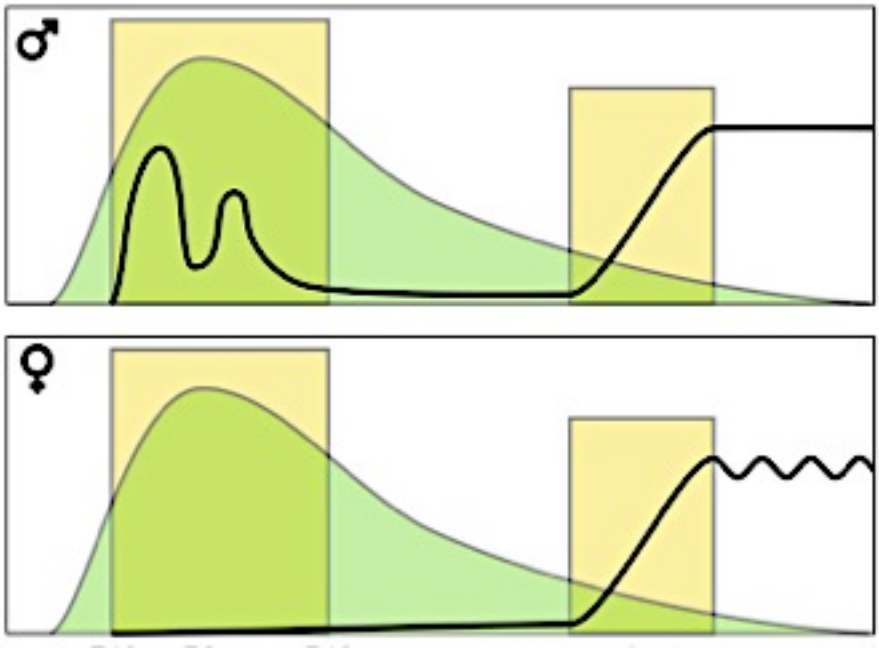

Figure 2. Model that shows the organizational/activational hypothesis of brain sex differentiation together with suggested rising and declining sensitivity to sex hormones. The line depicts sex steroid exposure, i e the prenatal and pubertal surges of testosterone and other gonadal hormones, the yellow blocks correspond to periods during gestation, and postnatal potential critical periods and the green curve the window of sensitivity to sex steroids.

1.2 VARIATIONS IN SEX DEVELOPMENT

Variations in sex development occur spontaneously by mechanisms such as new mutations, by inheritance or by environmental disruption of different origin. Individuals whose bodies do not match societies expectations of typically male and typically female have always existed. The norms of what is considered to be typical bodily features for the two sexes are not fixed and can change somewhat between countries and over time, as does the normative conception of what signifies masculinity and femininity (29).

Most babies born are assigned to either sex by assessment of their genitals at birth or by ultrasound during gestation. Every year in Sweden, around twenty children are born who cannot be assigned to either sex at birth before further investigations, or where there is discrepancy between prenatal karyotype test and the appearance of genitalia at birth. When this occurs, an investigation is carried out including assessment of karyotype, hormonal situation and of internal and external genitalia, specifically gonadal status. A genetic screening for known mutations is also made. Based on this information a decision is made as to which sex the child will be assigned to grow up in. When making this decision, aspects such as need for life long hormone substitution, surgical options, fertility potential and lately, also an estimation of prenatal androgen exposure of the brain are taken into account. The aim

of the decision is to make a choice that will match gender identity later in life while minimizing the need for medical interventions. Sometimes, but not always, a molecular diagnosis can be given.

1.2.1 DSD nomenclature

Since a consensus conference was held in Chicago in 2005, where representatives of patient organizations and medical practitioners attended, the nomenclature has been standardized with regards to medical terminology with the aim of describing the somatic condition and to avoid stigmatizing and imprecise labeling. The categorization is based on sex chromosome complement, gonadal status, with diagnoses according to the identified affected gene or hormone dysfunction when known (30).

Table 1. Schematic overview of medical classification and nomenclature of DSD conditions

Sex Chromosome DSD	**46,XY DSD**	**46,XX DSD**
45,X Turner syndrome and variants	46,XYGD Disorders of gonadal (testicular) development	46,XXGD Disorders of gonadal (ovarian) development
46,XXY Kleinefelter syndrome and variants	Disorders of androgen synthesis or action CAIS, 5α-reductase deficiency, 17β-HSD deficiency	Androgen excess CAH
45,X/46,XY Mixed GD, ovotesticular DSD	Other: genital malformations, hypospadias	Other: MRKH
46,XX/46,XY Chimeric, ovotesticular DSD		

1.2.2 Health care in DSD - historical outlook

There are early accounts of medical doctors encountering patients with DSD conditions, concluding that the person had physical characteristics of the opposite sex post mortem (31). Records exist describing interventions concerning the physical sex characteristics as corrections of hypospadias among gladiators described by their physician Galen (32).

The healthcare approach towards DSD in the beginning of the 20th century has been described as the ”true sex policy” (33) where the conviction was that if a person’s true sex could be determined by some key biological criterion, this would form the basis for sex assignment. The role of the gonads in sex differentiation was shown in experiments with rabbits (34) and sex hormones, particularly androgens, were identified as key actors in this development. The medical discipline of endocrinology has evolved during the first half of the 20th century, describing the actions and interplay of hormones and their importance for physical growth and functioning over all. When karyotype tests became available and technical advances made the imaging of internal genitalia possible, the picture became more complex. In other parts of healthcare, karyotyping has made for an easy way to “determine sex” of children before birth. In the field of DSD, the medical profession could utilize this as one of several parameters in the assessments made before sex assignment.

Around 1950, at Johns Hopkins University clinic, Maryland, USA, a program for gender assignment in patients with ambiguous genitalia was developed by the psychologist John Money and his colleagues. The policy emerging there, often referred to as the “optimal gender policy”, put a priority not only to assigning gender according to genital appearance or karyotype, but also took into consideration the preservation of fertility, available hormonal and surgical treatments, and the expected sexual function. Based on this, the treatment consisted of aligning the physical appearance of genitalia by surgical interventions and/or hormonal treatment with the assigned sex. It was believed that gender identity would mainly be determined by sex of rearing, thereby emphasizing the importance of being brought up in a clear gender role (35). Knowledge of the condition was considered a risk factor for psychosocial adjustment and adult patients today, often describe that they have not been fully informed of their condition or that they have had to search for appropriate information themselves.

1.2.3 Present day healthcare policies

There are as of yet no evidence based guidelines for DSD care. Healthcare practices thus vary considerably depending on region or type of clinic. The current guideline for care of patients with DSD recommends full disclosure of information for the patient and the family. It also stresses the importance of working in multidisciplinary teams and puts emphasis on the functional outcome of surgery rather than surgery for primary cosmetic reasons (36). This paradigm struggles with the same issues as before but also brings new challenges like informing parents and children of the basic yet sometimes complex information regarding

diagnoses, treatment options and outcome. Whole genome sequencing has brought possibilities in terms of precise molecular diagnoses, thereby increasing complexity in terms of knowledge of carrier and the implications of inheritance for the individual and other family members. Since some of the treatment options used include irreversible hormonal and surgical procedures, there is a need for psycho-education in order to support the families and individuals in understanding the alternatives at hand to be able to participate more actively in decisions concerning the medical management of their condition. DSD healthcare at present has been described as being in a state of transition, moving towards a "minimal medical interventions" policy (37).

Recent and ongoing changes in treatment practices

In the last half of the twentieth century many individuals with XY karyotype were assigned female sex as the importance of physical appearance on upbringing was emphasized and cosmetic surgery was carried out accordingly. The accumulating knowledge of sex differentiation of the brain and behavior, acknowledging the role of prenatal exposure to androgens for neurobehavioral sex differentiation, has made for a shift in sex assignment practices. The assignment of male gender to individuals with XY DSD when they present in the newborn period with ambiguous genitalia, if they can be assumed to have had considerable amounts of androgen exposure, in utero, has increased (38). The practice of surgery in the newborn period is successively being postponed to a period when the patient can play a more active part in this process. Surgical techniques are continuously changing and as surgery is being delayed, new data is emerging on feasibility of not intervening surgically on DSD conditions (39).

1.2.4 The patient perspective

Discourse around DSD conditions raise questions of its impact on identity development, the boundaries of normality and human rights issues. The last couple of years have seen an increase in qualitative studies that take the patient perspective and evaluate aspects of living with a DSD condition, capturing the more subtle aspects and the subjective experience (40-44). There are also many patient representatives involved in advocacy and activism to educate and create public awareness around these conditions.

Generally, most advocate groups, activists and non profit organizations that reach out in media are propagating for healthcare to be *less active in effectuating medical interventions and more active in offering psychological support for all affected by these conditions.*

1.2.5 Characterization of the patient groups included in studies I-IV.

Men and women with congenital adrenal hyperplasia, CAH

CAH is a group of autosomal recessive disorders of cortisol and aldosterone metabolism. It constitutes of a block in one of the five enzymatic steps in the synthesis of cortisol by the adrenal glands. The incidence varies in different populations most often between 1/10000 – 1/15000 (45,46). The most frequent form, referred to as "classic CAH", affecting more than 90% of cases, is caused by deficiency of the 21-hydroxylase (21-OH) enzyme that also catalyzes the conversion of progesterone to deoxycorticosterone, an aldosterone precursor. Aldosterone is a hormone that maintains sodium balance in the body without which salt loss and adrenal crisis occurs.

Untreated CAH can be life threatening; therefore, CAH is included in newborn screening programs worldwide. The level of 17-hydroxyprogesterone, the metabolite before the enzyme block, is measured within the first days to detect individuals that need treatment to survive. The screening programs usually do not identify milder forms of CAH, so called "non-classic" forms of the disease, a form where cortisol production is lowered but present and symptoms of androgen excess lead to clinical diagnosis, if it is detected at al. Cortisol interacts in several of the hormonal axis responsible for basic regulation of sleep, stress, immune function, and growth. Symptoms and affected outcome of the disease are manifold and might affect several central physiological functions, some visible but most not. There is a good correspondence between genotype (*CYP21A2* mutation) and clinical manifestations such as disease severity (45).

Due to accumulation of precursors and due to HPA axis dysregulation, increased androgen levels are present already prenatally. This may lead to varying degrees of virilisation of the external genitalia in girls with CAH; fusion of labia and clitoromegaly, while the internal genitalia are unaffected. In extreme cases girls can initially be perceived as boys in the newborn period, and assigned male sex of rearing (47). The development of boys with 46XY and CAH is male typical. Several other physical complications can present in untreated or not optimally treated individuals connected with growth, obesity, cardiometabolic riskfactors and infertility (48).

Treatment involves lifelong glucocorticoid replacement (hydrocortisone) and mineralocorticoid replacement (Florinef). Surgical correction of genitalia in girls with CAH used to be carried out in the newborn period but is at present increasingly more often postponed due to suboptimal results from surgery follow up studies. Outcome for non-operated cases shows that a spontaneous reduction in clitoral size occurs after the treatment has been initiated and optimized (39).

Pubertal development on hydrocortisone treatment is typically female for girls and typically male for boys. If compliance problems arise or treatment is not optimal, pubertal development can be affected with pseudo-precautious puberty in boys and androgen symptoms including amenorrhea in girls.

Women with complete androgen insensitivity, CAIS

CAIS has an estimated incidence that ranges from 1-5 in 100 000 live births (49,50). The condition is due to a mutation in the androgen receptor (AR) gene that causes complete insensitivity to androgens. The inheritance is X-linked, recessive meaning that 46,XX individuals will be carriers and that the disorder is expressed in 46,XY individuals. Individuals with this condition are phenotypically female but have XY chromosomes and intra abdominally located testes. Development of typical female internal genitalia is absent due to anti-Müllerian hormone (AMH) produced by the Sertoli cells of the testes, and these women can therefore not carry a pregnancy. The condition is most often discovered during puberty due to primary amenorrhea, at surgery for inguinal hernia, or as the result of prenatal karyotype test not matching phenotype at birth. While testosterone does not have any direct effect due to the lack of androgen receptor, it is converted to estrogen by aromatisation. Therefore, spontaneous pubertal development occurs, including breast development with the exception of menarche (51). Due to the lack of androgen effect, body hair is sparse and skin complications like acne is absent (52). If vaginal agenesia is present, treatment options vary with self-dilation as first choice or more rarely vaginoplasty.

Treatment consists of estrogen replacement to preserve bone density and promote general health. Due to the anticipated risk of germ cell tumour development, gonads were previously surgically removed as a standard procedure. Recently, this practice has become questioned and gonads are now monitored for malignancies rather than removed (53, 54). These patients are known to live in a female gender role and identify as female in terms of gender identity (55,56) with few exceptions (57).

Women with XY and XX Gonadal dysgenesis, GD

Underdeveloped gonads resulting in deficient gonadal steroid production characterize gonadal dysgenesis, GD. The condition can be caused by mutations in several genes important for gonadal formation (4,58,59). 46, XY GD with complete absence of gonads is also referred to as Swyers syndrome (60). In this condition it is common that a uterus and fallopian tubes develop, due to lack of AMH although variations exist. With ovum donations these women can usually carry a child (61). If dysgenetic gonads exist they are often removed due to the risk for malignancies (62). The resulting lack of gonadal hormones has

several implications. Infertility and estrogen deficiency are almost always present. The condition is sometimes discovered due to primary amenorrhea and absence of secondary sex characteristics, which typically implies complete gonadal dysgenesis, CGD. The condition can also be partial with low prenatal exposure to androgens. Clinical presentation varies but the majority of individuals with complete XYGD are phenotypically female as well as those with XXGD (63). Few studies exist regarding gender identity and gender role behavior but female identification is described (64). Hormone replacement is often prescribed to preserve bone density and promote general health (59).

Women with premature ovarian insufficiency, POI

POI is clinically manifested as premature depletion of ovarian follicles and onset of menopause before the age of 40. Etiologies differ and include genetic, autoimmunity and inflammatory processes affecting gonadal function (65). It can be due to enzyme deficiencies, metabolic or idiopathic circumstances but can also remain unidentifiable. This leads to estrogen deficiency that has wide spread and varying effects on the female body. The women are otherwise healthy but receive hormonal substitution in the form of estrogens and cannot produce oocytes for own biological children, which is equivalent to the situation for some of the women with DSD (66).

Men with hypospadias

Hypospadias is one of the most common genital malformations with a reported prevalence of 1/125 newborn boys in Sweden (67). It ranges from mild forms causing mostly cosmetic problems with minor displacement of the urethra, to more severe forms with the urethral opening at the base of the penis or in the perineum, which may lead to uncertain sex at birth. Severe forms can cause problems with sexual function, fertility and in addition, all forms of hypospadias can have urinary problems. The etiology is sometimes not known in the individual case, causes can be genetic or environmental and are mostly multifactorial (7,68). Male genital development is to some extent driven, sustained and supported by testicular hormones such as androgens (69), therefore consideration of endocrine factors contribute to the understanding of causative mechanisms. Surgery is usually carried out during the first year of life. Surgical procedures are sometimes carried out in several steps. If complications occur, repeated surgery can be experienced while growing up and in adulthood.

1.3 SEX DIFFERENCES; MEASUREMENTS, CONCEPTUALIZATIONS AND FINDINGS

The study of neurobehavioral sex differences takes place in different scientific fields and encompasses brain anatomy, basic functions like connectivity and neurotransmission, development, cognition and behavior which in turn can be further subdivided into different types of behavior on different types of arenas. Social psychology and psychotherapy each has research fields devoted to the investigations of sex differences and these fields are probably best summed up elsewhere.

Sex – Gender

The word sex stems from the latin word sexus, the state of being man or woman, which may derive from "seco" which refers to -half the race which in turn can be connected to "secare", -to divide (http://www.thesaurus.com). In different research traditions the words sex and gender are used but not interchangeably. The ways they are used also to some extent indicate the discourse. Sex typically refers to the biological attributes, the differences where biological contributions can be discerned or assumed. Gender on the other hand is used to refer to the social or cultural roles differing between the sexes and also to describe the experience of the individual as being male or female. Early 20th century research concerning gender development, which had influence from studies in DSD populations, was using both concepts interchangeably. This ceased in the end of the 1970s when scholars were encouraged not to refer to sex or biology in an attempt to refrain from determinism in describing gender development (70). Since then the academic use of these two concepts have been more distinct and separated.

Since this thesis does not concern the social shaping forces of gendered behavior there will be no overview of theories or evidence on the subject included. This should not be interpreted as disregarding the importance of these factors that are intertwined with biological effects. No biological mechanisms can act independently; we are all born into a social environment and become who we are by interaction within a social context throughout life.

In the literature concerned with biological influences on sex differences, much focus has been on investigating the impact of early exposure to sex hormones. Typically the rise in testosterone that developing male fetuses are exposed to, that coincides with a critical period for development of genitalia, is hypothesized to have an organizing effect. But while sexual differentiation and brain development begins and parallels, it does not end at the same time as the formation of genitalia during the first trimester. Therefore, in theory, it can be affected differently by factors contributing to sex differentiation of other parts of the body (71). Contemporary models of the background of sex differences incorporate both biological effects in interaction with environmental effects.

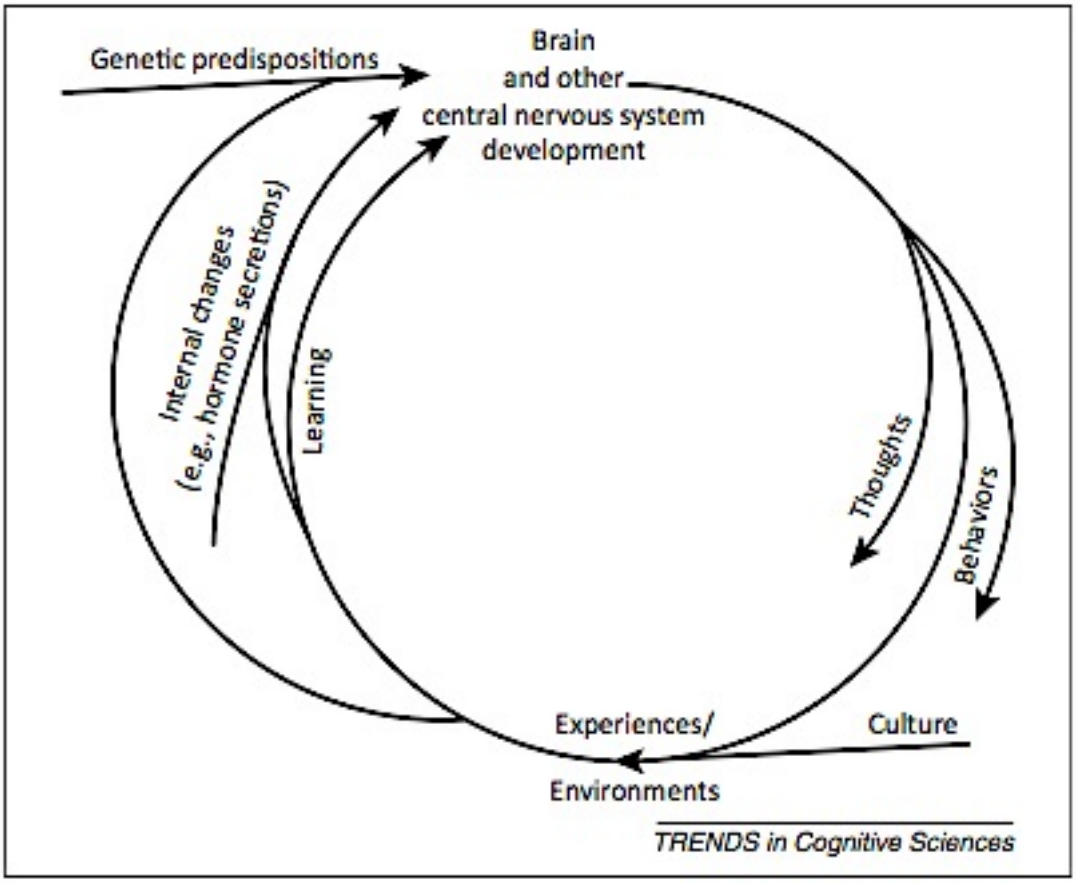

Fig 3. Biopsychosocial model depicting influences of biology and environment that exert interactive effects. This loop can create, increase or reduce sex differences.

Reprinted from Trends in Cognitive Sciences, Vol 18, Miller D., Halpern. D., (2014) The new science of cognitive sex differences 1 37-45, *First published in* Sex differences in cognitive abilities, Halpern, D. *Copyright 2012 Psychology press, New York, NY*

Sex differences in disease

One of the urgent reasons to study neurobehavioral sex differences is that men and women differ in incidence, onset, severity and symptoms in disorders affecting brain development, function and aging (16,72). Neuropsychiatric morbidity is more prevalent in boys and men (71,73) while mood disorders are overrepresented in women (74,75). Men and women are also differently affected by disorders like schizophrenia (76), Alzheimer's disease (77,78) and Parkinson's disease (79) both in terms of onset and differences in symptoms. These differences in prevalence tend to be large compared to differences in behavior that are sometimes modest in magnitude.

1.3.1 Sex differences in brain anatomy

Although a classification of brains as either male or female is a categorization with low explanatory value (80) the brains of men and women do in fact differ anatomically. On the level of individual cells, each cell contains either of the sex chromosomes of XX, XY or other, even though the significance of this difference is not yet clear (81).

In terms of volume, men's brains are overall heavier and larger than women's although this difference is smaller, when adjusting for body size (82). When brain size is adjusted for, regional differences in size and volume are found such as higher gray matter concentrations in women with regional and individual differences (83).

Although many reasonably large differences have been found in several brain structures, like the amygdala, cerebral cortex and the substantia nigra (16), contradicting results are also abundant, as reviewed in (27). The measurement method, focus of investigation and strategies to adjust for individual brain volumes contribute to inconsistent findings (84).

While they may be significant, and paralleling sex differences in behavior, anatomical differences cannot predict the nature, magnitude and direction of differences in behavior and cognition. These factors, therefore have to be studied as an autonomous entity (16). The metaphor of computer size and function serves to illustrate this phenomenon.

1.3.2 Sex differences in cognition

While men and women do not differ on global composite parameters like intelligence tests, there are systematic group level differences for particular abilities that while they may vary with socioeconomic development and cultural aspects (85), the pattern of differences show stability across the globe (86-88) and across the life span (89) in a long term perspective. Women are typically superior on verbal abilities (90) and some memory functions (91) while men excel on spatial tasks (92,93). Women also have an advantage when reading the emotional state in someone's facial expression (94,95).

From a developmental perspective, sex differences in children like the discrepancy in verbal abilities have been argued to depend to some extent on differences in maturation (96) which also provides girls and boys with different periods of developing superior skills. Throughout life these differences contribute to differences in choice of education and occupational careers (97).

1.3.3 Sex differences in behavior

Despite overlap in most behaviour, men and women differ systematically in some preferences and actions. To describe and conceptualize facets of psychosexual development the terms gender role behavior, sexual orientation and gender identity are commonly used.

Gender role behavior that is, sex typical behavior, describes the psychological attributes a person expresses in daily life that differs between the sexes in a majority of the general population. This construct is investigated by different direct and indirect methods. Children's play style, choice of best friend and toy preferences are known to show large sex differences with variations during development (98). This can be measured by self-report, parental proxy reports or by direct observation. Gender role behavior in adults is often investigated by self report questionnaires that inquire about everyday habits, what gender role one thinks others regard one as, and other behaviors that are carried out in society and that differs systematically between men and women. In some studies, more objective measures are used, for example choice of occupation.

Sexual orientation refers to the direction of a person's romantic interest, desires and actions. This is most often measured by self-report. Homosexual, heterosexual and bisexual are concepts most often used in the measurements while literature on sexual orientation also include categories like asexual, gynecophilia or androphilia (35).

Gender identity (GI) indicates which sex a person self identifies as in the persons own mind. GI was defined by John Money as "the private manifestation of gender role" (99) and has been defined by Kohlberg as "the cognitive self-categorization as a boy or a girl" (100). Other conceptualizations include felt pressure for gender conformity and felt compatibility aspects of gender identity (101). A contemporary model put forth by Tobin include five dimensions; knowledge of a gender category, gender centrality, gender contentedness, felt gendered conformity and gender typicality (102).

Gender identity development is a dynamic process throughout life with important milestones in the preschool years, during puberty and early adulthood (101,103). Traditionally it has been measured on a dichotomous spectrum from male to female but at present multidimensional assessments also exist.

These three developmental trajectories do not always correspond and might develop and change over time. Likewise variation within groups and a permanent overlap between sexes exists for most sex typical behaviors. Psychosexual development is often conceptualized as male or female or other. All possible variations cannot be acknowledged in research settings or on other arenas, but the individual perception can be unreflected, undecided or nonbinary and may well be a situation better described as female and male, ambiguous or in transition.

There is often a somewhat superficial dichotomous nature introduced by the measurement methods in order to make the phenomena under study possible to analyze by quantitative statistical methods. For this sake it is worthwhile to emphasize that overlap between the sexes and variation within each sex in most aspects studied is greater than the differences between them and that predictions about individuals in one category cannot be made on the basis of group means (104) .

1.3.4 Sex differences related to sex chromosomes – learning from animal studies

For direct, non gonadal effects on sex differentiation of behavior, very little evidence exist in humans. The traditional view of gonads and gonadal hormones being the determining agent for most biological sex differences was challenged by some classical experiments on zebra finch. The male zebra finch sings a special courtship song to his female counterpart. Many attempts were made to change this behavior by experimental manipulation of steroid levels (105) but failed. Since then, several sex chromosome related effects in different species contributing to sex differentiation have been identified (106). These effects can act through genes on the Y chromosome either causing masculinizing effects (107) or inhibiting feminizing effects.

The role of sex chromosome complement is investigated in different models. One paradigm is the four core genotype, (FCG) mice that includes XY or XX individuals with either testes or ovaries or the XY model, varying the number of X and Y chromosomes independently (16,108). Type of sex chromosome complement has been shown to be of importance in mice models for behaviours such as aggressiveness and parenting behaviour (109), behavioural modelling of drug addiction, and social behaviours (17). Hypotheses on mechanisms by which these effects arise include non-gonadal effects of Sry or other genes located on the Y chromosome (110), X chromosome gene dosage effects and parental imprinting of the X gene (15). Besides more or less direct genetic effects, epigenetic effects can give rise to permanent sex differences. This also gives firm evidence for the role of environmental factors for the emergence of sex differences in many species (106).

1.4 OUTCOME STUDIES IN DSD POPULATIONS

1.4.1 Cognitive outcome studies in individuals with DSD conditions

The research field on sex differences in cognitive abilities struggles with issues related to interactional effects of biological mechanisms versus those of the environment. Therefore, inviting individuals with DSD conditions to participate in research is one possible mode to study biological sex differentiation, as sex chromosomal disposition, hormonal situation and sex of rearing sometimes may be decoupled.

Individuals with Turner (45,X DSD) and Klinefelter (47,XXY DSD) syndrome often have cognitive dysfunction, which is a common finding in groups of individuals with sex chromosome aneuploidy (Hong 2014). It is therefore difficult to draw conclusions even though many outcome studies regarding cognitive abilities exist (111-114).

CAH

Most of what is known concerning early androgen exposure effects on cognition is learned through studies in women with CAH. The results concerning effects on spatial ability are mixed with some reporting no effect (115-118), or no effects for mental rotation tasks but targeting (119), while others report enhanced spatial ability (120,121,122). Taken together, higher spatial performance in comparison with control girls was supported in a meta study (123). Disease severity moderates the effect (124) and biomarkers for prolonged exposure for androgens correlates with improved performance in a computerized water maze task (125). In contrast, boys with CAH have been found to have normal or impaired spatial abilities (119-121).

CAIS

There are few studies exploring cognitive functions in patients with CAIS. The first two (126,127), compared women to their relatives, on tests measuring general intelligence. Women with CAIS performed inferior to controls (127) on tests of visuo-spatial ability, but not overall intellectually or verbally (126). The largest study investigating spatial cognition with fMRI in CAIS (n21) failed to detect a difference in performance between patients and female or male controls, although differences in activation where noted, with men having increased activation in the inferior parietal regions. The activation pattern of women with CAIS resembled those of control women more than men, thereby indicating a role for hormonal influences rather than chromosomal effects on brain activation patterns (128). Another study (n=11) of performance on a virtual analogue of the water maze task showed that women with CAIS performed intermediary between men and women (129). To our knowledge there are no previous studies in individuals with hypospadias or complete gonadal dysgenesis on cognitive functions.

1.4.2 Psychosexual outcome in DSD

Many studies in DSD patient groups investigating psychological endpoints in DSD patient groups have focused on psychosexual development, posing research questions pertaining to the biological shaping forces of sex differences (130). In conditions characterized by androgen excess, such as CAH, it has been shown that girls and women more often prefer activities and interests in male dominated areas (131) and choose more male dominated occupations as adults (132,133) and that there is an overrepresentation of women with sexual orientation other than heterosexual (134,135). This is associated with the higher than usual prenatal exposure to androgens that also has effect on play style. Girls with CAH play more similar to boys (136) with a dose response effect, operationalized as disease severity (137). A study that included measurement of socialization showed no crucial role for socialization on psychosexual development in girls with CAH, although some evidence supports individual variation in terms of the size of the role for different influencing factors (138).

In the majority of individuals with conditions characterized by deficiencies in androgen function or synthesis, such as CAIS and Complete GD, gender role and gender identity development is typically female (56,59). In DSD conditions, where individuals with XY sex chromosomes and partial or typical androgen function is present, the picture is more varied (139,140). Men with hypospadias are typically reported to develop and live in a male gender role (141-143). In many DSD conditions, sex of rearing is still the best predictor of gender identity (101,144).

1.4.3 Psychosocial outcome in DSD populations

Studies investigating mental health issues in DSD present varying outcomes. Investigations of quality of life (QoL) have revealed acceptable QoL for groups with a mixture of diagnoses when assessed by generic QoL instruments in comparison to the rest of the population (145-147) and to control group (40,148). When wellbeing is assessed by more multifaceted or sensitive approaches, impaired outcome is reported with respects to psychiatric symptoms, wellbeing and relationships (149), fatigue, attention and memory problems as well as emotional and behavioral problems (150).

Essential to outcome in DSD is also the outcome of genital surgeries. Body satisfaction and sexual wellbeing is varied and often affected, particularly in conditions where early surgical interventions have been performed. The scope of investigations of psychosexual outcome in DSD cohorts with clinical objectives is sometimes not limited to the individual's wellbeing. It also has the objective to assess outcome of practices surrounding sex assignment and early treatments. The majority of individuals with DSD are generally content with their sex of rearing (151) but in comparison to the general populations there is a slight increase in the frequency of gender dysphoria (152) and gender transitions (144) which is sometimes difficult to predict based on information from the clinical diagnosis (153,154).

CAH

The few studies investigating mental health issues in children with CAH show increased prevalence of ADHD, particularly for boys, and increased prevalence of anxiety disorders (155). Self rated QoL for children with CAH differ slightly from the proxyratings by their parents (156). The areas affected, as rated by the children, is fatigue, whereas their parents state academic underachievement, impaired physical and emotional health and psychosocial outcomes (157). For adults, the outcome results are mixed. Some reports show unaffected psychological adjustment measured by self-assessment instruments, (158,159) other report impaired QoL and wellbeing (133) for patients with CAH. Health related QoL outcome domains that are most often affected is general health and vitality (160). Increased prevalence of psychiatric morbidity is present for both men (161) and women, particularly regarding anxiety, depression and substance abuse (162). Compared to other chronic conditions, QoL

investigations have showed better outcome than for patients with diabetes (163) or adrenal insufficiency (164). For males with CAH, QoL has been less often assessed but found to be acceptable, with differences between groups with different treatment strategies. Factors shown to affect QoL and psychosocial outcome are type of glucocorticoid therapy (165,166), suboptimal surgery outcome (167,168) and trauma from investigations in childhood (169).

Sexual function for women with CAH is associated to surgical outcome and is often negatively affected (133,168), particularly in severe genotype groups (170). Patients own perceptions of surgical outcome is not always assessed but have been shown to differ somewhat from clinicians (171).

The prevalence of gender dysphoria has been assessed in girls and women with CAH showing mixed results. Studies show consistently that a majority of girls or women with CAH experience a core female identity (172) but may still have experienced that they "…wanted to be of the opposite sex" (134). Gender identity issues are prevalent also in individuals with 46,XX karyotype and CAH raised as males although these results must be treated with caution since there is a scarcity of studies (172,47). With respect to recalled childhood gendered play behavior, sexual preferences, and core gender identity, 46,XY boys with CAH did not differ from other boys (134,173). Most women with CAH report heterosexual orientation but between 10-50% report other than heterosexual orientation (135), compared to around 2.5 % in the Swedish population (133).

Qualitative studies in groups of women with CAH focus on the impact of the condition on relationships, sexuality, gender identity, gender role and healthcare experiences. Negative impact of the condition vary, but includes suboptimal outcome of surgery, and problems in forming relationships (42,44).

Patients report isolation and embarrassment over information concerning the condition as well as discomfort with aspects of the medical treatment, anxiety about sexual contacts and impaired body image (41).

CAIS

There are a few reports on psychosocial wellbeing and QoL in CAIS, but outcomes are contradictory, possibly because of small patient numbers and participation bias (174). Some studies indicate better outcome compared to other individuals with DSD diagnoses (116) or unaffected compared to controls (56). An Italian study in a larger cohort reported good psychosocial adjustment, superior academic achievement and QoL, while at the same time higher frequency of psychological distress such as depression, anxiety, internalizing and externalizing symptoms.

The few reports of gender identity/gender role behavior from populations of CAIS individuals show female identification and female gender role behavior (175), although singular cases of gender dysphoria have been reported (57).

Qualitative studies of women with CAIS report distress related to infertility, deviation from norms around female, low self esteem and fear of devaluation on the basis of having CAIS (176). A German follow up study of eleven participants with CAIS described difficulties coping with the disorder and its psychological consequences and others report high percentages that had utilized psychological care or support of some kind (57). Typically, most of the women with CAIS receive the diagnosis in the teenage years. This experience is described as traumatic while reactions and coping abilities have been shown to depend partly on life experiences before receiving the diagnoses (43).

Hypospadias

Many studies of psychosocial outcome in hypospadias show acceptable QoL (142) despite some indications of adverse outcomes like dissatisfaction with sexual wellbeing and negative penile perception post surgery (177,178). A large cohort study based on registry data showed that in most psychosocial outcome parameters studied (education level, income level, and marriage), men with hypospadias did not differ from controls (67). They did however have an increased risk of receiving disability pension; this was particularly pronounced in men with proximal hypospadias and associated with increase in psychiatric morbidity and neurodevelopmental disorders such as autism spectrum disorder (ASD) and intellectual disability (67).

2 AIM

The aim of this thesis is to increase knowledge on outcome in DSD patient groups with regards to psychosocial outcome, psychiatric comorbidity, cognitive abilities and gender role behavior. Furthermore it discusses the contribution of biological factors to sex differences in cognition and gender role behavior.

2.1 RESEARCH QUESTIONS

- Does psychosocial outcome for men and women with CAH differ from population controls? (Study I)
- Are there sex differences and does disease severity moderate psychosocial outcome for men and women with CAH? (Study I)
- What is the prevalence of psychiatric morbidity in women with CAIS, GD, POI?(Study II)
- Are there differences between women with congenital or acquired hormone dysfunction in the prevalence of psychiatric morbidity? (Study II)
- Do factors related to variation in sex chromosomes and/or androgen function affect performance on cognitive tasks that typically yield sex differences? (study III)
- Does selfreported gender role behavior and performance on cognitive tasks that typically yield sex differences differ between men with and without hypospadias or between men with different severity of hypospadias? (study IV)

2.2 DESCRIPTION OF THE PROJECT

These studies are part of a larger follow-up program of adult patients with DSD in Sweden including a clinical retrospective follow-up, a prospective study of children assessed for sex assignment in the new born period and registry studies of patient cohorts.

3 OVERVIEW OF STUDY I-IV

3.1 STUDY I

Suboptimal psychosocial outcomes in patients with CAH.

3.1.1 Background

This is a matched case cohort study comparing the existing and historical cohort of CAH patients in Sweden to a matched control group from the general Swedish population investigating psychosocial outcome. Measures were chosen in order to replicate previous research findings from other countries on psychosocial outcome parameters and to reflect areas of daily life of importance for quality of life.

3.1.2 Methods

Setting

The study was conducted in collaboration with the institute of medical epidemiology and biostatistics (MEB) at Karolinska Institutet.

Data sources - registers

All patients with a confirmed CYP21A2 deficiency born from 1910 and onward are included in the *national CAH register* (46) held by the newborn screening laboratory in the Centre for Inherited Metabolic Diseases CMMS, Karolinska Hospital. *The national patient register (NPR)* contains information on patient, geographical, administrative and medical data from inpatient care at public hospitals. The register is updated once a month from each of the county councils in Sweden. The register used to contain data on inpatient care only, but incorporates outpatient visits for surgery and psychiatric care since 2001. Primary care is not yet covered in the NPR. It was used in study I to identify patients not included in the national registry which had the diagnosis of CAH. *The total population register* contains information on births, civil status, citizenship, migration and place of residence. It is held by Statistics Sweden. *The multi-generations register* contains information on relationships between people born after 1932 and their parents/adoptive parents. Both were used in study I to identify controls, assess marital status and the number of children of patients. *Longitudinal integration database for health and labor market studies, (LISA*) is a database containing information on income, education, employment, and data from the social insurance agency. Data is available from 1990 and onward. This database was the main sources of outcome data in study I. *The education register* contains further information on education from 1985 and

onward and was used in study I to assess eligibility for higher education for the part of the cohort born after 1989.

Study population

Women and men with congenital adrenal hyperplasia (CAH)

CAH patients (n=588) either included in the national CAH registry (46) (n=545) or detected through the National Patient registry (n=43) constitute the study group.

The patients identified through the national patient registry were those that had been given the diagnosis of CAH (ICD-8: 255.01, 255.08; ICD-9: 2552, 255C; ICD-10: E25.0) on more than two occasions and that had no other endocrine diagnoses (i.e. Cushings syndrome, acromegaly) or malignancies requiring glucocorticoid treatment.

All patients with a confirmed CYP21A2 deficiency, born between 1910-2009, were included. The patients were matched for sex, year and place of birth with 100 controls per patients from the general population. The patients' identities were anonymized before linkage in registries and analyses were performed on the cohort as a whole and on subgroups based on clinical severity (salt wasting SW, simple virilising SV, non classic NC) and type of mutation. This classification of genotype group was made depending on the severity of the mildest *CYP21A2* allele. The classification in groups of clinical severity was made either by that predicted by genotype or by the authors (AN, HF) through medical information.

Table 2. Classification of clinical severity based on genotype and number of participants in each genotype group. NR= identified in national registry only. NPR=identified in national patient registry only.

SW n=240	**SV n=167**	**NC n=75**	**Unknown n=106**
Null n=100	I172N n=130	V281L n=56	NR n= 63
I2splice n=122	P30L n=24		NPR n=43
Clinical n=18	Clinical n=13	Clinical n=19	

Table 3. Outcome measures for psychosocial outcome

Measures

Complete education	Eligibility for secondary education (LISA) [1]
Primary education	To have basic education as the highest level of education.
Higher education	To have attained education after primary education.
Working 3-7 years	To have been employed more than 3, less than 7 years consecutively.
Working >7 years	To have been employed for more than 7 years consecutively.
High income	Disposable family income in the top 80th percentile.
Low income	Disposable family income below the 20th percentile. Income was including earned income and allowances.
Sick leave	To have had sick leave periods (longer than 14 consecutive days) during a period of two years.
Disability pension	To ever have received compensation in the form of early retirement given to those not able to participate in the open labor market (LISA).
Social welfare	Dependency on social welfare for anyone in the family for more than one year (LISA).
Marriage	The first registered marriage or partnership (Multigenerations register).
Children	Whether one had biological children, first child. (Multigenerations register).

[1] The eligibility to pursue secondary education change over time and between secondary educations preparatory for university or work related programs. Generally it includes having passed in 8-12 subjects, particularly Swedish, English and Math.

Statistical methods

All outcome measures were modeled as dichotomous and a logistic regression was used to calculate odds ratios (OR). ORs were calculated with 95% confidence intervals (CI). An OR with a CI not surpassing 1 was considered significant. Calculations were performed using SAS version 9.3 (Statistical Analyses Systems).

3.1.3 Results

Patients with CAH were less often eligible for secondary education than controls. They had more sick leave periods and had received disability pension to a higher degree and were less prone to have children than controls. Less women with CAH than controls were eligible for secondary education. Men more often had steady employment and also more sick leave periods than controls. Both women and men with CAH were less likely to have children than controls (table 4).

Table 4. ORs (CI) for all parameters, comparisons between all individuals with CAH and controls, women with CAH and female controls and men with CAH with male controls.

	All CAH n=588	♀ **n= 335**	♂ **n=253**
Complete education	**0.5 (0.3-0.9)**	**0.3 (0.2-0.6)**	0.9 (0.4-2.1)
Primary education	0.8 (0.6-1.1)	0.8 (0.5-1.4)	0.8 (0.5-1.3)
Higher education	0.7 (0.4-1.2)	0.9 (0.4-1.7)	0.5 (0.2-1.3)
Working 3-7 yrs	1.3 (0.7-2.2)	1.4 (0.7-2.8)	1.3 (0.5-3.6)
Working >7 yrs	1.8 (0.99-3.2)	1.6 (0.8-3.2)	**3.1 (1.1-8.8)**
High income	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.8 (0.6-1.2)
Low income	0.9 (0.6-1.4)	0.8 (0.5-1.4)	1.0 (0.5-2.0)
Sick leave	**1.7 (1.2-2.4)**	1.3 (0.8-2.0)	**2.8 (1.6-4.8)**
Disability pension	**1.5 (1.0-2.2)**	1.4 (0.9-2.4)	1.6 (0.8-3.2)
Social welfare	1.0 (0.7-1.4)	1.1 (0.7-1.7)	0.9 (0.5-1.6)
Marriage	1.0 (0.8-1.4)	0.7 (0.5-1.0)	**1.6 (1.0-2.5)**
Children	**0.3 (0.2-0.3)**	**0.2 (0.1-0.3)**	**0.4 (0.2-0.6)**

Comparisons between individuals with SW CAH and controls

Patients with SW CAH had received disability pension more often than controls and both men and women had less children than controls. Women with SW were less likely to be eligible for secondary education but were more likely to have income in the top quintile compared to female controls (table 5).

Table 5. ORs (CI) for all parameters, comparisons on the level of patient groups subdivided by severity and sex between women and men with SW CAH**.**

	All SW n=240	**SW♀ n=105**	**SW♂ n=135**
Complete education	1.4 (0.4-5.2)	**0.3 (0.1-0.7)**	1.2 (0.3-4.6)
Primary education	1.3 (0.8-2.2)	1.4 (0.7-2.9)	1.2 (0.5-2.5)
Higher education	0.7 (0.5-1.1)	0.7 (0.4-1.1)	0.9 (0.5-1.7)
Working 3-7 yrs	0.9 (0.3-2.5)	0.7 (0.2-2.6)	1.4 (0.3-6.7)
Working >7 yrs	2.3 (0.6-13.6)	2.0 (0.6-6.7)	2.9 (0.6-13.6)
High income	0.9 (0.5-1.4)	**2.0 (1.0-4.2)**	1.0 (0.5-1.9)
Low income	0.9 (0.4-1.9)	1.2 (0.5-3.1)	0.5 (0.1-1.9)
Sick leave	1.6 (0.9-3.0)	1.6 (0.9-3.0)	1.7 (0.7-4.4)
Disability pension	**2.0 (1.0-3.9)**	1.7 (0.7-4.0)	2.2 (0.7-6.9)
Social welfare	0.8 (0.4-1.4)	0.6 (0.3-1.4)	1.1 (0.4-2.6)
Marriage	0.9 (0.5-1.5)	0.5 (0.2-1.1)	1.6 (0.7-3.5)
Children	**0.1 (0.1-0.2)**	**0.05 (0.0-0.1)**	**0.4 (0.2-0.8)**

Table 6. ORs (CI) for all parameters, comparisons on the level of patient groups subdivided by severity and sex between women and men with SV CAH.

	All SV n=167	**SV♀ n=91**	**SV♂ n=76**
Complete education	0.6 (0.2-1.5)	0.3 (0.1-1.1)	1.0 (0.2-4.9)
Primary education	0.5 (0.2-1.1)	0.5 (0.1-1.6)	0.5 (0.2-1.5)
Higher education	**1.5 (1.0-2.3)**	1.4 (0.8-2.4)	1.7 (0.9-3.4)
Working 3-7 yrs	1.5 (0.5-5.0)	1.7 (0.4-7.5)	1.7 (0.2-15.2)
Working >7 yrs	1.5 (0.4-5.3)	1.0 (0.2-4.7)	7.3 (0.7-79.8)
High income	1.3 (0.7-2.2)	1.0 (0.6-2.0)	0.5 (0.2-1.0)
Low income	0.5 (0.1-1.7)	0.6 (0.1-2.7	0.3 (0.0-2.9)
Sick leave	**2.8 (1.4-5.4)**	**2.6 (1.1-6.4)**	**3.4 (1.3-9.4)**
Disability pension	0.8 (0.4-1.9)	0,9 (0.3-2.6)	0.9 (0.2-3.5)
Social welfare	0.7 (0.3-1.5)	0.7 (0.3-1.8)	0.7 (0.2-3)
Marriage	1.4 (0.8-2.3)	1.1 (0.6-2.2)	1.8 (0.8-4.4)
Children	**0.4 (0.2-0.7)**	**0.4 (0.2-0.7)**	**0.3 (0.2-0.8)**

Comparisons between individuals with SV CAH and controls

Both women and men with SV had pursued secondary education more often than controls and had more sick leave periods. Both women and men had children less often (table 6).

Comparisons between individuals with NC CAH and controls

Patients with NC CAH had steady jobs more often and less sick leave periods than controls. They were more prone to receive disability pension. Women with NC more often had a steady job and were more likely to have received social welfare (table 7).

Table 7. ORs (CI) for all parameters, comparisons on the level of patient groups subdivided by severity and sex between women and men with NC CAH.

	All NC n=75	**NC♀ n=56**	**NC♂ n=19**
Complete education	0.5 (0.1-1.9)	0.5 (0.1-2.5)	0.5 (0.0-6.0)
Primary education	0.6 (0.3-1.2)	0.4 (0.1-1.9)	1.9 (0.2-19.5)
Higher education	1.8 (0.9-3.5)	1.9 (0.8-4.1)	1.7 (0.4-7.7)
Working 3-7 yrs	**7.6 (1.5-37.4)**	**6.5 (1.2-35.1)**	◊
Working >7 yrs	4.5 (0.8-25.4)	3.5 (0.6-20.8)	◊
High income	2.1 (0.9-4.9)	2.0 (0.8-5.3)	2.7 (0.3-23)
Low income	1.0 (0.9-4.9)	1.3 (0.4-4.4)	◊
Sick leave	**0.3 (0.1-0.7)**	0.3 (0.1-1.1)	0.5 (0.1-8.5)
Disability pension	**3.3 (1.0-11.1)**	3.4 (0.9-11.8)	◊
Social welfare	2.0 (0.9-4.9)	**2.4 (1.0-6.2)**	1.2 (0.1-10.8)
Marriage	1.7 (0.7-4.3)	1.4 (0.5-3.9)	3.9 (0.5-32.7)
Children	0.9 (0.3-2.4)	0.9 (0.3-2.7)	0.9 (0.1-7.1)

◊ ORs cannot be calculated.

3.1.4 Conclusion

The study showed that the patient group in many aspects did not differ from controls when compared on a whole cohort level. When divided by sex and severity, psychosocial outcome differed from controls for some groups. Women, particularly those with SW form, were more negatively affected in terms of being less often married and not being eligible for secondary education while men differed from controls both regarding employment and sick leave. Both men and women have decreased fertility.

3.2 STUDY II

Increased psychiatric morbidity in women with complete androgen insensitivity syndrome or complete gonadal dysgenesis.

3.2.1 Background

Previous research on psychosocial and psychological outcome for women with CAIS and GD is mixed with some studies indicating minimal impact on psychosocial while at the same time larger impact in the form of mental health issues related to the condition. There are few previous studies of psychiatric symptomatology in CAIS, GD and POI. The aim of this study was to assess the prevalence of psychiatric morbidity in a group of women with CAIS and GD and to see whether it differs from that in women with POI.

3.2.2 Methods

Setting

This study was performed in a clinical setting as a cross-sectional follow-up taking place alongside routine annual or bi annual checkups at the department of obstetrics and gynecology, Karolinska University hospital. The study was part of a larger project assessing medical and mental health, psychosocial and cognitive outcome including gynecological, endocrine and anatomical asessments. The patients spent one or two days at the clinic and received economic compensation for loss of earnings.

Study population

The participants were women with CAIS (n=20), complete GD (XYGD n=6, XXGD n=7) and women with POI (n =21).

Women were recruited from all parts of Sweden through their treating physician; the majority of the cohort from the department of obstetrics and gynecology, Karolinska University Hospital and Uppsala University hospital. The control group (n=61) were matched on age and sex with the participating female patients and recruited from the national patient registry by letter of invitation (table 8).

Table 8. Characteristics of the study population, age, level of education and relationship status.

			Highest level of education	
	Mean age (range)	In a relationship	Secondary	University
♀ DSD n33	33 (21-57)	21 (64%)	15 (45%)	18 (55%)
♀ POI n21	21 (21-44)	15 (71%)	9 (43%)	12 (57%)
♀ Control n61	32 (21-57)	46 (75%)	22 (36%)	38 (62%)
Subgroups ♀ DSD				
CAIS n20	34 (21-57)	10 (50%)	11 (55%)	8 (40%)
46 XY CGD n6	34 (24-47)	3 (50%)	2 (33%)	5 (71%)
46 XX CGD n7	28 (23-36)	5 (71%)	2 (29%)	4 (67%)

Measurements

The lifetime prevalence of psychiatric morbidity was assessed using a validated clinical interview, the Mini International Neuropsychiatric Interview (MINI+), a structured diagnostic interview developed to assess the 17 most common diagnoses in DSM-IV and ICD-10, for use as a clinical screening tool or within research settings. This is a frequently used method to assess psychiatric morbidity with high sensitivity, specificity and test retest reliability as well as acceptable inter-rater reliability (179,180).

In addition survey questions regarding previous use of psychiatric healthcare and psychotropic drug use were analyzed. The questions were part of a larger online survey that all patients and controls filled out prior to participation containing questions on educational level and marital status. A psychiatrically trained physician or psychologist performed the interviews that lasted 15-60 minutes.

Questions regarding utilization of psychiatric healthcare, psychotropic drugs:

Self reported utilization of healthcare, medication for mental health problems.
Have you ever been in contact with health care services because of mental health problems?
Do you take any medication (psychotropic drugs) at the moment?
Have you ever taken any medication for mental health problems?

3.2.3 Results

On the self-report questions, 64% of the women with DSD answered that they had been in contact with mental health services compared to 43% among those with POI and 40% of controls. 15% of women with DSD were currently on medication as compared to 10% of women with POI and 13% of controls. Previous use of medicine was reported for 39% of women with DSD, 33% of women with POI and 31% of controls. None of these differences were significant.

Table 9. P-values and odds ratios for differences in psychiatric morbidity for women with DSD (CAIS, XYGD, XXGD) and POI and compared to female controls.

	♀DSD vs ♀POI	♀DSD vs ♀Controls		♀POI vs ♀Controls
	p	p	OR (CI 95%)	p
At least one psychiatric disorder	0.476	0.003*	**5.1 (1.7, 14.9)**	0.07
At least two psychiatric disorders	0.451	0.008*	**3.2 (1.3, 7.8)**	0.29
Major depressive episode	0.775	0.030*	**2.7 (1.1, 6.5)**	0.14
At least one anxiety disorder	0.403	0.003*	**4.2 (1.7, 10.3)**	0.16
Obsessive Compulsive disorder	0.131	<0.000*	**21.1 (2.5, 175.3)**	0.26

Comparing women with CAIS or GD with, either women with POI, or population controls showed that both groups of women with conditions affecting prenatal steroid production or sensitivity and the group of women with acquired deficiency of steroid production had increased risk for psychiatric morbidity. Among the women with CAIS/GD, 85% fulfilled the criteria for one or more psychiatric disorder, which was higher but not significantly so than the 76% of women with POI, but significantly more than controls where 54% had at least one psychiatric disorder. In both patient groups (CAIS/GD,POI), 67% of women had experienced at least one mood disorder and 58% of women with CAIS/GD and 42% of women with POI had experienced at least one anxiety disorder (table 9).

3.2.4 Conclusion

There is an increased prevalence of psychiatric morbidity in women with CAIS and GD especially regarding anxiety disorders. Due to this, increased attention to the presence of mental health issues is warranted in the care for all women with DSD and POI. The basic care of DSD women should include a screening for psychiatric symptoms and also include an offer for psychological support.

3.3 STUDY III

Cognitive abilities in complete androgen insensitivity, CAIS or gonadal dysgenesis, GD

3.3.1 Background

Many questions remain unanswered regarding mechanisms behind sex differences in cognitive abilities. Prevailing theories concerning biological contributions to these differences in performance and/or ability focus on early and prolonged exposure to sex hormones. There is however evidence of direct effects of sex chromosomes on sex typical behavior in other domains and in other species. This is a research area where interaction between effects of socialization, and different biological contributions challenge research design. Including individuals with DSD as subjects is one way to investigate biological contributions to sex differences.

3.3.2 Methods

Setting

The subjects were recruited and assessed in the same cross-sectional follow up setting as study II, alongside the annual or biannual checkup.

Study population

The participants were women with CAIS (n=18) or XYGD (n=6) or XXGD (n=7). Women were recruited from all parts of Sweden through their treating physician; the majority of the cohort was from the department of obstetrics and gynecology, Karolinska University Hospital and Uppsala University Hospital. The control groups were matched on age (day of birth) and sex, recruited from the national patient registry by letter of invitation. The female controls were matched with the women with CAIS, CGD while the men were matched with a cohort of men with hypospadias, the participants in study IV. In the female control group from the general population many women turned out to be nonnative Swedish speakers. To compensate for this some additional female controls (n=18) in the same age range were recruited from groups of students and administrators at KI and Stockholm University.

The female controls completed the assessment at a clinic visit, except for those recruited at a later point in time. Most of the men in the male control group participated online, from home.

Measurements

The outcome parameter was performance on cognitive tasks that typically yield sex differences. A battery of online tests was constructed for this study. The tasks were chosen because they had previously been shown to yield group level sex differences in studies performed in the general population (181). The tasks in which men often perform at a higher level than women were mental rotation task (182) and a version of the judgment of line angle task, (183). The tasks where women typically outperform men were verbal fluency (FAS), episodic memory with faces as stimuli (91) and an emotion recognition task "The Reading the Mind in the Eye" test (184). A vocabulary test (SRB) was included typically not yielding sex differences and was used to indicate level of verbal ability in the different groups. The tests were adapted for online use with minor adjustments and Swedish instructions.

Statistical analysis

When analyzing performance on tests where language proficiency could affect results, all controls born abroad were excluded from the analysis. Comparisons with regards to differences in performance were made with one-way ANOVA with group (CAIS/XYGD, female controls, male controls) as fixed factor. The women with CAIS and XY were combined into one group as they were assumed to have similar exposure to androgens and sex chromosome complement. In order to explore differences between patient groups for hypothesis generating purposes, a one-way ANOVA with group (XYGD, XXGD, CAIS) as fixed factor was computed. When the effect of group was significant, Tukey post hoc tests were computed. All analyses were two tailed, with alpha set to <.05.

3.3.3 Results

Table 10. P-values for comparisons between female and male controls, women with CAIS or XYGD and between patient groups women with CAIS, XYGD and XXGD on all tasks.

	Vocabulary	Spatial direction	Mental rotation	Episodic memory	Word fluency	Typing speed	Emotion recognition
♀ controls vs ♂ controls	0.03*	0.00**	0.00**	0.02*	0.00**	0.65	0.02*
CAIS/XYGD vs ♀ Controls	0.74	1.00	0.99	1.00	0.03*	0.92	0.02*
CAIS/XYGD vs ♂ Controls	0.49	0.01*	0.01*	0.16	0.28	0.98	0.68
Patient groups contrasts							
CAIS vs XYGD	0.90	0.73	0.69	0.97	0.04*	0.80	0.59
CAIS vs XXGD	0.99	0.97	0.69	0.39	0.09	0.34	0.00**
XYGD vs XXGD	0.95	0.69	1.00	0.68	0.36	0.27	0.00**

Note[(a)] Typing was included as a covariate when analyzing differences in word fluency performance

All women and men in the control groups differed on all tasks except typing speed. Women with CAIS and XYGD underperformed relative to female controls on the emotion recognition and verbal fluency task. Women with CAIS and XYGD underperformed relative to the male control group on the spatial direction and mental rotation tasks. In the comparisons between patient groups, women with CAIS underperformed relative to women with XYGD on the word fluency task and there was a trend in the same direction relative to women with XXGD. Finally women with XXGD outperformed both women with CAIS and XYGD on the emotion recognition task (table 10).

3.3.4 Conclusion

The results support theories of androgen influence on cognitive abilities as women with dysfunction in androgen sensitivity or production perform lower than male controls and on the same level as female controls. There are also indications of effects more directly linked to sex chromosomes on variation in emotion recognition performance as women with CAIS/XYGD perform on the same level as male controls. The superior performance of women with XXGD could also be related to their special hormonal and chromosomal situation, but due to the small participant numbers this needs to be replicated before conclusions can be drawn. The conclusions hitherto do not exclude the possibility of parallel effects of socializing processes.

3.4 STUDY IV

No difference in cognitive performance or gender role behavior between men with and without hypospadias

3.4.1 Background

Hypospadias is a common genital malformation of different severity, affecting 1/125 boys born in Sweden yearly. The incidence has been reported to be increasing, etiology is multifactorial and the exact cause is often not known. Since male genital development is dependent on androgen action the question has been raised of whether hypospadias is also associated with variations in psychosexual outcome or if other aspects of development are affected. The few previous studies investigating gender role behavior in hypospadias have been inconclusive indicating increase of cross-gendered behavior (185) or no differences (141). There were no previous studies of cognitive abilities in boys or men with hypospadias.

3.4.2 Methods

Setting

A cohort of men with hypospadias (n=83), who were operated on at Karolinska when they were children, who lived in and around the Stockholm area, and had agreed to participate in the larger cross sectional follow up study (178) were invited to participate in cognitive assessment. The majority of men with proximal form of hypospadias participated in cognitive testing as part of an outpatient clinic visit. All the men with distal hypospadias, and all controls were offered the possibility to participate online.

Patients and controls

Men with hypospadias of different severity participated. The majority had distal form (n=73) and a small group of men had proximal hypospadias (n=10). A female control-group (the same as in study III) was included to ensure the validity of the cognitive tests and gender role questions.

Measurements

The cognitive battery of tests accessible online were the same as in study III. Participants also completed a web survey containing instruments measuring psychosocial outcome such as questions inquiring about childhood gender role behavior. These questions were used to assess childhood toy and activity preferences and sex of best friend in childhood, in adolescence or in adulthood.

Statistical analysis

Comparisons with regards to differences in performance on the cognitive tests were made with one-way ANOVA with group (female controls, male controls, men with hypospadias) as fixed factor. In order to explore differences between patient groups based on severity, for hypothesis generating purposes, a one-way ANOVA with group (men with distal hypospadias/men with proximal hypospadias) as fixed factor was computed. When the effect of group was significant, Tukey post hoc tests were computed. All analyses were two tailed, with alpha set to <.05. When analyzing performance on tests where language proficiency could affect results, all patients and controls born abroad were excluded from the analysis.

Comparisons with regards to self reported gender role behaviour were not expected to be normally distributed, therefore nonparametric statistical methods were applied: The Kruskal-Wallis test to compare the three groups (female controls/ male controls/ men with

hypospadias) and a Mann-Whitney U test for post hoc comparisons of significant results. For comparison between men with distal (n=73) and proximal hypospadias (n=10) Mann-Whitney U test was used.

3.4.3 Results

Background factors such as age and education were equal between the different groups. Both the cognitive tests and the questions on gender role behavior showed sex differences between the male and female control group in the expected directions. No significant differences were found between men with hypospadias and men in the control groups neither on the cognitive tasks, nor on self reported gender role behavior in childhood. Men with proximal hypospadias performed generally lower on the cognitive tasks, exept for on the emotion recognition task. This reached significance only on the test of episodic memory (p=.031) and word fluency (p=0.038).

3.4.4 Conclusion

Hypospadias in general is not associated with differences in performance on cognitive tests that typically yield sex differences, or with altered gender role behaviour in childhood. This indicates that the disturbance of genital growth occurring in the prenatal period, a possible sensitive period for brain development when sex differentiation of brain anatomy and function takes place, is not reflected in adult life in terms of differences in cognitive abilities or recalled childhood gender role behaviour. The lower results on cognitive measures overall in boys and men with proximal hypospadias warrant further studies.

3.5 ETHICAL CONSIDERATIONS

There are a number of ethical issues currently discussed in the field of DSD patient care and research (186). With regards to clinical care, the debate has for some time centered on the timing of surgery, patients right to full disclosure of information and right to make decisions about irreversible cosmetic surgery and hormonal treatments. Healthcare providers, researchers, patient organizations and government offices are attending to these questions attempting to produce knowledge and mapping of the healthcare issues but also to influence policy. In Sweden the issues concerning early surgery has been acknowledged by SMER, the Swedish Medical Etic advisory board (187) and other aspects of health cares approach to individuals with DSD as a patient group have been described by the Swedish National Board of Health and Welfare (188). Attention has also been received from non profit organizations like Amnesty (189), particularly concerning the question of timing of surgical and other irreversible interventions.

The Council of Europe issued a paper in 2015 describing human rights of intersex people in Europe (190) and has recently issued a statement concerning healthcare approaches to DSD patients both of which has been responded to by members of the professions (191,192).

Since medical healthcare in itself, with the best of intentions, has contributed to problems in these patient groups in the past, there is no guarantee that treatments of today are completely free from unexpected adverse effects. While this thesis incorporates and touches upon the questions concerning treatment and while the program overall includes investigations of sexual health and other intimate topics, the need for ethical considerations have been somewhat more restricted in this particular project.

Efforts to make this project as efficient and non-intrusive as possible were made. First a screening carried out by a patient representative of our survey and study design for potentially negative or offensive framings of problems or questions. Second, we tried to do as much as possible at one visit, working multi-professionally. Third, we have provided patients that were not in a follow up program with medical advice and referrals (men with hypospadias) and psycho-education concerning the condition, if wished for (women with DSD and POI) and limited psychological counseling and facilitation of access to mental health care if needed (all patient groups). The offer to receive psychological counseling was not extended to controls. In hindsight this might seem regrettable, since there was a comparably high prevalence of psychiatric comorbidity in this group too.

4 DISCUSSION

This thesis set out to investigate psychosocial, psychological and psychiatric aspects of living with a DSD condition and also to test theories on the biological contributions to sex differences in behavior and cognition.

4.1 MAIN FINDINGS

Briefly stated

- There were suboptimal psychosocial outcomes for patients with CAH regarding education, ability to participate in worklife and fertility that differed depending on disease severity and sex.
- Psychiatric morbidity was increased in women with CAIS, GD and POI.
- Women with CAIS and GD perform more similar to women than men on cognitive tasks that usually yield sex differences.
- There were no differences between men with hypospadias and male controls on cognitive tasks that usually yield sex differences or on self-reported gender role behavior.

4.2 PSYCHOSOCIAL AND PSYCHIATRIC ISSUES IN THE STUDIED POPULATIONS

Sex and severity moderate outcome

We found that while psychosocial outcome was comparable to controls in some aspects it was negatively affected for men and women with CAH. This was particularly evident regarding education and ability to participate in work life, resulting in dependency on social insurance (sick leave and disability pension), and fertility was also decreased.

The lower academic achievement particularly for the female patients with the severe type of the condition was one of the findings in study I. Chronic conditions in general are associated with poorer academic achievement (193) which has been accredited in part to the success of pediatric medicine in improving long term survival (194). Adolescents living with chronic conditions sometimes have a double disadvantage that affects academic achievement (195) although the reasons behind this may vary between conditions. In CAH, the treatment has to be managed everyday in order to optimize physical and mental capacities to cope with internal and external stressors, as well as maintain basic functions. This in itself can be a

stressor and a burden. Problems with adherence are not much studied in CAH but if present, may contribute to the impaired ability to cope with stressors. It is known from stress research that glucocorticoid deficiency, as well as overexposure, affects cognitive function (196,197). In CAH, higher doses have been linked to cognitive, particularly executive dysfunction (198). In study I however, since lower academic achievement (being less likely to be eligible for secondary education) was only true for girls, we did not assume that cognitive dysfunction was the primary reason for suboptimal outcomes. It may however contribute to a spectrum of risk factors.

We interpreted the suboptimal academic achievement as being primarily dependent on other psychological factors. Indeed, in subsequent studies in the same cohort, increased psychiatric morbidity was found for women (162) and men (161) with CAH, where the increased risk in women with the more severe forms of the condition was not consistently found for men. This pattern corresponds to the results in Study I where women with SW were least likely to be eligible for secondary education.

To have CAH increased the odds of having periods of sick leave or to receive disability pension indicating that capacity for full time participation in work life is reduced. This replicates findings from a Norwegian study (160). While the reasons for being on sick leave or to be entitled to disability pension in the individual case can be manifold, it is possibly affected by ability to cope with stress and demands of daily life. Sick leave and disability pension in the Swedish social insurance system is at present two forms of allowances, administered by the same authority and can be seen as a continuum as one often precedes the other. It may be that these two outcomes reflect the same underlying vulnerability and that disease severity moderates the outcome where patients with more severe forms (SW) more often had disability pension and less severe (SV) more often were on sick leave.

Fertility in women with CAH have previously found to be impaired (160,170,199) particularly for those with SW and SV form (200) whereas those with NC form have been reported to be normal (201). Reasons for infertility have not been sufficiently clarified but hypothesized to relate to factors related to hyperandrogenism, outcome of genital surgery and subsequent impaired sexual health and reduced motivation to become a parent (200). Fecundity for those who have attempted and persisted to get pregnant is also reported to be normal (202) which indicates that healthcare interventions make a difference for those who wish to conceive.

The lower fertility in men with CAH, which was not present in men with non-classic CAH, has been the subject of previous and present research. Lower fecundity for men with CAH, despite reporting living in a relation and/or equal or higher marriage frequency, has been noted also in other cohorts (203) and has been hypothesized to result from both hyperandrogenism and also suboptimal psychosocial adaptation (204). Secondary problems related to CAH that have been connected to lower fertility are benign adrenal rest tumors and

suppression of gonadotrophin secretion. The latter may lead to hypogonadotropic hypogonadism resulting from under treatment and may be corrected with at least partial restitution of fecundity (203). Further exploration of fertility in the whole cohort of Swedish men with CAH comparing those born before and after newborn screening showed that older men have children by adoption to a higher degree than younger men who more often have biological children (205) indicating that improvement in disease management early in life makes a difference.

Women with DSD conditions have more adverse outcome

Suboptimal outcomes were present for both women and men with CAH, yet the women seemed to fare worse. Particularly this was true for women with the most severe genotype, i. e. SW form of CAH. They were married much less often than female controls and even though the rate of registered partnerships was increased, this did not compensate for the discrepancy since men were married to a larger extent than controls. Lower rates of marriage have been reported in groups living with chronic conditions (194) and there are also sex differences but in the opposite direction for individuals with growth hormone deficiency (206). The individual reasons for this may perhaps best be found in qualitative research based on interviews. One such report summed up and interpreted the challenges faced by women with CAH as "….a struggle for dignity, individuality and self agency on the one hand and shame, on the other" (44). However, the most obvious difference between men and women with CAH is perhaps that while the men have a chronic condition affecting daily living, the women also experience the aspects of the condition associated with DSD, i. e. those affecting psychosexual development. This seems to constitute a somewhat more complex risk factor for adverse psychosocial outcome.

In study II we found a larger prevalence of psychiatric morbidity in a group of women with CAIS and Gonadal dysgenesis compared to controls. This indicates that a DSD condition or some factor related to it constitute a risk factor for the development of psychiatric symptoms in women. We hypothesized that congenital adverse hormonal situation would constitute a risk factor compared to acquired hormonal deficiency but found no support for this as the prevalence of psychiatric diagnoses did not differ between women with DSD and women with POI. Shared factors between women with CAIS and POI and many other women with DSD are that the knowledge of the condition is received during critical periods of identity formation (teenage years or childbearing years). Many patients describe a trauma connected to receiving a diagnosis and finding out about physical differences that impact fertility and putting one in a position that differs from peers at a point when this is crucial; not receiving your menses during the teenage years, or to not be able to have children when your friends are forming families. Previous trauma of other kinds less related to the DSD condition could also be present. We did not include a measurement of previous traumatic experience but qualitative interviews with some of the participants showed that this had an impact on the way the diagnosis was understood and how coping strategies were developed (43). Being part

of a minority might also play a role as a psychosocial stress factor. The concept of minority stress (207) has not yet been investigated systematically in DSD but is sometimes mentioned as a mediator of psychosocial outcome (208).

Socioeconomic status was not evaluated in our studies and could impact the results. Previous studies in CAIS and women with other XYDSD diagnoses have however observed superior socioeconomic outcomes in a Danish population study where women with XYDSD had higher income than controls (209) and an Italian study of women with CAIS where educational and professional achievement were superior to that of controls (210). Other possible factors acknowledged as risk factors in women with DSD in general is poor outcome of surgical interventions (167) contributing to suboptimal sexual wellbeing and function (211,212), the removal of gonads, substitution with synthetic hormones and exposure to repeated genital examinations (169).

Our results, indicating increased vulnerability for female patients with DSD conditions are not unique. Previous research has also concluded that women with DSD fare worse than men in terms of psychosocial adjustment. Is this a phenomenon occurring in DSD conditions only or are there general factors with impact? Women are in general more affected by mood disorders. Epidemiological studies estimate the lifetime prevalence for women to be 21.3%, which is almost twice that of men 12.7% (213). This sex gap emerges in adolescence and wanes in midlife. Both biological background factors and social background factors are hypothesized to contribute to this discrepancy and both include multifactorial explanations. Genetic vulnerability together with fluctuations in sex hormones (74) and HPA and HPG axis dysregulation have been discussed (75). Sex hormones, particularly progesterone and estradiol, are important modulators of neurotransmission that have impact on both serotonergic and dopaminergic pathways. These are also pathways showing sex differences (16). The abundance of glucocorticoid and gonadal steroid receptors in large parts of the brain is also an indication that sex steroids and glucocorticoids impact many transmitter substance systems by a variety of mechanisms (214).

Biological vulnerability alone cannot explain these differences but may be understood as an important risk factor. Psychosocial aspects such as role-stress, trauma, internalizing coping strategies and disadvantages in social status are all acknowledged as contributing factors (213) to psychiatric morbidity. Many biological and social factors are accentuated and further complicated in women with DSD. Therefore increased vulnerability is present and may contribute to understanding why adult patients with DSD in some studies have shown psychological distress on levels comparable to women with a history of physical or sexual abuse (215).

Knowing this, what can be done to prevent or ameliorate the increased prevalence of psychiatric morbidity? The strengthening of resilience factors, increased psychosocial support and changes in medical interventions are requested from lobbyists and patient advocate groups. There are very few studies of psychotherapeutic interventions for patient groups

within the DSD spectrum but recommendations based on qualitative studies (42) and some tailored cognitive behavioral interventions have been published (216).

While replicating and extending results that show increased risk of adverse outcome we also found what seem to be some advantages. In study I, the analyses on subgroup level showed that women with SV CAH pursued higher education more often than controls and women with SW had higher income than controls. The small sample sizes on the level of sex and severity made it difficult to control for confounders such as having a family or not and socioeconomic status of original family. Despite these limitations we concluded that it might be factors related to the condition that contributes to this superior outcome related to academic achievement for women with SV CAH and income for women with SW CAH.

Also in study III, we unexpectedly found that women with XXGD performed superior to both men and women on a test of emotion recognition despite not performing differently on any other tests of cognitive function. While refraining to interpret this finding before it has been replicated, there seems to be some evidence that factors related to DSD conditions can enhance some aspects of functioning.

4.2.1 Methodological limitations

In study I, many compromises were made when selecting parameters and planning analyses due to limitations in the data sources and the size of patient groups. We wanted to compare outcome for those identified by newborn screening to those that were discovered by clinical presentation to assess if screening had improved outcome. While this had been possible for some parameters like marriage or level of education, only sick leave and disability pension differed between patients and controls on the level of the whole patient group. Newborn screening for CAH was initiated in 1986. The register data for disability pension was available from 1990-2004 making this comparison impossible, as those identified by screening could not yet have received this form of allowance. The comparison would have been possible for the sick leave parameter. However, the rules and regulations for health insurance and labor market politics have changed the pattern of sick leave over the time period covered and this was judged to be an analysis that required more correction of other demographic and historical factors than were deemed possible given the timeframe of the study. Comparison of fertility has been performed in another study with a slightly different selection of the group, showing improved fertility after newborn screening (205).

4.3 STUDIES OF COGNITION IN DSD POPULATIONS

Women with XYDSD perform more similar to female than male controls

In study III, we found that women with CAIS and GD, both with XY karyotype but minimal exposure to or receptivity for androgens, performed more similar to female controls on most cognitive tasks but not on verbal fluency and emotion recognition.

Research in clinical populations bring a number of biases, such as small patient numbers, non typical variations in general intellectual performance, working memory or other cognitive abilities (217). Variation in results can also depend on the use of measures that do not show sex differences, lack of information on general intellectual ability and when cognitive measures are applied, on roof and ceiling effects. Previous studies of cognitive abilities in CAIS have struggled with all these issues and our study is one of the first that clearly show that women with CAIS perform more like female controls on most cognitive tasks that yield sex differences in the control groups.

The lower performance by women with CAIS and GD on word fluency and emotion recognition, on the same level as the male control group, was contradicting the pattern of performing like the female controls. Word fluency is a task dependent on several cognitive abilities, and is mostly used in clinical practice as a measure of executive functions. However it is also dependent on language abilities. As some individuals in the patient group were nonnative, albeit fluent, Swedish speakers we found it difficult to draw conclusions based on this finding as it may be related to small differences in language proficiency. The results on the emotion recognition task were surprising. They were not in line with the previous published study (218) that uses the same task. In that study, emotion recognition was investigated in groups with variations in testosterone synthesis and sensitivity (CAH, 5α-reductase deficiency, CAIS and male and female controls). The results showed that performance was associated with androgen exposure such that the group with least exposure/effect (CAIS) performed better than all patient groups and also better than control males. Female controls were superior in performance. The mean results in this study for all groups however, are on a much lower level than in our study, potentially indicating that the test has a somewhat different function in groups of different cultural and geographic background, which is discussed by the authors (218). The mean performances in our groups are also higher than in the original study and in the Swedish validation study (table 11).

Table 11. Reading the mind in the Eye, mean performance in studies carried out in the United Kingdom, Sweden and Iran.

Revised version, Baron Cohen 2001	Söderstrand et al 2012 Swedish validation study	Strandqvist et al 2018	Khorazad et al 2018
♀ General pop 26.0	♀♂ 27.2	♀ 28.3	♀ 23.5
♂ General pop 26.4		♂ 26.5	♂ 21.0
♀ Students 28.6		♀CAIS 26.2	♀CAIS 22.9
♂ Students 27.3		♀XYGD 24.7	♀CAH 19.8
♀♂AS[1]/HFA[2] 21.9		♀XXGD 31.1	♀5αRD2 18.1
♀♂IQ matched [3]30.9			

[1] Autism spectrum group [2] High functioning autism group [3] Individuals that were IQ matched to the autism spectrum group.

Distribution of results on the emotion recognition task

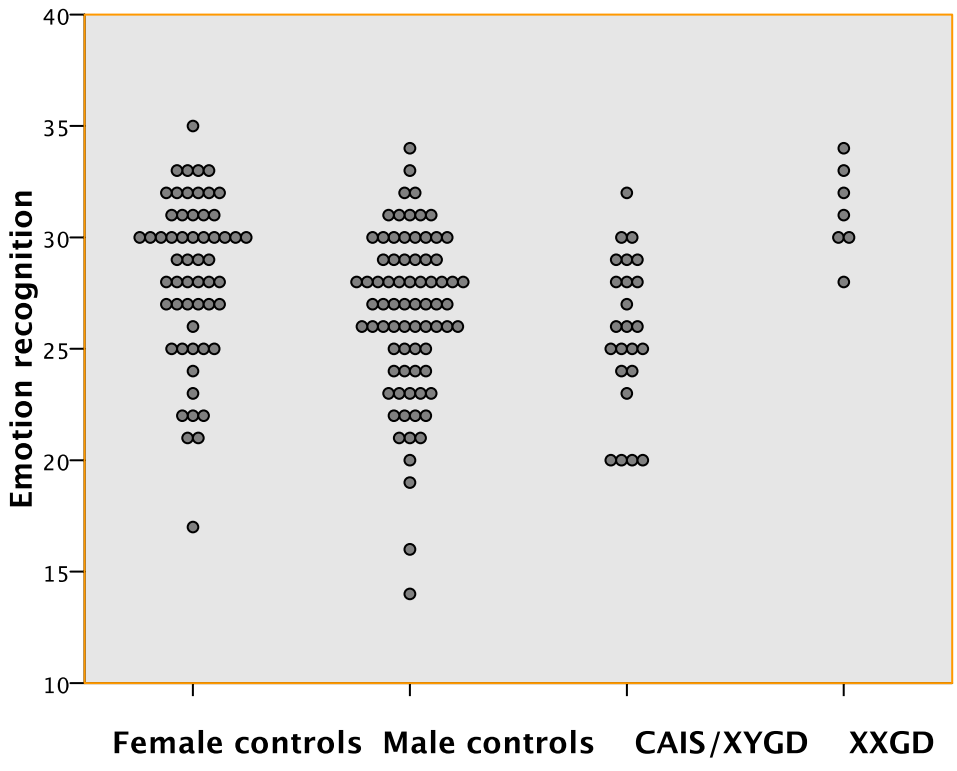

Fig 4. Distribution of test results for female controls, male controls, the combined group of women with CAIS and XYGD and the group of women with XXGD on the reading the mind in the Eyes test.

The distribution of results for the different groups are spread out and the distribution of results for the group of women with XYDSD could match that of the group of male controls but it also did not differ much from that of female controls, except for a cluster of four individuals (fig 4). These four individuals contribute to the lower average and if they are excluded the difference to female controls ceases to reach significance. The reason for the lower performance of these four women is not known. They are not afflicted with neuropsychiatric disorders, are not born abroad and do not have low vocabulary performance. Assuming the results are not due to motivational issues or chance findings, this indicates that factors related more directly to sex chromosome disposition may be important for performance on emotion recognition tasks. Further research is warranted.

While androgens have been shown to enhance spatial ability, less is known about what explains variation in abilities where women typically excel. We found that women with XX GD outperformed all other groups on the emotion recognition task. Unless this is a chance finding or the result of some other shared factor between the women we may assume that the optimal prerequisites for development of superior emotion recognition skills is related to XX chromosomes and low pre and postnatal exposure to sex steroids but may also be related to the timing of puberty, which has been suggested to impact ability to read emotion in faces (219). As the group of XXGD consists of only seven women, significantly younger with a higher education level than controls and other patient groups, the reason behind this superior performance need replication in a larger group before conclusions can be drawn.

The impact of oral contraceptives (OC) on brain function and mood is a growing field of research. OC use have been previously associated with both increased attention, enhanced and impaired function in experimental studies of perception of emotional faces as well as unaltered performance for emotion recognition (220). Whether long term OC use can affect cognition performance needs to be taken into account in future studies of cognition in DSD patients.

Men with hypospadias perform similar to male controls

There were to our knowledge, no prior studies on cognitive functions or profiles in men with hypospadias and there is also a lack of studies on psychosexual outcome (142). The results from cognitive tests and surveys in our cohort of men with hypospadias showed no association between the condition and self-reported gender role behavior or cognitive functions. This implies that whether or not there are differences between the groups in terms of early hormonal exposure/ situation, or genetic effects that impact genital growth and formation, these possible differences have no or negligible effect on gender typical behavior or cognitive function in a group of men with mixed background reasons for hypospadias. To be able to reason around the impact of androgen exposure or receptivity per se, the selection of men with hypospadias would have to be restricted to a group where known dysfunction of androgen synthesis or receptivity was present. The results from the small group of men with

proximal hypospadias showing lower cognitive performance may be an indication of comorbidity affecting cognitive function for individuals within the group and may contribute to understanding of the adverse psychosocial outcome seen within this group in other studies (67).

A reflection on socialization

In our studies the social impact during development was not assessed but we assume that the social impact in terms of growing up in a female or male gender role is approximately the same for women with DSD and men with hypospadias as for female and male controls respectively, recruited from the general population. One aspect of socialization is that it is dependent on the environment. The individual however, by seeking out and following his or her own preferences, participate in selecting and perhaps co-creating that environment. Therefore, biological effects that impact preferences can indirectly shape environment, interacting with opportunities and create training effects. For women with CAH it has been shown that they have better geographical knowledge and knowledge of how mechanical tools are used, skills that are learned by training rather than being innate (122). That early exposure to sex hormones would in fact have an effect on preferences rather than innate ability, leading to increased activity that may lead to better achievement on some tasks, is put forth by some as an important explaining factor for the variance in sex differences (221).

Critical periods for brain sex differentiation induced by hormonal exposure

Androgens play a crucial role for prenatal genital development, and the brain is also influenced by the fluctuations in sex hormone levels but while genital development has a sensitive window during the first half of gestation, the entire period from before birth to around puberty has been suggested to constitute a sensitive period for brain development (22). Most research done hitherto on critical periods was carried out in animals and the evidence in humans for sensitive/critical periods either regarding gender role behavior, gender identity or sexual preferences is scarce and understudied.

Previous research in girls and women with CAH, together with twin studies (222) show support for organizing effects of prenatal androgen exposure. Girls with CAH receive treatment from birth and onward, with the aim of correcting the cortisol deficiency and lowering androgens. Therefore, given that treatment is initiated and adherence is good, they do not have elevated postnatal androgen exposure. This is in contrast to boys that typically experience a postnatal surge of testosterone, which could contribute to the predisposition to develop superior skills in mental rotation.

Studies in girls and boys from the general population have shown the significance of postnatal exposure, by relating testosterone levels pre and postnatal to play behavior in

toddlers, controlling for prenatal exposure and postnatal growth (223-225). During puberty, somatic effects of sex steroids are evident like deepening of the voice and general growth. Whether there are organizational effects of sex steroids on brain and behavior during puberty is debated. Some studies show changes of brain anatomy and function associated to androgen levels during puberty at least for boys (28,226) while previous studies fail to show any change in sex differences pre and post puberty (227). There is also evidence of negative effects of androgen levels on spatial behavior following puberty, favoring a different hypothesis that state late pubertal maturation as important for good spatial abilities (228).

Sex chromosomes

Experimental animal research shows a clear case for sex chromosome complement and/or sex specific gene expression to sometimes modulate, sometimes enhance or act together with hormone exposure to increase or decrease sex differences. In human studies, the evidence is not yet substantial enough to conclude a significant effect of sex chromosome complement on either cognitive or behavioral development. There are however circumstances in some of the DSD conditions natural history that indicates that karyotype makes a difference. One example is that gender identity development in individuals with 46,XY and partial androgen insensitivity or a defect of synthesis, is often in the male direction, given some androgen effect. However the paradigm change that has emerged, directing focus on the uniform effect of these two aspects, hormones and karyotype, acting in concert driving and directing sex differentiation together with the environment will direct future research and have clinical implications for DSD healthcare.

4.3.1 Methodological considerations

Translating research from animal studies

Experimental research in animals has built the theoretical framework, conceived and tested the main hypotheses and continue to expand knowledge concerning sex differentiation. In translating these findings to humans there are however serious limitations. For one, biological mechanisms of sex differentiation differ between species. Masculinization of rodent behavior is induced by the aromatization of estradiol into testosterone whereas in primates and presumably in humans, testosterone and dihydrotestosterone induce defeminisation and masculinization (11). An illustration of this difference is knockout mice with CAIS that are masculine in their behavior, which devaluates comparison with humans in both sickness and health. Besides this, one of the most important aspects of psychosexual development, namely gender identity, is not possible to observe in animals. Therefore, to know how these effects present in humans, given our complex social environment and in many aspects shown plasticity of brain anatomy, we have to refrain from drawing conclusions before relevant studies have been made.

The limitations of the model disorder paradigm

The social environment, personal experiences and inborn predispositions interact for psychosexual and cognitive development on an individual level. On a group level however, both biological and environmental influences can be studied given that one can tease apart the influence of one on the other to some extent. DSD conditions are sometimes referred to as "model disorders" to study the impact of the biological contributions while keeping constant the impact of gender socialization. This model of studying is not without flaws but it is one of very few ways of gaining knowledge on a hard to reach and important subject. One of the issues with this approach is that other effects of the conditions may constitute bias in the investigation. In studies on CAH and cognition this has become increasingly evident as working memory (118,229) and executive dysfunctions have been documented, possibly affecting outcome in other cognitive measures as well.

Another limitation relates to the possibility to assess the biological parameters on which assumptions are made in studies investigating prenatal influences on brain development. Many parameters like levels of prenatal exposure to testosterone are not possible to observe or measure directly. When investigating this in girls with CAH we infer the elevated androgen exposure during gestation as revealed by the level of masculinization of genitalia that takes place during the first trimester of gestation or by known *CYP21A2* genotype. It is generally assumed that the levels of androgens for girls with CAH are somewhat higher than that of typically developing boys and also constant, instead of showing the first trimester peak, present in typically developing boys. The activity of other enzymatic pathways leading to increased production of DHT may also be of importance (230).

In addition it is not known how the body, placenta and other tissues compensates, adapts or fails to adapt to this hormonal situation. To add to this, the sensitivity of the androgen receptor is a factor that is difficult to evaluate in the clinical and research situation. Therefore, in studies observing the association between for example early androgen exposure and three-dimensional mental rotation the outcome is being studied while the exposure is only estimated.

In our studies, similar limitations are present. In study II for example, we do not know the cause of ovarian dysfunction in women with POI and can therefore not be certain that they had not had some difference in sex hormone stimulation or response earlier or that their hormonal situation is due to another factor, also contributing to the susceptibility to psychiatric morbidity. Autoimmune reactions with increased inflammatory responses are possible alternative biological explanations to the increase in psychiatric symptomatology. In addition we also could not control for previous or present use of hormonal substitution, as we did not have full retrospective information of this.

Hence, different mechanisms may congregate in the study of clinical conditions. There are possible psychosocial confounding factors unevenly distributed between the groups like possible gender identity issues, trauma of receiving diagnosis at different ages and unresolved fertility issues. To better account for these differences and comparisons, some measure of perceived trauma may have improved the background demographic descriptions and contributed to better understanding of psychiatric morbidity in study II.

4.4 CLINICAL IMPLICATIONS

Need of psychological interventions in routine healthcare for DSD populations

Psychosocial outcome for DSD patients is often compromised and we know that information, counselling and support is helpful both as prevention and to overcome difficulties (231). The suboptimal outcomes for women with CAH in study I indicate that as a group, they have needs for psychological interventions tailored to their situation, general and individual needs. While it may be appropriate at many times while growing up it is especially urgent for adolescent and young adults facing challenges in everyday life in the educational situation, in developing a healthy body image, sexual identity as well as a personal identity overall. The high prevalence of psychiatric comorbidity in study II indicates that women with CAIS and gonadal dysgenesis should be screened at some point for psychiatric symptoms and offered psychosocial support. From previous literature it seems urgent when the diagnosis is new as this may constitute a trauma in itself but one clinical experience from this project is that the trauma can also be re experienced at a later point when issues around fertility or forming of stable relationships emerge at stages of life when this becomes important, for example during childbearing age.

While genital malformations can be the primary source of psychological burden, it is essential not to overlook other issues like body image and other attitudes and perceptions about the condition. The impact this may have on self-esteem, feeling different and also the potential trauma from healthcare experiences may constitute a problem in different situations in life. In DSD, the surgical interventions have been standard whereas psychological support and screening for cognitive and other developmental problems have been optional and more difficult to get access to (231). Despite recommendations, not all centers offer psychological support. This is regrettable as the potential risks with psychological interventions are negligible compared to other alternatives.

The results in this thesis support the claims made from patients and caregivers alike to increase psychological support in care for DSD populations and to include screening for psychiatric symptoms in the care for women with DSD, neurodevelopmental problems in boys with proximal hypospadias and perhaps also screening for both cognitive deficits and psychiatric symptoms in women with severe forms of CAH.

Cognitive abilities are not impaired in women with CAIS and GD

The results of study III that women with CAIS and GD perform like female controls further corroborates previous research that shows predominantly female psychosexual development in women with CAIS. The slightly lower result on word fluency and emotion recognition is not necessarily indicating impaired performance as it is on the level of the mean performance in the general population in studies from other countries.

Hypospadias in general, is not associated to variation in cognitive abilities and gender role behaviour

The clinical implication of study IV is information to clinicians and parents of children with hypospadias concerning questions around psychosexual development and cognitive development. There is no reason to believe that mild hypospadias is associated with variations in development to a larger degree than in the general population.

4.5 FUTURE PERSPECTIVES

To further the knowledge of risk factors for negative psychosocial and psychiatric outcome

In study I and II we found compromised psychosocial outcome and increased psychiatric morbidity for the patient groups under study. The vulnerability to psychiatric disease point to serious side effects of the disorders but we do not know enough about the risk and resilience factors in development of psychiatric comorbidities, or the relative contribution of the known risk factors. Researchers in DSD and related fields have emphasized the importance of the factors that mediate and moderate psychosocial outcome rather than to have a narrow focus on psychosexual aspects of DSD or the outcomes per se (232) since the mediators highlights and directs needs for psychological intervention as well as preventive measures.

To further knowledge of outcome on rare disorders, transnational and international cooperation is a possibility to assess the same parameters in groups large enough to reach sufficient statistical power. DSD life is one such endeavor (233) and perhaps the future will see more such joint projects between different clinics/research groups.

The mediating aspects of psychosocial outcome in women and men with CAH

Many of the results in study I could be elaborated further like whether the periods of sick leave for patients with CAH that to some extent depend on disease severity is also associated to mental health status. That women with CAH to a larger extent do not have complete basic education at the appropriate age is worrying and needs further clarification. Are the reasons for this found in aspects of functioning related to mental health, executive or intellectual

capacities, adherence to treatment, psychosocial environment like bullying, loneliness or is it a side effect of going through secondary surgery at the age when you are also finishing school? For women with CAH, overtreatment with glucocorticoids is sometimes suggested as a cause of negative side effects of treatment. As girls risk masculinizing effects of cortisol deficiency and this is not as feared in boys, girls may receive slightly higher doses of hydrocortisone as a preventive intervention, but to what extent this is related to various outcomes has not been studied. There is also emerging evidence of sex dimorphic effects of glucocorticoids (234), and genetic functions (235) that in the future may be further clarified. Adolescence is a period when adherence to treatment is more dependent on the person him or her self rather than on parents, managing the daily administration of medication. For some individuals, this may be a difficult task, particularly in the prescence of other problems like executive functions.

It would be of great value to better understand the reasons behind school underachievement and absence from work life in order to better tailor treatment and support so that this chronic condition has least possible impact on daily life. Likewise, the results of greater variation in cognitive outcome for men with proximal hypospadias should be further investigated. We detected lower cognitive performance that may harmonize with findings of increased risk of adverse psychosocial outcome in men with proximal hypospadias (236).

Interaction and training effects on cognitive sex differences

The relative importance of biological and social factors is difficult to evaluate and cannot easily be teased apart as they as they act in concert in the emergence of sex differences. In stark contrast to this symbiosis stands the different research fields investigating and sometimes debating the importance of nature vs nurture (237). Present models of sex differentiation capture these interactional processes (181) although few studies to date take into account interactional effects and more empirical evidence is needed. Training effects and other ways of capturing both aspects under different hormonal and chromosomal circumstances, is one of the future directions in sex difference research.

The association of androgen insensitivity to cognitive abilities in men with hypospadias

In study IV the men with hypospadias had different and sometimes unknown background to the hypospadias and we cannot therefore draw any conclusions from this study related to theories of sex steroid impact on cognition. Including men with hypospadias with an etiology of defects of androgen synthesis or receptivity could bring further light to this question. The results of gender role behavior should also be replicated in a group of boys with hypospadias using direct observational methods.

To further the knowledge on what contributes to enhanced cognitive abilities in women

The findings of enhanced emotion recognition in women with 46, XXGD should be further studied and may reveal possible mechanisms behind variation in social cognitive skills where women outperform men.

5 SVENSK SAMMANFATTNING

Bakgrund: Atypisk eller annorlunda könsutveckling (disorders of sex development, DSD) är benämningen på ett spektrum av tillstånd där en individs könskörtlar (äggstockar, testiklar) är annorlunda eller hens kromosomala, anatomiska eller hormonella situation skiljer sig från det typiska. Diagnoser inom denna grupp varierar vad gäller risk för somatisk sjuklighet från komplett benigna till livshotande och många tillstånd påverkar möjligheterna till fertilitet. Sjukvårdens insatser har varierat historiskt sett och har under modern tid praktiserat metoder som kritiserats av såväl patientorganisationer som samhälleliga instanser för att vara i strid mot mänskliga rättigheter. Det gäller främst kirurgi i nyföddhetsperioden men även andra behandlingsalternativ som medför icke reversibla somatiska och psykologiska effekter. Överväganden som i vissa länder görs av föräldrarna och i andra länder av medicinsk expertis i samråd med föräldrar kan vara både komplexa och medföra svåra etiska ställningstaganden. Nutida riktlinjer föreskriver multidisciplinärt omhändertagande, betonar vikten av att få fullständig information om sitt tillstånd och innefattar en målsättning om optimering av individens psykiska och fysiska hälsa och samtidig minimering av medicinska interventioner. Frågeställningar kvarstår kring optimal tidpunkt för interventioner. Forskning kring utfall visar på suboptimala kirurgiska resultat, överrepresentation av psykisk ohälsa med en stor variation inom och mellan patientgrupper.

Den klassiska modellen för biologisk könsdifferentiering betonar könskörtlarnas roll, dvs äggstockarnas eller testiklarnas, och påföljande hormon exponering för utvecklingen i manlig eller kvinnlig riktning. Könshormon som androgener och östrogener har under kritiska perioder i livet; pre och postnatalt, under pubertet, graviditet och vid menopaus effekter på framför allt fysisk utveckling men även andra beteenden, bland annat de som uppvisar könsskillnader. Även könskromosomuppsättning har i djurstudier visat sig ha betydelse för uppkomsten av könsskillnader då närvaro/frånvaro av genetiskt material föregår eller bidrar till könsdifferentiering.

Dessa biologiska faktorer ger sammantaget upphov till fysiska könsskillnader men bidrar även till skillnader i preferenser och beteenden, kognitiva funktioner och könsrollsbeteende. Vilka mekanismer som ligger bakom, och hur stor inverkan de har är svårt att undersöka då socialiseringsprocesser interagerar och påverkar könsskillnader i hög utsträckning. Genom att inkludera individer med variation i könsutvecklingen med avseende på karyotyp och hormonell situation kan detta studeras till viss del.

Syfte: Dessa studier kartlägger psykosocialt utfall och förekomst av psykiatrisk morbiditet för grupper av patienter med variationer i könsutvecklingen; kvinnor med CAH, CAIS, GD, POI och män med Hypospadi. Den belyser även frågor kring betydelsen av hormonell och kromosomal/genetisk påverkan för utvecklingen av könsskillnader i kognitiva förmågor och könsrollsbeteende.

Studie I

I denna registerstudie undersökte vi psykosocialt utfall för män och kvinnor med kongenital binjurebarkshyperplasi, CAH med avseende på utbildningsnivå, inkomst, utnyttjande av sjukförsäkringssystemet, giftermål och fertilitet.

Metod: Informationen inhämtades från flera svenska nationella register och patienterna matchades och jämfördes med 100 kontroller per person från befolkningsregistret. Analyserna gjordes både på gruppen som helhet men uppdelningar gjordes även med avseende på svårighetsgrad (SW, SV, NC) och mellan kvinnor och män.

Resultat: Sammantaget visade resultaten att gruppen individer med CAH var heterogen beroende på svårighetsgrad av CAH och det fanns könsskillnader. Både kvinnor och män erhöll sjukersättning eller förtidspension oftare och hade mer sällan barn än kontroller. Kvinnor saknade i högre utsträckning behörighet till gymnasiet och var mer sällan gifta. Män var oftare fast anställda och gifta i högre utsträckning än kontroller. Kvinnor med SW hade oftare inte behörighet till gymnasiet men hade också oftare inkomst i övre kvintilen jämfört med kvinnliga kontroller.

Studie II

I den här tvärsnittsstudien undersöktes förekomst av psykiatrisk sjuklighet hos en grupp kvinnor med komplett androgenokänslighetssyndrom (CAIS), eller komplett gonaddysgenesi (GD) och jämfördes med friska åldersmatchade kontroller från normalbefolkningen samt en grupp kvinnor med prematur ovarial svikt (POI). Kvinnorna intervjuades med en klinisk strukturerad intervju, MINI-IV och fick besvara kompletterande frågor via en enkät.

Resultat: Kvinnor med CAIS och GD hade mer psykiatrisk sjuklighet än kontroller men inte signifikant mer än kvinnor med POI. Det fanns en ökad förekomst av psykiatrisk sjuklighet i alla patientgrupper främst vad gäller ångest och nedstämdhet.

Slutsatser studie I och II: Patientgrupper med DSD har en ökad risk för suboptimalt psykosocialt och psykiatriskt utfall vilket betyder att psykologiskt stöd bör vara en del av den ordinarie vården och den bör innefatta screening för psykiatrisk problematik. Orsaker bakom suboptimalt psykosocialt utfall behöver identifieras för att förbättra medicinskt och psykologiskt omhändertagande vid CAH.

Studie III

I den här studien undersöktes betydelsen av karyotyp och hormonell situation för kognitiva förmågor hos kvinnor med DSD med syftet att undersöka skillnader mellan grupper med XY karyotyp med låg och hög androgenexponering samt att jämföra kvinnor med XY resp XX kromosomuppsättning för att resonera kring betydelsen av hormonexponering och karyotyp.

Metod: Kognitiva tester som i andra studier uppvisar könsskillnader; spatialt tänkande, verbalt flöde, ansiktsminne och emotionsigenkänning administrerades till en grupp kvinnor med CAIS och XYGD samt en mindre grupp kvinnor med XXGD och en grupp åldersmatchade kvinnliga och manliga kontroller.

Resultat: Kvinnor med CAIS och XYGD presterade mer likt kvinnliga än manliga kontroller på tester som traditionellt visar könsskillnader. På tester av igenkänning av emotioner presterade kvinnor med XY kromosomer mer likt den manliga kontrollgruppen medan kvinnor med XXGD överpresterade gentemot alla övriga grupper.
Slutsats: Resultaten ger stöd för teorier rörande betydelsen av exponering för androgener för könstypisk påverkan på kognitiva funktioner medan det i vissa fall som vid emotionsigenkänning finns indikation för att könskromosomuppsättning eller till denna relaterade faktorer har betydelse.

Studie IV

I denna studie undersöktes kognitiv prestation hos män med hypospadi. Hypospadi är en relativt vanlig genital missbildning som drabbar en av 125 män årligen. Manlig genital utveckling är i vissa faser beroende av normal androgenfunktion. Därför har frågan om huruvida hypospadi kan förknippas med ökad variation i könsrollsbeteende och andra aspekter ställts.
Metod: Män med hypospadi genomförde samma batteri av kognitiva tester som i studie III och en retroaktiv självskattning av könsrollsbeteende i barndomen, och jämfördes med kvinnliga och manliga kontroller.
Resultat: Män med hypospadi skiljer sig inte från manliga kontroller vad gäller kognitiva tester eller självskattat retrospektivt könsrollsbeteende. De skiljer sig däremot från kvinnliga kontroller i samma utsträckning som män i kontrollgruppen. Jämförelser mellan män med olika svårighetsgrad indikerar att män med proximal hypospadi presterar något lägre generellt på kognitiva tester men skattar könsrollsbeteende likt övriga män med hypospadi och manliga kontroller.
Slutsats: Faktorer som bidrar till hypospadi påverkar inte i mätbar utsträckning kognition eller könsrollsbeteende. Kognitiva förmågor hos män med proximal hypospadi bör kartläggas vidare.

6 ACKNOWLEDGEMENTS

First of all, I wish to thank all the patients and controls that participated, without you this project would not be.

Anna Nordenström, my main supervisor for all the laughs and good times. To accept someone as a doctoral student is to create an opportunity for that person for both personal development, career possibilities, fun, sweat and pain. Thank you for opening that door, for the persistence with which you convinced me to take on this project, and for sharing your great knowledge and expertise in DSD. Your creativity, perseverance, and enthusiasm for the research questions is definitely contagious.

Agneta Herlitz, co-supervisor, for being invaluable as an authority on sex differences in cognition or anything else that matters, but also for good guidance, advice and patience during so many phases of the project. Thank you also for providing me with a workplace in the section for psychology at the clinical neuroscience department.

Agneta Nordenskjöld, co-supervisor, for playing so many parts in this project, bringing structure and continuity to the DSD research group on a local and national level which has led to both interesting learning opportunities and social occasions. I am also grateful to have worked with you in the clinic, as you are the best pediatric urologist working in a DSD team ever.

Louise Frisén, co-supervisor, for teaching me things that are not included in any educational curriculum and a lot about the psychological aspects in DSD. Your knowledge is immense and your work in creating structures for clinical care and research for this group of patients is impressive. I hope we will join forces in the future to continue what you started.

Angelica Lindén Hirschberg for being an important person this project in so many ways, providing our patients with the most excellent care and support, for collegial cooperation and guidance. Thank you also for trusting me with your patients in the clinic.

My fellow PhDs in this project, Hedvig Engberg, Lisa Örtqvist and Anna Skarin Nordenvall for beeing your sweet and very unique selves, and for good companionship all the way. Hanging out with you in Trysil, Miami, Glasgow, Ghent or at home have made this journey a meaningful and fun endeavour. You all have a special place in my heart and the way you handled all the hardships that came our way made me stronger.

Thank you Charlotte af Ekenstam, Emilia Johansson, Lotta Blomberg, Berit Legestam och Siv Rödin Andersson for cooperation in planning, data gathering and taking care of all the participants. Carl Lundeberg and Mathilde Annerstedt for assistance with data handling and Freddie Karlbom for programming of the web based tests, Martin Asperholm for support in different software matters, being the best roommate and all of you for good company.

All the participants of the National research school for clinicians in psychiatry for sharing so many ideas and good times. Charlotte Haglind for beeing a great overseas co-worker in other research endeavors in parallell projects.

All the people at the section for psychology, department of clinical neuroscience for providing the social environment for my everyday academic experience, I will miss ”network-cake” particularly. Thank you all clinical collegues in research; Riika Lovio, Anna Miley Åkerstedt, Lena Backström Eriksson, Maria Bragesjö och Maria Helander, spending a lot of lunches together with you has enriched my days.

The former and present heads of the department of medical psychology at Karolinska sjukhuset. Ylva Novak for serving as a mentor, Louise Lettholm, Agneta Julinder, Richard Wicksell, Caroline Björk, Linda Holmström and last but not at all least, Pernilla Bergman for showing interest in my research and being generally supportive. The head of staff of the child endocrinology department, that has supported my participation in research, Svetlana Lajic for being a superwoman. Tatia Hirvikoski and Marika Möller for being excellent role models as female researcher-psychologists, Marika also for serving on the halftime committee.

Thank you Niklas my little brother for being both my biggest critic, a fun companion in cooking, eating, running, talking, and for solving some of the practical problems by lending me your network of excellent people. Thank you Mia, my big little sister that is both a role model as a researcher and a great person to hang and reason with. Tim and Christoph for being their chosen ones, the best of their kind. Thank you mom and dad for providing me with good enough genes and environment to pursue this education, and for basic support with everything from Apple products to babysitting.

My father in law Kjell Strandqvist, that not only is the kindest and best grandfather anyone can dream of but also for providing a picture to the most known part of the thesis, the front cover.

Thank you Annika Haglund for withstanding everyday complaints, and for taking on the role of coach from time to time. Thank you all other friends that keep in touch despite the fact that I haven’t been so good at that lately, and cheers to the brain-book-heart club for intellectual stimulation of a different kind.

Last but not least I want to thank the family; Kristoffer, Dorotea and Ferdinand, for being my reason for everything, I love you to death.

7 REFERENCES

1. Biason-Lauber A. Control of sex development. Best Practice & Research Clinical Endocrinology & Metabolism. 2010;24(2):163–86.
2. Nielsen J, Wohlert M. Chromosome abnormalities found among 34910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Human Genetics. 1991;87(1):81–3.
3. Bojesen A, Hertz JM, Gravholt CH. Genotype and phenotype in Klinefelter syndrome - impact of androgen receptor polymorphism and skewed X inactivation. International Journal of Andrology. 2011 Oct 7;34(6pt2):e642–8.
4. Arboleda VA, Sandberg DE, Vilain E. DSDs: genetics, underlying pathologies and psychosexual differentiation. Nature Reviews Endocrinology. Nature Publishing Group; 2014 Oct 1;10(10):603–15.
5. Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. Best Practice & Research Clinical Endocrinology & Metabolism. 2015 Aug;29(4):607–19.
6. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature. 1990 Jul 19;346(6281):240–4.
7. Bouty A, Ayers KL, Pask A, Heloury Y, Sinclair AH. The genetic and environmental factors underlying hypospadias. Sex Dev. 2016 Jan 29;9(5):239–59.
8. Witchel SF. Disorders of sex development. Best Pract Res Clin Obstet Gynaecol. 2018 Apr;48:90–102.
9. Hiort O. The differential role of androgens in early human sex development. BMC medicine. 2013;11(1):930.
10. Barbaro M, Wedell A, Nordenström A. Disorders of sex development. Seminars in Fetal and Neonatal Medicine. 2011;16(2):119–27.
11. McCarthy M. Sex and the developing brain. Colloquium Series on the Developing Brain. 2010. Morgan & Claypool life sciences series.
12. Skuse DH. X-linked genes and mental functioning. Human Molecular Genetics. 2005;14(suppl_1):R27–R32.
13. Carrel L, Cottle AA, Goglin KC. A first-generation X-inactivation profile of the human X chromosome. 1999. pp. 14440–4.
14. Brown CJ, Greally JM. A stain upon the silence: genes escaping X inactivation. TRENDS in Genetics. 2003;19(8):432–8.
15. de Vries GJ, Rissman EF, Simerly RB. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. Journal of Psychosomatic Research. 2002;187(12):977–85.
16. Ngun TC, Ghahramani N, Sánchez FJ, Bocklandt S, Vilain E. The genetics of sex differences in brain and behavior. Frontiers in Neuroendocrinology. Elsevier Inc; 2011 Apr 1;32(2):227–46.
17. Arnold AP, Chen X, Link JC, Itoh Y, Reue K. Cell-autonomous sex determination outside of the gonad. Dev Dyn. 2013 Apr;242(4):371–9.
18. Almey A, Milner TA, Brake WG. Estrogen receptors in the central nervous system and their implication for dopamine-dependent cognition in females. Hormones and Behavior. 2015;74:125–38.
19. Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the Hypothalamic-Pituitary-Gonadal Axis in Infancy: Minipuberty. Horm Res Paediatr. 2014;82(2):73–80.
20. Nelson R J. An Introduction To Behavioral Endocrinology, Third Edition. 2005. Oxford university press.

21. Chrousos GP. Stress and disorders of the stress system. Nature Reviews Endocrinology. 2009 Jul;5(7):374–81.

22. de Vries GJ, Fields CT, Peters NV, Whylings J, Paul MJ. Sensitive periods for hormonal programming of the brain. Curr Top Behav Neurosci. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014;16(Chapter 286):79–108.

23. Tobet S, Knoll JG, Hartshorn C, Aurand E, Stratton M, Kumar P, et al. Brain sex differences and hormone influences: a moving experience? Journal of Neuroendocrinology. 2009 Mar;21(4):387–92.

24. Phoenix C, Goy R, Gerall A, Young W. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology. 1959;65:369–82.

25. Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: the organizational–activational hypothesis adapted to puberty and adolescence. Hormones and Behavior. 2009;55(5):597–604.

26. Thornton J, Zehr JL, Loose MD. Effects of prenatal androgens on rhesus monkeys: a model system to explore the organizational hypothesis in primates. Hormones and Behavior. 2009 May;55(5):633–45.

27. Giedd JN, Raznahan A, Mills KL, Lenroot RK. Review: magnetic resonance imaging of male/female differences in human adolescent brain anatomy. Biology of Sex Differences. BioMed Central Ltd; 2012;3(1):19.

28. Nguyen TV, Lew J, Albaugh MD, Botteron KN. Sex-specific associations of testosterone with prefrontal-hippocampal development and executive function. Science. 2017;76:206–17.

29. Dreger AD. A history of intersexuality: from the age of gonads to the age of consent. J Clin Ethics. 1998;9(4):345–55.

30. Hughes IA, Houk C, Ahmed SF, Lee PA, Lawson Wilkins Pediatric Endocrine Society/European Society for Paediatric Endocrinology Consensus Group. Consensus statement on management of intersex disorders. Journal of Pediatric Urology. 2006 Jun;2(3):148–62.

31. de Crecchio L. Sopra un caso di apparenze virili in una donna. II Morgagni. 1865;:7:151.

32. Hadidi AT, Azmy AF. In: Hypospadias Surgery. Berlin, Heidelberg: Springer Berlin Heidelberg; 2004. pp. 99–105.

33. de Vries ALC, Doreleijers TAH, Cohen-Kettenis PT. Disorders of sex development and gender identity outcome in adolescence and adulthood: understanding gender identity development and its clinical implications. Pediatr Endocrinol Rev. 2007 Jun;4(4):343–51.

34. Jost A. The age factor in the castration of male rabbit fetuses. Proc Soc Exp Biol Med. 1947 Nov;66(2):302.

35. Berenbaum SA, Meyer-Bahlburg HF. Gender development and sexuality in disorders of sex development. Hormone and Metabolic Research. 2015;47(05):361–6.

36. Lee PA, Houk CP, Ahmed SF, Hughes IA, International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. 2006. pp. e488–500.

37. Federer EK. Making Sense of Intersex: Changing Ethical Perspectives in Biomedicine. Bloomington Indianapolis; 2014.

38. Kolesinska Z, Ahmed SF, Niedziela M, Bryce J. Changes over time in sex assignment for disorders of sex development. Pediatrics. 2014;134(3):e710–5.

39. Bougnères P, Bouvattier C. Deferring surgical treatment of ambiguous genitalia into adolescence in girls with 21-hydroxylase deficiency: a feasibility study. International Journal of Pediatric Endocrinology. 2017;2017(1):5110.

40. Kuhnle U, Bullinger M, Schwarz HP. The quality of life in adult female patients with congenital adrenal hyperplasia: a comprehensive study of the impact of genital malformations and chronic disease on female patients life. European Journal of Pediatrics. 1995;155(7):620–1.

41. Guth LJ, Witchel RI, Witchel SF, Lee PA. Relationships, Sexuality, Gender Identity, Gender Roles, and Self-Concept of Individuals Who Have Congenital Adrenal Hyperplasia: A Qualitative Investigation. Journal of Gay & Lesbian Psychotherapy. 2006;10(2):57–75.

42. Malouf MA, Inman AG, Carr AG, Franco J, Brooks LM. Health-Related Quality of Life, Mental Health and Psychotherapeutic Considerations for Women Diagnosed with a Disorder of Sexual Development: Congenital Adrenal Hyperplasia. International Journal of Pediatric Endocrinology. 2010;2010(3):1–11.

43. Lundberg T, Roen K, Hirschberg AL, Frisén L. ” It“s part of me, not all of me”: Young women”s experiences of receiving a diagnosis related to diverse sex development. Journal of Pediatric and Adolescent Gynecology. 2015.

44. Engberg H, Möller A, Hagenfeldt K, Nordenskjöld A, Frisén L. The experience of women living with Congenital Adrenal Hyperplasia: impact of the condition and the care given. Clinical Endocrinology. 2016 Apr 13;85(1):21–8.

45. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. The Lancet. 2005 Jun;365(9477):2125–36.

46. Gidlöf S, Falhammar H, Thilén A, Döbeln Von U, Ritzén M, Wedell A, et al. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. The Lancet Diabetes & Endocrinology. 2013 Sep;1(1):35–42.

47. Lee PA, Houk CP, Husmann DA. Should Male Gender Assignment be Considered in the Markedly Virilized Patient With 46,XX and Congenital Adrenal Hyperplasia? Journal of Urology. Elsevier Inc; 2010 Oct 1;184(S):1786–92.

48. Falhammar H, Thorén M. Clinical outcomes in the management of congenital adrenal hyperplasia. Endocrine. 2012 Jan 7;41(3):355–73.

49. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, MacDougall J. Androgen insensitivity syndrome. The Lancet. 2012;380(9851):1419–28.

50. Berglund A, Johannsen TH, Stochholm K, Viuff MH, Fedder J, Main KM, et al. Incidence, Prevalence, Diagnostic Delay, and Clinical Presentation of Female 46,XY Disorders of Sex Development. The Journal of Clinical Endocrinology & Metabolism. 2016 Dec;101(12):4532–40.

51. Cheikhelard A, Morel Y, Thibaud E, Lortat-Jacob S, Jaubert F, Polak M, et al. Long-Term Followup and Comparison Between Genotype and Phenotype in 29 Cases of Complete Androgen Insensitivity Syndrome. The Journal of Urology. 2008;180(4):1496–501.

52. Boehmer ALM. Genotype Versus Phenotype in Families with Androgen Insensitivity Syndrome. Journal of Clinical Endocrinology & Metabolism. 2001;86(9):4151–60.

53. King TFJ, Wat WZM, Creighton SM, Conway GS. Bone mineral density in complete androgen insensitivity syndrome and the timing of gonadectomy. Clinical Endocrinology. 2017 Jun 8;87(2):136–40.

54. Tadokoro-Cuccaro R, Hughes IA. Androgen insensitivity syndrome. Current Opinion in Endocrinology & Diabetes and Obesity. 2014;21(6):499–503.

55. Wisniewski AB, Migeon CJ, Meyer-Bahlburg HF, Gearhart JP, Berkovitz GD, Brown TR, et al. Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. The Journal of Clinical Endocrinology & Metabolism. 2000 Aug;85(8):2664–9.

56. Hines M, Ahmed SF, Hughes IA. Psychological Outcomes and Gender-Related Development in Complete Androgen Insensitivity Syndrome. Arch Sex Behav. Kluwer Academic Publishers-Plenum Publishers; 2003;32(2):93–101.

57. Brunner F, Fliegner M, Krupp K, Rall K, Brucker S, Richter-Appelt H. Gender Role, Gender Identity and Sexual Orientation in CAIS (“XY-Women”) Compared With Subfertile and Infertile 46,XX Women. The Journal of Sex Research. 2015;53(1):1–16.

58. Aittomäki K. The genetics of XX gonadal dysgenesis. Am J Hum Genet. 1994 May;54(5):844–51.

59. McCann-Crosby B, Mansouri R, Dietrich JE, McCullough LB, Sutton VR, Austin EG, et al. State of the art review in gonadal dysgenesis: challenges in diagnosis and management. International Journal of Pediatric Endocrinology. BioMed Central; 2014;2014(1):4.

60. Swyer G. Male pseudohermaphroditism: a hitherto undescribed form. British medical journal. 1955.

61. Michala L, Goswami D, Creighton SM. Swyer syndrome: presentation and outcomes. Journal of Obstetrics and Gynaecology 2008;115(6):737–41.

62. Looijenga L, Hersmus R, de Leeuw BH. Gonadal tumours and DSD. Best Practice & Research Endocrinology and Metabolism 2010;24(2):291–310.

63. Meyers CM, Boughman JA, Rivas M, Wilroy RS, Simpson JL. Gonadal (ovarian) dysgenesis in 46,XX individuals: Frequency of the autosomal recessive form. American Journal of Medical Genetics. 1996;63(4):518–24.

64. McCarty BM, Migeon CJ, Meyer Bahlburg HFL, Zacur H, Wisniewski AB. Medical and psychosexual outcome in women affected by complete gonadal dysgenesis. Journal of Pediatric Endocrinology and Metabolism. 2006 Jul;19(7):873–7.

65. Luisi S, Orlandini C, Regini C, Pizzo A, Vellucci F, Petraglia F. Premature ovarian insufficiency: from pathogenesis to clinical management. J Endocrinol Invest. Springer International Publishing; 2015 May 7;38(6):597–622.

66. Podfigurna-Stopa A, Czyzyk A, Grymowicz M. Premature ovarian insufficiency: the context of long-term effects. Journal of Psychosomatic Research. 2016;39(9):983–90.

67. Nordenvall AS, Frisén L, Nordenström A. Population based nationwide study of hypospadias in Sweden, 1973 to 2009: incidence and risk factors. 2014;191(3):783–9.

68. van der Zanden L, van Rooij Human reproduction I. Aetiology of hypospadias: a systematic review of genes and environment. academicoupcom. 2012 Feb 26;18(3):260–83.

69. Sharpe RM. Pathways of endocrine disruption during male sexual differentiation and masculinisation. Best Practice & Research Clinical Endocrinology & Metabolism. 2006 Mar;20(1):91–110.

70. Zosuls KM, Miller CF, Ruble DN, Martin CL, Fabes RA. Gender development research in sex roles: Historical trends and future directions. Sex Roles. 2011;64(11-12):826–42.

71. Bao A-M, Swaab DF. Sexual differentiation of the human brain: Relation to gender identity, sexual orientation and neuropsychiatric disorders. Frontiers in Neuroendocrinology. Elsevier Inc; 2011 Apr 1;32(2):214–26.

72. Mueller SC, Grissom EM, Dohanich GP. Assessing gonadal hormone contributions to affective psychopathologies across humans and animal models. Psychoneuroendocrinology. Elsevier Ltd; 2014 Aug 1;46:114–28.

73. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. Current Opinion in Neurology. 2013 Apr;26(2):146–53.

74. Noble RE. Depression in women. Metabolism. 2005 May;54(5):49–52.

75. Goldstein JM, Holsen L, Handa R, Tobet S. Fetal hormonal programming of sex differences in depression: linking women's mental health with sex differences in the brain across the lifespan. Front Neurosci. 2014;8:247.

76. Goldstein JM, Cherkerzian S, Tsuang MT, Petryshen TL. Sex differences in the genetic risk for schizophrenia: History of the evidence for sex-specific and sex-dependent effects. Am J Med Genet. 2013 Oct 17;162(7):698–710.

77. Fratiglioni L, Viitanen M, Strauss E Von, Tontodonati V, Herlitz A, Winblad B. Very Old Women at Highest Risk of Dementia and Alzheimer's Disease: Incidence Data from the Kungsholmen Project, Stockholm. Neurology. 1997 Jan 1;48(1):132–8.

78. Gao S, Hendrie HC, Hall KS, Hui S. The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. Arch Gen Psychiatry. 1998 Sep;55(9):809–15.

79. Miller IN, Cronin-Golomb A. Gender differences in Parkinson's disease: Clinical characteristics and cognition. Mov Disord. 2010 Oct 5;25(16):2695–703.

80. Joel D, Berman Z, Tavor I, Wexler N. Sex beyond the genitalia: The human brain mosaic. 2015. pp. 15468–73.

81. Arnold AP, Burgoyne PS. Are XX and XY brain cells intrinsically different? Trends Endocrinol Metab. 2004 Jan;15(1):6–11.

82. Lenroot RK, Giedd JN. Sex differences in the adolescent brain. Brain and Cognition. 2010;72(1):46–55.

83. Luders E, Gaser C, Narr KL, Toga AW. Why sex matters: brain size independent differences in gray matter distributions between men and women. Journal of Neuroscience. 2009;29(45):14265–70.

84. Luders E, Narr KL, Thompson PM, Rex DE, Jancke L, Steinmetz H, et al. Gender differences in cortical complexity. Nat Neurosci. Nature Publishing Group; 2004 Aug 1;7(8):799–800.

85. Else-Quest NM, Hyde JS, Linn MC. Cross-national patterns of gender differences in mathematics: a meta-analysis. Psychological Bulletin. 2010;136(1):103–27.

86. Guiso L, Monte F, Sapienza P, Zingales L. DIVERSITY: Culture, Gender, and Math. Science. 2008 May 30;320(5880):1164–5.

87. Lippa RA, Collaer ML, Peters M. Sex differences in mental rotation and line angle judgments are positively associated with gender equality and economic development across 53 nations. Arch Sex Behav. 2nd ed. 2010;39(4):990–7.

88. Weber D, Skirbekk V, Freund I, Herlitz A. The changing face of cognitive gender differences in Europe. Proceedings of the National Academy of Sciences. 2014;111(32):11673–8.

89. Geiser C, Lehmann W, Eid M. A note on sex differences in mental rotation in different age groups. Intelligence. 2008 Nov;36(6):556–63.

90. Hyde JS, Linn MC. Gender differences in verbal ability: A meta-analysis. Psychological Bulletin. 1988;104(1):53–69.

91. Herlitz A, Nilsson LG, Bäckman L. Gender differences in episodic memory. Mem Cognit. 1997 Nov;25(6):801–11.

92. Linn MC, Petersen AC. Emergence and Characterization of Sex Differences in Spatial Ability: A Meta-Analysis. Child Development. 2nd ed. 1985;56(6):1479–98.

93. Voyer D, Voyer S, Bryden MP. Magnitude of sex differences in spatial abilities: A meta-analysis and consideration of critical variables. Psychological Bulletin. American Psychological Association; 1995 Mar 1;117(2):250–70.

94. Hall JA, Matsumoto D. Gender Differences in Judgments of Multiple Emotions From Facial Expressions. Emotion. American Psychological Association; 2004 Jun 1;4(2):201–6.

95. McClure EB. A meta-analytic review of sex differences in facial expression processing and their development in infants, children, and adolescents. Psychological Bulletin. 2000;126(3):424–53.

96. Etchell A, Adhikari A, Weinberg LS, Choo AL, Garnett EO, Chow HM, et al. A systematic literature review of sex differences in childhood language and brain development. Neuropsychologia. 2018 Apr 11;114:19–31.

97. Dekhtyar S, Weber D, Helgertz J, Herlitz A. Sex differences in academic strengths contribute to gender segregation in education and occupation_ A longitudinal examination of 167,776 individuals. Intelligence. Elsevier; 2018 Apr 1;67:84–92.

98. Hines M. Sex-related variation in human behavior and the brain. Trends in Cognitive Sciences. 2010;14(10):448–56.

99. Money J, Ehrhardt AA. Man and woman, boy and girl: Differentiation and dimorphism of gender identity from conception to maturity. Johns Hopkins U. Press; 1972.

100. Maccoby EE. The role of gender identity and gender constancy in sex-differentiated development. New Directions for Child and Adolescent Development. US: Jossey-Bass Publishers, Inc; 1990;1990(47):5–20.

101. Steensma TD, Baudewijntje P.C. Kreukels, de Vries ALC, Cohen-Kettenis PT. Gender identity development in adolescence. Hormones and Behavior. Elsevier Inc; 2013 Jul 1;64(2):288–97.

102. Tobin DD, Menon M, Spatta BC. The intrapsychics of gender: a model of self-socialization. Psychological Review. 2010;117(2):601–22.

103. Ruble DN, Taylor LJ, Cyphers L, Greulich FK. The role of gender constancy in early gender development. Child Development. 2007;78(4):1121–36.

104. Hyde JS. Sex and cognition: gender and cognitive functions. Current Opinion in Neurobiology. 2016 Jun;38:53–6.

105. Chen X, Agate RJ, Itoh Y, Arnold AP. Sexually dimorphic expression of trkB, a Z-linked gene, in early posthatch zebra finch brain. Proceedings of the National Academy of Sciences. 2005 May 24;102(21):7730–5.

106. McCarthy MM, Arnold AP. Reframing sexual differentiation of the brain. Nat Neurosci. 2011 May 25;14(6):677–83.

107. Kopsida E, Stergiakouli E, Lynn PM, Wilkinson LS, Davies W. The Role of the Y Chromosome in Brain Function. Open Neuroendocrinol J. 2009;2:20–30.

108. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, de Vries GJ. Sex Differences in the Brain: The Not So Inconvenient Truth. Journal of Neuroscience. Society for Neuroscience; 2012 Feb 15;32(7):2241–7.

109. Gatewood JD, Wills A, Shetty S, Xu J, Arnold AP, Burgoyne PS, et al. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. J Neurosci. Society for Neuroscience; 2006 Feb 22;26(8):2335–42.

110. Direct Regulation of Adult Brain Function by the Male-Specific Factor SRY. Curr Biol. 2006 Feb;16(4):415–20.

111. Ross J, Roeltgen D, Zinn A. Cognition and the Sex Chromosomes: Studies in Turner Syndrome. Horm Res. 2006;65(1):47–56.

112. Burnett AC, Reutens DC, Wood AG. Social cognition in Turner's Syndrome. Journal of Clinical Neuroscience. 2010;17(3):283–6.

113. Verri A, Cremante A, Clerici F. Klinefelter's syndrome and psychoneurologic function. Molecular Human Reproduction. 2010;16(6):425–33.

114. Van Rijn S, Stockmann L. Social cognition and underlying cognitive mechanisms in children with an extra X chromosome: a comparison with autism spectrum disorder. Genes, Brain and Behavior. 2014;13(5):459–67.

115. Helleday J, Bartfai A, Ritzen EM, Forsman M. General intelligence and cognitive profile in women with congenital adrenal hyperplasia (CAH). Psychoneuroendocrinology. 1994;19(4):343–56.

116. Johannsen TH, Ripa C, Reinisch JM. Impaired cognitive function in women with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology & Metabolism. 2006;91(4):1376–81.

117. Malouf MA, Migeon CJ, Carson KA, Petrucci L, Wisniewski AB. Cognitive Outcome in Adult Women Affected by Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. Horm Res. 2006;65(3):142–50.

118. Collaer ML, Hindmarsh PC, Pasterski V. Reduced short term memory in congenital adrenal hyperplasia (CAH) and its relationship to spatial and quantitative performance. Psychoneuroendocrinology. 2016;64:164–73.

119. Hines M, Fane BA, Pasterski VL, Mathews GA. Spatial abilities following prenatal androgen abnormality: Targeting and mental rotations performance in individuals with congenital adrenal hyperplasia. Psychoneuroendocrinology. 2003;28(8):1010–26.

120. Resnick SM, Berenbaum SA, Gottesman II, Bouchard TJ. Early hormonal influences on cognitive functioning in congenital adrenal hyperplasia. Developmental Psychology. 1986;22(2):191–8.

121. Hampson E, Rovet JF, Altmann D. Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Developmental Neuropsychology 1998;14(2-3):299–320.

122. Berenbaum SA, Bryk KLK, Beltz AM. Early androgen effects on spatial and mechanical abilities: Evidence from congenital adrenal hyperplasia. Behavioral Neuroscience. 2012;126(1):86–96.

123. Puts DA, McDaniel MA, Jordan CL, Breedlove SM. Spatial Ability and Prenatal Androgens: Meta-Analyses of Congenital Adrenal Hyperplasia and Digit Ratio (2D:4D) Studies. Arch Sex Behav. 2007;37(1):100–11.

124. Hampson E, Rovet JF. Spatial function in adolescents and young adults with congenital adrenal hyperplasia: Clinical phenotype and implications for the androgen hypothesis. Psychoneuroendocrinology. 2015;54:60–70.

125. Mueller SC, Temple V, Oh E, VanRyzin C, Williams A, Cornwell B, et al. Early androgen exposure modulates spatial cognition in congenital adrenal hyperplasia (CAH). Psychoneuroendocrinology. 2008;33(7):973–80.

126. Masica DN, Money J, Ehrhardt AA, Lewis VG. IQ, fetal sex hormones and cognitive patterns: studies in the testicular feminizing syndrome of androgen insensitivity. Johns Hopkins Med J. 1969 Jan;124(1):34–43.

127. Imperato-McGinley J, Plchardo M, Gautier T, Voyer D, Bryden MP. Cognitive abilities in androgen-insensitive subjects: comparison with control males and females from the same kindred. Clinical Endocrinology. 1991;34(5):341–7.

128. van Hemmen J, Veltman DJ, Hoekzema E, Cohen-Kettenis PT, Dessens AB, Bakker J. Neural Activation During Mental Rotation in Complete Androgen Insensitivity Syndrome: the Influence of Sex Hormones and Sex Chromosomes. Cerebral Cortex. 2014 Dec 1;:1–10.

129. Mueller SC, Verwilst T, Van Branteghem A. The contribution of the androgen receptor (AR) in human spatial learning and memory: a study in women with complete androgen insensitivity syndrome (CAIS). Hormones and Behavior 2016;78:121–6.

130. Stout SA, Litvak M, Robbins NM, Sandberg DE. Congenital Adrenal Hyperplasia: Classification of Studies Employing Psychological Endpoints. International Journal of Pediatric Endocrinology. 4 ed. 2010;2010(9):1–11.

131. Berenbaum SA, Bailey JM. Effects on Gender Identity of Prenatal Androgens and Genital Appearance: Evidence from Girls with Congenital Adrenal Hyperplasia. The Journal of Clinical Endocrinology & Metabolism. 2003;88(3):1102–6.

132. Cohen-Bendahan CCC, van de Beek C, Berenbaum SA. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neuroscience & Biobehavioral Reviews. 2005;29(2):353–84.

133. Frisén L, Nordenström A, Falhammar H. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. The Journal of Clinical Endocrinology & Metabolism. 2009;94(9):3432–9.

134. Hines M, Brook C, Conway GS. Androgen and psychosexual development: Core gender identity, sexual orientation, and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia. Journal of Sex Research. 2004;41(1):75–81.

135. Meyer Bahlburg HFL, Dolezal C, Baker SW, New MI. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. Arch Sex Behav. 10 ed. 2008 Feb;37(1):85–99.

136. Pasterski V, Geffner ME, Brain C, Hindmarsh P, Brook C, Hines M. Prenatal hormones and childhood sex segregation: Playmate and play style preferences in girls with congenital adrenal hyperplasia. Hormones and Behavior. Elsevier Inc; 2011 Apr 1;59(4):549–55.

137. Nordenström A, Servin A, Bohlin G. Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia. The Journal of Clinical Endocrinology & Metabolism. 2002;87(11):5119–24.

138. Pasterski VL, Geffner ME, Brain C, Hindmarsh P, Brook C, Hines M. Prenatal Hormones and Postnatal Socialization by Parents as Determinants of Male-Typical Toy Play in Girls With Congenital Adrenal Hyperplasia. Child Development. 2005;76(1):264–78.

139. Mazur T. Gender dysphoria and gender change in androgen insensitivity or micropenis. Arch Sex Behav. 2005;34(4):411–21.

140. Warne GL. Long-term outcome of disorders of sex development. Sex Dev. 2008;2(4-5):268–77.

141. Sandberg DE, Meyer Bahlburg HFL, Yager TJ, Hensle TW, Levitt SB, Kogan SJ, et al. Gender development in boys born with hypospadias. Psychoneuroendocrinology. 1995;20(7):693–709.

142. Schönbucher VB, Landolt MA, Gobet R, Weber DM. Health-Related Quality of Life and Psychological Adjustment of Children and Adolescents with Hypospadias. The Journal of Pediatrics. 2008 Jun;152(6):865–72.

143. Örtqvist L, Fossum M, Andersson M, Nordenström A, Frisén L, Holmdahl G, et al. Sexuality and fertility in men with hypospadias; improved outcome. Andrology. 2017 Mar;5(2):286–93.

144. Baudewijntje P.C. Kreukels PhD, PhD BKM, PhD ANM, MSc RR, PhD UTM, MD CB, et al. Gender Dysphoria and Gender Change in Disorders of Sex Development/Intersex Conditions: Results From the dsd-LIFE Study. J Sex Med. Elsevier Inc; 2018 May 1;15(5):777–85.

145. Cassia Amaral R, Inacio M, Brito VN, Bachega TASS, Oliveira AA Jr, Domenice S, et al. Quality of life in a large cohort of adult Brazilian patients with 46,XX and 46,XY disorders of sex development from a single tertiary centre. Clinical Endocrinology. 2014 Sep 1;82(2):274–9.

146. Wang C, Tian Q. The investigation of quality of life in 87 Chinese patients with disorders of sex development. Biomed Res Int. 2015;2015(4):342420–6.

147. Rapp M, Mueller-Godeffroy E, Lee P, Roehle R, Baudewijntje P.C. Kreukels, Köhler B, et al. Multicentre cross-sectional clinical evaluation study about quality of life in adults with disorders/differences of sex development (DSD) compared to country specific reference populations (dsd-LIFE). Health Qual Life Outcomes. 3rd ed. Health and Quality of Life Outcomes; 2018 Apr 3;16(1):1–13.

148. Fagerholm R, Mattila AK, Roine RP, Sintonen H, Taskinen S. Mental health and quality of life after feminizing genitoplasty. Journal of Pediatric Surgery. Elsevier Inc; 2012 Apr 1;47(4):747–51.

149. Johannsen TH, Ripa C, Mortensen EL. Quality of life in 70 women with disorders of sex development. European Journal of Endocrinology. 2006;155(6):877–85.

150. de Neve Enthoven N, Callens N. Psychosocial well-being in Dutch adults with disorders of sex development. Journal of Psychosomatic Research. 2016;83:57–64.

151. Cohen-Kettenis PT. Psychosocial and psychosexual aspects of disorders of sex development. Best Practice & Research Clinical Endocrinology & Metabolism. 2010;24(2):325–34.

152. Lee PA, Witchel SF. 46,XX Patients with Congenital Adrenal Hyperplasia: Initial Assignment as Male, Reassigned Female. Journal of Pediatric Endocrinology and Metabolism. 2005;18(2).

153. Meyer-Bahlburg H, Gruen RS, New MI, Bell JJ. Gender change from female to male in classical congenital adrenal hyperplasia. Hormones and Behavior. 1996;30(4):319–32.

154. Cohen-Kettenis PT. Gender Change in 46,XY Persons with 5α-Reductase-2 Deficiency and 17β-Hydroxysteroid Dehydrogenase-3 Deficiency. Arch Sex Behav. Kluwer Academic Publishers-Plenum Publishers; 2005 Aug;34(4):399–410.

155. Mueller SC, Ng P, Sinaii N, Leschek EW, Green-Golan L, VanRyzin C, et al. Psychiatric characterization of children with genetic causes of hyperandrogenism. European Journal of Endocrinology. 2010 Oct 7;163(5):801–10.

156. Halper A, Hooke MC, Gonzalez-Bolanos MT, Vanderburg N, Tran TN, Torkelson J, et al. Health-related quality of life in children with congenital adrenal hyperplasia. Health Qual Life Outcomes. 2017 Oct 6;15(1):194.

157. Gilban DLS, Junior PAGA, Beserra ICR. Health related quality of life of children and adolescents with congenital adrenal hyperplasia in Brazil. Health Qual Life Outcomes. 2014 Aug 13;12(1):1–9.

158. Berenbaum SA, Korman Bryk K, Duck SC, Resnick SM. Psychological adjustment in children and adults with congenital adrenal hyperplasia. The Journal of Pediatrics. Elsevier; 2004 Jan 6;144(6):741–6.

159. Kanhere M, Fuqua J, Rink R, Houk C, Mauger D. Psychosexual development and quality of life outcomes in females with congenital adrenal hyperplasia. International Journal of Pediatric Endocrinology. 2015;2015(1).

160. Nermoen I, Husebye ES, Svartberg J, Løvås K. Subjective health status in men and women with congenital adrenal hyperplasia: a population-based survey in Norway. European Journal of Endocrinology. 2010 Sep;163(3):453–9.

161. Falhammar H, Butwicka A, Landén M. Increased psychiatric morbidity in men with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. 2013.

162. Engberg H, Butwicka A, Nordenström A. Congenital adrenal hyperplasia and risk for psychiatric disorders in girls and women born between 1915 and 2010: a total population study. Psychoneuroendocrinology. 2015;60:195–205.

163. Warne G, Grover S, Hutson J, Sinclair A, Metcalfe S, Northam E, et al. A Long-term Outcome Study of Intersex Conditions. Journal of Pediatric Endocrinology and Metabolism. 18(6):945.

164. Reisch N, Hahner S, Bleicken B, Flade L, Pedrosa Gil F, Loeffler M, et al. Quality of life is less impaired in adults with congenital adrenal hyperplasia because of 21-hydroxylase deficiency than in patients with primary adrenal insufficiency. Clinical Endocrinology. 2011 Jan 6;74(2):166–73.

165. Han TS, Krone N, Willis DS, Conway GS, Hahner S, Rees DA, et al. Quality of life in adults with congenital adrenal hyperplasia relates to glucocorticoid treatment, adiposity and insulin resistance: United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE). European Journal of Endocrinology. 2013;168(6):887–93.

166. Falhammar H, Nyström HF, Thorén M. Quality of life, social situation, and sexual satisfaction, in adult males with congenital adrenal hyperplasia. Endocrine. 2014 Sep;47(1):299–307.

167. Nordenskjöld A, Holmdahl G, Frisén L, Falhammar H, Filipsson H, Thorén M, et al. Type of Mutation and Surgical Procedure Affect Long-Term Quality of Life for Women with Congenital Adrenal Hyperplasia. The Journal of Clinical Endocrinology & Metabolism. 2008 Feb;93(2):380–6.

168. Crouch NS, Liao L-M, Woodhouse CRJ, Conway GS, Creighton SM. Sexual Function and Genital Sensitivity Following Feminizing Genitoplasty for Congenital Adrenal Hyperplasia. The Journal of Urology. 2008 Feb;179(2):634–8.

169. Meyer Bahlburg HFL, Khuri J, Reyes-Portillo J, New MI. Stigma in Medical Settings As Reported Retrospectively by Women With Congenital Adrenal Hyperplasia (CAH) for Their Childhood and Adolescence: Table I. Journal of Pediatric Psychology. 2016 May 16;47:jsw034–8.

170. Gastaud F, Bouvattier C, Duranteau L, Brauner R, Thibaud E, Kutten F, et al. Impaired Sexual and Reproductive Outcomes in Women with Classical Forms of Congenital Adrenal Hyperplasia. The Journal of Clinical Endocrinology & Metabolism. 2007 Apr;92(4):1391–6.

171. Nordenström A, Frisén L, Falhammar H, Filipsson H, Holmdahl G, Janson PO, et al. Sexual Function and Surgical Outcome in Women with Congenital Adrenal Hyperplasia Due to CYP21A2Deficiency: Clinical Perspective and the Patients' Perception. The Journal of Clinical Endocrinology & Metabolism. 2010 Aug;95(8):3633–40.

172. Dessens AB, Slijper FME, Drop SLS. Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. Arch Sex Behav. Kluwer Academic Publishers-Plenum Publishers; 2005 Aug;34(4):389–97.

173. Berenbaum SA. Effects of Early Androgens on Sex-Typed Activities and Interests in Adolescents with Congenital Adrenal Hyperplasia. Hormones and Behavior. 1999;35(1):102–10.

174. Wisniewski A, Mazur T. 46,XY DSD with Female or Ambiguous External Genitalia at Birth due to Androgen Insensitivity Syndrome, 5-Reductase-2 Deficiency, or 17-Hydroxysteroid Dehydrogenase Deficiency: A Review of Quality of Life Outcomes. International Journal of Pediatric Endocrinology. 2009;2009(1):567430.

175. Wisniewski AB. Gender Development in 46,XY DSD: Influences of Chromosomes, Hormones, and Interactions with Parents and Healthcare Professionals. Scientifica. 2012;2012:1–15.

176. Alderson J, Madill A, Balen A. Fear of devaluation: Understanding the experience of intersexed women with androgen insensitivity syndrome. British Journal of Health Psychology. 2004;9(1):81–100.

177. Snodgrass W, Bush N. Recent advances in understanding/management of hypospadias. F1000Prime Rep. 2014;6:101.

178. Örtqvist L, Fossum M, Andersson M, Nordenström A, Frisén L, Holmdahl G, et al. Long-Term Followup of Men Born with Hypospadias: Urological and Cosmetic Results. The Journal of Urology. 2015;193(3):975–82.

179. Lecrubier Y, Sheehan DV, Weiller E, Amorim P, Bonora I, Sheehan KH, et al. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. European Psychiatry. Éditions scientifiques et médicales Elsevier, Paris; 1997;12(5):224–31.

180. Pettersson A, Modin S, Wahlström R, Hammarberg SAW, Krakau I. The Mini-International Neuropsychiatric Interview is useful and well accepted as part of the clinical assessment for depression and anxiety in primary care: a mixed-methods study. BMC Fam Pract. BMC Family Practice; 2018 Jan 23;19(1):1–13.

181. Miller DI, Halpern DF. The new science of cognitive sex differences. Trends in Cognitive Sciences. 2014;18(1):37–45.

182. Vandenberg SG, Kuse AR. Mental rotations, a group test of three-dimensional spatial visualization. Percept Mot Skills. 1978 Oct;47(2):599–604.

183. Collaer ML, Reimers S, Manning JT. Visuospatial performance on an internet line judgment task and potential hormonal markers: sex, sexual orientation, and 2D: 4D. Arch Sex Behav. 2007;36(2):177–92.

184. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. J Child Psychol Psychiatry. 2001 Feb;42(2):241–51.

185. Sandberg DE, Meyer-Bahlburg HF, Aranoff GS, Sconzo JM, Hensle TW. Boys with hypospadias: a survey of behavioral difficulties. Journal of Pediatric Psychology. 1989 Dec;14(4):491–514.

186. Wiesemann C, Ude-Koeller S, Sinnecker GHG, Thyen U. Ethical principles and recommendations for the medical management of differences of sex development (DSD)/intersex in children and adolescents. European Journal of Pediatrics. Springer-Verlag; 2018 Jan 26;169(6):671–9.

187. Kokko J, Eriksson L. Vården av intersexuella barn – etiska aspekter på tidiga kirurgiska ingrepp. SMER kommenterar. 2017 Mar pp. 1–5.

188. Bodin M. Vård och behandling av personer med intersexuella tillstånd – Kartläggning av det tidiga omhändertagandet. socialstyrelsens rapportserie. 2017 Jan 23;:1–94.

189. Amnesty International. First do no harm: Ensuring the rights of children born intersex. [Internet]. Available from: https://www.amnesty.org/en/latest/campaigns/2017/05/intersex-rights/

190. Human rights commissioner Conseil de l'Europe. Human rights and intersex people. 2017. 62

191. Mouriquand P. Commentary to ``Attitudes towards `disorders of sex development' nomenclature among affected''. Journal of Pediatric Urology. Journal of Pediatric Urology Company; 2017 Dec 1;13(6):609.

192. Cools M, Simmonds M, Elford S, Gorter J, Ahmed SF, D'Alberton F, et al. Response to the Council of Europe Human Rights Commissioner's Issue Paper on Human Rights and Intersex People. Eur Urol. European Association of Urology; 2016 Sep 1;70(3):407–9.

193. Crump C, Rivera D, London R, Landau M, Erlendson B, Rodriguez E. Chronic health conditions and school performance among children and youth. Ann Epidemiol. 2013 Apr;23(4):179–84.

194. Maslow GR, Haydon A, McRee AL, Ford CA, Halpern CT. Growing Up With a Chronic Illness: Social Success, Educational/Vocational Distress. JAH. Elsevier Inc; 2011 Aug 1;49(2):206–12.

195. Sawyer SM, Drew S, Yeo MS, Britto MT. Adolescents with a chronic condition: challenges living, challenges treating. The Lancet. 2007;369(9571):1481–9.

196. Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain and Cognition. 2007 Dec;65(3):209–37.

197. Erickson K, Drevets W, Schulkin J. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. Neuroscience & Biobehavioral Reviews. 2003 May;27(3):233–46.

198. Webb EA, Elliott L, Carlin D, Wilson M, Hall K, Netherton J, et al. Quantitative MRI brain in congenital adrenal hyperplasia: in vivo assessment of the cognitive and structural impact of steroid hormones. The Journal of Clinical Endocrinology & Metabolism. 2017 Nov 20.

199. Słowikowska-Hilczer J, Hirschberg AL, Claahsen-van der Grinten H, Reisch N, Bouvattier C, Thyen U, et al. Fertility outcome and information on fertility issues in individuals with different forms of disorders of sex development: findings from the dsd-LIFE study. Fertility and Sterility. 2017 Nov;108(5):822–31.

200. Meyer-Bahlburg HFL. What Causes Low Rates of Child-Bearing in Congenital Adrenal Hyperplasia? Journal of Clinical Endocrinology & Metabolism. 1999;84(6):1844–7.

201. Bidet M, Bellanné-Chantelot C, Galand-Portier M-B, Golmard J-L, Tardy V, Morel Y, et al. Fertility in women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. The Journal of Clinical Endocrinology & Metabolism. 2010 Mar;95(3):1182–90.

202. Casteràs A, De Silva P, Rumsby G, Conway GS. Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate. Clinical Endocrinology. 2009 Jun;70(6):833–7.

203. Bouvattier C, Esterle L, Renoult-Pierre P. Clinical outcome, hormonal status, gonadotrope axis, and testicular function in 219 adult men born with classic 21-hydroxylase deficiency. A French national survey. The Journal of Clinical Endocrinology & Metabolism. 2015;100(6):2303–13.

204. Jääskeläinen J, Tiitinen A, Voutilainen R. Sexual function and fertility in adult females and males with congenital adrenal hyperplasia. Horm Res. Karger Publishers; 2001;56(3-4):73–80.

205. Falhammar H, Frisén L, Norrby C, Almqvist C, Hirschberg AL, Nordenskjöld A, et al. Reduced Frequency of Biological and Increased Frequency of Adopted Children in Males With 21-Hydroxylase Deficiency: A Swedish Population-Based National Cohort Study. The Journal of Clinical Endocrinology & Metabolism. 2017 Nov 1;102(11):4191–9.

206. Mitra MT, Jönsson P, Åkerblad A-C, Clayton P, Kołtowska-Häggström M, Korbonits M, et al. Social, educational and vocational outcomes in patients with childhood-onset and young-adult-onset growth hormone deficiency. Clinical Endocrinology. Wiley/Blackwell (10.1111); 2017 Feb 3;86(4):526–33.

207. Hendricks ML, Testa RJ. A conceptual framework for clinical work with transgender and gender nonconforming clients: An adaptation of the Minority Stress Model. Professional Psychology: Research and Practice. 2012;43(5):460–7.

208. van de Grift TC, Cohen-Kettenis PT, de Vries ALC, Kreukels BPC. Body image and self-esteem in disorders of sex development: A European multicenter study. Health Psychology. 2018 Apr;37(4):334–43.

209. Berglund A, Johannsen TH, Stochholm K, Viuff MH, Fedder J, Main KM, et al. Morbidity, mortality, and socioeconomics in females with 46,XY disorders of sex development: a nationwide study. The Journal of Clinical Endocrinology & Metabolism. 2017 Nov 20.

210. D'Alberton F, Assante MT, Foresti M. Quality of life and psychological adjustment of women living with 46, XY differences of sex development. The journal of sexual medicine 2015;12(6):1440–9.

211. Köhler B, Kleinemeier E, Lux A, Hiort O, Grüters A, Thyen U, et al. Satisfaction with Genital Surgery and Sexual Life of Adults with XY Disorders of Sex Development: Results from the German Clinical Evaluation Study. The Journal of Clinical Endocrinology & Metabolism. 2012 Feb;97(2):577–88.

212. Schönbucher V, Schweizer K, Rustige L. Sexual quality of life of individuals with 46, XY disorders of sex development. J Sex Med. 2012;9(12):3154–70.

213. Kessler RC. Epidemiology of women and depression. Journal of Affective Disorders. 2003;74(1):5–13.

214. Steiner M, Dunn E, Born L. Hormones and mood: from menarche to menopause and beyond. Journal of Affective Disorders. 2003;74(1):67–83.

215. Schützmann K, Brinkmann L, Schacht M, Richter-Appelt H. Psychological Distress, Self-Harming Behavior, and Suicidal Tendencies in Adults with Disorders of Sex Development. Arch Sex Behav. 2nd ed. 2007 Oct 18;38(1):16–33.

216. Heller-Boersma JG, Edmonds DK. A cognitive behavioural model and therapy for utero-vaginal agenesis (Mayer-Rokitansky-Küster-Hauser syndrome: MRKH). Behavioural and Cognitive Psychotherapy. 2009;37(04):449.

217. Hines M. Gender development and the human brain. Annual review of neuroscience. 2011.

218. Khorashad BS, Khazai B, Roshan GM, Hiradfar M, Afkhamizadeh M, van de Grift TC. Prenatal testosterone and theory of mind development_ Findings from disorders of sex development. Psychoneuroendocrinology. Elsevier; 2018 Jan 29;:1–6.

219. Lawrence K, Campbell R, Skuse D. Age, gender, and puberty influence the development of facial emotion recognition. Front Psychol. 2015 Jun 16;6(e20989):195.

220. Montoya ER, Bos PA. How Oral Contraceptives Impact Social-Emotional Behavior and Brain Function. Trends in Cognitive Sciences. Elsevier Ltd; 2017 Feb 1;21(2):125–36.

221. Valla JM, Ceci SJ. Can sex differences in science be tied to the long reach of prenatal hormones? Brain organization theory, digit ratio (2D/4D), and sex differences in preferences and cognition. Perspectives on Psychological Science. 2011;6(2):134–46.

222. Vuoksimaa E, Kaprio J, Kremen WS. Having a male co-twin masculinizes mental rotation performance in females. Psychological Science. 2010;21(8):1069–71.

223. Lamminmäki A, Hines M, Kuiri-Hänninen T, Kilpeläinen L, Dunkel L, Sankilampi U. Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls. Hormones and Behavior. 2012 Apr;61(4):611–6.

224. Pasterski V, Acerini CL, Dunger DB, Ong KK, Hughes IA, Thankamony A, et al. Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: Evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. Hormones and Behavior. 2015;69:98–105.

225. Hines M, Spencer D, Kung KT, Browne WV, Constantinescu M, Noorderhaven RM. The early postnatal period, mini-puberty, provides a window on the role of testosterone in human neurobehavioural development. Current Opinion in Neurobiology. 2016 Jun;38:69–73.

226. Nguyen T-V, McCracken JT, Albaugh MD, Botteron KN, Hudziak JJ, Ducharme S. A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood. Psychoneuroendocrinology. 2016 Jan;63:109–18.

227. Herlitz A, Reuterskiöld L, Lovén J, Thilers PP, Rehnman J. Cognitive sex differences are not magnified as a function of age, sex hormones, or puberty development during early adolescence. Dev Neuropsychol. 5 ed. 2013;38(3):167–79.

228. Vuoksimaa E, Kaprio J, Eriksson C. Pubertal testosterone predicts mental rotation performance of young adult males. Psychoneuroendocrinology. 2012;37(11):1791–800.

229. Browne WV, Hindmarsh PC, Pasterski V. Working memory performance is reduced in children with congenital adrenal hyperplasia. Hormones and Behavior. 2015;67:83–8.

230. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. Androgen excess is due to elevated 11-oxygenated androgens in treated children with congenital adrenal hyperplasia. J Steroid Biochem Mol Biol. 2018 Apr;178:221–8.

231. Bennecke E, Werner-Rosen K, Thyen U, Kleinemeier E, Lux A, Jürgensen M, et al. Subjective need for psychological support (PsySupp) in parents of children and adolescents with disorders of sex development (dsd). European Journal of Pediatrics. 2015 Oct;174(10):1287–97.

232. Sandberg D, Gardner M, Cohen-Kettenis P. Psychological Aspects of the Treatment of Patients with Disorders of Sex Development. Semin Reprod Med. 2012 Oct 8;30(05):443–52.

233. Röhle R, Gehrmann K, Szarras-Czapnik M, Claahsen-van der Grinten H, Pienkowski C, Bouvattier C, et al. Participation of adults with disorders/differences of sex development (DSD) in the clinical study dsd-LIFE: design, methodology, recruitment, data quality and study population. BMC Endocr Disord. 2017 Aug 18;17(1):e488–1.

234. Wallensteen L, Zimmermann M. Sex-Dimorphic Effects of Prenatal Treatment With Dexamethasone. The Journal of Clinical endocrinology and Metabolim 2016;101(10):3838–46.

235. Bramble MS, Lipson A, Vashist N, Vilain E. Effects of chromosomal sex and hormonal influences on shaping sex differences in brain and behavior: Lessons from cases of disorders of sex development. Cahill L, editor. Journal of Neuroscience Research. 2017 Jan 2;95(1-2):65–74.

236. Skarin Nordenvall A, Norrby C, Butwicka A, Frisén L, Nordenström A, Almqvist C, et al. Psychosocial outcomes in adult men born with hypospadias: A register-based study. Nishimura W, editor. PLoS ONE. 2017 Apr 6;12(4):e0174923–10.

237. Jannini EA, Burri A, Jern P. Genetics of human sexual behavior: where we are, where we are going. Sexual Medicine 2015;3(2):65–77.