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

REGISTER-BASED STUDIES OF HYPOSPADIAS

Anna Skarin Nordenvall

Karolinska Institutet

Stockholm 2017

Published by Karolinska Institutet.
Cover illustration: Arvid Steen
Printed by E-Print AB 2017

ISBN 978-91-7676-714-6

Karolinska Institutet

Institutionen för kvinnors och barns hälsa

Register-based studies of hypospadias

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Leksellsalen, Eugeniahemmet, Karolinska Universitetssjukhuset

Fredagen den 9 juni 2017 klockan 9.00

av

Anna Skarin Nordenvall

Huvudhandledare:
Professor Agneta Nordenskjöld
Karolinska Institutet
Institutionen för kvinnors och barns hälsa

Fakultetsopponent:
Professor Ragnhild Emblem
Universitetet i Oslo
Avdeling for gastro- og barnekirurgi

Bihandledare:
Docent Louise Frisén
Karolinska Institutet
Institutionen för klinisk neurovetenskap

Docent Anna Nordenström
Karolinska Institutet
Institutionen för kvinnors och barns hälsa

Betygsnämnd:
Docent Ulrik Kvist
Karolinska Institutet
Institutionen för medicin

Docent Helene Engstrand Lilja
Uppsala Universitet
Institutionen för kvinnors och barns hälsa

Professor Anders Hjern
Karolinska Institutet
Institutionen för medicin

Stockholm 2017

To my family

ABSTRACT

Hypospadias is a common genital malformation of complex origin. It is characterized by misplacement of the urethral orifice, proximal to the tip of the glans penis, and is most often accompanied by a cleaved prepuce and varying degrees of ventral penile curvature. The phenotype ranges from distal hypospadias, where the misplacement of the urethral orifice is small, to more proximal cases where the urethral orifice may be located in the perineum and lead to uncertain sex at birth.

This thesis aims to elucidate aspects of the etiology, and increase the knowledge concerning the consequences of being born with hypospadias. All studies are based on information collected from national population-based Swedish registers, containing healthcare and demographic data.

In ***Study I*** we investigated associated risk factors, in terms of parental and perinatal characteristics, and the prevalence of hypospadias. We found an increased prevalence of boys assigned with hypospadias during the late 20th century. Further, we found that boys born small for gestational age, as twins, as a result of ART, or by parents from greater Europe (excluding the Nordic countries) or Asia were at an increased risk of being registered with a diagnosis of hypospadias. The trend in prevalence was not attributable to temporal changes in the investigated risk factors.

Study II highlighted the association between hypospadias and the neuromuscular disorder known as spinal-bulbar muscle atrophy (SBMA), for which the common denominator is a CAG repeat expansion in the androgen receptor (*AR*) gene. We described one clinical case; a boy born with proximal hypospadias who was found to have 42 CAG repeats in the *AR* gene, which is a mutation known to cause SBMA later in life. In Swedish health care registers we found four potential cases of SBMA and hypospadias in individuals and within families.

In ***Study III*** we investigated socioeconomic outcomes in men born with hypospadias as a proxy of well-being in adulthood. We found that men born with hypospadias displayed a similar level of education and income, and were as likely to be married as non-affected men. Men with proximal hypospadias did, however, suffer a greater risk of receiving a disability pension. This risk may be due to the effect of unmeasured psychiatric comorbidity, conditions related to androgen deficiency, or hypospadias as a part of unrecognized syndromes.

In ***Study IV*** we aimed to assess the fertility of adult men born with hypospadias. We found a lower probability of registered paternity among men with hypospadias; the association was most prominent in men with proximal hypospadias and of small magnitude in distal hypospadias. Men with hypospadias were more likely to being diagnosed with male infertility and of conceiving through ART. The overall results imply that fertility is impaired in men with distal and proximal hypospadias, probably as a result of anatomic features, gonadal dysfunction, psychological, or genetic factors.

SVENSK SAMMANFATTNING

Hypospadi är en vanlig medfödd genital missbildning hos pojkar. Missbildningen karaktäriseras av att urinröret inte mynnar på toppen av ollonet, utan proximalt längs penis undersida. Utöver den avvikande lokalisationen av urinrörsmynningen, återfinns ofta en kluven förhud och en varierande grad av ventral kurvering av penis. Fenotypen varierar stort, från fall med liten grad av missbildning hos vilka urinrörsmynningens lokalisation är litet avvikande, till mer uttalade fall i vilka urinrörsmynning kan återfinnas i perineum och som ibland resulterar i svårigheter att vid födseln avgöra vilket kön barnet har.

Syftet med denna avhandling var att öka kunskaperna om orsakerna till att hypospadi uppstår samt att undersöka hur vuxna mäns välmående och fertilitet påverkas av att vara född med hypospadi. Samtliga studier baserades på information hämtad från svenska nationella populationsbaserade register.

Studie I syftade till att undersöka vilka riskfaktorer som är associerade med hypospadi samt till att studera om prevalensen av hypospadi förändrats över tid. Vi fann att pojkar födda små för tiden, som tvillingar, som ett resultat av IVF-behandling och av föräldrar från icke-nordiska Europa och Asien hade större risk att vara drabbade av hypospadi. Vi fann därtill en ökning av antal pojkar som diagnostiserats med hypospadi under senare delen av 1900-talet.

I ***Studie II*** uppmärksammade vi sambandet mellan hypospadi och den neuromuskulära sjukdomen spinobulbär muskelatrofi (SBMA), vars gemensamma nämnare utgörs utav en förlängd CAG sekvens i androgenreceptor (*AR*) genen. Studien beskriver ett fall som utgörs av en pojke med proximal hypospadi, som i samband med molekylär utredning befanns vara bärare av 42 CAG-kopior i *AR* genen, vilket är patognomont med framtida SBMA. Med hjälp av registerbaserad data fann vi fyra fall av hypospadi och misstänkt SBMA hos enskilda individer och inom familjer

I ***Studie III*** undersökte vi socioekonomiska utfall i syfte att värdera välmående i vuxen ålder. Vi fann att män med hypospadi uppnådde likvärdig utbildnings- och inkomst-nivå, samt gifte sig i lika stor utsträckning som män som inte var drabbade av hypospadi. Vi fann dock en ökad risk för förtidspensionering bland män med proximal hypospadi, vilket kan vara en effekt av psykiatrisk sjuklighet och odiagnostiserade syndrom eller vara en konsekvens av sjuklighet associerad med androgenbrist.

Studie IV syftade till att bedöma fertiliteten hos män med hypospadi, med hjälp av register-baserad data. Vi fann att män med hypospadi var registrerade som fäder i mindre omfattning än män utan hypospadi, skillnaden var störst hos män med proximal hypospadi och mycket liten hos män med distal hypospadi. Därtill fann vi att män med hypospadi i större utsträckning fått barn med hjälp av assisterad befruktning och oftare hade erhållit diagnosen manlig infertilitet. Sammantaget tyder dessa resultat på att fertiliteten hos män med hypospadi är nedsatt.

PREFACE

In the beginning there is nothing more than that tiny, single cell. From the outside it may not look that interesting, but when exploring what hides within its wall you find an enormous and neatly packed register crammed with data; an individual and unique biological code, the DNA. The content of that register provides the basis for what comes next, the continuation of life. Some of the data is unconditional and may not be influenced by the way we live our lives, such as the code that determines the color of our eyes or the presence of a third copy of chromosome 21. Other data may merely provide cornerstones that through intricate interplay with behaviors and exposures generate what makes us unique, such as our personality traits and risk of numerous illnesses.

This thesis provides four scientific papers in which we have investigated how one single event in life, being born with hypospadias, may arise and what consequences it may lead to. In order to do so we have grouped large numbers of diverse individuals, with unique biological codes, only because they share one anatomical trait. The results of our studies provide knowledge and answers that may be helpful in the meeting between healthcare professionals and patients, but let us remember that what's measurable on a group level may not be true for the individual and that there is so much more that defines a human being than a phenotypic variation.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals (I-IV).

I. **Nordenvall AS,** Frisén L, Nordenström A, Lichtenstein P, Nordenskjöld A.
Population based nationwide study of hypospadias in Sweden, 1973 to 2009: incidence and risk factors.
The Journal of Urology. 2014;191:783-9.

II. **Nordenvall AS,** Paucar M, Almqvist C, Nordenström A, Frisén L, Nordenskjöld A.
Hypospadias as a novel feature in spinal bulbar muscle atrophy.
Journal of Neurology. 2016;263:703-6.

III. **Nordenvall AS,** Norrby C, Butwicka A, Frisén L, Nordenström A, Almqvist C, Nordenskjöld A.
Psychosocial Outcomes in Adult Men Born with Hypospadias: A Register-Based Study.
PLOS ONE. 2017;12. e0174923.

IV. **Nordenvall AS,** Norrby C, Chen Q, Frisén L, Nordenström A, Almqvist C, Nordenskjöld A.
Fertility in hypospadias: a nationwide register-based cohort study with sibling analysis.
Manuscript

LIST OF ABBREVIATIONS

AMH	Anti-Müllerian Hormone
AR	Androgen Receptor
ART	Assited Reproductive Technologies
BMI	Body Mass Index
CAH	Congenital Adrenal Hyperplasia
CI	Confidence Interval
DHT	Dihydrotestosterone
DSD	Disorders (or differences) of sex development
EDC	Endocrine Disrupting Chemical
EUROCAT	European Surveillance of Congenital Abnormalities and Twins
FoB	The Population and Housing Censuses
FSH	Follicle-Stimulating Hormone
hCG	Human Chorionic Gonadotropin
HR	Hazard Ratio
ICSI	Intracytoplasmic Sperm Injection
ICD	International Classification of Disease
IVF	In Vitro Fertilization
LH	Luteinizing Hormone
LISA	Longitudinal Integration database for Health and Labor Market Studies
MBR	Medical Birth Register
MGR	Multi-Generation Register
NBHW	National Board of Health and Welfare
NPR	National Patient Register
OR	Odds Ratio
PIN	Personal Identification Number
SBMA	Spinal-Bulbar Muscle Atrophy
SES	Socioeconomic Status
SGA	Small for Gestational Age

SRY	Sex-determining Region Y
TDS	Testicular Dysgenesis Syndrome
TPR	Total Population Register
UREG	Register of Education

CONTENTS

1 INTRODUCTION

1.1 Male sex development

The first determinant of sex differentiation, the chromosomal sex, is established at fertilization, when the ovum with its X chromosome fuses with a sperm carrying an additional sex chromosome, either X or Y. Thereafter, the embryo stays anatomically undifferentiated until fetal week seven, when the gonadal tissues begin to differentiate to testes or ovaries, depending on which sex chromosomes are present. The type of gonad then influences the development of the phenotypic sex, male or female, which is defined by the internal and external genitalia and secondary sex characteristics [1].

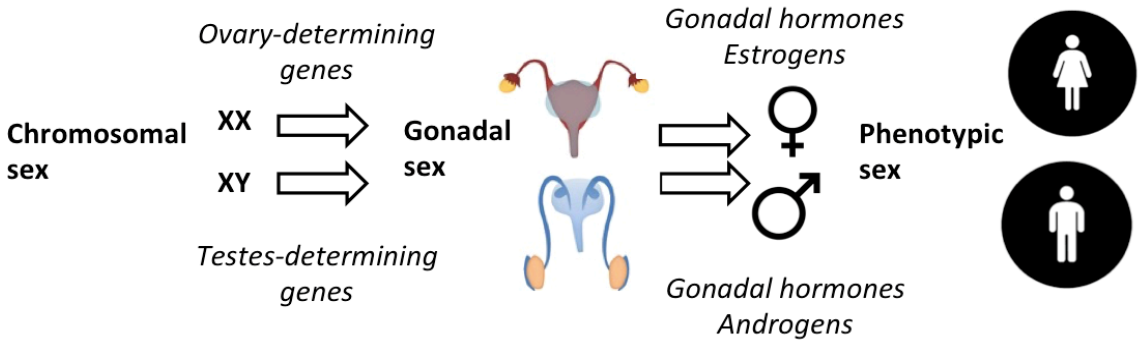

Figure 1. *Simplified sex differentiation in humans: chromosomal sex dictates the development of the bipotential gonads towards either testes or ovaries, the gonadal sex thereafter influences the phenotypic sex development.*

The undifferentiated stage

Approximately four weeks after fertilization, the urogenital ridges, the precursors of the urogenital system, develop in the still sexually undifferentiated embryo. The urogenital ridge gives rise to two duct systems, the Wollfian and Müllerian ducts, which are the primordial parts of the inner genitalia in males and females, respectively. The most distal parts of these ducts fuse together in the cloaca, which is surrounded by the inner and outer genital folds at the orifice. The genital folds join at the anterior end of the cloaca and are delimited by the genital tubercle that later gives rise to either the penis or clitoris. Rudimentary gonads arise through immigration of primordial germ cells into the gonadal ridges in the sixth week and are at this point still bipotential [1, 2].

Differentiation of male internal genitalia

If a Y chromosome is present in the 7th fetal week, a complex interplay between genes will generate testes and subsequent hormonal action will lead to male phenotypic sex development.

The main factor for male sex development, located on the Y chromosome, is the *SRY* gene (*S*ex-determining *R*egion of the *Y* chromosome). This gene is expressed in the gonadal ridges and initiates the development of the gonads towards testicular tissue. The *SRY* gene induces a

genetic cascade, including actions by the *NR5A1* and *SOX9* genes, which drives the gonad towards Sertoli cell differentiation. The Sertoli cells of the testis secrete anti-müllerian-hormone (AMH) that leads to regression of the Müllerian ducts (the primordial part of female internal genitalia). Placental hCG (Human Chorionic Gonadotropin), fetal pituitary luteinizing hormone (LH), and factors secreted from Sertoli cells stimulate the development of the Leydig cells of the testis. The Leydig cells are the main providers of testosterone that, by binding to the androgen receptor (encoded by the *AR* gene), induce the differentiation of the Wollfian ducts to internal male genitalia: the seminal vesicles, vas deferens, and epididymides. In the absence of a Y chromosome, and subsequent absence of testes, AMH and androgen action, the internal and external genitalia will typically develop towards the female phenotype [1, 3-5].

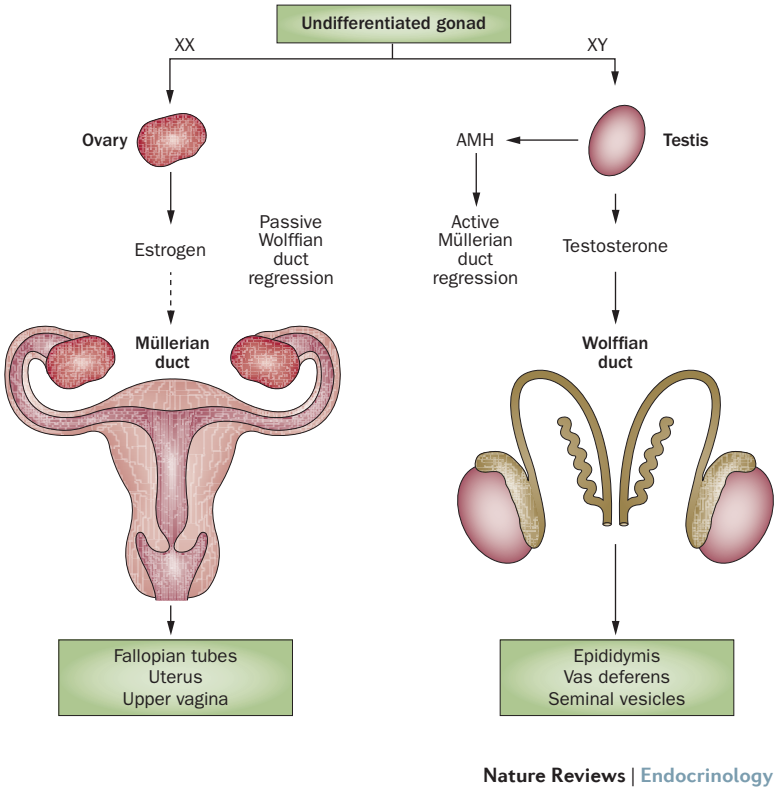

Figure 2. *Schematic illustration of the development of internal genitalia in the male and female. Published with the permission of Nature publishing group.*

Differentiation of male external genitalia

In the primordial parts of the external male genitalia, testosterone is further converted to dihydrotestosterone (DHT) by steroid 5 alfa-reductase enzyme (encoded by the *SRD5A2* gene). DHT is a more potent androgen required for the development of male external genitalia. It binds to the androgen receptor in the target tissues and thereby drives the differentiation towards male development of the external genitalia.

The virilization of the male external genitalia begins in fetal week nine with the fusion of the labioscrotal folds that leads to lengthening of the anogenital distance and elongation of the

genital tubercle into the phallus. The cloaca is at this point subdivided into the urogenital sinus and rectum [1, 2, 6].

As the genital tubercle elongates, the urethral groove develops on its ventral aspect, defined by urethral folds and lateral genital swellings, the latter giving rise to the scrotum in the male. An epithelial layer, referred to as the urethral plate, covers the lining of the urethral groove. The prostatic and membranous parts of the urethra originate from the urogenital sinus, whilst the penile part of the urethra develops through DHT acting on the urethral folds. The urethral folds follow the elongation of the genital tubercle and fuse medially over the urethral groove in a proximal to distal direction like a zipper, thereby creating a tube that communicates with the urogenital sinus and runs to the base of the glans.

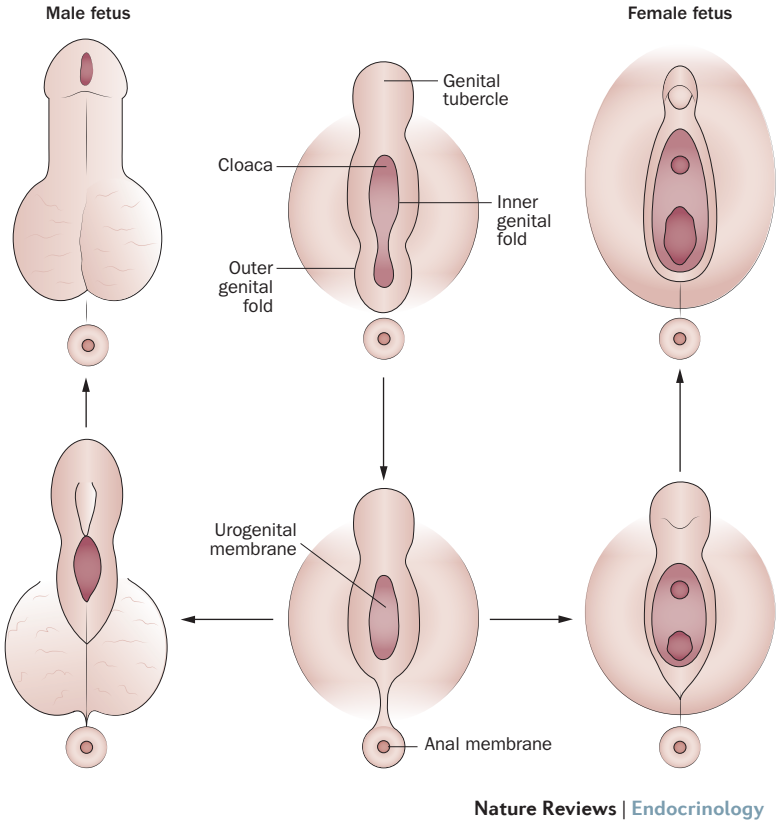

Figure 3. *Schematic illustration of the development of external genitalia in the male and female. Published with the permission of Nature publishing group.*

This fusion is often completed by the end of the first trimester. The most distal glandular part is the last step in the formation of the urethra, completed by the end of the fourth month. It is thought to arise through ectodermal ingrowth from the glans, with the formation of a short cord connecting to the penile urethra, but other mechanisms such as canalization of a solid urethral plate have been proposed. The corpus spongiosum and corporeal bodies form after the complete fusion of the urethra through the fusion of the outer genital folds in a proximal to distal direction. The final formation of male external genitalia with the closure of the prepuce is completed by fetal week 20 [6-8].

1.2 Characteristics of hypospadias

Most commonly, the differentiation into male and female phenotypic sex follows the textbook example described above but, in some cases, the development of chromosomal, gonadal or phenotypic sex is atypical. Conditions where the development of sex is different are collected under a common umbrella term: disorders (or differences) of sex development (DSD). Diagnoses included in the concept of DSD are diverse, both regarding physical traits and molecular diagnoses. The sub-classifications of the conditions collectively named as DSD are made upon chromosomal sex. Congenital adrenal hyperplasia in girls presents as 46,XX DSD, with virilized external genitalia, including fusion of the labioscrotal folds and clitoromegaly, and proximal hypospadias is a physical trait that may be present in the under-masculinized child with 46,XY DSD [9].

Hypospadias is an inborn error in the development of the male external genitalia and is characterized by three main anatomical features; an abnormal location of meatus somewhere alongside the ventral part of the penis, a ventral curvature of the penis, and a cleaved hood-like prepuce assembled on the dorsal side of the penis. Another important feature that may be present, and should be evaluated early on, is meatal stenosis [10].

The abnormally positioned, and sometimes narrow, meatus may result in a ventrally deflected and splayed stream of urine. This can make the stream difficult to control and patients may experience trouble voiding while standing. The small child may sometimes suffer from a meatal stenosis with subsequent difficulties for completely emptying the bladder. Other symptoms mainly affect older boys and adult men, such as the ventral curvature that can result in painful erections and inhibit sexual intercourse. Further, the overall appearance of hypospadias may lead to cosmetic dissatisfaction.

Hypospadias develops due to failure of the normal, zipper like, tubularization of the urethra in fetal weeks 9-16. The abnormal position of the meatus depends on when the failure of ventral fusion occurs. The later the failure occurs the more distal the meatus will be positioned.

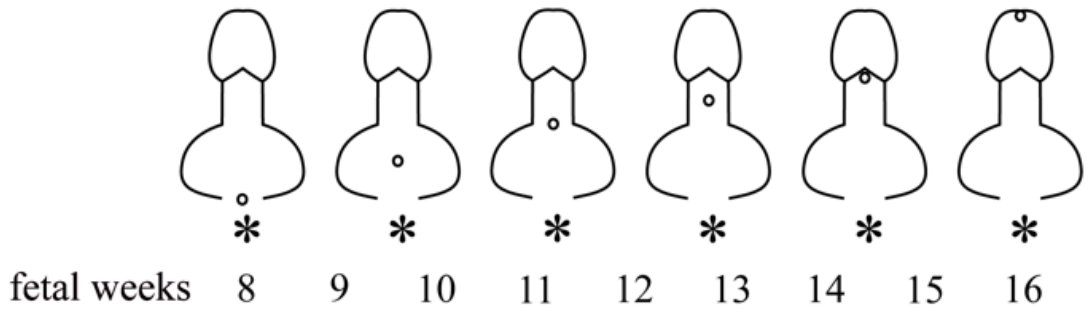

Figure 4. *Fusion of the urethral folds, in a proximal to distal direction during fetal weeks 9-16.*

If the failure occurs in the later part of urethral development the boy presents with a distal hypospadias with a slightly misplaced meatus, close to the tip of the glans or at the subcoronal margin. An early failure may result in incomplete fusion of both urethral and genital folds leaving the boy with a much more proximally located meatus located in the perineum and a bifid scrotum and may even result in ambiguous genitalia at birth.

Ventral curvature is present in 10 to 30% of cases of hypospadias [11]. The degree of penile curvature varies and is often associated with the position of the meatus. Just proximal to the aberrant meatus the spongiosal body, that should surround the entire penile urethra, is split into two diverging branches. This is usually where the ventral penile curvature occurs. A distal hypospadias may present with none or a small degree of curvature, whilst a more proximally located meatus is most often associated with a severe penile curvature. In distal hypospadias the curvature is primarily caused by deficient periurethral growth or skin length, whilst the curvature associated with proximal hypospadias is due to apoptosis of the urethral plate and corpus spongiosum in lack of sufficient androgen action [10]. An important aspect of the penile curvature is that when it is released, it may reveal that the meatus is in a much more proximal position than first expected. With the arrest in development there is often also an impaired quality of the skin and supportive tissue on the ventral side of the penis and there may be a lack of corpus spongiosum proximal to the meatus. Most commonly, the prepuce fails to encircle on the ventral side of the penis, but in rare cases hypospadias presents with an intact prepuce, and may then be diagnosed during circumcision or after puberty. Other characteristics of hypospadias that may be present are penoscrotal transposition, penile torsion, webbed or concealed penis, and glans tilt, and in some cases there is only penile curvature without an aberrant meatal position [10].

The phenotype is diverse and depends on all the anatomical features described above. A correct and uniform description of each patient is desirable in determining what type of repair is possible and in order to enable research on surgical outcomes. At present, the most common classification is based on the location of the meatus on physical examination prior to surgery, the distal form being the most common and the proximal being the least frequent [10, 12].

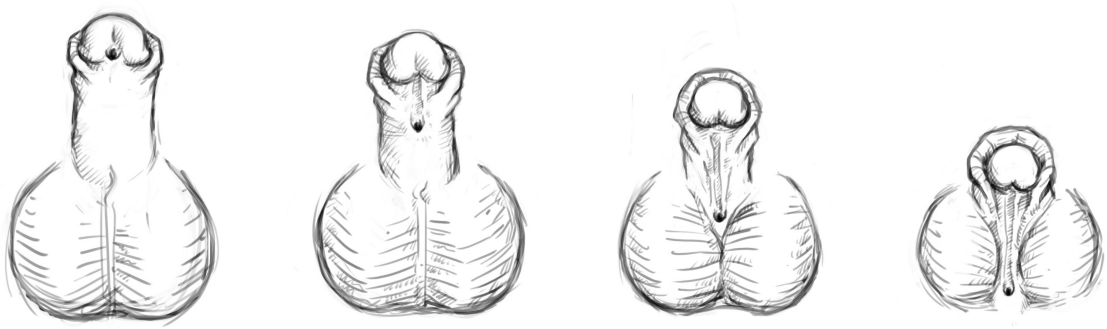

Figure 5*. Distal hypospadias, with the meatus located on the glans or at the subcoronal margin, affects 60-65% of the patients. Boys with penile hypospadias constitute 20-30% of the patients, and approximately 10-15% of the patients present with proximal hypospadias, with the meatus located in a penoscrotal, scrotal or perineal position. Illustration: Arvid Steen.*

1.3 Associated malformations and conditions

Hypospadias is most often an isolated feature but may present with concomitant cryptorchidism or inguinal hernia. Undescended testes are more frequent in proximal hypospadias compared with distal cases. Extra-urogenital malformations associated with hypospadias are congenital heart disease, cleft palate, and musculoskeletal and anorectal malformations [13]. Chromosomal aberrations, mainly involving sex chromosomes, have been described in up to 7% of cases of hypospadias and are even more common in boys with a co-occurrence of hypospadias and cryptorchidism [14, 15]. Further, hypospadias is part of several syndromes and shares a genetic etiology with other conditions that may be present at birth or arise later in life [16, 17].

1.4 History of hypospadias

The name hypospadias is derived from the Greek “hypo” that means under and “spadon” for fissure. Hypospadias and its potential consequences have been documented throughout history and one of the earliest descriptions is that from the Alexandrian surgeons Antyllus and Helidorus in the 1st and 2nd centuries [18].

Figure 6. *The ancient Pharos of Alexandria, depicted by Johann Barnhard Fisher von Erlach (1656-1723).*

In the early descriptions various surgical techniques were presented, ranging from partial amputation of the penis to the level of the urethral orifice to less drastic methods such as skin stretching to the tip of the penis [18, 19].

The potential implications of penile curvature, a feature commonly associated with hypospadias, on fertility has been described historically. It is said that Henry II, the King of France (1547-1559), lived in a childless marriage for ten years until he had his penile curvature corrected and thereafter fathered ten children with his wife [18].

Hypospadias surgery has evolved throughout the centuries. Until the eighteenth century one of the most important reference books on surgery was a medical encyclopedia called *Al Tasreef*, written by Albacusis of Cordoba (930-1013). In this book Albacusis described tunneling or incision of the glans in hypospadias repair and further mentioned the importance

of dilating a narrow meatus. In 1837, a Scottish surgeon, Professor Robert Liston (1794–1847), described the need of extra tissue in order to elongate and keep the canal permanently open after tunneling. In order to do so he used parts of the prepuce to line the tunnel, and further described how this technique relieved the patients from unpleasant symptoms, such as urgency [18].

The introduction of ether anesthesia in the 1840s revolutionized surgery and more sophisticated procedures could be carried out with much less suffering for the patient. In 1874, the surgeon Theophil Anger reported on the successful treatment of a penoscrotal hypospadias by using two lateral skin flaps in order to create a neourethra. This technique denoted the modern era of hypospadias repair. And even though methods have been refined and more than 300 different surgical techniques of hypospadias repair have been described since, the idea of using flaps to create a neourethra has remained [18, 19].

1.5 Etiology

Although hypospadias is common and has been extensively investigated the etiology in individual cases is still largely unknown. As the development of male internal and external genitalia is mainly driven by testosterone and DHT, a defect in androgen stimulation is often thought to be the ultimate cause of hypospadias. The reason for this lack of androgen action may be caused by environmental or genetic factors, or a combination thereof. In most cases the specific cause remains unknown, but in some individuals, in particular those with proximal hypospadias, the specific cause may be identified.

Non-genetic and environmental risk factors for hypospadias

One of the most well established risk factors for hypospadias is low birth weight. The weight is only measurable after birth and may thus only be a reflection of some other event that takes place during early pregnancy at the time of the urethral development. The underlying mechanism behind the association is probably placental insufficiency and impaired placental hCG secretion leading to subsequent fetal growth restriction and inadequate testosterone production in the XY fetus. Several studies have shown an association between placental pathology and hypospadias and the hypothesis is further strengthened by the fact that hypospadias is more common in multiple births and that in monozygotic twins the twin with the lower birth weight is more often affected by hypospadias [20-23]. In addition, pre-eclampsia and maternal hypertension during pregnancy may impair placental function and have consistently been shown to be associated with hypospadias [24]. Low birth weight seems to be more associated with proximal than distal cases of hypospadias [25, 26].

A positive association between preterm birth and hypospadias has been demonstrated in most, but not all, studies [24, 27-31]. Maternal smoking increases the risk of preterm delivery and fetal growth restriction, but most studies show no association between hypospadias and prenatal tobacco exposure [32].

A higher maternal age and both lower or higher paternal age have been proposed to be associated with hypospadias, but most studies show no association with parental age. Endogen estradiol levels increase with an increasing body mass index (BMI) and a high maternal BMI is associated with hypospadias in some studies, but results are conflicting [28, 33-35]. However, the maternal use of oral contraceptives during early pregnancy, i.e. a potent estrogen exposure, does not seem to be associated with a higher risk of hypospadias in offspring [24, 28, 36, 37].

Assisted reproductive technologies (ART) may involve hormonal stimulation and are associated with an increased risk of multiple birth and congenital malformations, and currently account for 3% of births in Sweden. Hypospadias has been shown to be associated with ART in many but not all studies, and often most specifically with the use of intracytoplasmic sperm injection (ICSI) [24, 35, 38]. The risk linked to ICSI may reflect an association to paternal subfertility and the inheritance of a genetic abnormality from the father, rather than a risk associated with the ART protocol per se. However, a recent meta-

analysis did not find any significant difference in the risk of hypospadias when comparing ICSI and IVF conceptions [39]. This might be due to temporal changes in the indication of performing ICSI.

Hypospadias, cryptorchidism, testicular cancer, and male infertility have been proposed to be symptoms of a common disorder, the Testicular Dysgenesis Syndrome (TDS), and it has further been suggested that this syndrome is partly due to increased levels of endocrine-disrupting chemicals (EDC) in the environment [40]. There are reports on how chemicals may influence the development of genitalia in animals, and they may possibly have similar effects in humans [41-43]. The impact of EDCs on androgen action is likely to be modulated by individual susceptibility and the effects of different compounds may be additive, which complicates the interpretation of studies in humans [44]. At present, there is however limited epidemiological evidence on whether the level of EDCs in the environment is sufficient to affect the development of human male external genitalia [45, 46].

Genetic risk factors for hypospadias

There is strong support for genetic influence on the development of hypospadias. Familial clustering is seen in 4-25% of cases, predominantly in distal hypospadias, and the recurrence risk in male siblings ranges between 9 and 17%. Many candidate genes have been investigated in the quest of mutations causative of hypospadias. Mutational findings are, however, uncommon and a monogenic cause is most frequently found in patients with proximal hypospadias [47].

Genes influencing androgen synthesis and activity are of obvious interest when investigating the genetic origins of hypospadias. Patients with DSD may present with complete or partial gonadal dysgenesis, or with gonads containing both ovarian and testicular tissue, due to mutations in genes affecting gonadal development [4]. Androgen deficiency, for example due to mutations in genes encoding 17β-HSD, an important enzyme in steroidogenesis, may lead to hypospadias. Mutations or genetic variants in the androgen receptor gene, crucial for development of the male phenotype, as well as in the gene encoding steroid 5 alpha-reductase (*SRD5A2*), the enzyme responsible for converting testosterone to the more potent DHT, have been found in hypospadias [24, 48, 49]. In addition, an excess of androgens from the adrenals due to congenital adrenal hyperplasia, may result in a situation where the external genitalia of the 46,XX fetus becomes virilized and gives the appearance of hypospadias with unpalpable gonads. Mutations in other genes important in the development of the gonad/testis and thereby the male genitalia, such as the *NR5A1* and Wilm's tumor 1 (*WT1)* genes, as well as mutations in the LH receptor gene and *MAMLD1* gene can cause hypospadias [48, 50, 51]. The *NR5A1* gene encodes the steroidogenic factor 1 (SF1), a protein that is crucial for adrenal, gonadal and reproductive development and function, and mutations in *NR5A1* may result in gonadal dysgenesis and ambiguous genitalia [52, 53]. Further proof of genetic origin is that hypospadias is part of many genetic syndromes, such as the hand-foot-genital syndrome due to mutations in the *HOXA-13* gene, and Opitz syndrome characterized by mutations in the *MID1* gene [54, 55].

Complex etiology

As stated above, a monogenic cause of hypospadias is seldom identified and, if so, most often in proximal hypospadias. The familial clustering and lack of a Mendelian inheritance pattern in distal hypospadias suggests a complex etiology, a hypothesis strengthened by segregation analyses [56]. Complex traits are caused by the interactions of multiple genes, and the effect may be further influenced by environmental factors. Large numbers of polymorphisms and aberrations in many different genes have been shown to be associated with hypospadias, but the individual effect of the majority of genetic variants is hard to estimate. Polymorphism may increase, as well as reduce, the risk of hypospadias and has been proposed to modulate individual susceptibility to environmental factors, such as EDC´s, but to what extent remains largely unknown [57, 58].

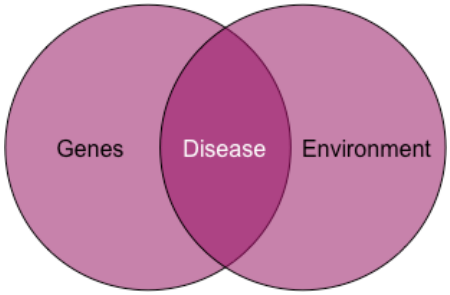

Figure 7. *Complex traits are believed to be a result of genetic variations within multiple genes and interaction with environmental factors.*

1.6 Epidemiology

Incidence and prevalence are two common measures of disease frequency, the former usually provides information on the risk of contracting a disease and the latter indicates how widespread it is. Incidence, or incidence rate, is defined as the number of new cases per population at risk during a given timeframe. Prevalence, on the other hand, provides information on the total number of cases in a population at a given time.

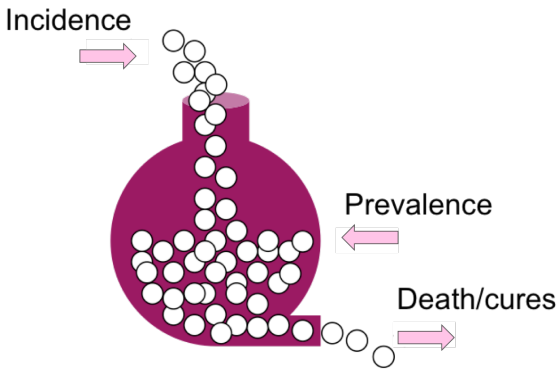

Figure 8. *Incidence is most commonly used as a measure of the risk of contracting a disease, i.e. the number of new cases in a population. Prevalence usually refers to how widespread a condition is, i.e. the total number of cases within a population, and changes as new cases arise, or are lost due to death or cure.*

There is however an exception, when prevalence rather than incidence, is the preferred measurement of new cases, and that is congenital malformations. In order to investigate the incidence one must be able to accurately identify the total population at risk, which is hardly possible in the case of congenital malformations since not all embryos survive until birth.

Thus prevalence, measured at the time of birth, is a more accurate measurement and will be used in this text henceforth [59].

The reported prevalence of hypospadias does vary largely between countries and ethnicities, ranging between extremes such as 0.6/10.000 live births in Malaysia to 464/10.000 live births in Denmark [60]. This may be explained by actual differences in the prevalence of hypospadias, but these variations may also mirror an effect of heterogeneous study designs and different definitions and regimes of reporting hypospadias. A particularly high prevalence of hypospadias has been found in Denmark and North America [61-63].

Since the 1970s numerous studies have shown an increasing prevalence of boys diagnosed with hypospadias, but there are many conflicting reports, however, and the phenomenon is debated. As with the prevalence of hypospadias, the studies on temporal trends are heterogeneous with regards to study design and definition of hypospadias [60]. The European Surveillance of Congenital Abnormalities and Twins (EUROCAT), established in 1979, is a network of population-based European registries for the surveillance of congenital anomalies. The EUROCAT report from 2011, including the years 1999-2008, showed an increasing prevalence of hypospadias [64]. In contrast to that study, a report from 2015, which included data from 23 EUROCAT registers, presented large geographic variations, but overall no statistically significant temporal trends in the prevalence of hypospadias between 2001 and 2010 [65].

Regardless of the huge amount of studies that address the prevalence of hypospadias, the question, however, remains unanswered. Is the actual prevalence increasing, or do the studies just reflect a statistical phenomenon that is due to improved reporting and an increased awareness of hypospadias? Apart from the more complete registration of hypospadias, the change in prevalence has been proposed to be an effect of surgical success and subsequent improved fertility in men with hypospadias. Further, the temporal trend has been suggested to be due to an increased influence of EDCs [40] but, as previously stated, there is a lack of evidence supporting a causal effect of these compounds in humans [24, 46].

1.7 Management

Hypospadias is most often diagnosed at birth but in rare cases abnormal sexual development may be suspected during prenatal ultrasound examination. Distal hypospadias may, however, remain undiagnosed until adult age, especially if the prepuce is not cleaved. The management of hypospadias largely depends on the phenotype.

Management of the new born child with uncertain sex at birth

The development of the external genitalia may be so deviant that the child presents with uncertain sex at birth. If there is any uncertainty in sex assignment, a prompt referral to the regional DSD team is recommended [66, 67]. The attending physician or midwife should always avoid any hasty or uncertain gender assignment but rather inform the parents that these conditions occur and that a more thorough investigation is needed to find the child´s sex instead of just looking at the external genitalia. Importantly, investigations on whether the child suffers from congenital adrenal hyperplasia (CAH) should be carried out immediately, since this is a potentially life threatening condition. Additional molecular diagnostics that are recommended at an early stage in children with ambiguous genitalia include karyotyping, mutational screening of genes involved in sex determination and differentiation, and hormonal analyses. The physical examination of children with ambiguous genitalia should include an evaluation for the signs of adrenal insufficiency, such as skin pigmentation and dehydration. Examination of external genitalia should determine the size of the phallus, the degree of fusion of inner (urethra) and outer (labia majora/scrotum) genital folds, the localization of the urethral or urogenital orifice and whether there are palpable gonads. The evaluation of internal genitalia often includes radiological investigations of the pelvic region as well as cystoscopy, vaginoscopy and gonadal biopsy [68-71]. Apart from providing the best care available to the child, the healthcare personnel must ascertain the coping abilities of the parents. Most parents of newborn children experience some level of stress, and much more so if the child is born with a congenital malformation or suffer from chronic illness [72]. Parents of children born with DSD have shown an increased risk of posttraumatic-stress [73].

The surgical management of boys born with hypospadias

Most cases of hypospadias do not, however, present with ambiguous genitalia, and sex assignment can be carried out regardless of hypospadias. The boy may then be referred for less acute surgical consultation by a pediatric urologist. In Sweden, pediatric urologists and surgeons responsible for the treatment of hypospadias are situated in pediatric surgery clinics in Stockholm, Uppsala, Göteborg and Lund, and in plastic surgery clinics in Malmö and Linköping. In proximal cases, and when there is a family history of hypospadias or when a syndrome is suspected, additional molecular diagnostics such as mutational screening of the *AR*, *SRD5A2*, *NR5A1, HSD17B3* and *WT1* genes or array CGH (comparative genomic hybridization) may be carried out.

Prior to surgery all patients should be examined regarding: 1) penile length; 2) position, shape and size of the urethral meatus; 3) appearance of the prepuce (cleaved or not), glans (size, cleft or flat) and scrotum; 4) the degree of penile curvature and rotation; 5) the position of the testes. Additional malformations, such as inguinal hernia, should be recorded but there is no need for routine screening for renal or ureteric anomalies [10]. Classification of hypospadias mainly relies on the position of the meatus since this, in combination with the degree of penile curvature, dictates what surgical method is most suitable [10, 11].

In Sweden, hypospadias surgery is usually performed between one and two years of age. The guidelines from the European Association of Urology (EAU) and American Academy of Pediatrics (AAP) states that surgical intervention is preferably performed between 6 and 18 months of age [74, 75]. In distal hypospadias, where surgery may be indicated only for cosmetic purposes, some favor delayed genital surgery in order to be able to involve the patient in the decision regarding elective surgery. The discussions on whether age at surgery is associated with the healing process and affect the risk of complications is ongoing [76, 77]. A recent study including 5000 patients with hypospadias, found that the risk of secondary cystoscopy and urethral dilatation/incision increased with 15% and 21%, respectively, for each additional year of age at surgery in patients with distal hypospadias [78].

The aim of surgical intervention in boys with hypospadias is to create a urethra with good function, correct any ventral curvature and attain a cosmetically acceptable penis. The choice of surgical method is mainly based on the degree of hypospadias, classified according to meatal position and ventral curvature [18]. Internationally, circumcision is most often performed simultaneously with hypospadias repair, whilst parents of patients in Sweden often chose to have a prepuce reconstruction performed if possible.

In glandular cases, with an absence of ventral curvature, hypospadias may be satisfactorily corrected using the *MAGPI* procedure (Figure 9), which seldom requires hospitalization. The *MAGPI* procedure includes meatal advancement through incision of the bridge between the aberrant meatus and the urethral groove, and glanuloplasty combined either with prepuce reconstruction or circumcision [10].

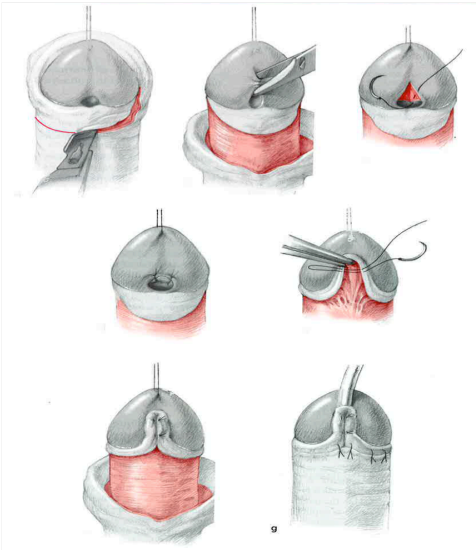

***Figure 9**. The MAGPI procedure is often used in patients with distal hypospadias and absence of ventral curvature. Meatal advancement is achieved through meato- and glanuloplasty. Picture from Hypospadias surgery by professor A.T. Hadidi. Published with the permission of Springer.*

Penile hypospadias is more frequently associated with ventral curvature and often requires urethroplasty in order to advance the meatal position to the tip of the glans penis. This is most commonly performed with the *TIP (tubularized incised plate)* procedure (Figure 10), introduced by W Snodgrass, in which the urethral plate is incised in the dorsal midline and tubularized around a stent, thereby creating a neourethra [79]. The neourethra is covered with adjacent dartos tissues in order to minimize the risk of fistulas, and the procedure is finished with a glanuloplasty and skin closure [10]. The procedure is combined with a dripping stent that is removed after a week.

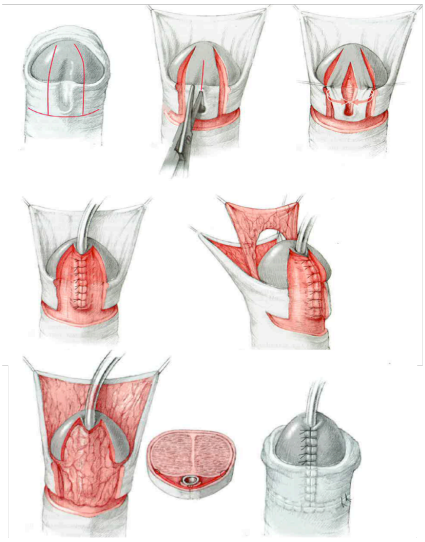

Figure 10. *The Snodgrass/TIP procedure. The urethral plate is incised vertically, thereby making it wider, which enables tubularization and the creation of a neourethra of sufficient width. Picture from Hypospadias surgery by professor A.T. Hadidi. Published with the permission of Springer.*

Proximal hypospadias still remains the ultimate challenge in hypospadias repair and is often corrected either through the *Duckett* procedure or a two-stage repair according to *Bracka* after correction of the ventral curvature. In the *Duckett* procedure, a pediculed preputial flap is used to create the neourethra around a dripping stent. The two-stage *Bracka* repair involves a free transplantation of a prepuce flap to the ventral side of the penis that is circulated to a urethra after at least a six-month period. Proximal hypospadias is frequently characterized by a small glans that often has to be incised in the midline in order to encircle the neourethra and bring the meatus to the tip of the penis [10].

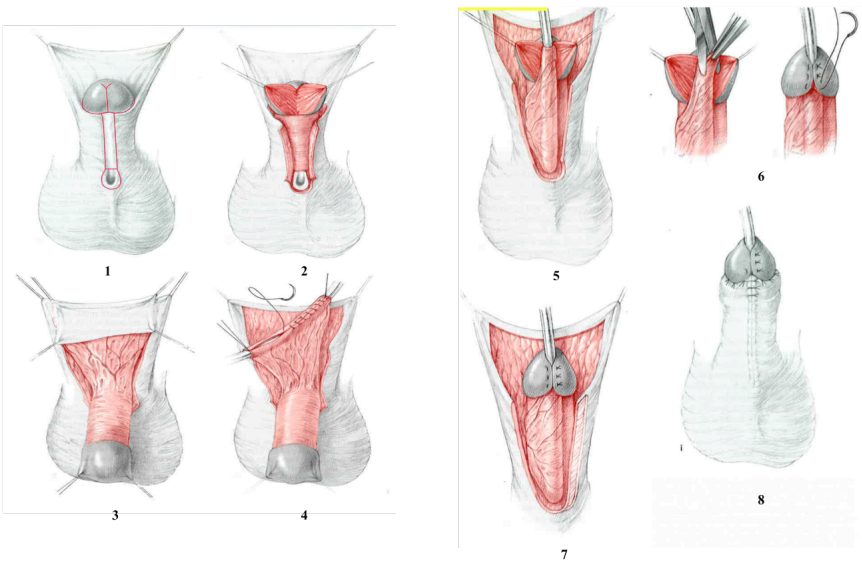

Figure 11. *The Duckett procedure. A neourethra is created by encircling a pediculed preputial flap around a dripping stent. Picture from Hypospadias surgery by professor A.T. Hadidi. Published with the permission of Springer.*

1.8 Outcomes

Surgical complications

Early complications in hypospadias repair are rare but include hemorrhage, wound dehiscence, and infections. Many of these complications may be avoided with the appropriate surgical technique and correct post-operative care. Urethrocutaneous fistula is the most common late complication after hypospadias repair, estimated to affect 7.5% of patients who have undergone single-stage hypospadias surgery [10, 80]. Fistulas may arise due to suturing techniques and material, inadequate amounts/highly tensioned or poorly vascularized tissues covering the neourethra, and distal stenosis. Fistula repair includes excision, suturing and coverage with several layers of tissue to avoid recurrence [10]. Meatal stenosis may lead to stranguria, urinary retention and infection, and is found in approximately 4% of hypospadias repairs [11, 80]. Urethral diverticula are a rare complication that may be diagnosed at long-term follow-up, presenting as a ballooning on the ventral side of the penis during micturition. It may be a result of the construction of an excessively wide neourethra during primary surgery or secondary to meatal stenosis [10]. Other late complications include persisting or recurrent ventral curvature and glandular dehiscence [10, 11]. Although hypospadias repair aims to reduce urinary symptoms and satisfactory cosmetic results, some patients continue to report voiding problems and dissatisfaction with penile appearance in adulthood [81].

Sexual function and fertility

The long-term effects of hypospadias on sexual function have been investigated in many studies and evaluated by a range of measures. Delayed sexual debut has been reported [82], as well as ejaculatory and erectile dysfunction and dissatisfaction with the cosmetic results [83-85]. Nonetheless, many patients report satisfactory sexual function [85, 86].

Although overall sexual functioning is described as good, fertility may still be impaired in patients with hypospadias due to several different reasons. Common genetic origins, such as mutations in the *AR* and *SRD5A2* genes may induce hypospadias as well as reduce fertility [87, 88]. As previously mentioned, low birth weight is highly associated with hypospadias, but has also been proposed to impair future fertility in men [89, 90]. Male subfertility and the use of ART are suggested risk factors for hypospadias, and it is plausible that subfertility traits in the father may be transferred to the son [91, 92]. Further, anatomic features such as persisting ventral curvature, lack of spongiosal tissue around the urethra and meatal stenosis may inhibit intercourse and affect the ejaculation process, and dissatisfaction with penile appearance may affect willingness to seek intimate relationships [93, 94].

Regardless of the many possible causes of impaired fertility in men with hypospadias, methodologically correct studies on this matter are scarce. According to the World Health Organization, infertility is defined as the inability of a sexually active and non-contraceptive using couple to achieve spontaneous pregnancy in one year [95]. In approximately 50% of infertile couples, abnormal semen parameters are found together with male-infertility-factors (e.g. maldescended testes and other congenital or acquired urogenital abnormalities,

malignancies, immunological factors such as sperm autoantibodies, urogenital infections, endocrine disturbances and chromosomal abnormalities such as Klinefelter's syndrome). Infertility in a couple may be due to male or female factors, or a combination thereof. In 30-40% no male infertility factor is found. Cases of idiopathic male infertility are supposed to be caused by a combination of factors such as epigenetic abnormalities, reactive oxygen species, and endocrine disruption due to environmental pollution [96].

The diagnostic evaluation of infertility must include an investigation of both partners, and the male investigation to include medical history, physical examination and semen analysis (volume, count, concentration, motility and morphology). If at least two semen analyses show abnormal results, such as oligozoospermia, asthenozoospermia or teratozoospermia, further andrological investigation, including hormonal analysis, is indicated. A common cause of reduced male fertility is testicular deficiency and impaired spermatogenesis, these men may present with high levels of follicle-stimulation hormone (FSH) and luteinizing hormone, indicating hypogonadism. In addition to semen- and hormone analysis, genetic screening, ultrasonography and biopsies of testes may be carried out [96].

The proper investigation of fertility in men involves active patient involvement and time consuming testing, besides being costly, this may be the explanation for why it has been scarcely investigated in men with hypospadias. However, investigations from a Danish study, including both hormone and semen analysis in samples from 108 men with hypospadias, revealed that semen characteristics in isolated hypospadias were similar to those of controls, whilst men with concomitant cryptorchidism had higher levels of abnormal sperm motility, count and morphology. Higher levels of FSH, LH and lower levels of free testosterone were found both in men with isolated hypospadias and men with hypospadias in combination with cryptorchidism, compared with controls [97]. Similar results are available from Kumar et al. [98]. Although different from controls, hormonal levels were within the normal range and not as deviant as in patients with overt hypogonadism [99]. In yet another study, endocrine testicular dysfunction was found in 57% of boys and adolescents with hypospadias accompanied by additional genital malformations, compared with 14% in isolated hypospadias [100]. Overall, these three studies support the belief that men with distal or isolated hypospadias have similar fertility compared with the general population.

Calculations of birth rates and reported paternity are other ways of estimating fertility in men with hypospadias. In a recent Swedish investigation of 167 men with hypospadias, only those with proximal hypospadias had a lower reported fertility compared with distal hypospadias and controls [86], and a Finnish study reported on a similar number of children in men with hypospadias (n=46) and controls (n=43) [101]. In contrast to these results, Asklund et al. found lower paternity rates in hypospadias (n=1043) compared with age-matched controls, despite similar semen characteristics [97].

Well-being and psychosocial outcomes

Well-being is a subjective term and may be described as feeling good and judging life in positive terms. Personality traits, genetic factors and individual physical and mental health status are all related to well-being. Having supportive relationships has a notably positive effect on happiness and is a strong determinant of well-being and general health. Employment and income ensure access to basic resources such as shelter and food, and are fundamental to well-being. Level of income has some effect on happiness, probably by enabling a higher standard of living, leisure time, and access to medicine and thus mediating better health. Education is closely linked to both income and occupational status, but is also independently associated with level of happiness. However, the direction of causality and the level of interaction between the above-mentioned associations is hard to determine [102].

There are several reasons to consider whether the well-being of boys and men born with hypospadias is impaired. Having a child with a congenital malformation may have negative effects on the parents' mental health with subsequent effects on the cognitive and socio-emotional development of the offspring [72, 103-105]. Further, there are concerns regarding the effect of surgery and anesthesia in childhood on later cognitive function. However, a recent population-based Swedish study only found a very small association between exposure to surgery before the age of four and school grades and IQ level at conscription. The effects of early surgery were much less than the effects related to sex, birth month and parental educational level [106].

Early studies on small populations suggested that boys with hypospadias had more behavioral problems, poorer school performance, were bullied more, and showed shyness more often than controls [82, 107]. In addition Berg et al. found that men with hypospadias had less qualified occupations compared with controls [108]. However, later and larger studies have not been able to reproduce these findings but have suggested a good psychosocial development in boys and men with hypospadias [94, 109, 110]. Further, a comparable level of education and income has been reported in more recent studies [94, 101, 109-111]. Available studies on health-related quality of life in boys, adolescents, and men with hypospadias show contradictory results [110, 112, 113]. Studies on hypospadias and psychiatric disorders are rare, but an increased risk of neurodevelopmental disorders, intellectual disability, anxiety, depression, and behavioral and emotional disorders has been described [114, 115].

2 AIMS

The overall aim of this thesis was to increase the knowledge on the etiology and consequences of hypospadias.

The specific objectives were:

Study I

- To describe the prevalence of hypospadias in Sweden and investigate if trends were due to temporal changes in risk factors.

Study II

- To describe the association between hypospadias and spinal-bulbar muscle atrophy (SBMA) and investigate the co-occurrence in subjects and within families.

Study III

- To increase the knowledge on psychosocial outcomes in adult men born with hypospadias.

Study IV

- To assess the fertility in adult men born with hypospadias by register-based methods.

3 MATERIAL AND METHODS

3.1 At a glance

	Population	Material	Method
I	Cohort 1: All men born between January 1973 and December 2009 in Stockholm, Malmö or Uppsala counties. Cohort 2: All men born in Sweden between January 1987 and December 2009.	*Exposure:* Parental and perinatal characteristics as recorded in the MBR. *Outcome:* Registered diagnosis of hypospadias.	Descriptive statistics of prevalence. Simple and multivariable logistic regression when investigating the association between hypospadias and risk factors. Logistic regression model, with significant risk factors as covariates, when investigating temporal trends in prevalence.
II	One clinical case from Karolinska University Hospital. In registers: All men with a registered diagnosis of hypospadias born in Swedish counties with full coverage in the NPR and their male first- and second-degree relatives on the maternal side.	One clinical case. Register-based screening of concomitant hypospadias and suspected diagnosis of SBMA in individuals and within families.	Mutational screening. Cross linkage of data from the MBR, NPR and MGR in order to detect SBMA within male first- and second-degree relatives of patients with hypospadias. Descriptive data only.
III	All men with a registered diagnosis of hypospadias born between 1969 and 1993 in Swedish counties with full coverage in the NPR, matched with 100 unaffected men by birth year and birth county. Followed until December 2009.	*Exposure*: Hypospadias, sub-grouped according to phenotype. *Outcome*: Socioeconomic outcomes collected from LISA, UREG and Grade 9 School marks Register. *Covariates*: Perinatal characteristics from MBR and NPR.	Conditional logistic regression. Sensitivity analysis investigating the effects of psychiatric illness.
IV	All men born in Sweden between 1964 and 1998, in counties with full coverage in the NPR and who had not died or emigrated before the age of fifteen. Followed until outcome event, migration, death or 31st December 2013.	*Exposure*: Hypospadias, sub-grouped according to phenotype. *Outcome:* Age at childbirth, childbirth conceived through ART and diagnosis of male infertility from MBR and NPR. *Covariates*: Maternal and perinatal characteristics from MBR and NPR.	Cox proportional hazard with robust standard errors in the full cohort. Stratified Cox proportional hazard model, conditional on sibling group, in sibling analyses. Sensitivity analyses exploring the effect of cryptorchidism, and other additional malformations.

3.2 Data sources

Since 1947 all residents in Sweden have been assigned a personal identification number (PIN), consisting of their birth data and a unique four-digit identification number [116]. Via the PIN, data from Swedish nationwide population-based registers may be linked together [117]. The studies in this thesis take advantage of registers providing health care data held by the National Board of Health and Welfare (NBHW) and registers held by Statistics Sweden, containing demographic data. Additional data on twins were collected from the Swedish Twin Register held by the Department of Medical Epidemiology and Biostatistics at Karolinska Institutet.

The National Patient Register

In 1964 the NBHW began documenting individual hospital discharges in the National Patient Register (NPR). The register expanded gradually and only included data from only a few counties when it began, to full nationwide coverage from 1987 and onwards. Each recording corresponds to one in-hospital event and contains data on admission and discharge and up to eight diagnoses. Since 2001 additional diagnoses given at outpatient specialist clinics have been collected. No data from primary care are recorded in the NPR.

Diagnoses are coded according to the International Classification of Disease (ICD) system and retrieved from records written by physicians. Data withdrawals may therefore include spelling errors or irregularities nevertheless, the accuracy of recordings has been estimated to be more than 98%, with an overall positive predictive value of 85-95% [118, 119]. Within this thesis, ICD codes from four different time periods were used: ICD-7 (1964-1968), ICD-8 (1969-1986), ICD-9 (1987-1996) and ICD-10 (1997 and onwards). From the NPR we collected information on diagnosis of hypospadias, used in all four studies, information on additional malformations used in *Studies III* and *IV*, and presumed diagnosis of SBMA used in *Study II.*

The Medical Birth Register

The Medical Birth Register (MBR) was established in 1973 by the NBHW and provides data on 98% of all births in Sweden. The register contains information on mother and child recorded in the antenatal-, delivery- and neonatal care, such as maternal age, weight and smoking habits, ART, the child's sex, gestational age, birth weight, and diagnoses [120]. Diagnoses on mother and child are recorded according to the International Classification of Disease (ICD) system. Within this thesis we used the MBR to collect data on diagnosis of hypospadias, used in all four studies, and additional data on outcomes and covariates used in *Studies I, III* and *IV*.

The Cause of Death Register

The Cause of Death Register, held by NBHW, was established in 1952 and records data on the deaths of all Swedish residents. The register contain data on date and cause of death according to the ICD classification on all residents, regardless of whether the death occurred

inside or outside the country [121]. Data from the Cause of Death Register was used in *Studies II, III* and *IV*.

The Total Population and Multi-Generation Register

Statistics Sweden established The Total Population Register (TPR) in 1968 [122]. The register provides information on births, civil status, citizenship, migration, and place of residence and was used in *Study I, III and IV*.

The Multi-Generation Register (MGR) was established as part of the TPR in 1991 and enables linkage between individuals and parents [123]. It includes all individuals registered in Sweden since 1961, born 1932 or later. Information on the mother is available in 97% of children born and information on the father, most often established as the husband of the mother or by acknowledgement, is present for 95% of children born in Sweden. The MGR was used to establish familial relationships in *Studies I, II* and *III*.

Longitudinal Integration database for Health and Labor Market Studies

The Longitudinal Integration database for Health and Labor Market Studies (Swedish acronym, LISA) is held by Statistics Sweden and contains information on income, education, employment and compensation from social insurance. Data on all Swedish residents, aged 16 years or older, has been collected annually since 1990 [124]. Earlier data on income and educational level is available from The Population and Housing Censuses (Swedish acronym, FoB) that were conducted every fifth year between 1960 and 1990, based on questionnaires that all Swedish residents who had attained the age of fifteen were obliged to fill in, the response rate ranges between 97.5 and 99.2% [125].

Additional data on educational level and eligibility for high school are available from the Register of Education (Swedish acronym, UREG; 1985 and onwards) and the Grade 9 School Marks Registers (1998 and onwards) [126]. Information from LISA, FoB 75-85, UREG and Grade 9 School Marks Registers was used in *Study IV*.

3.3 Defining hypospadias

The most accurate way to identify, and properly classify, individuals with hypospadias is in the clinical setting with direct access to the patient. This is a time- and cost consuming activity from a researchers point of view, and seldom provides study populations as large as population-based registers. The results within this thesis are solely based upon register data, thus relying on the reports of others. Data on a diagnosis of hypospadias were collected from the NPR and MBR as recorded according to the ICD system. One diagnosis of hypospadias in either the NPR or MBR was sufficient to be classified as a case with hypospadias. The studies include men born in 1964-2009 and hypospadias was accordingly defined on the basis of four different ICD coding systems (ICD-7: 757.21, ICD-8: 752.20, 752.21, 752.22, 752.29, ICD-9: 752.6 and ICD-10: Q54.0, Q54.1, Q54.2, Q54.3, Q54.8, Q54.9). Hypospadias was classified according to phenotype as far as possible but the codes in ICD-7 and ICD-9 do not

provide any additional information on the severity of hypospadias, as do the codes in ICD-8 and ICD-10. If codes corresponding to more than one phenotype were recorded, the most severe definition of hypospadias was used in analyses.

ICD-7 (1964-1968)	ICD-8 (1969-1986)	ICD-9 (1987-1996)	ICD-10 (1997-
Hypospadia (757.21)*	Hypospadia glandis (752.20)**	Hypospadi (752.6)*	Glandular hypospadias (Q54.0)**
	Hypospadia scrotalis (752.21)***		Penile hypospadias (Q54.1)**
	Hypospadia totalis (752.22)***		Penoscrotal hypospadias (Q54.2)***
	Hypospadia alia sive NUD (752.29)*		Perineal hypospadias (Q54.3)***
			Hypospadias not other specified (Q54.8, Q54.9)*

* Sub-grouped as hypospadias not other specified (NOS)
** Sub-grouped as distal/mild hypospadias
*** Sub-grouped as proximal/severe hypospadias

3.4 Study populations and designs

Researchers are often interested in establishing causal relationships, but when interpreting the scientific results of observational studies such as those within this thesis, one must remember that mere associations do not always imply causality. Although a significant association is the present between two variables, it does not automatically mean that the outcome is caused by the exposure. Associations may be present due to the effect of bias, such as confounding. A confounder is defined as a variable that is a common cause of both exposure and outcome, but is not on the causal pathway. Study designs and statistical modeling may strive to reduce the effect of bias due to confounding by adjusting for the effect of these variables in analyses. If a covariate lies on the causal pathway in between exposure and outcome it is referred to as a mediator.

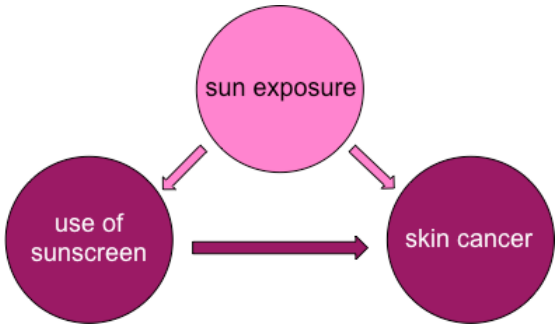

Figure 12. *Illustration of an association confounded by a factor associated with both exposure and outcome. The association between the use of sunscreen and skin cancer is present due to the confounding effect of sun exposure.*

Study I

This study aimed to investigate whether there has been any change in the prevalence of hypospadias in Sweden and to establish risk factors for hypospadias in the Swedish population.

As previously mentioned, the diagnosis of hypospadias was collected from both the MBR and NPR and the latter did not reach nationwide coverage until 1987. In order to obtain a longer observational time, the population was split into two cohorts. Cohort 1 included men born in large Swedish counties (Stockholm, Uppsala and Malmö) between 1973 and 2009, and the second, nationwide, cohort 2 consisted of all men born between 1987 and 2009. A subset of parental (age, weight, ethnicity, the use of ART to conceive) and perinatal (born small for gestational age (SGA) or as twin) characteristics was selected as putative risk factors. Analyses comparing the risk of being born with hypospadias in different birth cohorts, i.e. years with high prevalence vs. years with low prevalence, were carried out in order to investigate if trends in prevalence were due to temporal changes in risk factors.

Study II

Study II aimed to highlight the association between hypospadias and the neuromuscular condition spinal-bulbar muscle atrophy (SBMA), also known as Kennedy's disease.

The study includes the description of one clinical case from Karolinska University Hospital and an attempt to investigate the co-occurrence of hypospadias and SBMA in individuals and within families in the Swedish registers. An obvious obstacle when evaluating SBMA by register-based methods is that the condition has no specific ICD code. In order to identify cases with suspected SBMA the following codes covering motor neuron disease were included (ICD-8: 348.9, ICD-9: 335W, 335X, ICD-10: G12.2). The criteria used in order to exclude possibly misdiagnosed or very uncertain diagnoses of SBMA and patients with ALS are presented in Table 1.

Exclusion criteria
<6 years follow-up
Deceased <6 years after first diagnosis of motor neuron disease
Diagnosis at <20 or >65 years of age
Motor neuron disease as cause of death
Presence of additional neurological diagnoses:
Stroke (prior to presumed SBMA diagnose)
Late effect of cerebrovascular disease
Multiple sclerosis
ALS

Table 1. *Exclusion criteria for identifying suspected cases of SBMA in the NPR.*

Study III

The third study aimed to obtain an overall picture of well-being through different socioeconomic outcomes in men born with hypospadias and was performed as a matched cohort study.

The socioeconomic outcomes were: highest attained educational level (elementary, upper secondary or college) and income level (categorized as low, median or high. Range of categories derived from yearly calculations of income levels in the total male population, divided into quintiles), eligibility for high school (i.e. passing grades in eight subjects, including mathematics, Swedish and English), getting married, and ever receiving disability pension. In addition, sensitivity analyses investigating the effects of psychiatric illness as a putative mediator were conducted. In order to stretch the observational period, all men with a hypospadias diagnosis that were born in counties with full coverage in the NPR from 1969 were included. Since the cohort was followed until 2009 and outcome variables were mainly available from LISA, where individuals aged 16 and older are included, only men born before 1994 were included. Eligibility for high school was investigated in analyses restricted to men born in 1983 and onwards.

The matching of subjects may be performed in order to obtain similar characteristics in those exposed and unexposed and thus reduce bias due to confounding by these factors. In *Study III*, men diagnosed with hypospadias were matched with 100 unaffected men, by birth year (as a proxy of age), and birth county.

Study IV

Study IV aimed to assess fertility in men with hypospadias and was conducted as a nationwide cohort study.

The study investigates the associations between hypospadias and 1) having biological children, 2) conceiving through ART and 3) receiving a diagnosis of male infertility. All men born in Swedish counties with full coverage of the NPR, between 1964 and 1998, and who had not died or emigrated before the age of fifteen, were included. Individuals were followed until outcome event, death, migration or 31 December 2013.

As previously stated, observational studies may identify associations between exposures and outcomes, but this may not be due to a causal effect. Adjustments for confounding factors may help elucidate causal relationships, but can only be addressed if known and measured. Unmeasured confounders may evoke non-causal spurious associations between exposures and outcomes. Confounders, in terms of genetic and behavioral factors are often shared within families. Full siblings share approximately half of their genes and are most often exposed to the same parental factors or intrauterine exposures. In a within-family design individuals are compared with siblings or twins within the same family, thus adjusting for shared familial factors that may account for confounding affects. If the association between an exposure and outcome is causal, the additional within-family comparisons will expand

the possibilities to make causal inference by adjusting for shared confounders such as common genetics and environmental exposures [127].

To further investigate the causal relationship between being born with hypospadias and subsequent fertility, we estimated the associations both within the full cohort and between full brothers, thereby adjusting for familial confounding. The study further includes sensitivity analyses in which men with cryptorchidism, additional other malformations, or psychiatric illnesses are excluded.

3.5 Statistical methods

Statistics, in terms of collecting and interpreting data, is an ancient science. As early as in the second millennium BC the Pharaohs of Egypt required periodic censuses, which were used for tax gathering and recruitment to military services. Statistics may be descriptive, e.g. summarized data from censuses, or inferential. Inferential statistics are based upon probability theory and are used to draw conclusions from data that are subject to random variation. In epidemiology, descriptive statistics may be used to describe the available data in detail while statistical analyses are used to describe the relationship between exposures and outcomes. The calculations are based upon a sample of data, from which patterns of associations are estimated with mathematical methods. The number of different statistical models is vast, and the choice of model depends on which data is analyzed and what research question is to be answered [128, 129].

Logistic regression

Logistic regression models are used to study the association between exposure and outcome, and may be applied when the outcome is either binary (as in the case of hypospadias, yes/no) or categorical (as the highest level of income; low/median/high). Multiple, or multivariate, logistic regression analysis may be used to examine the impact of multiple exposures on a binary outcome and covariates may be included in the logistic regression model to adjust for confounding factors. The effect estimate is an Odds Ratio (OR), with corresponding 95% Confidence Intervals (95% CI). When studying categorical outcomes one category of the outcome must be chosen as a reference, the OR then reflects the change in odds when being in a particular category compared with the reference category. Odds are not the same as risk, but the OR may be similar to the relative risk if the outcome of interest is rare [128, 129].

Logistic regression models were used in *Studies I* and *III*. In *Study I* single and multivariate logistic regression models were used when estimating the associations between risk factors and hypospadias. Further, we investigated the cause of temporal changes in prevalence by comparing the risk of hypospadias in different birth cohorts and including the risk factors as covariates.

Conditional logistic regression models may be used when dealing with matched data such as in *Study III*. The models in *Study III* were further adjusted by including gestational age, birth

weight and additional malformations as covariates. All computations including logistic regressions were performed in SAS.

Time-to-event analysis and Cox proportional hazard models

Survival analysis, or time-to-event, analysis is used when study participants are followed from a start time to an endpoint. It requires a precise and unambiguous definition of start and end of follow-up, event of interest (e.g. disease occurrence or death) and time scale (i.e. attained age, time-in-study or calendar time).

In *Study IV* we used Cox proportional hazard models to perform time-to-event analysis. In contrast to logistic regression models, in which the *proportion* of events are investigated, Cox proportional hazard models compare the *rate* of events, thus taking time under exposure into account. Whilst a odds ratio provides information on the proportion of events in the end of the study period, the hazard ratio tells one the risk of an event at any particular point in time (e.g. at any age or at any time since diagnosis). It is most intuitively applied when studying survival but is beneficial in situations when follow-up is different and time-at-risk should be taken into account. The model adjusts for the effect of time very efficiently and if any particular time scale is a strong confounder of the association between exposure and outcome, it should be chosen as the underlying time scale. The actual rate may vary over time, but the model demands proportionality, i.e. that the difference in rate between the exposed and unexposed (hazard ratio) is constant over time. This assumption may be evaluated visually by plotting the log cumulative hazard functions or by investigating the Shoenfeld's residuals [129].

In *Study IV*, attained age was chosen as the underlying time scale, and the possible endpoints were the three different outcome events (age at: first childbirth, first childbirth conceived through ART, diagnosis of male infertility). Subjects were censored at death, migration or end of study period. Birth weight, gestational age and maternal ethnicity were added as additional covariates to the models. In sibling comparison, a stratified Cox proportional hazards model, conditional on sibling group, was used to estimate the associations in exposed and unexposed full brothers. In sensitivity analyses the effect of psychiatric disease, additional malformations and cryptorchidism were investigated. All computations including Cox proportional hazard models were done in STATA version 14.

4 MAIN RESULTS AND DISCUSSION

4.1 More boys are assigned with a diagnosis of hypospadias

In *Study I* we found an increased prevalence of boys who had received a diagnosis of hypospadias during the late 20th century. The increase in prevalence was present for both distal and proximal hypospadias (Figure 13).

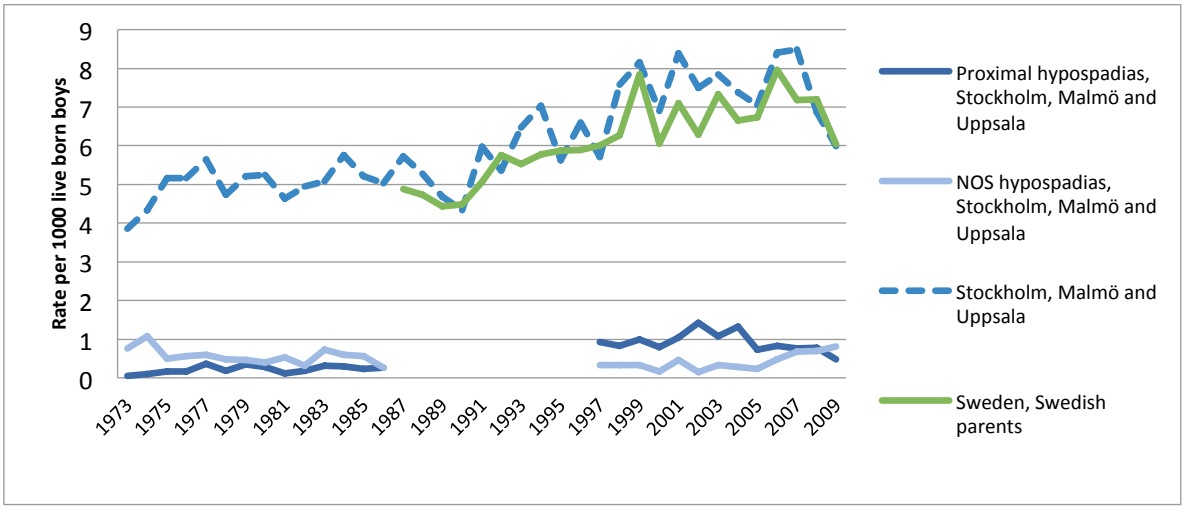

***Figure 13**. Prevalence of hypospadias in Sweden 1987-2009 and Stockholm, Malmö and Uppsala 1973-2009.*

Out of the parental and perinatal factors investigated, being born small for gestational age, as a twin, by parents from greater Europe and Asia or as a result of ART treatment was significantly associated with having the diagnosis of hypospadias (Table 2).

	Univariate -87 OR (95% CI)	Multivariate -87 OR (95% CI)	Multivariate -91 OR (95% CI)
Parental ethnicity			
Swedish	0.81 (0.78-0.86)	0.94 (0.87-1.02)	0.94 (0.87-1.02)
Nordic	0.77 (0.58-1.02)	0.99 (0.71-1.39)	1.17 (0.82-1.69)
Greater European	1.52 (1.37-1.68)	1.46 (1.26-1.68)	1.41 (1.21-1.63)
Asian	1.65 (1.51-1.80)	1.51 (1.33-1.72)	1.45 (1.27-1.65)
African	1.35 (1.16-1.57)	1.20 (0.98-1.46)	1.13 (0.92-1.39)
Other	0.52 (0.35-0.78)	0.50 (0.30-0.83)	0.41 (0.22-0.74)
Pre-pregnancy BMI			
< 19	0.95 (0.88-1.03)	0.95 (0.87-1.04)	0.99 (0.89-1.09)
20-24	0.90 (0.86-0.95)	0.94 (0.87-1.02)	0.95 (0.87-1.03)
25-29	1.03 (0.98-1.09)	1.00 (0.92-1.08)	0.99 (0.91-1.07)
> 30	1.19 (1.10-1.28)	1.13 (1.02-1.25)	1.10 (0.98-1.22)
Parental age			
Mother >35 y.a	1.02 (0.95-1.09)	1.04 (0.95-1.14)	1.01 (0.92-1.11)
Father >35 y.a	0.97 (0.92-1.03)	1.00 (0.93-1.07)	0.98 (0.91-1.06)
SGA*	4.17 (3.85-4.52)	4.15 (3.87-4.56)	4.34 (3.94-4.78)
Twin*	1.84 (1.66-2.04)	1.90 (1.69-2.13)	1.80 (1.59-2.04)
ART **	1.34 (1.22-1.48)	N.A	1.15 (1.02-1.29)
Non-ICSI ART	1.29 (1.16-1.42)	N.A	1.16 (1.02-1.31)
ICSI	1.65 (1.31-2.1)		1.06 (0.75-1.48)

***Table 2**. Uni- and multivariate models on the association of parental and perinatal factors on hypospadias. *Separate models for SGA and twin. ** Subjects born 1991 and onward included. ART included as either binary or categorical (ref: no ART) exposure.*

Further, the temporal variation in prevalence could not be explained by changes in the investigated risk factors (Table 3).

	Crude OR (95% CI)	Adjusted OR (95% CI)
Nationwide *2006-2007 vs. 1992-1993*[a]	1.42 (1.29-1.57)	1.37 (1.22-1.54)*
Stockholm, Malmö and Uppsala *2006-2007 vs.1973-1974*[b]		
Hypospadias as an aggregate	1.76 (1.47-2.1)	1.67 (1.39-2.02)**
Distal hypospadias	1.86 (1.52-2.26)	1.80 (1.47-2.21)**
Proximal hypospadias	5.76 (2.25-14.77)	4.02 (1.51-10.71)**

Table 3. *Comparison of birth cohorts and risk of hypospadias. a. Born 2006-2007: N=110.604, cases=899 (0.81%). Born 1992-1993: N=123.943, cases=710 (0.57%). b. Born 2006-2007: N= 46.749, cases=395 (distal 83.8%, proximal 9.4%). Born 1973-1974: N=38.018, cases=180 (distal 80.4%, proximal 16.8%). *European, Asian or African parentage, SGA, ART as a categorical covariate, maternal BMI >30 as a binary covariate. **European, Asian or African parentage or being a twin.*

This study confirmed the previous notion that being born small for gestational age is highly associated with having hypospadias [24]. Asian and greater European parentage was associated with hypospadias, probably reflecting genetic variations. We could not detect any significant association between hypospadias and ICSI. Previous Swedish studies have shown temporal variations in the effect of ART on a subsequent risk of hypospadias [130-132], and a recent meta-analysis could not detect any certain increase in risk of hypospadias when comparing ICSI with non-ICSI ART [39]. Temporal variations in the risk of hypospadias associated with ICSI may be due to improved protocols, reduced twinning rates or changes in treatment criteria (i.e. paternal subfertility).

Similar to many other studies, we found an increasing prevalence of boys with a diagnosis of hypospadias. The highest observed frequencies were found in 2006-2008 and ranged between 400-450 cases per year. The majority of cases had more than one registered diagnosis of hypospadias. The prevalence in our study is high compared with many other studies, but similar to the results of several European and American register-based studies [61, 133, 134]. There are many potential causes of the observed trend in our and other studies. Firstly, the increase may be due to previous under-reporting, and possibly later over-reporting, of hypospadias. In the 1970s and 1980s many boys with mild glandular hypospadias were left without surgical intervention or only underwent circumcision, and were thus seen at outpatient visits and, further, they may not even have been referred to a pediatric urologist for evaluation. In addition, there has been an increased awareness of hypospadias throughout the study period, which may have led to a gradually more complete registration of distal hypospadias. We did, however, detect an increasing prevalence of boys who had received a diagnosis corresponding to proximal hypospadias as well. We find it less likely that the finding of proximal hypospadias suffers from under-reporting in live-born boys. Studies on the association between preterm birth and hypospadias show somewhat conflicting results [24], nonetheless, proximal hypospadias seems to have a stronger association with preterm

birth than distal hypospadias [25]. Obstetric and neonatal care has evolved substantially over the last few decades, and children who previously were lost in late pregnancy, may now present as preterm survivors. It is possible that the observed trend is explained by the decreasing frequencies of stillbirths, and especially so in proximal hypospadias. Further, the progress in surgical techniques and access to ART may have improved fertility outcomes in men with hypospadias. With a greater chance of affected men having children, the load of genetic risk factors for hypospadias in the population may have increased. It has also been proposed that the increased prevalence of genital malformations may partly be due to endocrine disrupting chemicals.

4.2 Hidden comorbidities and the impact of molecular diagnostics

In the second study we described a clinical case of a boy born in Sweden and diagnosed with isolated proximal hypospadias at birth. There was no history of neuromuscular disorders or hypospadias in the family. Routine mutational screening of five genes; *AR*, *SRD5A2*, *HSD17B3*, *WT1* and *SF1*, was performed and a pathological expansion of 42 CAG repeats in exon 1 in the *AR* gene, located on the X chromosome, was unexpectedly found (normal range 17-34 CAG repeats). Previous studies have shown that CAG expansion in the *AR* gene is associated with hypospadias and this mutation may thus have contributed to the development of hypospadias in this patient [135, 136]. Further, a CAG expansion of this size is known to cause the rare disorder of spinal-bulbar muscle atrophy (SBMA) later in life [137, 138]. The mother was tested and was found to be a carrier of the mutation. The screening of Swedish national registers identified one male with hypospadias and suspected SBMA, and three cases of potential SBMA in the male relatives (compatible with an X-linked recessive inheritance pattern) of men with a diagnosis of hypospadias.

There are only two previous reports describing the co-occurrence of either hypospadias or chordee and a CAG expansion in the range that predisposes for future development of SBMA [139, 140]. This may be due to the fact that hypospadias is an under reported feature of SBMA, since the association has not been acknowledged by the neurological community and neurologists may simply fail to enquire about childhood diagnoses. It is also likely that hypospadias is very rare in men with SBMA.

Spinal-bulbar muscle atrophy (SBMA) shows consistent X-linked inheritance. The elongated CAG repeat alters the conformation of the androgen receptor, which gains toxic functions and accumulates in the nuclei of neurons. The main histopathological findings in patients with SBMA are the loss of motor neurons in brainstem motor nuclei and motor neurons in anterior horns of the spinal cord [141]. The elongation of the CAG repeat in the *AR* is associated with an attenuated transactivation function, and may thereby reduce the effect of androgens during the formation of male external genitalia, and be responsible for symptoms of androgen insensitivity in SBMA [142, 143]. It is unlikely that the elongation of the CAG repeat in the *AR* gene alone is causative of hypospadias, since in that case all men with SBMA would present with hypospadias. More likely is that elongation of the CAG repeat modulates the

risk of hypospadias, and in combination with other genetic variations and environmental influences results in under-masculinized genitalia [135].

Molecular diagnostics in proximal hypospadias are important since they may tell us more about recurrence risk within the family and future fertility in the subject, and in addition help the scientific community elucidate the genetic origins of hypospadias. Molecular testing for SBMA in asymptomatic individuals younger than 18 is not considered appropriate, since it is of little benefit and negates the autonomy of the child [138]. In a case like ours, the psychological burden of the diagnosis primarily falls on the parents that have to cope not only with the fact that their child is born with a congenital malformation, but also that their child is the carrier of a mutation that will inevitably lead to future morbidity. However, regardless how unexpected the finding might be, the health care system has a responsibility to give full disclosure to the patients.

Molecular diagnostics have led to great progress in terms of detecting, treating and monitoring risk of disease. With that success follows a great responsibility for the health care system to inform patients on the possible outcomes of genetic testing and help them cope with the impact of discouraging results.

4.3 Socioeconomic outcomes and aspects of well-being

In *Study III* we found that men with hypospadias, as an aggregate, were similar to non-affected men with regards to income level, educational level and marriage status. They were, however, at a greater risk of receiving a disability pension, and the risk remained after adjustments for confounding factors and in sensitivity analyses excluding men with psychiatric comorbidity. A subset of the population born 1983 and onwards was less likely to be eligible for upper secondary school, but the upper boundary of the CI was close to 1, after adjustment for confounding factors (Table 4).

	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2** OR (95% CI)	Model 3*** OR (95% CI)
Entered marriage	1.03 (0.92-1.14)	1.05 (0.95-1.17)	0.99 (0.88-1.12)	1.02 (0.90-1.14)
Disability pension	2.01 (1.75-2.30)	1.46 (1.27-1.69)	1.87 (1.62-2.15)	1.39 (1.20-1.61)
Eligible for upper secondary†	0.81 (0.72-0.90)	0.82 (0.74-0.92)	0.86 (0.77-0.96)	0.87 (0.78-0.98)
Highest level of education				
Elementary school	1.15 (1.04-1.27)	1.12 (1.01-1.24)	1.11 (1.00-1.23)	1.09 (0.99-1.20)
Upper secondary school	1	1	1	1
College	1.04 (0.96-1.13)	1.05 (0.97-1.14)	1.06 (0.98-1.16)	1.07 (0.98-1.17)
Highest level of income				
Low income	1.01 (0.91-1.13)	1.00 (0.90-1.11)	1.01 (0.90-1.12)	0.99 (0.89-1.10)
Median income	1	1	1	1
High income	0.90 (0.82-0.99)	0.93 (0.84-1.01)	0.93 (0.84-1.02)	0.95 (0.86-1.04)

Table 4. *Logistic regression models for the associations between hypospadias and socioeconomic outcomes, regardless of phenotype, conditional on birth year and birth county. † Men born 1983 and onwards included. *Adjusted for additional malformations, **adjusted for gestational age and birth weight, ***adjusted for additional malformations, gestational age and birth weight.*

When assessing the same outcomes in distal hypospadias, the results were similar except that the association with disability pension was no longer evident after adjustments for additional malformations and other perinatal characteristics (Table 5).

	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2** OR (95% CI)	Model 3*** OR (95% CI)
Entered marriage	1.03 (0.91-1.16)	1.05 (0.93-1.19)	1.01 (0.89-1.15)	1.03 (0.91-1.18)
Disability pension	1.72 (1.42-2.09)	1.28 (1.06-1.56)	1.60 (1.31-1.95)	1.20 (0.98-1.48)
Eligible for upper secondary†	0.71 (0.60-0.85)	0.73 (0.61-0.87)	0.75 (0.63-0.90)	0.77 (0.64-0.92)
Highest level of education				
Elementary school	1.08 (0.94-1.24)	1.05 (0.91-1.21)	1.06 (0.92-1.22)	1.03 (0.89-1.19)
Upper secondary school	1	1	1	1
College	1.03 (0.93-1.13)	1.04 (0.94-1.14)	1.03 (0.94-1.15)	1.04 (0.94-1.15)
Highest level of income				
Low income	0.97 (0.82-1.16)	0.96 (0.81-1.15)	0.97 (0.81-1.16)	0.96 (0.81-1.15)
Median income	1	1	1	1
High income	0.89 (0.80-0.98)	0.91 (0.82-1.01)	0.92 (0.82-1.02)	0.93 (0.84-1.05)

Table 5. *Logistic regression models for the associations between distal hypospadias and socioeconomic outcomes, conditional on birth year and birth county. † Men born 1983 and onward included. *Adjusted for additional malformations, **adjusted for gestational age and birth weight, ***adjusted for additional malformations, gestational age and birth weight.*

In men with proximal hypospadias, the only association that remained significant after adjustment for confounding factors was that indicating an increased risk of receiving a disability pension (Table 6).

	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2** OR (95% CI)	Model 3*** OR (95% CI)
Entered marriage	0.82 (0.46-1.46)	0.87 (0.48-1.55)	0.78 (0.40-1.52)	0.82 (0.42-1.60)
Disability pension	4.24 (2.59-6.93)	2.32 (1.37-3.91)	3.13 (1.85-5.31)	1.94 (1.11-3.37)
Eligible for upper secondary†	1.96 (0.91-4.20)	1.98 (0.92-4.28)	2.21 (1.00-4.77)	2.23 (1.03-4.82)
Highest level of education				
Elementary school	1.26 (0.79-2.01)	1.16 (0.72-1.86)	1.14 (0.69-1.86)	1.06 (0.65-1.75)
Upper secondary school	1	1	1	1
College	1.06 (0.71-1.60)	1.07 (0.71-1.61)	1.12 (0.73-1.72)	1.13 (0.74-1.75)
Highest level of income				
Low income	1.15 (0.71-1.86)	1.13 (0.69-1.83)	1.09 (0.67-1.76)	1.07 (0.65-1.76)
Median income	1	1	1	1
High income	0.72 (0.45-1.16)	0.80 (0.49-1.28)	0.87 (0.53-1.46)	0.93 (0.56-1.56)

Table 6. *Logistic regression models for the associations between proximal hypospadias and socioeconomic outcomes, conditional on birth year and birth county. † Men born 1983 and onwards included. *Adjusted for additional malformations, **adjusted for gestational age and birth weight, ***adjusted for additional malformations, gestational age and birth weight.*

Although it only affects a small group of patients, the finding regarding disability pension in men with proximal hypospadias is worrying. It reflects an impaired work ability, but as a consequence of what remains unclear. The two main reasons for sick leave and receiving a disability pension in Sweden are psychiatric disorders and musculoskeletal diagnoses. It is

possible that men with hypospadias suffer from more musculoskeletal problems in adulthood, due to the association with limb anomalies. Being born preterm or with low birth weight is associated with both hypospadias and adverse health outcomes and developmental impairment later in life. However, after adjustments for additional malformations, birth weight and gestational age at birth, the association with disability pension remained significant. The effect of psychiatric illness was taken into account in sensitivity analyses, but did not diminish the association substantially. The association is thus mediated or confounded by some other, at present, unknown factors. The risk was present primarily in men with proximal hypospadias, who may have androgen insensitivity as a result of mutations in the *AR* gene or gonadal dysfunction with subsequent androgen deficiency for other reasons. A low testosterone level, or a reduced response to testosterone, is associated with a number of symptoms in men. The boy with a pre-pubertal onset of androgen deficiency may present with delayed puberty, reduced sexual desire, and low bone mass. Symptoms associated with androgen deficiency in the adult male are insulin resistance, obesity, metabolic syndrome, osteoporosis, depression, and diminished cognitive function [99]. It is possible that the increased risk of receiving a disability pension in men with proximal hypospadias is due to such conditions related to androgen deficiency.

Apart from the increased risk of a disability pension, most socioeconomic outcomes were similar. The subjects with hypospadias achieved similar level of education and income, which are both important aspects in well-being. Further, the men with hypospadias were as likely to being registered as married as non-affected men. Marriage served as a proxy of having a supportive relationship, but may lack specificity in a society like the Swedish, where only 70% of cohabiting couples enter marriage[144]. The result of this study is by no means an exact measure of equal happiness in men with and without hypospadias, but we do hope that it reflects a similar level of well-being.

4.4 Assessment of fertility by register-based methods

In *Study IV,* fertility was assessed by three different outcomes; registered paternity, conceiving through ART, and a registered diagnosis of male infertility. The crude cumulative incidence of paternity by phenotype is presented in Figure 14.

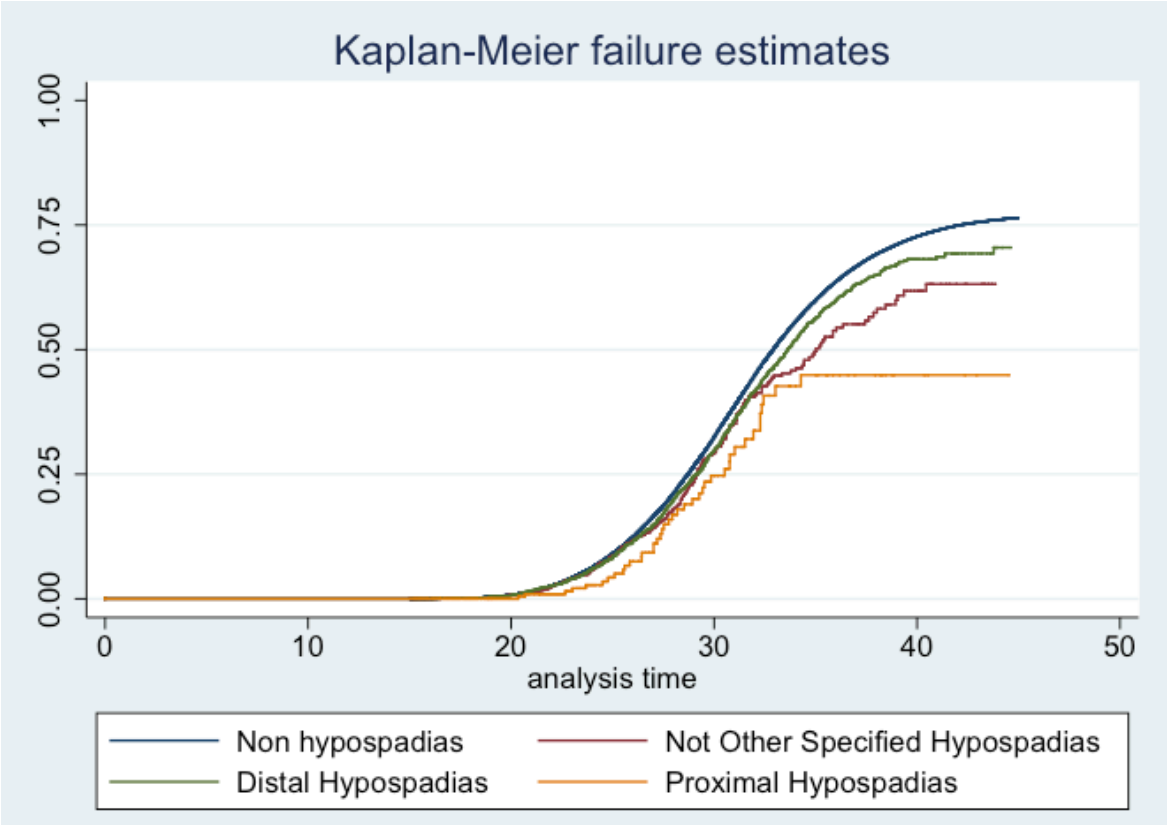

Figure 14. *Crude cumulative incidence of registered paternity, by phenotype.*

In time-to-event analyses, a significant inverse association between registered paternity and hypospadias was present in all subgroups. The reduction in probability was nearly non-significant in men with distal hypospadias and most prominent in men with proximal hypospadias (Table 7).

	Crude -64 HR (95% CI)	Model 1 -64 HR (95% CI)	Crude -73 HR (95% CI)	Model 2 -73 HR (95% CI)
All hypospadias	0.87 (0.82-0.92)	0.87 (0.83-0.92)	0.89 (0.84-0.94)	0.90 (0.85-0.96)
NOS hypospadias	0 .84 (0.75-0.94)	0.84 (0.75-0.94)	0.84 (0.74-0.96)	0.86 (0.76-0.98)
Distal hypospadias	0.90 (0.84-0.96)	0.90 (0.85-0.96)	0.92 (0.86-0.98)	0.93 (0.87-0 .99)
Proximal hypospadias	0.58 (0.42-0.81)	0.59 (0.42-0.81)	0.64 (0.44-0.89)	0.65 (0.46-0.92)

Table *7. Cox regression models for the association between hypospadias and being registered as a biological father in the full cohort, by phenotype. Model 1 adjusted for maternal ethnicity. Model 2 adjusted for maternal ethnicity, weight and gestational age at birth.*

In sibling comparison estimates remained similar, but CIs surpassed 1.0 in most comparisons. When excluding men with hypospadias, and comparing their non-affected brothers with the remains of the full cohort, no differences in probability of paternity remained.

As an aggregate, men with hypospadias were at an increased risk of conceiving through ART (Table 8). The associations remained in both distal and proximal hypospadias after adjustment for confounding factors and in sensitivity analyses excluding men with concomitant cryptorchidism.

	Crude -64 HR (95% CI)	Model 1 -64 HR (95% CI)	Crude -73 HR (95% CI)	Model 2 -73 HR (95% CI)
All hypospadias	1.49 (1.23-1.81)	1.49 (1.22-1.81)	1.56 (1.27-1.91)	1.56 (1.27-1.92)
NOS hypospadias	1.49 (0.93-2.38)	1.49 (0.93-2.38)	1.43 (0.78-2.62)	1.49 (0.81-2.73)
Distal hypospadias	1.39 (1.12-1.74)	1.39 (1.11-1.74)	1.46 (1.17-1.83)	1.45 (1.15-1.82)
Proximal hypospadias	4.60 (2.27-9.31)	4.56 (2.25-9.23)	6.23 (3.20-12.15)	6.77 (3.47-13.20)

***Table 8**. Cox regression models for the association between hypospadias and conceiving through ART, by phenotype. Model 1 adjusted for maternal ethnicity. Model 2 adjusted for maternal ethnicity, weight and gestational age at birth.*

Men with proximal hypospadias were at an increased risk of having a registered diagnosis of male infertility (Table 9). The association remained significant in analyses adjusting for confounding factors and in sensitivity analyses excluding men with cryptorchidism.

	Crude -64 HR (95% CI)	Model 1 -64 HR (95% CI)	Crude -73 HR (95% CI)	Model 2 -73 HR (95% CI)
All hypospadias	1.34 (1.11-1.62)	1.33 (1.10-1.60)	1.29 (1.06-1.57)	1.26 (1.03-1.55)
NOS hypospadias	1.60 (1.08-2.36)	1.52 (1.03-2.26)	1.59 (1.03-2.45)	1.57 (1.00-2.44)
Distal hypospadias	1.17 (0.93-1.49)	1.17 (0.93-1.46)	1.13 (0.89-1.43)	1.10 (0.86-1.40)
Proximal hypospadias	3.59 (1.97-6.53)	3.57 (1.96-6.49)	3.62 (1.84-7.13)	3.79 (1.92-7.46)

***Table 9.** Cox regression models for the association between hypospadias and having a registered diagnosis of male infertility, by phenotype. Model 1 adjusted for maternal ethnicity. Model 2 adjusted for maternal ethnicity, weight and gestational age at birth.*

As previously stated, fertility in men is most accurately assessed with anatomical examination together with semen and hormonal analyses. There are however several different ways of investigating fertility in men by epidemiological methods. Birth rates may be used but do not reflect the true reproductive potential in societies where reproduction is influenced by family planning. The standardized fertility ratio (SFR) may be applied if the observed and expected fertility ratio can be calculated before and after the exposure of interests is introduced, e.g. before and after the introduction of a chemical in a work place environment. This method is not applicable in a situation such as ours, where the exposure is present from birth. Investigating the use of ART is another measure, but may suffer from bias since not all couples with fertility problems seek medical help. In addition, twinning rates may be used to study male fertility [145].

In this study we used three different outcome measures to assess the fertility in men with hypospadias. Our results imply that men with hypospadias, and especially so those with the proximal phenotype, have impaired fertility. Some of the plausible causes of reduced fertility and altered reproductive patterns in men with hypospadias, other than etiological genetic factors and the malformation itself, are concomitant cryptorchidism, low birth weight and

possibly psychiatric illness. The overall results did, however, not change after adjustment for these factors. In distal hypospadias, the inverse association with registered paternity was almost non-significant, supporting the general opinion that distal hypospadias have little to no effect on reproductive abilities. The men with distal hypospadias were, however, just as those with the proximal phenotype, at an increased risk of conceiving through ART, regardless of concomitant cryptorchidism. We suggest that this finding indicates impaired fertility in both distal and proximal hypospadias.

Although previous studies found semen parameters in men with isolated hypospadias, of whom most had the distal phenotype, to be similar to those of controls, hormonal analyses showed higher levels of FSH and LH compared with controls [97, 98]. This might indicate low-grade gonadal dysfunction that could have effects on fertility. Other possible causes of impaired fertility in men with hypospadias are anatomical features, probably with the greatest impact in men with proximal hypospadias, psychological factors and genetic background, and maybe most probably a combination thereof. It has also been proposed that hypospadias, cryptorchidism, testicular cancer and male infertility are part of a syndrome of common origin, and that the prevalence of this syndrome is influenced by the presence of endocrine disrupting chemicals [146]. Such exposures, and other factors that may influence reproduction, e.g. genetics and psychosocial circumstances, are often shared within families. In this study we found no convincing evidence that non-affected brothers of patients with hypospadias demonstrate the same risk of not having children, suggesting that the finding is not due to factors shared between full brothers.

5 METHODOLOGICAL CONSIDERATIONS

5.1 Internal validity

Internal validity refers to what extent the results of a study reflect causal relationships and whether results are representative of the source population. Internal validity relies on both systematic and random errors. Random errors arise due to random variability in data and affect the precision of the estimates. Statistical analyses aim to distinguish between findings that arise due to chance and those that are true and may be replicated. The precision of analyses may be described in terms of confidence intervals or p-values. Increasing the size of the study population may reduce the effect of random errors. Systematic errors may arise as a result of different types of bias or residual confounding, and will be discussed in the following sections.

Selection bias

Selection bias arises when the association between exposures and outcomes differs between those who do and do not participate in the study, making the study participants' non-representative of the population that is intended to be analyzed. Selection bias may arise due to non-random sampling or self-selection, and is most common in case-control studies but may also be present in population-based cohort studies. In *Study I*, inevitable selection bias was introduced by the fact that only live births were included, and it is not possible to attain data on all conceptions through register data.

Information bias

Information, or misclassification, bias arises when the data on exposures, outcomes or covariates in the study participants are incorrectly recorded, measured or categorized. Misclassification may be either differential, i.e. the error rate differs across comparison groups, or non-differential, i.e. an equal error rate in all study participants. Differential information bias results in over- or underestimation of the association between exposure and outcome.

It is not possible to estimate the extent of misclassification of hypospadias in Sweden without conducting a validation study. It is possible that children that were included as cases, were actually non-affected by hypospadias. Boys may have received a diagnosis in the maternity ward, which was later discarded by a pediatric urologist. It may, however, be just as likely that boys with very distal hypospadias were not registered with a diagnosis. Some physicians neglect to register minor malformations, arguing that a malformation diagnosis may affect the possibility to take out private health insurance for the child. Further, there is a risk of misclassification of the phenotype. It is possible that we have overestimated the number of subjects with proximal hypospadias by using the code corresponding to the most proximal phenotype when classifying hypospadias. However, when discarding hypospadias NOS, the proportion of proximal hypospadias in the studies within this thesis, corresponds to the frequencies reported by others (10-15%) [12].

Further, the highest observed frequencies of hypospadias in *Study I*, are similar to the number of new patients with hypospadias during 2016, as reported at the latest Swedish national meeting on hypospadias. The overall risk of misclassification regarding the diagnosis of hypospadias is hopefully random, and thus non-differential. There is, however, an exception where non-differential misclassification probably is present. In *Studies III* and *Study IV*, patients born before 1973 were identified through diagnosis in the NPR, meaning that only those who had received a diagnosis during inpatient visits were registered with hypospadias. The risk of such misclassification is probably especially high among distal cases with little or no surgical intervention. Nonetheless, since the results in the analyses restricted to men born 1973 and onwards remained similar the effect is probably small.

The actual number of subjects affected by psychiatric illness within *Studies III* and *IV* may be underestimated, since many patients with such conditions are treated in outpatient clinics or in primary care, and often by other medical professionals than physicians. Such misclassification is however most likely non-differential. Differential misclassification may, however, have occurred in *Study IV*; the rate of paternal discrepancy may be higher in men with hypospadias, thereby diluting the true association between hypospadias and biological paternity.

Detection, or surveillance bias, may be regarded as another type of information bias. It occurs when the presence of an exposure leads to closer surveillance and earlier detection regarding covariates or outcomes. In *Studies III* and *IV* cryptorchidism, and additional malformations in general, were used in sensitivity analyses. It is possible that those covariates suffer from detection bias, since individuals with one malformation may undergo more frequent and careful examinations in order to detect associated conditions. The other parental and perinatal characteristics in *Studies I, III* and *IV*, are less likely to suffer from detection or recall bias since they were prospectively collected. Detection bias is possibly also a problem in *Study IV,* since men with hypospadias may be more prone to seek medical help if fertility issues arise. This may explain the increased risk of conceiving through ART and being diagnosed with male infertility.

Confounding

As previously stated, association is not the same as causation. An inherent problem in observational studies is that the observed associations may arise as an effect of unmeasured, uncontrolled or unknown residual confounding. In the studies within this thesis we have tried to avoid spurious associations by controlling for confounders and further elucidate relationships between exposures and outcomes by conducting sensitivity analyses. However, the register data is not always as specific as one could wish and some variables are used as best available proxies of the true association.

In *Study I,* the majority of parental and perinatal factors included serve as proxies of other biological events, e.g. parental ethnicity was used as a proxy of genetic susceptibility, being born small for gestational age was used as a proxy for early intrauterine growth restriction. It

is possible that the observed associations were confounded by unmeasured factors. Other factors associated with being born small for gestational age, such as maternal smoking and chronic hypertension may have confounded some of the association between hypospadias and SGA [147]. Hypospadias may not be associated with parental ethnicity per se, but rather factors associated with ethnicity, such as socioeconomic status. Further, when investigating the risk of hypospadias in different birth cohorts, analyses on whether the elevated risk in the later time period was associated with more children being born pre-term were not performed.

Children born by parents with low socioeconomic status (SES) are more likely to be born small for gestational age and suffer from illness during childhood, and parental SES further affects cognitive performance during childhood. In adolescents, low socioeconomic status is associated with impaired health status [148]. In *Study III* we investigated the association between hypospadias and different socioeconomic outcomes, and adjusted for potential confounders such as gestational age, birth weight and concomitant malformations. We did however not adjust for parental SES. We investigated the putative effect of parental educational level, which together with occupation represent the best measure of SES, but found no convincing effect on the outcomes and therefore chose to omit it as a confounding factor in analyses. The association between hypospadias and disability pension is most probably confounded or mediated by some other factor; possibly unmeasured psychiatric comorbidity, undiagnosed chromosomal aberrations or syndromes, or conditions related to androgen deficiency.

In *Study IV* we found a decreased rate of having biological children among men with hypospadias, compared with the rate in non-affected men. The finding may reflect impaired spermatogenesis or testicular deficiency in these patients but may also be due to several other factors, which we have not been able to account for. It is possible that the lower birth rate reflects impaired sexual function, in terms of lower desire or arousal, or psychological aspects such as avoidance or shame. We do, however, consider that the finding of an increased risk of conceiving through assisted reproductive technologies indicates that there is more than psychological factors behind the associations and that the desire for having children is preserved in men with hypospadias. We have not yet investigated whether hypospadias is associated with any specific somatic comorbidities, but it is possible that the associations with lower birth rates, ART and male infertility are confounded or mediated by other conditions rather than hypospadias itself.

5.2 External validity

External validity, or generalizability, refers to what extent results may be applied to other situations, populations or locations. Adequate internal validity is crucial for external validity.

An inherent problem in the long-term follow-up of many surgical diagnoses is that the results may be a reflection of historical techniques and thus might not be possible to generalize to future generations. This is especially important when reviewing the results within *Study IV*. In addition, the individuals included in *Studies III* and *IV* were born no earlier than 1964. It is

possible that outcome status, and results, would change with a more complete follow-up. Further, results within *Studies III* and *IV* must be interpreted with the notion that Sweden has a well-developed, and publicly financed, welfare system. Inhabitants are ensured financial security in case of unemployment or illness, and healthcare and education is publicly funded. The impact of being born with hypospadias may be different in societies where welfare systems are less developed.

6 ETHICAL CONSIDERATIONS

Whether clinical or register-based, the benefits of medical research should always be balanced against the potential harm that the study participants may be exposed to. The studies within this thesis concern topics and answer questions asked by patients and their parents. However, regardless of the importance of the project, ethical approval must be obtained from the local ethics committee before a research project is started. If the benefit of the results is estimated to exceed the potential risks and the researchers have a strategy for how they will diminish the potential harm for the study participants, the ethical committee may approve of the research. The studies within this thesis attained approval from the regional ethical committee in Stockholm prior to execution. (DNR 2008/670-31/3, DNR 2008/1671-31/3, DNR 2009/939-31/5 and DNR 2013/862-31/5.)

6.1 Informed consent, integrity and data security

Medical research is regulated by both Swedish law and international directives, and acknowledges the right of the subjects to refuse to participate or withdraw their participation [149]. Since register-based research does not involve any direct contact with the study participants and integrity is therefore not at risk, informed consent is not needed to obtain permission from the local ethics committee. In addition, these studies do not include any risk of bodily harm. Indeed, informed consent would probably have dramatic effects on the benefit of large-scale observational studies by introducing selection bias and greatly reducing participation rates [150].

Although no individual consent was collected, great efforts were taken to protect the integrity and privacy of the study participants by accurate data handling and security. The data sets included anonymized information only; all data on personal identification numbers and names were removed and replaced with study participation numbers that enabled linkage across registers. Data were stored on secure servers with strict rules of access and available only to those involved in the projects.

7 CONCLUSION AND INTERPRETATIONS

The overall results of this thesis provide an improved understanding of the etiology and clinical characteristics of boys with hypospadias and increased knowledge about well-being and fertility in adult men born with hypospadias.

Study I

The prevalence of boys registered with a diagnosis of hypospadias has increased during the late 20th century. This trend could not be fully explained by temporal changes in the parental and perinatal risk factors investigated. The increased prevalence may be due to under- and possibly over-reporting of hypospadias in different time periods, changes in diagnostic criteria, increased survival of preterm children, improved fertility in men with hypospadias or the introduction of new risk factors, such as endocrine disrupting chemicals.

Study II

Hypospadias may be an under diagnosed feature in men with spinal-bulbar muscle atrophy. The co-occurrence of hypospadias and SBMA in subjects and within families is hard to evaluate by register-based methods, but the combination is estimated to be rare.

Study III

Men with hypospadias display comparable socioeconomic outcomes to non-affected men, possibly reflecting a similar level of well-being. Men with proximal hypospadias were, however, at an increased risk of receiving a disability pension. This may be due to the effects of unmeasured psychiatric comorbidity, conditions related to androgen deficiency or hypospadias as part of unrecognized syndromes.

Study IV

Compared with non-affected men, men with hypospadias display lower birth rates, and show a higher probability of conceiving through assisted reproductive technologies and being diagnosed with male infertility. The overall results imply that fertility is impaired in men with distal and proximal hypospadias, probably as a result of anatomic features, gonadal dysfunction, psychological factors, or genetic background.

8 ACKNOWLEDGEMENTS

Agneta Nordenskjöld, my main supervisor. You are a brilliant researcher and surgeon, a source of inspiration and a true role model for me! I am very grateful for the opportunity to work on this project and for the support, patience and generosity you have shown me during the past years. I sincerely hope that we will continue doing research together and that I may join you in the theater during the years to come.

Anna Nordenström and Louise Frisén, my co-supervisors. I am so impressed by you! The amount of energy that the two of you exhibit, and generously share, seems infinite. I am thankful for all the knowledge and thoughtful advice you have shared with me throughout the doctoral education. I hope we will continue doing research together in the near future.

Catarina Almqvist, co-author. I am very grateful for the possibility to be part of the CRIME project. Thank you for generously sharing your invaluable knowledge in epidemiology with me.

Paul Lichtenstein, co-author. Thank you for the support during my first tentative steps into the world of epidemiology. I have learned so much from our common project and much appreciate your help enabling the collaboration between the DSD research group and the CRIME project.

Christina Norrby, co-author, and outstanding data curator in the CRIME project. Thank you for making room for me in your office and making me feel welcome from the very first day. You have generously shared your knowledge in programming, registers and life in general, for that, and your friendship, I am immensely grateful.

Martin Paucar, co-author. Thank you for sharing your energy and vast knowledge during the SBMA project.

Agnieszka Butwicka, co-author. I am grateful for your help answering the infinite number of questions regarding epidemiology and biostatistics I have asked throughout the years.

Qi Chen, co-author. Thank you for generously sharing your knowledge in biostatistics and epidemiology.

Anna Strandqvist, **Hedvig Engberg and Lisa Örtqvist**, fellow PhD students in the DSD research group over the past five years. Your support, humor and friendship make research so much more fun! Thank you!

Magdalena Fossum, hypospadiologist. Thank you for sharing your positive spirit, energy and enthusiasm on hypospadias, and for taking the time to review this thesis.

Arvid Steen, friend and brilliant illustrator. Thank you for generously providing such amazing artwork!

Friends and colleagues at the pediatric surgery clinic, Astrid Lindgrens Barnsjukhus. Thank you for creating a work environment where science and research are premiered and for making my time at work so pleasant.

Fellow PhD students at ALB, thank you for inspiring meetings and good advice.

Astrid Häggblad and Anna Sandberg, Department of Women's and Children's Health, Karolinska Institutet. Thank you for kind support and patience.

Jia Cao, **Christina Nyström**, **Johanna Winberg**, thank you for being good friends and making time spent at the lab so much fun!

Gabriel Sandblom, thank you for good advice and support.

Margareta, Björn, Lalle and Marcus Nordenvall, thank you for being so supportive and making me feel welcome in the family! A special thank you to **Caroline Nordenvall** who has been supportive, and provided excellent answers, during my epidemiological crises.

Sara, Kalle, Sara, Andreas, Teja and Hedda. Thank you for being there, no matter what, and for helping me focus on other things than work once in a while.

Mom and Dad, thank you for the endless support throughout my life and for encouraging me to be a curious person.

Richard, the love of my life. You are truly amazing, I fall in love with you every day! Thank you for being there for me every step of the way and for being such a wonderful father to Oscar.

Oscar, my little sunshine. You make my life complete!

9 REFERENCES

1. Rey RA, Grinspon RP. Normal male sexual differentiation and aetiology of disorders of sex development. Best Pract Res Clin Endocrinol Metab. 2011;25(2):221-38. doi: 10.1016/j.beem.2010.08.013. PubMed PMID: 21397195.

2. Mitchell BS, Sharma RP. Embryology : an illustrated colour text. Edinburgh ; New York: Elsevier/Churchill Livingstone; 2005. vii, 81 p. p.

3. Biason-Lauber A. Control of sex development. Best Pract Res Clin Endocrinol Metab. 2010;24(2):163-86. doi: 10.1016/j.beem.2009.12.002. PubMed PMID: 20541146.

4. Eid W, Biason-Lauber A. Why boys will be boys and girls will be girls: Human sex development and its defects. Birth Defects Res C Embryo Today. 2016;108(4):365-79. doi: 10.1002/bdrc.21143. PubMed PMID: 28033664.

5. Hiort O. The differential role of androgens in early human sex development. BMC Med. 2013;11:152. doi: 10.1186/1741-7015-11-152. PubMed PMID: 23800242; PubMed Central PMCID: PMCPMC3706224.

6. Rey R, Josso N, Racine C. Sexual Differentiation. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Endotext. South Dartmouth (MA)2000.

7. Ammini AC, Sabherwal U, Mukhopadhyay C, Vijayaraghavan M, Pandey J. Morphogenesis of the human external male genitalia. Pediatric surgery international. 1997;12(5-6):401-6. PubMed PMID: 9244110.

8. Yamada G, Satoh Y, Baskin LS, Cunha GR. Cellular and molecular mechanisms of development of the external genitalia. Differentiation. 2003;71(8):445-60. doi: 10.1046/j.1432-0436.2003.7108001.x. PubMed PMID: 14641326.

9. Arboleda VA, Sandberg DE, Vilain E. DSDs: genetics, underlying pathologies and psychosexual differentiation. Nat Rev Endocrinol. 2014;10(10):603-15. doi: 10.1038/nrendo.2014.130. PubMed PMID: 25091731; PubMed Central PMCID: PMCPMC4441533.

10. Hadidi AT, Azmy AF. Hypospadias surgery. New york: Springer; 2004.

11. Snodgrass W, Bush N. Hypospadiology: Operation Happenis; 2015.

12. Giannantoni A. Hypospadias classification and repair: the riddle of the sphinx. Eur Urol. 2011;60(6):1190-1; discussion 1-2. doi: 10.1016/j.eururo.2011.08.057. PubMed PMID: 21917372.

13. Wu WH, Chuang JH, Ting YC, Lee SY, Hsieh CS. Developmental anomalies and disabilities associated with hypospadias. J Urol. 2002;168(1):229-32. PubMed PMID: 12050549.

14. Yamaguchi T, Kitada S, Osada Y. Chromosomal anomalies in cryptorchidism and hypospadias. Urol Int. 1991;47(2):60-3. PubMed PMID: 1686509.

15. Moreno-Garcia M, Miranda EB. Chromosomal anomalies in cryptorchidism and hypospadias. J Urol. 2002;168(5):2170-2; discussion 2. doi: 10.1097/01.ju.0000029562.62482.74. PubMed PMID: 12394752.

16. Hutson JM, Grover SR, O'Connell M, Pennell SD. Malformation syndromes associated with disorders of sex development. Nat Rev Endocrinol. 2014;10(8):476-87. doi: 10.1038/nrendo.2014.83. PubMed PMID: 24913517.

17. Nordenvall AS, Paucar M, Almqvist C, Nordenstrom A, Frisen L, Nordenskjold A. Hypospadias as a novel feature in spinal bulbar muscle atrophy. J Neurol. 2016;263(4):703-6. doi: 10.1007/s00415-016-8038-y. PubMed PMID: 26872663.

18. Hadidi AT. History of hypospadias: Lost in translation. Journal of pediatric surgery. 2017;52(2):211-7. doi: 10.1016/j.jpedsurg.2016.11.004. PubMed PMID: 27989535.

19. Lambert SM, Snyder HM, 3rd, Canning DA. The history of hypospadias and hypospadias repairs. Urology. 2011;77(6):1277-83. doi: 10.1016/j.urology.2010.10.031. PubMed PMID: 21497381.

20. Fredell L, Lichtenstein P, Pedersen NL, Svensson J, Nordenskjold A. Hypospadias is related to birth weight in discordant monozygotic twins. J Urol. 1998;160(6 Pt 1):2197-9. PubMed PMID: 9817368.

21. Fujimoto T, Suwa T, Kabe K, Adachi T, Nakabayashi M, Amamiya T. Placental insufficiency in early gestation is associated with hypospadias. Journal of pediatric surgery. 2008;43(2):358-61. doi: 10.1016/j.jpedsurg.2007.10.046. PubMed PMID: 18280290.

22. Arendt LH, Ramlau-Hansen CH, Wilcox AJ, Henriksen TB, Olsen J, Lindhard MS. Placental Weight and Male Genital Anomalies: A Nationwide Danish Cohort Study. Am J Epidemiol. 2016;183(12):1122-8. doi: 10.1093/aje/kwv336. PubMed PMID: 27257113; PubMed Central PMCID: PMCPMC4908208.

23. Chen Y, Sun L, Geng H, Lei X, Zhang J. Placental pathology and hypospadias. Pediatr Res. 2017. doi: 10.1038/pr.2016.246. PubMed PMID: 27855149.

24. van der Zanden LF, van Rooij IA, Feitz WF, Franke B, Knoers NV, Roeleveld N. Aetiology of hypospadias: a systematic review of genes and environment. Hum Reprod Update. 2012;18(3):260-83. doi: 10.1093/humupd/dms002. PubMed PMID: 22371315.

25. van Rooij IA, van der Zanden LF, Brouwers MM, Knoers NV, Feitz WF, Roeleveld N. Risk factors for different phenotypes of hypospadias: results from a Dutch case-control study. BJU Int. 2013;112(1):121-8. doi: 10.1111/j.1464-410X.2012.11745.x. PubMed PMID: 23305310.

26. Woud SG, van Rooij IA, van Gelder MM, Olney RS, Carmichael SL, Roeleveld N, et al. Differences in risk factors for second and third degree hypospadias in the national birth defects prevention study. Birth Defects Res A Clin Mol Teratol. 2014;100(9):703-11. doi: 10.1002/bdra.23296. PubMed PMID: 25181604; PubMed Central PMCID: PMCPMC4591539.

27. Chong JH, Wee CK, Ho SK, Chan DK. Factors associated with hypospadias in Asian newborn babies. J Perinat Med. 2006;34(6):497-500. doi: 10.1515/JPM.2006.096. PubMed PMID: 17140301.

28. Akre O, Boyd HA, Ahlgren M, Wilbrand K, Westergaard T, Hjalgrim H, et al. Maternal and gestational risk factors for hypospadias. Environ Health Perspect. 2008;116(8):1071-6. doi: 10.1289/ehp.10791. PubMed PMID: 18709149; PubMed Central PMCID: PMCPMC2516569.

29. Ghirri P, Scaramuzzo RT, Bertelloni S, Pardi D, Celandroni A, Cocchi G, et al. Prevalence of hypospadias in Italy according to severity, gestational age and birthweight: an

epidemiological study. Ital J Pediatr. 2009;35:18. doi: 10.1186/1824-7288-35-18. PubMed PMID: 19558700; PubMed Central PMCID: PMCPMC2717564.

30. Akin Y, Ercan O, Telatar B, Tarhan F, Comert S. Hypospadias in Istanbul: incidence and risk factors. Pediatr Int. 2011;53(5):754-60. doi: 10.1111/j.1442-200X.2011.03340.x. PubMed PMID: 21342360.

31. Akre O, Lipworth L, Cnattingius S, Sparen P, Ekbom A. Risk factor patterns for cryptorchidism and hypospadias. Epidemiology. 1999;10(4):364-9. PubMed PMID: 10401869.

32. Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update. 2011;17(5):589-604. doi: 10.1093/humupd/dmr022. PubMed PMID: 21747128; PubMed Central PMCID: PMCPMC3156888.

33. Adams SV, Hastert TA, Huang Y, Starr JR. No association between maternal pre-pregnancy obesity and risk of hypospadias or cryptorchidism in male newborns. Birth Defects Res A Clin Mol Teratol. 2011;91(4):241-8. doi: 10.1002/bdra.20805. PubMed PMID: 21462299; PubMed Central PMCID: PMCPMC3142793.

34. Marengo L, Farag NH, Canfield M. Body mass index and birth defects: Texas, 2005-2008. Matern Child Health J. 2013;17(10):1898-907. doi: 10.1007/s10995-012-1214-5. PubMed PMID: 23371247.

35. Carmichael SL, Shaw GM, Laurent C, Olney RS, Lammer EJ, National Birth Defects Prevention S. Maternal reproductive and demographic characteristics as risk factors for hypospadias. Paediatr Perinat Epidemiol. 2007;21(3):210-8. doi: 10.1111/j.1365-3016.2007.00809.x. PubMed PMID: 17439529.

36. Norgaard M, Wogelius P, Pedersen L, Rothman KJ, Sorensen HT. Maternal use of oral contraceptives during early pregnancy and risk of hypospadias in male offspring. Urology. 2009;74(3):583-7. doi: 10.1016/j.urology.2009.04.034. PubMed PMID: 19592074.

37. Wogelius P, Horvath-Puho E, Pedersen L, Norgaard M, Czeizel AE, Sorensen HT. Maternal use of oral contraceptives and risk of hypospadias - a population-based case-control study. Eur J Epidemiol. 2006;21(10):777-81. doi: 10.1007/s10654-006-9067-0. PubMed PMID: 17077991.

38. Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M, et al. Incidence of congenital malformations in children born after ICSI. Hum Reprod. 2000;15(4):944-8. PubMed PMID: 10739847.

39. Massaro PA, MacLellan DL, Anderson PA, Romao RL. Does intracytoplasmic sperm injection pose an increased risk of genitourinary congenital malformations in offspring compared to in vitro fertilization? A systematic review and meta-analysis. J Urol. 2015;193(5 Suppl):1837-42. doi: 10.1016/j.juro.2014.10.113. PubMed PMID: 25813561.

40. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972-8. PubMed PMID: 11331648.

41. Gray LE, Jr., Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci. 2000;58(2):350-65. PubMed PMID: 11099647.

42. Christiansen S, Kortenkamp A, Axelstad M, Boberg J, Scholze M, Jacobsen PR, et al. Mixtures of endocrine disrupting contaminants modelled on human high end exposures: an exploratory study in rats. Int J Androl. 2012;35(3):303-16. doi: 10.1111/j.1365-2605.2011.01242.x. PubMed PMID: 22372636.

43. Rider CV, Furr J, Wilson VS, Gray LE, Jr. A mixture of seven antiandrogens induces reproductive malformations in rats. Int J Androl. 2008;31(2):249-62. doi: 10.1111/j.1365-2605.2007.00859.x. PubMed PMID: 18205796.

44. Svechnikov K, Savchuk I, Morvan ML, Antignac JP, Le Bizec B, Soder O. Phthalates Exert Multiple Effects on Leydig Cell Steroidogenesis. Horm Res Paediatr. 2016;86(4):253-63. doi: 10.1159/000440619. PubMed PMID: 26559938.

45. Botta S, Cunha GR, Baskin LS. Do endocrine disruptors cause hypospadias? Transl Androl Urol. 2014;3(4):330-9. doi: 10.3978/j.issn.2223-4683.2014.11.03. PubMed PMID: 26816789; PubMed Central PMCID: PMCPMC4708138.

46. Bonde JP, Flachs EM, Rimborg S, Glazer CH, Giwercman A, Ramlau-Hansen CH, et al. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum Reprod Update. 2016;23(1):104-25. doi: 10.1093/humupd/dmw036. PubMed PMID: 27655588; PubMed Central PMCID: PMCPMC5155570.

47. Thorup J, Nordenskjöld A, Hutson JM. Genetic and environmental origins of hypospadias. Curr Opin Endocrinol Diabetes Obes. 2014;21(3):227-32. doi: 10.1097/MED.0000000000000063. PubMed PMID: 24722170.

48. Wang Y, Li Q, Xu J, Liu Q, Wang W, Lin Y, et al. Mutation analysis of five candidate genes in Chinese patients with hypospadias. Eur J Hum Genet. 2004;12(9):706-12. doi: 10.1038/sj.ejhg.5201232. PubMed PMID: 15266301.

49. Bouty A, Ayers KL, Pask A, Heloury Y, Sinclair AH. The Genetic and Environmental Factors Underlying Hypospadias. Sex Dev. 2015;9(5):239-59. doi: 10.1159/000441988. PubMed PMID: 26613581; PubMed Central PMCID: PMCPMC5012964.

50. Kohler B, Lin L, Mazen I, Cetindag C, Biebermann H, Akkurt I, et al. The spectrum of phenotypes associated with mutations in steroidogenic factor 1 (SF-1, NR5A1, Ad4BP) includes severe penoscrotal hypospadias in 46,XY males without adrenal insufficiency. Eur J Endocrinol. 2009;161(2):237-42. doi: 10.1530/EJE-09-0067. PubMed PMID: 19439508; PubMed Central PMCID: PMCPMC2754378.

51. Ogata T, Wada Y, Fukami M. MAMLD1 (CXorf6): a new gene for hypospadias. Sex Dev. 2008;2(4-5):244-50. doi: 10.1159/000152040. PubMed PMID: 18987498.

52. McElreavey K, Achermann JC. Steroidogenic Factor-1 (SF-1, NR5A1) and46,XX Ovotesticular Disorders of Sex Development:One Factor, Many Phenotypes. Horm Res Paediatr. 2017;87(3):189-90. doi: 10.1159/000454806. PubMed PMID: 27978531.

53. Lin L, Achermann JC. Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. Sex Dev. 2008;2(4-5):200-9. doi: 10.1159/000152036. PubMed PMID: 18987494; PubMed Central PMCID: PMCPMC2645687.

54. Innis JW. Hand-Foot-Genital Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. GeneReviews(R). Seattle (WA)1993.

55. Fontanella B, Russolillo G, Meroni G. MID1 mutations in patients with X-linked Opitz G/BBB syndrome. Hum Mutat. 2008;29(5):584-94. doi: 10.1002/humu.20706. PubMed PMID: 18360914.

56. Fredell L, Iselius L, Collins A, Hansson E, Holmner S, Lundquist L, et al. Complex segregation analysis of hypospadias. Hum Genet. 2002;111(3):231-4. doi: 10.1007/s00439-002-0799-y. PubMed PMID: 12215834.

57. Kalfa N, Philibert P, Baskin LS, Sultan C. Hypospadias: interactions between environment and genetics. Mol Cell Endocrinol. 2011;335(2):89-95. doi: 10.1016/j.mce.2011.01.006. PubMed PMID: 21256920.

58. van der Zanden LF, Galesloot TE, Feitz WF, Brouwers MM, Shi M, Knoers NV, et al. Exploration of gene-environment interactions, maternal effects and parent of origin effects in the etiology of hypospadias. J Urol. 2012;188(6):2354-60. doi: 10.1016/j.juro.2012.08.033. PubMed PMID: 23088992; PubMed Central PMCID: PMCPMC3602843.

59. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. x, 758 p. p.

60. Springer A, van den Heijkant M, Baumann S. Worldwide prevalence of hypospadias. J Pediatr Urol. 2016;12(3):152 e1-7. doi: 10.1016/j.jpurol.2015.12.002. PubMed PMID: 26810252.

61. Paulozzi LJ, Erickson JD, Jackson RJ. Hypospadias trends in two US surveillance systems. Pediatrics. 1997;100(5):831-4. PubMed PMID: 9346983.

62. Boisen KA, Chellakooty M, Schmidt IM, Kai CM, Damgaard IN, Suomi AM, et al. Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at three months of age. J Clin Endocrinol Metab. 2005;90(7):4041-6. doi: 10.1210/jc.2005-0302. PubMed PMID: 15870122.

63. Nelson CP, Park JM, Wan J, Bloom DA, Dunn RL, Wei JT. The increasing incidence of congenital penile anomalies in the United States. J Urol. 2005;174(4 Pt 2):1573-6. PubMed PMID: 16148654.

64. Loane M, Dolk H, Kelly A, Teljeur C, Greenlees R, Densem J, et al. Paper 4: EUROCAT statistical monitoring: identification and investigation of ten year trends of congenital anomalies in Europe. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S31-43. doi: 10.1002/bdra.20778. PubMed PMID: 21381187.

65. Bergman JE, Loane M, Vrijheid M, Pierini A, Nijman RJ, Addor MC, et al. Epidemiology of hypospadias in Europe: a registry-based study. World J Urol. 2015;33(12):2159-67. doi: 10.1007/s00345-015-1507-6. PubMed PMID: 25712311; PubMed Central PMCID: PMCPMC4655014.

66. Lee PA, Houk CP, Ahmed SF, Hughes IA, International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine S, the European Society for Paediatric E. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. Pediatrics. 2006;118(2):e488-500. doi: 10.1542/peds.2006-0738. PubMed PMID: 16882788.

67. Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. Horm Res Paediatr. 2016;85(3):158-80. doi: 10.1159/000442975. PubMed PMID: 26820577.

68. Hutson JM, Beasley SW. The surgical examination of children : an illustrated guide. Oxford: Heinemann Medical Books; 1988. 271 p. p.

69. Ashcraft KW, Holcomb GW, Murphy JP, Ostlie DJ. Ashcraft's pediatric surgery. Sixth edition / ed. London ; New York: Saunders/Elsevier; 2014. xxi, 1165 pages p.

70. McNamara ER, Swartz JM, Diamond DA. Initial Management of Disorders of Sex Development in Newborns. Urology. 2017;101:1-8. doi: 10.1016/j.urology.2016.08.010. PubMed PMID: 27538800.

71. Hiort O, Birnbaum W, Marshall L, Wunsch L, Werner R, Schroder T, et al. Management of disorders of sex development. Nat Rev Endocrinol. 2014;10(9):520-9. doi: 10.1038/nrendo.2014.108. PubMed PMID: 25022812.

72. Woolf-King SE, Anger A, Arnold EA, Weiss SJ, Teitel D. Mental Health Among Parents of Children With Critical Congenital Heart Defects: A Systematic Review. J Am Heart Assoc. 2017;6(2). doi: 10.1161/JAHA.116.004862. PubMed PMID: 28151402.

73. Pasterski V, Mastroyannopoulou K, Wright D, Zucker KJ, Hughes IA. Predictors of posttraumatic stress in parents of children diagnosed with a disorder of sex development. Arch Sex Behav. 2014;43(2):369-75. doi: 10.1007/s10508-013-0196-8. PubMed PMID: 24085468.

74. EAU Guidelines on Paediatric Urology [cited 2017]. Available from: http://uroweb.org/guideline/paediatric-urology/#3_5.

75. Timing of elective surgery on the genitalia of male children with particular reference to the risks, benefits, and psychological effects of surgery and anesthesia. American Academy of Pediatrics. Pediatrics. 1996;97(4):590-4. PubMed PMID: 8632952.

76. Garnier S, Maillet O, Cereda B, Ollivier M, Jeandel C, Broussous S, et al. Late surgical correction of hypospadias increases the risk of complications: a series of 501 consecutive patients. BJU Int. 2017. doi: 10.1111/bju.13771. PubMed PMID: 28083998.

77. Spinoit AF, Poelaert F, Van Praet C, Groen LA, Van Laecke E, Hoebeke P. Grade of hypospadias is the only factor predicting for re-intervention after primary hypospadias repair: a multivariate analysis from a cohort of 474 patients. J Pediatr Urol. 2015;11(2):70 e1-6. doi: 10.1016/j.jpurol.2014.11.014. PubMed PMID: 25797860.

78. Lee OT, Durbin-Johnson B, Kurzrock EA. Predictors of secondary surgery after hypospadias repair: a population based analysis of 5,000 patients. J Urol. 2013;190(1):251-5. doi: 10.1016/j.juro.2013.01.091. PubMed PMID: 23376710.

79. Snodgrass W. Tubularized, incised plate urethroplasty for distal hypospadias. J Urol. 1994;151(2):464-5. PubMed PMID: 8283561.

80. Hardwicke JT, Bechar JA, Hodson J, Osmani O, Park AJ. Fistula after single-stage primary hypospadias repair - A systematic review of the literature. J Plast Reconstr Aesthet Surg. 2015. doi: 10.1016/j.bjps.2015.07.024. PubMed PMID: 26272009.

81. 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. J Urol. 2015;193(3):975-81. doi: 10.1016/j.juro.2014.09.103. PubMed PMID: 25268894.

82. Svensson J, Berg R, Berg G. Operated hypospadiacs: late follow-up. Social, sexual, and psychological adaptation. Journal of pediatric surgery. 1981;16(2):134-5. PubMed PMID: 7241313.

83. Rynja SP, de Jong TP, Bosch JL, de Kort LM. Functional, cosmetic and psychosexual results in adult men who underwent hypospadias correction in childhood. J Pediatr Urol. 2011;7(5):504-15. doi: 10.1016/j.jpurol.2011.02.008. PubMed PMID: 21429804.

84. Jiao C, Wu R, Xu X, Yu Q. Long-term outcome of penile appearance and sexual function after hypospadias repairs: situation and relation. Int Urol Nephrol. 2011;43(1):47-54. doi: 10.1007/s11255-010-9775-y. PubMed PMID: 20556511.

85. Rynja SP, de Kort LM, de Jong TP. Urinary, sexual, and cosmetic results after puberty in hypospadias repair: current results and trends. Current opinion in urology. 2012;22(6):453-6. Epub 2012/08/25. doi: 10.1097/MOU.0b013e328357bc9e. PubMed PMID: 22918035.

86. Örtqvist L, Fossum M, Andersson M, Nordenström A, Frisén L, Holmdahl G, et al. Sexuality and fertility in men with hypospadias; improved outcome. Andrology. 2016:n/a-n/a. doi: 10.1111/andr.12309.

87. Hughes IA, Werner R, Bunch T, Hiort O. Androgen insensitivity syndrome. Semin Reprod Med. 2012;30(5):432-42. doi: 10.1055/s-0032-1324728. PubMed PMID: 23044881.

88. Kang HJ, Imperato-McGinley J, Zhu YS, Rosenwaks Z. The effect of 5alpha-reductase-2 deficiency on human fertility. Fertil Steril. 2014;101(2):310-6. doi: 10.1016/j.fertnstert.2013.11.128. PubMed PMID: 24412121; PubMed Central PMCID: PMCPMC4031759.

89. Boeri L, Ventimiglia E, Capogrosso P, Ippolito S, Pecoraro A, Paciotti M, et al. Low Birth Weight Is Associated with a Decreased Overall Adult Health Status and Reproductive Capability - Results of a Cross-Sectional Study in Primary Infertile Patients. PLoS One. 2016;11(11):e0166728. doi: 10.1371/journal.pone.0166728. PubMed PMID: 27893825; PubMed Central PMCID: PMCPMC5125617.

90. Liffner S, Hammar M, Bladh M, Nedstrand E, Martinez HR, Sydsjo G. Men becoming fathers by intracytoplasmic sperm injection were more often born small for gestational age. Asian J Androl. 2017;19(1):103-6. doi: 10.4103/1008-682X.178848. PubMed PMID: 27184547; PubMed Central PMCID: PMCPMC5227657.

91. Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. 2006;333(7570):679. doi: 10.1136/bmj.38919.495718.AE. PubMed PMID: 16893903; PubMed Central PMCID: PMCPMC1584372.

92. Fritz G, Czeizel AE. Abnormal sperm morphology and function in the fathers of hypospadiacs. J Reprod Fertil. 1996;106(1):63-6. PubMed PMID: 8667347.

93. Mieusset R, Soulie M. Hypospadias: psychosocial, sexual, and reproductive consequences in adult life. J Androl. 2005;26(2):163-8. PubMed PMID: 15713818.

94. Mondaini N, Ponchietti R, Bonafè M, Biscioni S, Di Loro F, Agostini P, et al. Hypospadias: incidence and effects on psychosexual development as evaluated with the Minnesota Multiphasic Personality Inventory test in a sample of 11,649 young Italian men. Urol Int. 2002;68(2):81-5. PubMed PMID: 11834895.

95. WHO. Infertility definitions and terminology [cited 2017]. Available from: http://www.who.int/reproductivehealth/topics/infertility/definitions/en/.

96. A. Jungwirth TD, G.R. Dohle, A. Giwercman, Z. Kopa, C. Krausz, H. Tournaye. EAU guidelines on male infertility 2015. Available from: http://uroweb.org/guideline/male-infertility/#1.

97. Asklund C, Jensen TK, Main KM, Sobotka T, Skakkebaek NE, Jorgensen N. Semen quality, reproductive hormones and fertility of men operated for hypospadias. Int J Androl. 2010;33(1):80-7. doi: 10.1111/j.1365-2605.2009.00957.x. PubMed PMID: 19281491.

98. Kumar S, Tomar V, Yadav SS, Priyadarshi S, Vyas N, Agarwal N. Fertility Potential in Adult Hypospadias. Journal of Clinical and Diagnostic Research : JCDR. 2016;10(8):Pc01-5. doi: 10.7860/jcdr/2016/21307.8276. PubMed PMID: 27656497; PubMed Central PMCID: PMCPMC5028481.

99. G.R. Dohle SA, C. Bettocchi, T.H. Jones, S. Kliesch. EAU guidelines on male hypogonadism 2015. Available from: http://uroweb.org/guideline/male-hypogonadism/#1.

100. Rey RA, Codner E, Iniguez G, Bedecarras P, Trigo R, Okuma C, et al. Low risk of impaired testicular Sertoli and Leydig cell functions in boys with isolated hypospadias. J Clin Endocrinol Metab. 2005;90(11):6035-40. doi: 10.1210/jc.2005-1306. PubMed PMID: 16131574.

101. Aho MO, Tammela OK, Somppi EM, Tammela TL. Sexual and social life of men operated in childhood for hypospadias and phimosis. A comparative study. Eur Urol. 2000;37(1):95-100; discussion 1. doi: 20107. PubMed PMID: 10671793.

102. Kahneman D, Diener E, Schwarz N. Well-being : the foundations of hedonic psychology. New York: Russell Sage Foundation; 1999. xii, 593 p. p.

103. Nagata S, Funakosi S, Amae S, Yoshida S, Ambo H, Kudo A, et al. Posttraumatic stress disorder in mothers of children who have undergone surgery for congenital disease at a pediatric surgery department. Journal of pediatric surgery. 2008;43(8):1480-6. doi: 10.1016/j.jpedsurg.2007.12.055. PubMed PMID: 18675639.

104. Kingston D, Tough S, Whitfield H. Prenatal and postpartum maternal psychological distress and infant development: a systematic review. Child Psychiatry Hum Dev. 2012;43(5):683-714. doi: 10.1007/s10578-012-0291-4. PubMed PMID: 22407278.

105. Shen H, Magnusson C, Rai D, Lundberg M, Le-Scherban F, Dalman C, et al. Associations of Parental Depression With Child School Performance at Age 16 Years in Sweden. JAMA Psychiatry. 2016;73(3):239-46. doi: 10.1001/jamapsychiatry.2015.2917. PubMed PMID: 26842307.

106. Glatz P, Sandin RH, Pedersen NL, Bonamy AK, Eriksson LI, Granath F. Association of Anesthesia and Surgery During Childhood With Long-term Academic Performance. JAMA Pediatr. 2017;171(1):e163470. doi: 10.1001/jamapediatrics.2016.3470. PubMed PMID: 27820621.

107. Sandberg DE, Meyer-Bahlburg HF, Aranoff GS, Sconzo JM, Hensle TW. Boys with hypospadias: a survey of behavioral difficulties. J Pediatr Psychol. 1989;14(4):491-514. PubMed PMID: 2607390.

108. Berg R, Svensson J, Aström G. Social and sexual adjustment of men operated for hypospadias during childhood: a controlled study. J Urol. 1981;125(3):313-7. PubMed PMID: 7206078.

109. Mureau MA, Slijper FM, Slob AK, Verhulst FC. Psychosocial functioning of children, adolescents, and adults following hypospadias surgery: a comparative study. J Pediatr Psychol. 1997;22(3):371-87. PubMed PMID: 9212554.

110. Ortqvist L, Andersson M, Strandqvist A, Nordenstrom A, Frisen L, Holmdahl G, et al. Psychosocial outcome in adult men born with hypospadias. J Pediatr Urol. 2016. doi: 10.1016/j.jpurol.2016.08.008. PubMed PMID: 28087231.

111. Sandberg DE, Meyer-Bahlburg HF, Hensle TW, Levitt SB, Kogan SJ, Reda EF. Psychosocial adaptation of middle childhood boys with hypospadias after genital surgery. J Pediatr Psychol. 2001;26(8):465-75. PubMed PMID: 11700331.

112. Schönbucher VB, Landolt MA, Gobet R, Weber DM. Health-related quality of life and psychological adjustment of children and adolescents with hypospadias. J Pediatr. 2008;152(6):865-72. doi: 10.1016/j.jpeds.2007.11.036. PubMed PMID: 18492533.

113. Ruppen-Greeff NK, Weber DM, Gobet R, Landolt MA. Health-related quality of life in men with corrected hypospadias: an explorative study. J Pediatr Urol. 2013;9(5):551-8. doi: 10.1016/j.jpurol.2013.04.016. PubMed PMID: 23731562.

114. Butwicka A, Lichtenstein P, Landen M, Nordenvall AS, Nordenstrom A, Nordenskjold A, et al. Hypospadias and increased risk for neurodevelopmental disorders. J Child Psychol Psychiatry. 2015;56(2):155-61. doi: 10.1111/jcpp.12290. PubMed PMID: 25048198.

115. Wang WW, Deng CH, Chen LW, Zhao LY, Mo JC, Tu XA. Psychosexual adjustment and age factors in 130 men undergone hypospadias surgery in a Chinese hospital. Andrologia. 2010;42(6):384-8. doi: 10.1111/j.1439-0272.2010.01061.x. PubMed PMID: 21105889.

116. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur J Epidemiol. 2009;24(11):659-67. doi: 10.1007/s10654-009-9350-y. PubMed PMID: 19504049; PubMed Central PMCID: PMCPMC2773709.

117. Ludvigsson JF, Almqvist C, Bonamy AK, Ljung R, Michaelsson K, Neovius M, et al. Registers of the Swedish total population and their use in medical research. Eur J Epidemiol. 2016;31(2):125-36. doi: 10.1007/s10654-016-0117-y. PubMed PMID: 26769609.

118. National Board of Health and Welfare. National Patient Register - Quality of coding 2010.

119. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450. doi: 10.1186/1471-2458-11-450. PubMed PMID: 21658213; PubMed Central PMCID: PMCPMC3142234.

120. National Board of Health and Welfare Centre for Epidemiology. The Swedish Medical Birth Register - A summary of content and quality. 2003 [cited 2017]. Available from: http://www.socialstyrelsen.se/publikationer2003/2003-112-3.

121. Dödsorsaksregistret [cited 2017]. Available from: http://www.socialstyrelsen.se/register/dodsorsaksregistret.

122. Registret över totalbefolkningen (RTB) [cited 2017]. Available from: http://www.scb.se/sv_/Vara-tjanster/Bestalla-mikrodata/Vilka-mikrodata-finns/Registret-over-totalbefolkningen-RTB/.

123. Ekbom A. The Swedish Multi-generation Register. Methods Mol Biol. 2011;675:215-20. doi: 10.1007/978-1-59745-423-0_10. PubMed PMID: 20949391.

124. Longitudinal integration database for health insurance and labour market studies (LISA by Swedish acronym) [cited 2017]. Available from: http://www.scb.se/en_/Services/Guidance-for-researchers-and-universities/SCB-Data/Longitudinal-integration-database-for-health-insurance-and-labour-market-studies-LISA-by-Swedish-acronym/.

125. Folk- och bostadsräkningar, FoB. Available from: http://www.scb.se/sv_/Vara-tjanster/Bestalla-mikrodata/Vilka-mikrodata-finns/Folk--och-bostadsrakningar-FoB/.

126. Registret över befolkningens utbildning. Available from: http://www.scb.se/sv_/Vara-tjanster/Bestalla-mikrodata/Vilka-mikrodata-finns/Registret-over-befolkningens-utbildning-/.

127. D'Onofrio BM, Class QA, Rickert ME, Sujan AC, Larsson H, Kuja-Halkola R, et al. Translational Epidemiologic Approaches to Understanding the Consequences of Early-Life Exposures. Behav Genet. 2016;46(3):315-28. doi: 10.1007/s10519-015-9769-8. PubMed PMID: 26590988; PubMed Central PMCID: PMCPMC4860044.

128. Campbell MJ, Walters SJ, Machin D. Medical statistics : a textbook for the health sciences. 4th ed. Chichester, England ; Hoboken, NJ: Wiley; 2007. xii, 331 p. p.

129. Rao CR, Miller JP, Rao DC. Epidemiology and medical statistics. 1st ed. Amsterdam ; Boston: Elsevier; 2008. xviii, 852 p. p.

130. Ericson A, Kallen B. Congenital malformations in infants born after IVF: a population-based study. Hum Reprod. 2001;16(3):504-9. PubMed PMID: 11228220.

131. Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. Birth Defects Res A Clin Mol Teratol. 2005;73(3):162-9. doi: 10.1002/bdra.20107. PubMed PMID: 15678490.

132. Finnstrom O, Kallen B, Lindam A, Nilsson E, Nygren KG, Olausson PO. Maternal and child outcome after in vitro fertilization--a review of 25 years of population-based data from Sweden. Acta Obstet Gynecol Scand. 2011;90(5):494-500. doi: 10.1111/j.1600-0412.2011.01088.x. PubMed PMID: 21306346.

133. Nissen KB, Udesen A, Garne E. Hypospadias: Prevalence, birthweight and associated major congenital anomalies. Congenit Anom (Kyoto). 2015;55(1):37-41. doi: 10.1111/cga.12071. PubMed PMID: 25040012.

134. Elliott CS, Halpern MS, Paik J, Maldonado Y, Shortliffe LD. Epidemiologic trends in penile anomalies and hypospadias in the state of California, 1985-2006. J Pediatr Urol. 2011;7(3):294-8. doi: 10.1016/j.jpurol.2011.03.006. PubMed PMID: 21527236.

135. Adamovic T, Nordenskjold A. The CAG repeat polymorphism in the androgen receptor gene modifies the risk for hypospadias in Caucasians. BMC Med Genet. 2012;13:109. doi: 10.1186/1471-2350-13-109. PubMed PMID: 23167717; PubMed Central PMCID: PMCPMC3560208.

136. Huang G, Shan W, Zeng L, Huang L. Androgen receptor gene CAG repeat polymorphism and risk of isolated hypospadias: results from a meta-analysis. Genet Mol Res. 2015;14(1):1580-8. doi: 10.4238/2015.March.6.5. PubMed PMID: 25867301.

137. Giorgetti E, Lieberman AP. Polyglutamine androgen receptor-mediated neuromuscular disease. Cell Mol Life Sci. 2016;73(21):3991-9. doi: 10.1007/s00018-016-2275-1. PubMed PMID: 27188284; PubMed Central PMCID: PMCPMC5045769.

138. La Spada A. Spinal and Bulbar Muscular Atrophy. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, et al., editors. GeneReviews(R). Seattle (WA)1993.

139. Ogata T, Muroya K, Ishii T, Suzuki Y, Nakada T, Sasagawa I. Undermasculinized genitalia in a boy with an abnormally expanded CAG repeat length in the androgen receptor gene. Clin Endocrinol (Oxf). 2001;54(6):835-8. PubMed PMID: 11422120.

140. Grunseich C, Kats IR, Bott LC, Rinaldi C, Kokkinis A, Fox D, et al. Early onset and novel features in a spinal and bulbar muscular atrophy patient with a 68 CAG repeat. Neuromuscul Disord. 2014;24(11):978-81. doi: 10.1016/j.nmd.2014.06.441. PubMed PMID: 25047668; PubMed Central PMCID: PMCPMC4252652.

141. Katsuno M, Banno H, Suzuki K, Adachi H, Tanaka F, Sobue G. Molecular pathophysiology and disease-modifying therapies for spinal and bulbar muscular atrophy. Arch Neurol. 2012;69(4):436-40. doi: 10.1001/archneurol.2011.2308. PubMed PMID: 22158719.

142. Lim HN, Chen H, McBride S, Dunning AM, Nixon RM, Hughes IA, et al. Longer polyglutamine tracts in the androgen receptor are associated with moderate to severe undermasculinized genitalia in XY males. Hum Mol Genet. 2000;9(5):829-34. PubMed PMID: 10749991.

143. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res. 1994;22(15):3181-6. PubMed PMID: 8065934; PubMed Central PMCID: PMCPMC310294.

144. Nästan halva befolkningen lever i parrelation. Available from: http://www.scb.se/sv_/Hitta-statistik/Artiklar/Nastan-halva-befolkningen-lever-i-parrelation/.

145. Olsen J, Ramlau-Hansen CH. Epidemiologic methods for investigating male fecundity. Asian J Androl. 2014;16(1):17-22. doi: 10.4103/1008-682X.122198. PubMed PMID: 24369129; PubMed Central PMCID: PMCPMC3901876.

146. Boisen KA, Main KM, Rajpert-De Meyts E, Skakkebaek NE. Are male reproductive disorders a common entity? The testicular dysgenesis syndrome. Ann N Y Acad Sci. 2001;948:90-9. PubMed PMID: 11795400.

147. McCowan L, Horgan RP. Risk factors for small for gestational age infants. Best Pract Res Clin Obstet Gynaecol. 2009;23(6):779-93. doi: 10.1016/j.bpobgyn.2009.06.003. PubMed PMID: 19604726.

148. Bradley RH, Corwyn RF. Socioeconomic status and child development. Annu Rev Psychol. 2002;53:371-99. doi: 10.1146/annurev.psych.53.100901.135233. PubMed PMID: 11752490.

149. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4. doi: 10.1001/jama.2013.281053. PubMed PMID: 24141714.

150. Ludvigsson JF, Haberg SE, Knudsen GP, Lafolie P, Zoega H, Sarkkola C, et al. Ethical aspects of registry-based research in the Nordic countries. Clin Epidemiol.

2015;7:491-508. doi: 10.2147/CLEP.S90589. PubMed PMID: 26648756; PubMed Central PMCID: PMCPMC4664438.