Etiological Aspects of Hypospadias

FROM THE GUEST EDITOR

Pierre Mouriquand, M.D.
Professor of Pediatric Urology, Head - Department of Pediatric Urology
Claude-Bernard University-Lyon, Debrousse Hospital, Lyon, France

A possible definition of hypospadias is the incomplete virilization of the genital tubercle causing an insufficient development of the tissues forming the ventral aspect of the penis. Four main protagonists are involved in the male genital construction (see table below).

1. The child himself with his gene bank, his endocrine machinery mainly represented by his gonads supervised by his central hormonal control, his target tissues with their protein platforms which may or may not respond to endocrine stimulation.

2. The placenta which is a complex endocrine machinery which orchestrates the hormonal climate especially during the first part of gestation. Its role is not fully understood and evaluated.

3. The mother who has her own hormonal production with its possible disorders which may affect the development of the child.

4. Finally the environment of the child and the mother which may also interfere on this fine balance.

Disruptors and promoters may interact in this complex play on which many other unidentified agents may have a role.

The place of hypospadias on the palette of Disorders of Sex Development (DSD) is not
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on the SPU website: www.spuonline.org or by contacting the Administrative Office at spu@prri.com.
There are possible associations between adverse health outcomes and environmental exposures. Wildlife studies has demonstrated an effect of proximity to sewage effluent on sexual differentiation in fish (107,108,109) and some amphibians. (110) A recent as yet unpublished study by this author based on a crude ecological exposure measure suggests the possibility of an association with water supply, however, further individual level study support is required.

Hypospadias has a strong genetic component. The Boston terrier offers an animal model. (111) In humans, hypospadias occurrence exhibits an elevated familial risk which increases with degree of relatedness to the index case. (8,11,30,31,36,51,112,113) Pedigree studies suggest evidence autosomal dominant, (6,90,115-117) autosomal recessive (6,117) and multi-factorial polygenic (49) modes of inheritance of hypospadias. Several studies have attempted to quantify the proportion of hypospadias cases with detectable chromosomal abnormalities. Estimates include 12.2% (95 CI 6.6 – 17.8) (23), 9.4% (95% CI 5.4 –13.3) (29) and 5.6% (95% CI 0 – 16%). (118) There are reports of hypospadias occurring as part of well described syndromes Robertsonian translocation(119), Beare Stevenson syndrome(120) and Opitz Friias G syndrome(121) among many others. (122-131)

Hypospadias appears to occur more frequently than expected with other congenital anomalies, (3,36,50,66) particularly urogenital anomalies, i.e., cryptorchidism. (22,66) This may point to a predisposition to genital urinary development abnormalities among affected families.

There is evidence of isolated hypospadias associated with abnormalities of androgen biosynthesis and of the androgen receptor. (133) Several authors have shown that androgen converting enzyme (3beta and 17beta-hydroxysteroid dehydrogenase) deficiency syndromes lead to hypospadias (126,134,135) These defects demonstrate Mendelian inheritance and are classically described as causing under-virilisation in males. (136) Reifenstein’s Syndrome which is also known as partial androgen insensitivity syndrome is associated with hypospadias. (137) Silver has reported that up to 10% of isolated hypospadias may be caused by a mutation in at least one allele of the 5-alpha reductase type 2 gene. Also, defects of the androgen receptor that may cause isolated hypospadias (138-142) and point mutations, in the androgen receptor gene have been reported, a la 596 → thr (143,144) and also ser 703 → gly. (145)

The use of the term testicular dysgenesis syndrome (TDS) to describe the novel concept that poor semen quality, testicular cancer, undescended testis and hypospadias are symptoms of an underlying entity which is more common due to adverse environmental influences. (145) As an ecological phenomenon the evidence is weak, based as it was on observations of trends in these disorders in the 1990s, subsequent trend data have not supported initial reports. As an entity occurring in individuals the evidence is even weaker. Yet the term has achieved a degress of acceptance.

Conclusion
Endocrine Disturbance

Experimental evidence, from as far back as the 1950s, also supports an endocrine aetiology for hypospadias. Jost et al. found that castration in male rabbit fetuses led to hypospadias but only if the castration was done before 23 days of gestation. (147) Hypospadias may have several causes, some simple (e.g., a single gene defect) or some complex (e.g., polygenic combinations with or without interaction with environmental factors). There is evidence of association with various risk factors, suggesting several biologically feasible etiologies, which are nevertheless compatible with each other. It is likely that endocrine disturbance is important but less likely that very low concentration exposure to chemicals causes it, even where they have endocrine activity in vitro.

Hypospadias has a genetic component and is likely to have an environmental component to its aetiology. It is, however, unlikely that current epidemiological research methods will identify a clinically significant environmental component which is amenable to public health action. It may be helpful to consider hypospadias as a field defect, (148) which is a discrete end point of a potentially multidimensional process.

CI calculated by this author from data in the original report.

Genetics of Hypospadias

Introduction

Hypospadias is a common genital anomaly defined as a midline fusion defect of the male urethra which results in misplaced urethral meatus. Genetic factors play a crucial role in the occurrence of this early developmental defect, in either isolated (non syndromic) or syndromic forms. Mutations in genes affecting the action and metabolism of androgens have been found in autosomal recessive hypospadias families. In addition, autosomal dominant forms of syndromic hypospadias are caused by mutations in genes involved in early genital development. Finally, hypospadias may be associated with various chromosomal abnormalities including gonosomal mosaicsisms and autosomal deletions. Whereas monogenic or chromosomal causes of hypospadias account for about 30% of all cases, etiological genetic factors remain unknown in the remaining cases. It is widely accepted that the best fitting mode of inheritance for idiopathic hypospadias is multifactorial, a model where the cumulative effects of minor genes and environmental factors result in the malformation when a threshold is passed. Deciphering the genetic causes of idiopathic hypospadias is a challenging task the near future. Various approaches, including animal studies and genetic linkage or chromosomal analyses in the human, are necessary in order to identify additional genetic factors involved in genital development.

The aim of this paper is to present the current understanding of the genetics of hypospadias rather than a comprehensive review. Malformations of the external genital system occur at a frequency second only to cardiac defects, currently occurring in approximately 1 in every 300 male live births. However, there is wide international variation in rates of hypospadias ranging from 0.03% in Japan to 0.4% in the United States. (1) Progressively increasing rates of hypospadias have been documented in some countries between the 1970s and the 1990s, thereby raising the question of the possible role of exogenous agents in its etiology. (1-3) In addition, a number of studies showed a significant association of hypospadias with poor intrauterine growth and suggested that the growth restriction was probably of early gestational cause and that common environmental factor(s) may cause both conditions. (3)

Hypospadias may be classified into glanular, penile, scrotal, and

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perineal types on the basis of the anatomical location of the misplaced urethral meatus, with the severity increasing from distal to proximal. This classical classification does not reflect the fact that the anomaly of the ventral aspect of the hypospadiac penis begins where the corpus spongiosum divides which can be significantly more proximal than the ectopic meatus. Some surgeons prefer to distinguish hypospadias with a distal division of the corpus spongiosum with little or no associated chordee and hypospadias with a proximal division of the corpus spongiosum with a significant chordee and a marked hypoplasia of the tissues forming the ventral aspect of the penis.\(^{10,14}\) Multiple or oversized urethral openings are sometimes observed. Isolated hooded foreskin may be a minor form of hypospadias and some consider an isolated penile chordee as a “missed” hypospadias. Glanular and penile (distal) hypospadias are the most common and often appear as an isolated anomaly, whereas about 20% are classified as scrotal and perineal (proximal) types. These latter forms frequently occur in association with other genital anomalies such as micropenis, bifid scrotum, penoscrotal transposition and cryptorchidism and may also occur in association with malformations of other organs. Although generally considered to be a malformation of male genitalia, hypospadias can occur in females and is characterized by an opening of the urethral meatus on the anterior wall of the vagina.

Gender is chromosomally determined at conception, although prior to the first 8 weeks of gestation, the external genitalia of the foetus remain undifferentiated. The urogenital sinus forms in the sixth week of gestation in the process of urethral development. The whole construction of the male urethra involves a tubularization of the horizontal segment of the urogenital sinus.\(^{19}\) Sex differentiation is almost complete by the sixteenth week, when the male glanular urethra forms together with the distal portion of the penis, until the preputial fold or foreskin covers the whole glans.\(^{19}\) Development of external genitalia in mammalian embryos requires tight coordination of a complex series of morphogenetic events involving outgrowth, proximodistal and dorsoventral patterning and epithelial tubulogenesis. It can be divided into two distinct phases: the first one involves initial outgrowth and patterning of the genital tubercle, which occurs in both male and female embryos; the second one, which is hormonally controlled, leads to the continued growth and differentiation of the penis or the arrest of outgrowth and differentiation of the clitoris. Hypospadias is characterized by failure of urethral tube closure often accompanied by agenesis of the tissues forming the ventral aspect of the penis, especially the corpus spongiosum.

### Genetics of hypospadias

#### Recurrence risks

Familial clustering, defined as patients with one or more first-, second-, or third-degree relative also affected with hypospadias, is seen in about 10% of cases.\(^{7-10}\) The recurrence risk of hypospadias in male siblings of an affected patient is about 15%, whereas 7% of the fathers of a child with hypospadias are also affected.\(^{11-13}\) The more severe the malformation of the index patient, the higher is the recurrence risk for the next male sibling.\(^{10}\)

#### Segregation analysis

Heritability for hypospadias has been estimated to be 0.99 using complex segregation analyses.\(^{10,12}\) The latter study, which tested more than two thousand pedigrees with at least one member affected with hypospadias, used different genetic models (additive, multifactorial, dominant and recessive) versus a sporadic model, and suggested that hypospadias might be due to monogenic effects in a small proportion of the families, whereas a multifactorial mode of inheritance was more likely in the majority of families.\(^{9}\)

### Steroid defects

Whereas early genital development is controlled by a genetic program that operates prior to production of steroid hormones, the second phase of penile development requires exposure to an androgen, either testosterone or dihydrotestosterone (DHT). Androgenic steroids, synthesized by the Leydig cells of the testes, are first seen just prior to the onset of androgen-induced genital differentiation.\(^{15}\)

Several hypospadias families were reported with an apparent monogenic inheritance pattern. Indeed, recessive inheritance has been suggested since there is a high incidence of hypospadias in ethnic groups with a high degree of inbreeding.\(^{14}\) In some of these, mutations have been identified in the 5-alpha reductase gene SRD5A2.\(^{15-17}\) Most of them have severe variants of hypospadias in combination with other genital anomalies, including penoscrotal hypospadias and some of them have partial androgen insensitivity syndrome (PAIS) and were raised as female. Conversely, a V89L polymorphism in the SRD5A2 gene appears to “protect” the male urethral development.\(^{18}\) In the human, 5-alpha reductase Type 2, an enzyme that converts testosterone to 5-alpha dihydrotestosterone (DHT), is highly expressed in the mesenchymal stroma surrounding the urethra, supporting the fact that SRD5A2 gene mutations are responsible for hypospadias.\(^{19}\)

In addition, mutations in the androgen receptor gene (AR) have been found in patients with severe forms of hypospadias, e.g., perineoscrotal hypospadias.\(^{20,21}\) hypospadias associated with cryptorchidism,\(^{22}\) micropenis,\(^{23}\) or partial or complete androgen insensitivity syndrome CAIS.\(^{24,25}\) In the developing external genitalia of mammals, androgen receptors (ARs) are abundant in the urethral epithelium, with lower concentrations found in the underlying stromal tissue.\(^{19}\) Interestingly, the Fibroblast growth factor receptor 2 gene (Fgfr2) is a transcriptional target of AR and appears to be essential for development of the urethral tube.\(^{26}\)

Heterozygous mutations in the Wilms tumour 1 gene (WT1) may also result in severe hypospadias associated with other genital anomalies.\(^{27,28}\) However, it is worth noting that mutations in the SRD5A2, AR and WT1 genes are not a common cause of isolated hypospadias.\(^{29,31}\) Recently, polymorphisms in the estrogen receptor beta gene (ESR2) have also been shown to be associated with hypospadias.\(^{32}\)

#### Syndromes with hypospadias

A search of the London Dysmorphology Database (LDDB), which excludes patent chromosomal abnormalities, identifies 198 syndromes with hypospadias. Some of these syndromes have known genetic bases and shed light on the molecular mechanisms involved in genital development. For example, Smith-Lemli-Opitz syndrome (SLOS), which includes mental retardation, microcephaly, facial dysmorphism, 2-3 syndactyly of the toes and, in males, hypospadias and a hypoplastic scrotum, is caused by a defect in steroid biosynthesis. SLOS is due to recessive mutations of the DHCR7 gene coding for 7-dehydrocholesterol reductase, localized on chromosome 11q13. There is recent evidence from Xenopus embryo studies that DHCR7 regulates Hedgehog (Hh) signaling,\(^{33}\) a pathway which involves the Sonic hedgehog gene (Shh). Shh is expressed in the endodermally derived urethral plate epithelium situated along the ventral side of genital tubercle and is required for outgrowth and patterning of the genital tubercle. Mice with a targeted deletion of Shh have penile and clitoral agenesis,\(^{34,35}\) consistent with the important role of Shh in genital development.

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Hand-foot-genital syndrome (HFGS) associates small hands, malformed thumbs with flat thenar eminence, small big toe and short first metacarpal and phalanx with genital anomalies including hypospadias in males. A variety of types of Homeobox A13 gene (HOXA13) mutations have been identified in HFGS.(36-38) In mice, members of the Hox paralogy group 13, Hoxd13 and Hoxa13, also play an essential role in external genital and limb development.(39,40) Indeed, loss of function of both genes results in agenesis of the genital tubercle, and heterozygosity for either causes patterning defects of the phallus. Hoxa13 is essential for normal expression of the Fibroblast growth factor 8 (Fgf8) and Bone morphogenetic protein 7 (Bmp7) genes in the urethral plate epithelium and Fgf8 appears to be sufficient to promote early proliferation of the genital tubercle, in the absence of Hoxa13.(39)

WAGR syndrome, which consists of Wilms’ tumor, aniridia, genital anomalies and growth and mental retardation, is considered as a contiguous gene syndrome and is due in most cases to an interstitial chromosomal deletion involving band 11p13. Several lines of evidence suggest that the WT1 gene, which maps within the deleted WAGR region and encodes a zinc-finger transcription factor involved in the development of the kidneys and gonads, may be responsible for the genital anomalies observed in WAGR syndrome.(41,42) Accordingly, WT1 point mutations may result in different, yet related, urogenital anomalies, depending on the nature and location of the mutation, e.g., Denys-Drash syndrome (mesangial sclerosis, gonadal dysgenesis and high risk of Wilms’ tumors),(43,44) Fraiser syndrome (focal glomerular sclerosis, gonadal dysgenesis,)(45) “atypical” Fraiser syndrome,(46) or severe hypospadias and Wilms tumor.(47)

G (Opitz-Frias) syndrome is characterized by midline anomalies including hypertelorism, hypospadias, and swallowing difficulties. Mutations in the Midline 1 gene (MID1) have been demonstrated in X-linked forms of G syndrome.(48) MID1 encodes a member of the B-box family of proteins, which contain protein-protein interaction domains including a RING finger. Middin, the gene product of MID1, is expressed in the genital tubercle,(49) but its precise role in the genital development remains as yet unknown. Finally, a novel autosomal dominant syndrome of unknown genetic cause, named “BILU”, an acronym for B cell Immunodeficiency, Limb malformations and Urogenital anomalies, has been reported in two unrelated families.(49,50) In the first family, the index patient had scrotal hypospadias whereas his affected father had epispadias, suggesting a common mechanism in both genital anomalies.

It is obvious from the high number of syndromes with hypospadias that multiple genes, possibly expressed in different spatiotemporal patterns, may act at different steps of molecular signalling pathways involved in genital development.

Chromosomal anomalies

A wide range of chromosomal anomalies are detected in about 7% of patients with hypospadias.(51) Several gonosomal aberrations may be associated with hypospadias, including Klinefelter’s syndrome, 47,XXY;(52) 48,XXYY,(53) and various mosaicism, e.g., 45,X/46,XY, which is a relatively common chromosomal anomaly known as mixed gonadal dysgenesis,(54) 45,X/46,XYq-,(55) 45,X/46,X,idi(YP)(56) 45,X/69,XY(57) and 46,X+mar, the long arm of the marker chromosome originating from the short arm of the Y chromosome.(58) Abnormal genital development in these patients may be related to a dosage effect (i.e., incomplete dose) of the SRY gene, which is located on the short arm of the Y chromosome and is involved in testis differentiation.(59) Accordingly, 46 XX patients without evidence for mosaicism but with the presence of SRY genomic DNA in peripheral blood may also prove to be males with genital ambiguities including hypospadias.(60) Interestingly, severe penoscrotal hypospadias was observed in a male patient with a de novo mosaic 46,XX/46,XX.dup(17)(q23.1q24.3), where the SRY-related HMG-Box gene 9 (SOX9) gene, which encodes a transcriptional activator acting immediately downstream of SRY, was duplicated on the rearranged chromosome 17. This suggests that in the absence of SRY, an extra dose of SOX9 is sufficient for testis differentiation.(61) Finally, since certain 46,XX male patients have no detectable SRY gene in peripheral leukocytes and no SOX9 detectable anomaly,(62) the presence of SRY should be checked in testicular cells when possible before diagnosing Y-negative 46,XX maleness.(63)

A number of autosomal abnormalities (e.g., translocations, deletions or duplications) have also been associated with syndromic hypospadias. They point to specific regions of the genome where genes involved in genital development may reside. For example, deletion syndromes with hypospadias have been observed on chromosomes 3q29,(64) 4p,(65,66) 9p23,(67,68) 9q34.3,(69) 11p13 (WAGR syndrome, WT1 gene), 10q26,(70) and 13q32-q34.(71,72) Interestingly, mice carrying mutations in ephrin-B2 and in the ephrin receptor EphB2 genes develop with variably penetrant severe hypospadias and incomplete midline fusion of the primitive cloaca demonstrating that these molecules also play major roles in cell adhesion events that tubularize the urethra and partition the urinary and alimentary tracts.(73) Thus, it is likely that the human ephrin-B2 gene (EFNB2), which is located at 13q33.3 within the critical 13q32-q34 deletion interval, plays a role in human hypospadias.

Finally, comparative genomic hybridization (CGH) microarrays, which can identify cryptic chromosomal rearrangements in patients with syndromic malformations, may prove to be a useful tool for mapping genes involved in genital development.

Idiopathic hypospadias

Isolated hypospadias of unknown cause account for about 70% of all cases.(74) An efficient method to map major disease-causing genes in monogenic disorders is genome-wide genotyping in families with a large number of affected individuals followed by parametric linkage analyses. However, no such study has been published yet, probably owing to the paucity of large families with clear monogenic inheritance of hypospadias. Another method referred to as “non parametric” (model free) linkage analysis may be used to map susceptibility genes in small families where the inheritance model cannot be simply inferred from observation of pedigrees. Indeed, a genome-wide model free linkage analysis in 69 multiplex (at least 2 affected patients) hypospadias families indicated possible linkage to chromosome 9q22 and to other chromosomal regions.(75) However, these results await further confirmation.

Another strategy consists of systematic screening of candidate genes in hypospadias patients. A recent study of gene expression profiles of the mouse genital tubercle using cDNA microarrays identified the up-regulation of genes involved in the transforming growth factor-beta (TGF-beta) and Wnt-Frizzled pathways and of thrombospondin (TSP) 4, a member of a cell-migration molecule family. All genes are, therefore, possible candidates for a role in urethral tube formation.(76) This is consistent with the recent report of the identification of 13 heterozygous variants of 4 different genes, namely, the BMP4 and BMP7 genes, involved in the TGF-beta signalling pathway, and in the HOXA4 and HOXB6 genes, involved in the development of skin, in 13 of 90 unrelated hypospadias patients. However, clear association between the variants and hypospadias remains to be made.(77)
Assisted reproduction technologies (ART)

The possible excess of congenital malformations in infants born after in vitro fertilization (IVF) has been largely discussed in the literature, with controversial conclusions. However, several studies, mainly arising from a Swedish team, show an increased risk for hypospadias after intra cytoplasmic sperm injection (ICSI), possibly related to paternal subfertility. (78-81) The genetic or epigenetic backgrounds for these observations are as yet unknown. In addition, a recent Danish longitudinal study showed that babies born to infertile couples after treatment had an increased prevalence of genital organ malformations compared with babies conceived naturally indicating that hormonal treatment for infertility may be related to the occurrence of malformations of genital organs. (82)

Conclusion

Several data favor the view that hypospadias has a genetic origin. Among them are syndromic forms of hypospadias caused either by mutations in genes involved in the early urogenital development or by chromosomal abnormalities, and autosomal recessive forms of hypospadias with mutations in genes playing a role in androgen biosynthesis. However, the underlying genetic causes of the remaining 70% “idiopathic” hypospadias are yet unknown. Exogeneous factors, discussed in an ac-

Endocrinology of Hypospadias (“Needle in a haystack” or “Tip of the iceberg”)

Bruno Ferraz-de-Souza M.D., John C. Achermann M.D., Developmental Endocrinology Research Group, Clinical & Molecular Genetics Unit, UCL Institute of Child Health, University College London

Introduction

Hypospadias is one of the most common congenital anomalies affecting up to 1:200 boys. In most cases, the degree of hypospadias is relatively mild and a specific endocrine cause is not sought or is not found. (1-3) However, endocrine events are clearly essential for normal phallic growth, and any child with hypospadias (precocious puberty, increased growth), so clinical (e.g., salt loss, hypoglycemia) or androgen excess may be related to the occurrence of malformations of genital organs. (4,5)

In contrast to the underandrogenized 46,XY male, hypospadias may rarely but importantly be the presenting feature of a severely androgenized 46,XX baby or child (e.g., 21-hydroxylase deficiency). “Testes” will not be palpable, but this sign may have been overlooked initially. Some of these children are at significant risk of life-threatening adrenal failure (e.g., salt loss, hypoglycemia) or androgen excess (precocious puberty, increased growth), so clinical vigilance needs to be maintained when presented with any child with hypospadias.

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Fig. 1. Overview of the pathways of testosterone biosynthesis and action. In the 46,XY fetus, impaired androgenization can result from an abnormality in A) testis development (and subsequent Leydig cell differentiation), B) a specific enzymatic block in the pathway to androgen biosynthesis, C) a target tissue defect in dihydrotestosterone (DHT) production and androgen receptor action, or D) an intrinsic defect in phallic growth (more likely due to a syndromic cause rather than a true endocrine etiology). LH, luteinizing hormone; hCG, human chorionic gonadotropin; STAR, steroidogenic acute regulatory protein; CYP11A1, P450 side-chain cleavage; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; CYP17, 17α- hydroxylase/17,20-lyase; POR, P450 oxidoreductase; HSD17B3, 17β-hydroxysteroid dehydrogenase type 3; SRD5A2, 5α-reductase; AR, androgen receptor.
The question that arises, therefore, is whether seeking endocrine causes of hypospadias is like looking for a “needle in a haystack” or are we currently seeing just the “tip of the iceberg”? To address this issue in more detail, we need to consider: 1) what endocrine disorders can present with hypospadias; 2) how might these endocrine conditions be distinguished clinically or biochemically from isolated hypospadias; and 3) does making an endocrine diagnosis matter?

**Endocrine causes of hypospadias**

Mild defects anywhere in the pathway of testosterone synthesis and action can result in hypospadias in 46,XY infants. An overview of this pathway is shown in Figure 1A-D, and some key factors involved in this pathway are listed in Table 1.

Severe abnormalities in testis development classically cause complete (pure) gonadal dysgenesis (Swyer syndrome) with marked underandrogenization and persistent Müllerian structures due to impaired müllerian inhibiting substance (MIS, AMH) secretion from Sertoli cells. However, gonadal dysgenesis can be viewed as a spectrum of disorders. Partial forms of gonadal dysgenesis are associated with normal Müllerian regression (i.e., no uterus) and varying degrees of testicular descent and genital ambiguity/hypospadias. Thus, milder loss of function mutations in established testis determining/promoting factors can all present with hypospadias (e.g., Wilms tumor-1 [WT1], steroidalogenic factor-1 [SF1], SRY), and population polymorphisms in SF1 have been described in association with micropenis or cryptorchidism. Finally, it is worth remembering that a very wide spectrum of testicular and genital phenotypes can occur in individuals with a 45,X/46,XY karyotype (mixed gonadal dysgenesis), so karyotype analysis has benefits beyond simply determining gender.

Milder defects in the androgen biosynthetic pathway have also now been described in association with hypospadias. Partial loss of LH receptor function (Leydig cell hypoplasia) can cause hypospadias or even micropenis. Defects in steroidalogenic acute regulatory protein, CYP11A1 (P450scce) and 3b-hydroxysteroid dehydrogenase deficiency usually cause a salt-losing adrenal phenotype and more severe underandrogenization, although hypospadias may theoretically be the presenting feature of these conditions in rare cases. Combined 17α-hydroxylase/17,20-lyase deficiency (or isolated 17/20-lyase deficiency) or P450oxidoreductase deficiency can present with varying degrees of hypospadias or micropenis, with progressive hypertension or skeletal abnormalities, respectively (7; Achermann, unpublished data). Although 46,XY individuals with 17b-hydroxysteroid dehydrogenase (type 3) or 5α-reductase deficiency usually have a female phenotype or only relatively mild clitoromegaly, milder variants can occur and a 5α-reductase gene polymorphism has been found to be associated with hypospadias in cohorts from different countries.(4,5) The spectrum of phenotypes associated with complete through partial androgen insensitivity syndrome (CAIS/PAIS) is well known to most urologists, and very mild defects in the androgen receptor can cause infertility alone. Basal endocrine investigations in these cases can be variable.(6,7) Finally, we reiterate that hypospadias may be a presenting feature of severely androgenized 46,XX infants or children with a severe steroidogenic disorder, such as 21-hydroxylase deficiency or 11b-hydroxylase deficiency, and further investigations are essential if the gonads cannot be palpated or if the child shows evidence of precocious puberty (Table 1).

**Distinguishing endocrine causes from isolated hypospadias**

Before examining the genitalia, there may be clues that could distinguish a developmental or endocrine disorder from an idiothetic form of hypospadias (Table 1). On balance, these conditions are likely to be rare, but should not be overlooked. For example, a child with dysorphic features or multi-system pathology warrants further thought as the hypospadias could reflect impaired testicular development rather than just a poor target tissue response, and long-term testosterone treatment might be needed at puberty. A detailed obstetric and perinatal history is important as hypospadias occurs more frequently in growth-retarded babies and certain medications (e.g., 5α-reductase inhibitors) could theoretically interfere with fetal endocrinology. The presence of hyperpigmentation (often of the scrotum) or evidence of salt-loss should never be overlooked, as it could herald potentially life-threatening primary adrenal failure in both 46,XY or 46,XX individuals. Finally, a family history of hypospadias, reproductive or adrenal disorders could be crucial in reaching a diagnosis or lowering the threshold for more detailed investigations. Gynecomastia in the adolescent boy may be an important sign.

Most urologists and endocrinologists would consider more detailed investigations in a child with hypospadias if there are additional features (above): a small phallus or flimsy corpora, poorly developed scrotum, and undescended or impalpable testes. The extent and timing of the tests include karyotype, basal gonadotropins (LH, FSH) and androgens (continued on the next page)

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**Table 1: Overview of some endocrine conditions that might present with hypospadias.**

A more detailed review of genetic / endocrine causes of disorders of sex development can be found in references 14 and 15. DOC, deoxycorticosterone; PAIS, partial androgen insensitivity syndrome.

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(DHEAS, androstenedione, testosterone, DHT), measurement of AMH/MS (which might be low in testicular dysgenesis or high in androgen insensitivity), and electrolytes, cortisol, steroid intermediates (e.g., 17-hydroxyprogesterone), and ACTH if a potential adrenal disorder needs evaluation. Pelvic imaging, urine steroid profiles, short (3 day) and prolonged (3 week) hCG stimulation tests, and ACTH stimulation tests - with measurement of appropriate steroids – can be useful in both assessing whether there is a problem and diagnosing a specific defect. However, age- and assay-specific normal ranges are often not readily available and some caution is needed when interpreting automated androgen assays especially in early life. Some key distinguishing biochemical findings are summarized in Table 1 and in key references (14 for 17 beta-HSD type 3, 9 for androgen insensitivity). Furthermore, tests such as prolonged hCG stimulation are relatively invasive and unpleasant, and it is not well established whether the differences in different hormone ratios (e.g., T:DHT ratio) are as marked in milder forms of these conditions. In addition to AMH, inhibin B may be a marker of testicular function in early life or at puberty, and insulin-like factor 3 (INSL3) may be developed in future as an additional marker of Leydig cell function. (12)

Finally, genetic testing is becoming increasingly available on a research or clinical basis and can be very useful in confirming a suspected diagnosis or for counseling individuals and families. A trial of testosterone (or DHT cream) can also be useful to assess target tissue responsiveness and improve phallic growth in a child being raised male.

Does making an endocrine diagnosis matter?

Achieving a balance between over- and under-investigating hypospadias is a real challenge, especially as we do not know whether important diagnoses are currently being missed. On the one hand, more thorough investigation for endocrine causes of hypospadias would have costs. There is added stress for the child and parents (and urologist who might have to interpret the results!), a relatively common condition becomes “medicalized”, and the financial and manpower costs of additional tests are not insignificant.

In contrast, not overlooking an adrenal defect is paramount. A child at risk of salt-loss or glucocorticoid insufficiency is prone to electrolyte imbalances and hypoglycemia and could die during infection or surgery. A salt-retaining form of congenital adrenal hyperplasia can cause hypertension and life-threatening hypokalemia with time. These conditions are treatable and – although rare – should always be considered, especially in a child with hyperpigmentation or clinical symptoms. Further, a diagnosis of partial gonadal dysgenesis, a mild steroidogenic defect, or partial androgen insensitivity can also be associated with a risk of gonadal tumorigenesis, impaired fertility and chronic hypoandrogenemia, which may have effects on libido, bone mineralization and adiposity with time. Thus, making the diagnosis and starting appropriate treatment could have important clinical implications throughout the individual’s life.

Finally, the real rarities – such as the young man with hypospadias who developed proteinuria and renal glomerulosclerosis at 20 years of age due to a Wilms tumor gene mutation – show how knowledge of the endocrinology of testis development and hypospadias can be important, and an open mind should be kept in all cases (13).

More systematic studies in large cohorts, and the development of combined metabolomic, proteomic and genetic analysis could improve diagnostic yield in the future. Until then it will be unknown how many individuals with “isolated/idiopathic hypospadias”, if any, might benefit from a more rigorous diagnostic and interventional work-up.

Environment and Hypospadias

Charles Sultan, M.D., Ph.D., Professor of Reproductive Medicine1-2, Pascal Philibert, Ph.D.2, and Nicolas Kalfa, M.D., Ph.D., Resident in Pediatric Surgery3

1Unité d’Endocrinologie et de Gynécologie Pédiatriques, Service de Pédiatrie I, Hôpital Arnaud de Villeneuve, CHU Montpellier
2Service d’Hormonologie et Inserm U. 540 (Groupe Pathologie Moléculaire des Androgènes)Hôpital Lapeyronie, CHU Montpellier
3Service de Chirurgie Pédiatrique, Hôpital Lapeyronie, CHU Montpellier

Over the last 30 years, male reproductive health has been marked by a deterioration of sperm count and an increasing number of undescended testes, testicular cancers and hypospadias. (37, 50) This phenomenon has raised some concerns regarding environmental chemicals such as by-products of industrial and agricultural development. (2, 36, 41, 42, 46)

I. Increasing prevalence of hypospadias in male newborns (7)

Hypospadias is one of the most common congenital anomalies (0.1-0.3%). Despite some inaccurate registers which under evaluate the number of hypospadias cases and their geographical distribution, (49) several reports suggest an increase in hypospadias over the last 20 years. (29) Boisen (4) in a prospective cohort study found a high prevalence (1%) of hypospadias in the Danish population of male newborns while the prevalence was reported at 0.73% in the Netherlands in a cross-sectional study (31). A prospective case-control study of 1442 male newborns identified 16 cases of middle and posterior (1.1%) in our area. (15)

II. Pathophysiology

The causes of hypospadias are essentially unknown and probably multifactorial. (1, 21, 40) The genital tubercle grows under the influence of androgens and any alteration of androgen production and receptors may
Various pollutants potentially involved in the abnormal development of the genital tubercle: chlorinated pesticides (DDT, Lindane); polychlorinated biphenyl; methoxychlor; phenolic derivatives; nonylphenol; endosulfan; atrazine; phthalates; dioxine; furans; xenooestrogens; phytooestrogens; mycooestrogens. These pollutants enter the body either by ingestion, inhalation, adsorption or may be conveyed through the placenta. Individual exposure to these substances is variable according to diet, style of life and work. Most of these pollutants are lipophilic and are stored in the body fat for lifetime. They are also found in breast milk and in the amniotic fluid. Since most of these chemicals use the same pathways as natural hormones, they have been named xenooestrogens and/or environmental disrupting chemicals (EDC). The molecular actions of xenooestrogens are listed in Table 1. Xenooestrogens have both an estrogenic and anti-androgenic actions and enter in competition with natural androgens for the ligand-binding domain (LBD) of the androgen receptor (AR). The conformation of LBD is, therefore, changed, the nuclear transfer of AR is altered as well as the transcriptional co-activators and the expression of the androgen-specific gene (Figure 1).

### Table 1. Molecular actions of xenooestrogens.

1. Binding of ERα / ERβ nuclear receptor, and transcription of activation (or repression) of specific genes expression.
2. Non-genomic actions mediated by a plasma membrane estrogen receptor.
3. Induction of more potent estrogenic metabolites.
4. Reduction of the binding of endogenous estrogens to SHBG.
5. Inhibition of transcription of androgen dependant-genes.
6. Potential additive effects.
7. Oncogenic effect (?)..

III. 2 Laboratory animals

The effects of prenatal xenooestrogens on male reproductive tract development have been studied by several groups. Male rats exposed to DES during gestation (at concentrations similar to those measured in first-trimester human fetal tissues) developed hypospadias. Hypospadias in male rodents was found after maternal treatment with vinclozolin (dose-response effect). Similar findings were recorded with prenatal exposure to PCB, phthalates or dioxin causes hypospadias.

Although several xenooestrogens consistently induce hypospadias in male offspring exposed in utero, extrapolation to human cannot be done as doses given to animals are not comparable to environmental exposure.

III. 3 Cell models

Bioengineering assays using stable transfected cell lines are powerful tools for studying ERα, ERβ and AR transactivated activity, as well as the binding potency of various xenooestrogens disruptors. A serum marker reflecting xenooestrogen exposure in human is also available.

III. 4 Human epidemiology (Figure 2)

To date, 3 epidemiologic studies reported the possible relationship between exposure to pesticides and hypospadias. Kristen published a moderate increase of odds ratio (OR) for hypospadias in individuals exposed to farm chemicals (Odds Ratio = 1.5%). Weidner stated that maternal farming or gardening led to a low risk of hypospadias (Odds Ratio = 1.27), Longnecker did not confirm any significant risk of hypospadias (Odds Ratio: 1.2) when mothers were exposed to DTT. The critical level of exposure to EDCs was not assessed in any of these epidemiologic studies.

Residence in the vicinity of hazardous waste-disposal sites has been associated with a high incidence of hypospadias. Similarly, an increased rate of hypospadias was reported in boys from parents exposed to dioxin after the Seveso industrial accident. Vegetarian diet in pregnant women is reported to have a significant risk of hypospadias (Odds Ratio=4.99)

IV. Conclusion

Although no single identified EDCs was identified to cause hypospadias in human, the significant increase of this anomaly warrants further research studies to establish the interference of environmental disruptors in the male genital construction.
Proteins and Hypospadias

The urethral tubularization during the first part of gestation involves complex mechanisms where regulators and effectors are closely interrelated. The molecular mechanisms and particularly those controlling the proteins involved in the hypospadiac penis (a very common anomaly characterized by an arrest of growth, formation of the genital tubercle) are poorly understood.

A failure of androgen signaling during the first term of gestation has long been the only known identifiable cause of hypospadias. However very few hypospadiac patients present with an abnormal endocrine screening affecting the hypothalamic-pituitary–gonadal axis, a deficient 5 alpha reductase activity or abnormal androgen receptors. Testicular functions are usually found to be normal in hypospadiac patients as well as their steroidogenesis. Although androgens are essential in the development of the genital tubercle, they could not act without upstream and downstream protein effectors and regulators which appear of considerable importance in the penis formation.

Several types of proteins including transcription factors, growth factors and their receptors, apoptotic and anti-apoptotic factors, proteases and their activators, subtracts and inhibitors are essential ingredients of this penile construction following a precise timing and space of action. Failure in the protein balance could potentially be a cause of hypospadias.

Mouse models have been used to identify some of these proteins involved in the male genital development although no correspondence with humans has been established. Several labelled urogenital as well as non-urogenital malformations can be associated with hypospadias suggesting a defect in proteins involved in the tissue formation. Several examples could illustrate this: Homeobox proteins are transcription factors expressed along the axis of urogenital tract. HOXA4 and HOXB6 play important roles in the skin development. HOXA13 was shown to cause Hand-Foot-Genitalia Syndrome and hypospadias of variable severity. HOXA13 is essential for the normal expression of Fibroblast Growth Factor (FGF) 8 and Bone Morphogenetic Protein 7 (BMP7) in developing urethral epithelium. It also acts in androgen receptor expression and in mediating the vascularization of the glans penis. In human, mutations of HOXA13 or WTAP are not commonly found in isolated hypospadias but this does not mean that they are not involved in the cascade of events. The sonic hedgehog (Shh), expressed in the epithelium of the male urogenital sinus, seems essential for the growth of the genital tubercle. Shh is an upstream regulator of FGF10 expression in the genital tubercle. Perturbations in BMP signalling are involved in the coronal and penile hypospadias in mice in providing an apoptotic signal to the urethral plate epithelium and surrounding mesenchyme. A screening of BMP4, BMP7, HOXA4 and HOXB6 mutations in patients with hypospadias showed anomalies in 14 of 90 cases. None of these anomalies were found in 380 control boys. These mutations are unlikely to be random events but further studies are needed to determine how these factors could be potentially involved in hypospadiac penis.

Growth factors and their receptors are also major actors for urogenital differentiation. Epidermal Growth Factor (EGF) is one of them. Its density was found reduced in hypospadiac preputial hood compared to foreskin controls. Androgen receptors mediate the role of EGF in male sexual differentiation. The fibroblast growth factors (FGFs) also play a role in genital development. Urethral epithelium is a source of FGF8. In FGF8 deficient mice, supplementation with FGF8 restores the development of the genital tract. FGF9 is expressed specifically in the mice XY gonads. FGF10 is a mitogen for urethral cells. Its absence in transgenic mice alters the development of external genital. The IIIb isoform of FGF Receptor2 (FGFR2IIIb) expressed in the urethral epithelium and prepuce of the mouse genital tubercle, is required for normal development of the urethra and ventral aspect of the phallus. Impaired FGFR2 IIIb or its ligand FGF result in severe proximal hypospadias. The formation of the urethral plate is also regulated by FGF10 signalling coming from the FGFRII IIIb found in the urethral epithelium. FGF-FGFR2 IIIb sustains the proliferation of basal cells in the urothelium. The cessation of cell division in this population causes an abrupt arrest of urethral differentiation resulting in a thin, disorganized urethral plate. Androgens are required for FGFR2 IIIb expression although FGFR2 IIIb also could have an early androgen-independent role in the development of the urethral plate. FGFR and FGFI0 null mutations have widespread effects on multiple organs, making highly unlikely that a large number of hypospadias carry mutations of these genes. The androgen regulation of FGFR2 IIIb in mouse genitalia raises the possibility of a specific local hormonal regulation and exposure to anti-androgenic molecules during pregnancy may lead to a diminished FGFR2 activity. Malformations of the urogenital tract in several syndromes are associated with mutations p63, an homologous of tumor suppressor p53. It is interesting to note that p63 regulates FGFR2 splicing and is required for generation of IIIb isoform.

Proteases and antiproteases represent regulators of differentiation, remodelling, cell migration, apoptosis and wound healing, and are highly expressed in reproductive organs. Their expression pattern changes with variations in hormonal and functional cycles. Our studies show an increased expression of metallo-proteases (MMP) activities in tissues forming the ventral aspect of hypospadiac penis compared to the preputial hood of the same patients or to the foreskin harvested in normal circumstances.

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**Figure 1.** Schematic relationship between structuring and destructuring proteins and hypospadias.

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An excess of MMPs can induce an extensive tissue loss and destruction. Upstream and downstream pathways of proteolytic activity are critical for defining inducers and protease targets in the penis. A decreased expression and abnormal subcellular localization of some adhesion and tight junction proteins (Cadherin E and Claudins) have been observed in human hypospadiac tissues. However more studies are needed to establish whether these unbalanced proteins are the cause or the consequence of hypospadias. As a matter of fact, very little is known about the cell-cell interaction that controls formation of midline penile urethra. Numerous proteins coordinate adhesion and repulsion actions. Cell surface molecule like ephrin–B2 and the ephrin receptors EphB2 and EphB3 play a role in cell adhesion which helps to tubularize the urethra.(17) The closure defects of the glanular urethra can be attributed to a failure of apoptosis regulation in the urethra. BMP7 provides an apoptotic signal to the urethral plate and surrounding mesenchyma. Activating Transcription Factor 3 (ATF3), normally expressed at a steady state in quiescent cells and probably involved in homeostasis, wound healing, cell adhesion or apoptosis, has recently been involved in hypospadias. ATF3 is up-regulated in the penile skin tissues of hypospadias.⁽¹⁸⁾ ATF3 is an estrogen-responsive protein. Estrogen receptors (ERs) are present in the developing male external genitalia and variations of ER2 might influence the onset of hypospadias.⁽¹⁹⁾

In conclusion, the molecular mechanisms explaining the construction of the penis are complex and interact with the gonadal development and the hormonal production. They represent the essential protein platform which supports the upstream and downstream regulation of hormonal activities. Their optimal action requires a fine balance between the various actors, an adequate timing and biodisponibility. Many disruptions may occur in this chain process which could explain some cases of hypospadias.

**Hypospadias and Testicular Dysgenesis**

Camilla Asklund, M.D., Katharina Main, M.D., Ph.D., Niels Jørgensen, M.D., Ph.D., and Niels E. Skakkebæk, M.D., University Department of Growth and Reproduction, University Hospital of Copenhagen, Denmark

**Introduction**

The birth prevalence of hypospadias has been reported to be increasing in certain regions.⁽¹⁻⁽³⁾ but the etiology is in most cases unknown. It has been hypothesized that hypospadias may be part of a symptom complex, the testicular dysgenesis syndrome (TDS), which encompasses other reproductive problems such as low semen quality, testicular cancer and cryptorchidism⁽⁴⁾ (Figure 1). These disorders may arise as a result of abnormal pre- and perinatal development of the testis and its constituent cell types (germ cells, Sertoli cells and Leydig cells).

The following review describes the current epidemiological evidence and the origin and biology of TDS that constitute the basis for a hypothesis of an etiological link between hypospadias and the other TDS symptoms.

**Perinatal risk factors**

In the majority of the patients with gonadal dysgenesis the etiology is unknown, but it is believed to be multi-factorial, involving both genetic and environmental factors. Severe but relatively rare genetic abnormalities which cause testicular dysgenesis and disorders of sex differentiation (e.g., 45X/46XY) are associated with a high risk of testicular cancer, often in combination with hypospadias and cryptorchidism.⁽⁵⁻⁽⁷⁾ Furthermore, rare gene mutations (e.g., partial androgen receptor mutation) have been described in a few cases of testicular dysgenesis.

Hypospadias, cryptorchidism, poor semen quality and testis cancer share risk factors, which are coupled to the intrauterine milieu, such as birth weight, prematurity, and low parity.⁽¹⁻⁽¹²⁾ In addition, each of the components of TDS is also a risk factor for one or more of the others, although the strength of scientific evidence may vary. Testicular cancer is associated with reduced semen quality and decreased fertility even in the years before the cancer is diagnosed.⁽¹²⁻⁽¹⁵⁾ In addition, it is well documented that the risk of harboring one or more of other male reproductive disorders (hypospadias, cryptorchidism, impaired semen quality and testicular cancer) increases in certain regions,⁽¹⁻⁽³⁾ but the etiology is in most cases unknown.

**Figure 1**

Schematic representation of pathogenetic links between hypospadias and the other components and clinical manifestations of the testicular dysgenesis syndrome. (Reprinted with permission from Skakkebæk et al. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16:972-8.)

**Testicular Dysgenesis Syndrome**

- **Environmental factors incl. endocrine disrupters**
  - Disturbed Sertoli cell function
  - Impaired germ cell differentiation
  - Reduced semen quality
  - Carcinoma in situ → Testicular cancer
  - Decreased Leydig cell function
  - Androgen insufficiency
  - Hypospadias
  - Testicular maldescent

- **Genetic defects incl. 45,XY/46,XY and point mutations**

**Table 1**

<table>
<thead>
<tr>
<th>Main topic</th>
<th>References</th>
<th>Study results</th>
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<tr>
<td>Testicular cancer</td>
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<td>hypospadias, RR=4.2 (95%CI=0.4-42.7), cryptorchidism, RR=5.2 (95%CI=2.1-13.0)</td>
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<td>The John Radcliffe Hospital Cryptorchidism Study Group, 1992</td>
<td>hypospadias, RR=3.3</td>
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<td>Hjertvik et al., 1989</td>
<td>hypospadias, RR=2.7 (95%CI=1.4-4.4)</td>
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<tr>
<td>Akre et al., 1999</td>
<td>hypospadias, OR=1.36 (95%CI=0.71-2.60)</td>
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<td>Khuri et al., 1981</td>
<td>5.5% cryptorchidism (distal/proximal penile forms)</td>
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<td>Wu et al., 2002</td>
<td>3.4% cryptorchidism (anterior forms)</td>
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<tr>
<td>Weidner et al., 1999</td>
<td>9.8% cryptorchidism (posterior forms)</td>
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<tr>
<td>Bauer et al., 1979</td>
<td>5% cryptorchidism</td>
<td></td>
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<td>Sweet et al., 1974</td>
<td>8% cryptorchidism</td>
<td></td>
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<tr>
<td>Bracka, 1989</td>
<td>impaired semen quality in a subgroup</td>
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mented that cryptorchidism is a strong risk factor for testicular cancer, including CIS.\textsuperscript{16-25} The association between hypospadias and other TDS symptoms is less well documented, partly because hypospadias is a rare disorder and partly due to unreliable reporting in registries, which is confounded by changes in diagnostic criteria and recording practices. Studies in selected populations of patients with testicular cancer;\textsuperscript{21} cryptorchidism\textsuperscript{26-28} and hypospadias\textsuperscript{24,29-33} revealed an association between hypospadias and the other TDS symptoms (Table 1). Previous reports on hormone levels in patients with hypospadias have shown conflicting results, most probably due to differences in severity of cases, assays, and different age groups.\textsuperscript{13,34,35} However, a recent study evaluated the endocrine testicular capacity in 61 patients with isolated hypospadias and 28 patients with hypospadias associated with micropenis, cryptorchidism or ambiguous genitalia; 16 (57\%) of 28 patients with associated genital abnormalities had a testicular endocrine pathology, whereas only 9 (15\%) of the 61 boys with isolated hypospadias had signs of testicular failure.\textsuperscript{36} Thus, isolated hypospadias may constitute a different etiological group from hypospadias with associated genital malformations.

Histological evidence of testicular dysgenesis (immature seminiferous tubules with undifferentiated Sertoli cells, microcalcifications and Sertoli cell only tubules, Leydig cell hyperplasia, morphologically distorted tubules and the presence of CIS cells) has been found scattered in biopsies of the contralateral testis of testicular germ cell cancer patients and in biopsies from patients with infertility, hypospadias or cryptorchidism (Figure 2).\textsuperscript{37-39} The changes are somewhat similar to those present in gonadoblastoma in patients with 46,XY pure gonadal dysgenesis where the dysgenetic histological features are much more pronounced.\textsuperscript{40,41}

Hypospadias and postnatal reproductive problems

Few publications have elucidated the consequences of hypospadias on semen quality and fertility. The results are often inconsistent, mainly due to methodological shortcomings, such as too small series, low participation rates, no reliable control groups and populations of different ages. However, psychosocial and physical factors may have an indirect impact on fertility in men diagnosed or operated for hypospadias. In 1953, Rahbek Sorensen found that men operated for hypospadias were married as often as control men.\textsuperscript{42} In a more recent case-control study only 62\% of men operated for hypospadias in contrast to 75\% of controls live with a partner.\textsuperscript{43} Men with hypospadias seemed to have slightly fewer children than controls (0.8 vs. 1.1), although this was not significant. Of men operated for hypospadias, 6-24\% have sexual problems such as genital pain, mechanical problems and dissatisfaction in sexual relations. Reported problems such as weak or dribbling ejaculation, retained or delayed ejaculation and in the most severe cases anejaculation were noted in 6-37\%.\textsuperscript{33,43-47} The only study of semen quality in men diagnosed or operated for hypospadias is a follow-up study of 169 patients with hypospadias, mean age 22 years and participation rate 79\%.\textsuperscript{45} In the group with proven fertility (n=32), 3\% had a semen concentration of < 20 million/mL (lower WHO limit) in contrast to 29\% in the group with non-proven fertility (n=137). This suggests that a subgroup had impaired semen quality, but the study had severe methodological limitations. Thus, standardized case control studies on semen quality in men with hypospadias are needed before firm conclusions can be drawn.

Both from a biological\textsuperscript{46} and an epidemiological\textsuperscript{24,29} point of view, hypospadias and cryptorchidism are interlinked. Whereas the link between hypospadias and semen quality and fertility needs still to be established, the association between cryptorchidism and an increased risk for subfertility is well known.\textsuperscript{12,48-50} Thus, some men with hypospadias may have an increased risk of having a reduced semen quality and fertility.

Temporal trends and geographical differences

The TDS hypothesis implies that populations with a high risk of hypospadias also have a greater frequency of men with poor semen quality, testicular cancer and cryptorchidism, and vice versa. Recent studies have reported a possible increase over a few generations time in the prevalence of hypospadias in some geographical regions.\textsuperscript{1,2,51-53} At the same time the incidence of testicular cancer in Caucasian men at least in Europe and North America, has increased,\textsuperscript{54} sperm counts appear to have declined\textsuperscript{55,56} and the prevalence of cryptorchidism seems to have increased, at least in certain regions.\textsuperscript{2,57} Additionally, there have been concerns about a low and decreasing birth rate in many industrialized countries, where up to 6\% of children today are born after assisted reproduction.\textsuperscript{58}

The increase in hypospadias and/or cryptorchidism may be causal or confounded by changes in diagnostic criteria and recording practices, which render registry data unreliable.\textsuperscript{2,53,55} However, recently published prevalences of hypospadias and cryptorchidism from systematic birth cohort studies conducted in the two Nordic countries, Finland and Denmark, found a significantly higher birth prevalence of these congenital disorders in Denmark. In addition, the Danish prevalences had increased significantly compared to a study conducted in the 1960’s with similar examination practices.\textsuperscript{51,57,59} Further support of the hypothesis that TDS conditions are interconnected is the East-West gradient in the Nordic-Baltic area with regard to semen quality, with a significantly better semen quality among the Finns than the Danes.\textsuperscript{60} Finally, Danish men have a four-fold higher incidence of testicular cancer than the Finnish men.\textsuperscript{61} Immigrants overtake the host country’s prevalence from the second generation onwards.\textsuperscript{61,62}

In conclusion, the epidemiological trends are consistent with the hypothesis that a common pathogenic factor is associated with hypospadias and the other TDS symptoms. The relatively rapid changes in the incidence and results from immigrant studies indicate that environmental factors contribute to the aetiology of TDS, e.g., endocrine disruptors.\textsuperscript{9,63}

The origin of testicular dysgenesis syndrome

The interrelation of the male reproductive tract disorders is reflected in the developmental biology. Between 6 and 7 weeks of gestation the male gonads differentiate under the influence of SRY gene products, resulting in the production of testosterone. One of the first signs of masculinization is an increase in the distance between the anus and the genital structures, followed by elongation of the phallus, formation of the penile urethra from the urethral groove, and development of the prepuce.\textsuperscript{64} The normal development of the male external genitalia during early gestation requires testicular determination of the bipotential primitive gonad, complete testicular differentiation, adequate levels of circulating testosterone and its conversion by intra-cellular 5-alpha reductase to dehydrotestosterone, and adequate expression and function of the androgen receptors in the target tissue.

Hypospadias is defined by an abnormal development of the urothral spongiosum and ventral prepuce along with an arrest in the normal embryological correction of penile curvature.\textsuperscript{64} The result is an abnormal location of the urethral meatus on the ventral part of the penis instead of the tip of the glans, thus hypospadias range from mild glandular cases to severe cases with ambiguous genitalia.

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The process of testicular descent occurs in two phases: the transabdominal phase, which is primarily mediated by the Leydig cell hormone insulin-like factor 3 (INSL3) and takes place in the second trimester, and the inguinoscrotal phase, which is mediated by androgens and occurs in the third trimester. Despite being manifested in young adults, testicular cancer is a result of disturbed gonadal development and germ cell differentiation. Both seminomas and non-seminomas, are derived from the common pre-invasive precursor, carcinoma in situ (CIS). The only exceptions are the spermatocytic seminoma (spermatocytoma), which arise from spermatogenic germ cells in older men. CIS cells are transformed gonocytes, which failed to differentiate and subsequently underwent non-random genomic aberrations facilitating their survival and further invasive progression. The primordial germ cell lineage, carcinoma in situ cells and testicular cancer cells have important characteristics in common, with respect to morphology, structure, enzyme markers and gene expression.

Poor semen quality can be caused by a number of postnatal factors, including infections, accidents, chemotherapy, drugs, life style factors (e.g., body mass index), but some cases of poor sperm quality may be due to testicular dysgenesis. Sertoli cells are the first cells to differentiate in the indifferent fetal gonad. Once formed, Sertoli cells facilitate formation of seminiferous cords and Leydig cells, induce Müllerian duct regression and support spermatogenesis. In adulthood, the capacity for sperm production is directly related to Sertoli cell number as each Sertoli cell can support only a limited number of sperm cells.

As evident from the above, any disturbance in early fetal life of the development and differentiation of the Sertoli and Leydig cells may result in impairment of both germ cell development and testicular hormone synthesis, the consequence of which can be irreversible testicular dysgenesis. This may result in hypospadias and cryptorchidism, as well as impaired spermatogenesis and testicular cancer later in life.

**Conclusion**

Hypospadias and other male reproductive health problems, including cryptorchidism, testis cancer and poor semen quality may be associated symptoms of a testicular dysgenesis syndrome (TDS), as a consequence of Sertoli cells and/or Leydig cell impairment in fetal life. Similar temporal trends and geographic differences, as well as the frequent combination of more than one of the above mentioned problems in the same individual, strongly suggest the existence of etiological and pathogenic links. The most severe cases of TDS may include all symptoms, i.e., hypospadias, undescended testis, testicular cancer and poor semen quality, whereas other cases may show no other signs of TDS than a modest reduction in semen quality. In a clinical setting it should be kept in mind that some patients with hypospadias could have a higher risk of harbouring one or more of the other disorders.

The relative roles of genetic mutations and environmental factors for development of TDS and hypospadias remain to be determined, however increasing trends in incidence of hypospadias and the other TDS symptoms over a few decades suggest that environmental factors are important in the causal pathway.

(Reprinted with permission from Skakkebaek et al. Association between testicular dysgenesis syndrome (TDS) and testicular neoplasia: Evidence from 20 adult patients with signs of maldevelopment of the testis. APMIS 2003;111:1-11.)
Gestation and Hypospadias
Agneta Nordenskjold, M.D., Ph.D., Associate Professor, Departments of Molecular Medicine and Surgery and Women and Child Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
Louise Frisen, M.D., Ph.D., Department of Molecular Medicine and Surgery, Karolinska Institutet, Karolinska University Hospital and Department of Clinical Sciences, Karolinska Institutet, Danderyds Hospital, Division of Psychiatry, Stockholm, Sweden

Hypospadias is a common malformation with an incidence of 1 in about 300 newborn boys. The phenotype differs due to different positions of the meatus on the ventral side of the penis, in the scrotum or in severe cases in the perineum. Hypospadias arises due to a premature arrest of the male urethra development during gestational week 8-16, the earlier arrest the more severe phenotype (Figure 1). This malformation is a complex disorder meaning that both the genetic predisposition and the environmental factors contribute to the developmental delay of the urethra during these 8 critical weeks.

Birth weight
The single most important gestational factor associated with hypospadias is low birth weight. Czeizel et al reported an association to low birth weight, twin births and first-borns based on 294 index cases with isolated hypospadias in Hungary. In addition, a familial clustering of 4% was described, thereby laying the ground for hypospadias as a complex disorder. Later, several epidemiological studies in Europe and America have confirmed the association with low birth weight. The importance of growth restriction as a factor associated with hypospadias was later confirmed when birth weight also was adjusted for gestational age. (8,12)

Gestational age per se in hypospadiac boys is slightly shortened. In addition, the incidence of hypospadias was more than ten times higher than expected in a neonatal unit (11%, 17/154 cases) with a tendency towards a lower placenta weight. (6)

The growth restriction was evidenced to be an early event during pregnancy since birth length and head circumference are also smaller in hypospadias boys. A clear correlation between birth weight and first trimester size is shown by ultrasound measuring of crown-rump length, especially if the growth is suboptimal. This growth restriction does not only affect gestational weight but persists at least up to three years of age. (12) The birth weight difference has been reported to be between 200 and 400 g in different studies. As a group, hypospadiac boys weigh less than girls and the birth weight is correlated with the severity of the phenotype. (8,12)

In an attempt to exclude the genetic impact on birth weight, hypospadiac boys were compared with their respective healthy brother (with whom they partly share genome). The hypospadiac boys weighed 3322 g at birth compared with 3485 g for their respective brothers. In a further study, in 16 of 18 monozygotic twin pairs, discordant for hypospadias, the affected boy had a significantly lower birth weight than the respective twin with a mean difference in birth weight almost 500 g. (17)

Androgens are crucial during the last part of the embryological development of the external genitals. In complete androgen insensitivity (CAIS), the birth weight in CAIS girls (XY) is comparable to girls and not to boys and in CAH girls the birth weight is comparable to boys indicating that androgens have a high impact on birth weight as well as on the risk for hypospadias. (18,19)

Multiple births
Growth differences in twins are not uncommon. In about 5% of twins there is more than 25% birth weight difference - described as the smallest twins weight of the largest. (20) Hypospadias has since long been recognized associated with twin births. This has been confirmed, but when adjusted for birth weight there is no (or negative) association, suggesting that the risk is due to the differences in birth weight only. Generally twins do not have more malformations than singletons. In hypospadias, the risk may also be affected by the sex of the twins, as male-male pairs have 1.9 times higher risk of hypospadias when compared to the male in male-female pairs that have a decreased risk. (21) This could indicate a relative lack of growth factor in male-male pairs. Further data on birth weight in twins has shown that a female in a male-female pair weighs more than a female in a female-female pair. (22) Taken together, this suggests that interactions in the placenta in multiple births affect the risk for hypospadias. Growth restriction in twins discordant for a malformation has been described in four disorders; hypospadias, esophageal atresia, heart defects and anencephaly. Since the other three malformations cause low birth weight per se this further supports low birth weight as a major etiological factor for hypospadias.

Placenta/ Preeclampsia
Another possible cause of growth restriction is placenta insufficiency. The association to low placenta weight was first recognized in 1990 by Stoll et al and later confirmed by Smith et al in 1998. The weight of the placenta has been shown to be on average almost 100 g less when a hypospadiac boy is born than in control boys. (12)

Placenta weight was also correlated to the severity of the phenotype when comparing penile and coronal hypospadias with glandular type (with a substantial proportion diagnosed after the prepuce was retractable at 3 years of age) 555 g and 573 g respectively compared to 658 g in controls. Efforts to measure placenta function by analyzing hCG in maternal serum before gestational week 18 failed to reveal any differences in mothers with normal boys compared to mothers of boys with hypospadias. (26)

Preeclampsia is associated with an increased risk of hypospadias in several studies with an odds ratio (OR) around 2. (11,12,17) (Table 1). Other factors that could be associated with decreased placenta function are bleeding and retained placenta, both associated with an increased risk for hypospadias. (11) All these studies indicate an important role of the placenta for growth of the fetus and thus the risk for hypospadias.

(continued on the next page)
Birth order

Hypospadias has often been described to be more common in first-borns and inversely correlated to maternal parity.\(^4,5,9,11,13,27,28\) Parity more than three children compared with primipara resulted in OR 0.52.\(^15\) Only in one smaller study this association was not confirmed.\(^7\) One possible explanation for why parity affects the risk for hypospadias is that first-born children are more likely to have growth restriction and a lower birth weight.\(^17\) An association with subfertility, hormone treatment or a more functional placenta in later pregnancies, could also explain this.

Syndromes and associated malformations

Hypospadias is often part of genetic syndromes; especially syndromes with growth restriction like Silver-Russel syndrome with intrauterine and postnatal growth restriction, limb asymmetry, triangular face and hypospadias. OMIM lists 166 syndromes with hypospadias.

Boys with other congenital malformations that are not part of a syndrome have been reported to exhibit an almost three times higher risk of hypospadias than normal boys.\(^11,14\) Malformations have been described in between 9 and 15% of hypospadias boys, the most common being cryptorchidism in up to 27%\(^2,3,5,9,13,25\). End deformities or midline defects, such as esophageal and anal atresia, cleft lip palate or omphalocele are often reported and may have a common mechanism.\(^11,30,31\)

Diabetes and other maternal health risk factors

Maternal health can affect the risk for hypospadias, probably due to low birth weight as a common mechanism. Prepregnancy diabetes mellitus increase the risk for congenital malformations, due to a higher blood glucose level or affected placenta circulation.\(^32\) Maternal diabetes mellitus is associated with malformations especially in CNS, cardiovascular and skeletal defects and causes a doubling of the risk for hypospadias (OR 2.18), while the association with gestational diabetes is not confirmed.\(^9,32,33,34\) The risk for malformation diminishes if the pre pregnancy care is optimized (Towner et al 1995). Other maternal disorders that can affect the risk for hypospadias are epilepsy, renal failure, asthma and influenza during the first trimester (Table 1).\(^9,35\) Another factor that has been considered to affect the risk for hypospadias is maternal age. The finding that boys of older mothers (>35 years) have an increased risk of hypospadias may be linked to subfertility, since subfertile women are likely to conceive later.\(^34,36,37,38\) However, this finding was not confirmed in other studies and the association remains unclear.\(^1,3,19,34\) A maternal vegetarian diet has been associated with hypospadias, explained as a result of increased amount of phytoestrogens.\(^35\) However, there are no conclusive epidemiological data that prenatal exposure to estrogen (e.g. diethylstilbestrol, oral contraceptives) causes hypospadias.\(^39\) Maternal smoking causes a growth restriction in the fetus and lower birth weight, but an association to hypospadias has not been established.\(^7,36,40\)

Assisted reproduction

An increased incidence of hypospadias after assisted reproduction was first reported in 1991.\(^41\) Since then, several studies have shown an increased risk of hypospadias after in vitro fertilization (IVF), particularly when intracytoplasmatic sperm injection (ICSI) is used.\(^42,44\) A meta-analysis of 22 studies found no increased risk for hypospadias after ICSI compared with other IVF methods,\(^44\) but later studies using large epidemiological materials show an increased risk for hypospadias in ICSI.\(^45,46\) The association with ICSI and hypospadias has been interpreted as a link to paternal subfertility.\(^45\) However, ICSI is also used without identified sperm deficiencies to improve the result of a standard IVF procedure, why it is believed that the specific effect of ICSI on hypospadias will be reduced in the future.\(^46\) Besides a causal relationship to infertility (i.e., by genetic factors), there are other explanations for the increased incidence of hypospadias in IVF and ICSI children. For example, the high levels of gonadotropic hormones used during the IVF procedure may interfere with androgen production, as well as progesterins administered after embryo transfer. Clomifene, a drug used for inducing ovulation, greatly increased the risk for hypospadias (odds ratio=6) in one study, but could not be confirmed in another.\(^37,47\)

In conclusion, most gestational risk factors affect fetal growth and/or fetal hormones during the critical time period of the urethra development.\(^48\)

| Table 1 | Odds ratio for risk factors |
| --- | --- | --- |
| **A** | **B** | **C** |
| **1** | **2** | **3** |
| **Risk factor** | **Odds ratio** | **References** |
| Associated malformation | OR 2.75 | Acre et al 1999 |
| Birth weight | OR 2.72 | Aochim et al 2004 |
| Birth weight | OR 4.1 if <3 kg | Pierik et al 2004 |
| Gestational age | OR 2.23 if <37 w | Weidner et al 1999 |
| Small for gestational age | OR 1.43 | Ahmed et al 2004 |
| Small for gestational age | OR 2.16 | Aochim et al 2004 |
| Small for gestational age | OR 5.5 | Pierik et al 2004 |
| Affected brother | OR 30 | Angerpointe TA 1984 |
| Affected father | OR 20.81 | Aochim et al 2004 |
| Other maternal health risk factors | OR 10.56 | Weidner et al 1999 |
| Maternal diabetes mellitus | OR 2.18 | Porter et al 2005 |
| Maternal diabetes melilitus | OR 2.0 | Sorensen et al 2005 |
| Maternal diabetes melilitus | OR 1.3 | Sorensen et al 2005 |
| Maternal diabetes melilitus | OR 1.4 | Sorensen et al 2005 |
| Maternal diabetes melilitus | OR 1.0 | Hussain et al 2002 |
| Maternal diabetes melilitus | OR 1.84 | Aochim et al 2004 |
| Maternal diabetes melilitus | OR 2.4 | Sorensen et al 2005 |
| Maternal diabetes melilitus | OR 2.1 | Acre et al 1999 |
| Maternal diabetes melilitus | OR 3.19 | North & Golding 2000 |
| Maternal smoking | no association | Kallen et al 2002 |
| Maternal smoking | OR 1.6 | Pierik et al 2004 |
| Maternal smoking | no association | Carmichael et al 2006 |
| Maternal smoking | OR 0.78 | North & Golding 2000 |
| Maternal smoking | OR 4.99 | North & Golding 2000 |
| Maternal smoking | no association | Pierik et al 2004 |
| Maternal smoking | OR 3.4 | Pierik et al 2004 |
| Maternal smoking | OR 5 | Silver et al 1999 |
| Maternal smoking | OR 3.0 | Wennstöm et al 2000 |
| Maternal smoking | OR 1.7 | Källén et al 2005 |
| Maternal smoking | OR 1.5 | Ericson et al 2001 |
| Maternal smoking | no association | Lie RT 2004 |
| Maternal smoking | OR 6.08 | Meijer et al 2006 |
| Maternal smoking | no association | Toft Sorensen et al 2005 |
ETIOLOGICAL ASPECTS OF HYPOSPADIAS

Guest Editor: Pierre Mouriquand, M.D.

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