Aetiology of hypospadias: A systematic review of genes and environment

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Background Hypospadias is a common congenital malformation of the male external genitalia. Most cases have an unknown aetiology, which is probably a mix of monogenic and multifactorial forms, implicating both genes and environmental factors. This review summarizes current knowledge about the aetiology of hypospadias.

Methods Pubmed was used to identify studies on hypospadias aetiology published between January 1995 and February 2011. Reference lists of the selected manuscripts were also searched to identify additional studies, including those published before 1995.

Results The search provided 922 articles and 169 articles were selected for this review. Studies screening groups of patients with hypospadias for single gene defects found mutations in WT1, SF1, BMP4, BMP7, HOXA4, HOXB6, FGF8, FGFR2, AR, HSD3B2, SRD5A2, ATF3, MAML1, MAML1, MID1 and BNC2. However, most investigators are convinced that single mutations do not cause the majority of isolated hypospadias cases. Indeed, associations were found with polymorphisms in FGF8, FGFR2, AR, HSD17B3, SRD5A2, ESR1, ESR2, ATF3, MAML1, DGKK, MID1, CYP1A1, GSTM1 and GSTT1. In addition, gene expression studies indentified CTGF, CYR61 and EGF as candidate genes.

Environmental factors consistently implicated in hypospadias are low birthweight, maternal hypertension and preeclampsia, suggesting that placental insufficiency may play an important role in hypospadias aetiology. Exogenous endocrine disrupting chemicals have the potential to induce hypospadias but it is unclear whether human exposure is high enough to exert this effect. Other environmental factors have also been associated with hypospadias but, for most, the results are inconsistent.

Conclusions Although a number of contributors to the aetiology of hypospadias have been identified, the majority of risk factors remain unknown.
KEY WORDS:

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Risk factors
INTRODUCTION

Hypospadias is a congenital hypoplasia of the penis, with displacement of the urethral opening along the ventral surface, often associated with dorsal hooded foreskin and chordee. More than 50% of cases have anterior hypospadias, with a small displacement of the meatus in the glandular region (Fredell et al., 2002b; van der Zanden et al., 2010b). Other patients have more substantial displacements, with middle (penile) or posterior (penoscrotal, scrotal and perineal) openings (Figure I). Hypospadias is usually diagnosed during physical examination of the newborn but localization is best established during surgery, after chordee release. Compared to healthy children, boys born with hypospadias more often have additional congenital anomalies (Latifoğlu et al., 1998; Akre et al., 1999; Aschim et al., 2004a; Nassar et al., 2007), an association that appears to be stronger for posterior compared to anterior cases (Latifoğlu et al., 1998; Wu et al., 2002; Nassar et al., 2007). Cryptorchidism in particular and other urogenital anomalies are frequently found with hypospadias (Weidner et al., 1999; Nassar et al., 2007; Schnack et al., 2009; Akin et al., 2011).

Even when patients receive surgery in their first two years of life, they may encounter severe medical, social and sexual problems later in life. After long-term follow-up (10 years) of mainly patients with anterior hypospadias who underwent 1-stage repair, different rates of complications in up to 50% of patients were reported, depending on inclusion of different aspects (Nuininga et al., 2005). Although most studies conclude that psychosocial development is not seriously altered, patients do suffer from negative genital appraisal, sexual inhibition, and more erection and ejaculation problems (Mieusset and Soulié, 2005; Schönbucher et al., 2008).

Prevalence

Figures on the birth prevalence of hypospadias vary considerably across countries, ranging from four to 43 cases per 10,000 births (Kurahashi et al., 2004; Nassar et al., 2007). Hypospadias occurs most frequently in whites, less frequently in blacks, and rates are lowest among Asians and Hispanics (Gallentine et al., 2001; Carmichael et al., 2003; Yang et al., 2004; Porter et al., 2005; Nelson et al., 2007).
There is debate about whether or not the prevalence of hypospadias is increasing. Some researchers reported increasing prevalences in China (Sun et al., 2009; Jin et al., 2010), Australia (Nassar et al., 2007), the USA (Paulozzi et al., 1997; Nelson et al., 2005) and Europe (Lund et al., 2009), whereas others did not find an increase in Canada, the USA (Fisch et al., 2001; Carmichael et al., 2003; Porter et al., 2005), Europe (Aho et al., 2000; Ahmed et al., 2004; Abdullah et al., 2007) and Japan (Kurahashi et al., 2004). However, results of different studies are difficult to compare because some are based on hospital discharge registries, including only surgically treated patients or all newborns diagnosed with hypospadias, whereas others are based on birth defects surveillance systems, including all registered hypospadias cases or excluding cases with glandular hypospadias. In addition, the diagnosis and definition of hypospadias may have changed over time.

Embryology of the male external genitalia

Indifferent stage

Early development of the external genitalia is similar for males and females. The embryonic cloaca, the far end of the hind gut, is separated from the amniotic cavity by the cloacal membrane. Early in the fifth week of development, a swelling develops on both sides of this membrane, the cloacal folds, which meet in the midline anterior to the cloacal membrane, forming the genital tubercle (Schoenwolf, 2009) (Figure II). At the same time, the genital ridges, the precursors of the gonads, develop. Studies in mice showed that this process requires Wilms tumour 1 (Wt1) activity, which activates splicing factor 1 (Sf1) (Wilhelm and Englert, 2002), thus preventing degeneration of the developing gonads (Luo et al., 1994). During the seventh week of human development, the urorectal septum fuses with the cloacal membrane, dividing the cloaca into the primitive urogenital sinus and the rectum, and dividing the cloacal membrane into the urogenital and the anal membrane. The swellings next to the urogenital membrane are then called the urogenital folds and a new pair of swellings, the labioscrotal swellings, appear on either side of these folds. In addition, the urogenital membrane breaks down (Schoenwolf, 2009).
**Early patterning**

The genital tubercle (GT) masculinises if exposed to androgens but early patterning is androgen-independent. Studies on genes and proteins involved in this patterning process have mainly been performed in mice and showed that the distal urethral plate epithelium is the signalling centre regulating GT outgrowth (Perriton *et al.*, 2002). Fibroblast growth factor protein (Fgf) and wingless-type MMTV integration site family member 5A (*Wnt5a*) signalling have a growth-promoting role in this outgrowth (Yamaguchi *et al.*, 1999), whereas bone morphogenetic proteins (Bmps) stimulate apoptosis (Morgan *et al.*, 2003; Suzuki *et al.*, 2003). Expression of *Fgf8* in the urethral plate is regulated by sonic hedgehog (Shh) and homeobox A13 (Hoxa13) (Haraguchi *et al.*, 2001; Perriton *et al.*, 2002; Morgan *et al.*, 2003), while Hoxa13 also regulates expression of *Bmp7* (Morgan *et al.*, 2003). Shh induces, either directly or via Fgf8 or other factors, expression of *Fgf10*, *Bmp2*, *Bmp4*, *Wnt5a*, *Patched 1* (*Ptch1*), *Msh homeobox 1* (*Msx1*) and *Hoxd13* (Haraguchi *et al.*, 2001; Perriton *et al.*, 2002). Shh thus modulates the balance between proliferation and apoptosis (Haraguchi *et al.*, 2001) and regulates the initiation of GT outgrowth (Perriton *et al.*, 2002). Immunohistochemical staining of human foetal penises showed expression of *SHH*, its receptor *PTCH1*, and its downstream genes smoothened, frizzled family receptor (*SMO*) and GLI family zinc finger 1 (*GLI1*) around the time of urethral closure (Shehata *et al.*, 2011). Studies in mice showed that Wnt-β-catenin signalling also seems to play a role in GT development, either in early androgen-independent GT development (Lin *et al.*, 2008) or as a downstream effector of androgen signalling essential for GT masculinisation (Miyagawa *et al.*, 2009).

**Masculinisation**

Subsequent masculinisation relies on hormones produced by the testes. Expression of the sex-determining region Y gene (*SRY*) induces a cascade of gene interactions, involving *SRY-box 9* (*SOX9*) (Schoenwolf, 2009), resulting in differentiation of the gonads into the testes (Sinclair *et al.*, 1990). *SRY* leads to the differentiation of Sertoli cells (Schoenwolf, 2009), which secrete anti-Müllerian hormone
Studies in mice showed that AMH secretion happens under the influence of Sf1 (Giuli et al., 1997). AMH causes regression of the Müllerian ducts that would otherwise form part of the female genital structures (Schoenwolf, 2009). HCG, produced by the placenta, controls foetal Leydig cell growth and stimulates foetal testicular steroidogenesis, the generation of steroids from cholesterol (Misrahi et al., 1998). The enzymatic steps of steroidogenesis, mainly taking place in the Leydig cell, are well documented and expression of key genes in this pathway is dependent on expression of SF1 (Scott et al., 2009) (Figure III). Testosterone leaves the Leydig cell and is converted into dihydrotestosterone (DHT) by steroid-5-alpha-reductase (SRD5A). Testosterone promotes formation of the internal reproductive structures from the Wolffian ducts, whereas DHT induces development of the external genitalia (Schoenwolf, 2009), both through their effect on the androgen receptor (AR). Expression of estrogen receptors (ESR) in male genital tissue during development suggests that the balance between androgens and estrogens is important as well (Crescioli et al., 2003).

During masculinisation of the external genitalia, between the 12th and 14th week after conception (Schoenwolf, 2009), the GT develops into the penis, the labioscrotal swellings fuse to form the scrotum (Ammini et al., 1997; Schoenwolf, 2009) and the urogenital folds close in a proximal to distal direction to form the penile urethra (Ammini et al., 1997; van der Werff et al., 2000; Schoenwolf, 2009; Yamada et al., 2003; Hynes and Fraher, 2004b) (Figure II). Several hypotheses have been proposed about formation of the glandular portion of the urethra. One of these states that, while the penile urethra is created by fusion and primary luminisation, the glandular urethra develops by fusion and secondary luminisation (van der Werff et al., 2000). According to another hypothesis, the complete urethra arises by fusion of the urogenital folds (Ammini et al., 1997; Baskin et al., 2001). Still others believe that the glandular portion of the urethra originates from a different set of folds (Hynes and Fraher, 2004a), ingrowth of surface cells (Jones, 1910) or canalization of the urethral plate (Schoenwolf, 2009).

As a result, the development of hypospadias is also controversial. From a clinical point of view, development of the urethra, corpora, glans and penile skin are directly correlated. In posterior
hypospadias, there is non-fusion of the labioscotal swellings with a distal dysplasia of the urethral plate and corpora, as well as non-fusion of the glans and skin in the midline. In middle hypospadias, the distal part of the penis shows a persistence of the urethral plate and non-tubularisation of the glans with disturbed penile skin formation. In glandular hypospadias, there is a dimple or a short tubular tract with a septum in between this tract and the urethral plate or tube and no closure of the skin in the midline. In the most minimal form, hypospadias sine hypospadias, only non-fusion of the preputial skin on the ventral side is seen, with dorsal hooded foreskin with or without some chordee.

Aim of this review
In 30% of the least frequently occurring posterior hypospadias cases a cause can be identified, for example, a complex genetic syndrome, partial androgen insensitivity related to AR mutations, or SRD5A type II deficiency (Albers et al., 1997; Boehmer et al., 2001). The aetiology of most other hypospadias cases, however, is not yet solved in spite of intensive research. In this review, we will summarize the current knowledge about the causes of the isolated, non-syndromic form of this common birth defect in humans, from both a genetic and an environmental point of view. In addition, we will provide recommendations for further research.
METHODS

Pubmed was used to identify all relevant manuscripts on the aetiology of hypospadias. We searched for papers published between January 1995 and February 2011 in the English language using the following keywords in the title or abstract: “(hypospadia OR hypospadias) NOT surgical NOT surgery NOT reconstruction NOT repair NOT incised NOT procedure”. This search provided 922 articles, of which we used the titles and abstracts to identify relevant papers. We focussed our review on the aetiology of isolated hypospadias in humans. Therefore, we excluded all animal studies (N = 99), articles that were not about hypospadias or the aetiology of hypospadias (N = 235), and articles or case-reports that described the phenotype of patients suffering from syndromes including hypospadias, or that investigated or described the most likely cause of the syndrome in these boys (N = 308). To systematically exclude articles with a lesser degree of evidence, we excluded all ecological studies (N = 11). For epidemiologic studies reporting negative findings for environmental factors, we took the power into consideration before reporting that it showed no association. In general, we excluded negative results on environmental factors from studies describing <100 cases, as these have, for example, only 37% power to significantly ($P < 0.05$) detect a two-fold increased risk, assuming a prevalence of 10% (15 studies were completely excluded because of this criterion). To guarantee that all information was included only once in the article, we excluded all reviews and meta-analyses (N = 79). In addition, when a study was supplemented with new data in a later publication (N= 3), we only included the article reporting the most complete data. Finally, all commentaries were excluded (N = 32). Reference lists of the selected manuscripts were searched to identify additional studies, including those published before 1995, although these were only included if they reported results that were not found in one of the more recently published articles (N = 29). This selection process resulted in 169 original articles that were included in this review and are described below.
RESULTS

Aetiology of hypospadias is multifactorial

Hypospadias shows familial clustering, with 7% of cases having affected first, second or third degree relatives (Fredell et al., 2002b). Familial occurrence seems to be more common for anterior and middle forms of hypospadias than for posterior types (Fredell et al., 2002b; Brouwers et al., 2010). The chance that a brother of an affected boy will also have hypospadias is 9 to 17% (Calzolari et al., 1986; Stoll et al., 1990; Schnack et al., 2008). In two family studies and one small twin study, the heritability of hypospadias was estimated to be 57 to 77% (Calzolari et al., 1986; Stoll et al., 1990; Schnack et al., 2008), meaning that 57 to 77% of the phenotypic variability can be attributed to genetic variability. Because hypospadias is equally transmitted through the maternal and paternal sides of the family and recurrence risks for brothers and sons of hypospadias cases are similar, genetic rather than shared environmental factors may play a principal role in familial hypospadias (Schnack et al., 2008). Segregation analysis, however, suggested that the majority of cases have a multifactorial aetiology, involving both genes and environmental factors (Fredell et al., 2002a).

Genes implicated in the aetiology of isolated hypospadias

Much of the genetic research on hypospadias has been focused on identification of causal mutations. In Table I, we summarize the exonic (including 3'-untranslated and splice acceptor site) mutations found in studies screening candidate genes in groups of patients with hypospadias, ordered according to the different stages of embryonic development. Whether these mutations have functional consequences remains unclear in most cases, as only few studies reported conservation and function of the region in which the mutation is located, or predicted potential influence of the mutation on protein function using bioinformatics. The majority of mutations were found only once and were identified in posterior or penile cases. The latter has contributed to the view that there is a difference in the genetic models underlying posterior versus anterior hypospadias, with posterior cases being more common in monogenic forms of hypospadias and anterior cases having a polygenic or multifactorial aetiology.
The studies investigating associations between genetic polymorphisms and hypospadias are summarized in Table II (following the same order as Table I).

**Indifferent stage**

All genes involved in the development of the male external genitalia are obvious candidate genes for hypospadias. Because *Wt1* and *Sf1* play major roles in early embryonic development of the kidneys and the urogenital system, mutations in these genes are likely to cause not only hypospadias but also more severe defects. Indeed, *SF1* mutations were found in severe penoscrotal hypospadias cases with cryptorchidism (Köhler et al., 2009), while a mutation in *WT1* was described in a boy with penoscrotal hypospadias and micropenis and also in three boys with isolated penile or glandular hypospadias (Wang et al., 2004) (Table I).

**Early patterning**

Genes involved in GT patterning are additional candidates for hypospadias. Mutation screening in hypospadias cases revealed mutations in *BMP4*, *BMP7*, *HOXA4*, *HOXB6*, *FGF8*, and the fibroblast growth factor receptor *FGFR2* (Chen et al., 2007; Beleza-Meireles et al., 2007c) (Table I), while associations with hypospadias were also observed for polymorphisms in *FGF8* and *FGFR2* (Beleza-Meireles et al., 2007c) (Table II).

**Masculinisation**

Expression of the *SRY* gene, located on the Y chromosome, is crucial for development of the testis from the indifferent gonad (Sinclair et al., 1990; Gubbay et al., 1990). Sex chromosome abnormalities were noticed in four out of 100 patients with hypospadias (Moreno-Garcia and Miranda, 2002) but no mutations in *SRY* were found in 90 patients in another study (Wang et al., 2004). In addition, screening Yq for microdeletions in 44 cases did not reveal any abnormalities (Tateno et al., 2000) and neither did screening the segments of the Y chromosome associated with infertility in 20 cases with middle or posterior hypospadias and cryptorchidism (Castro et al., 2004).
Genetic research has been focused on the hormone-dependent stage of sexual development as well. The gene encoding AR in particular was investigated extensively. AR is expressed in the developing human penis and urethra (Kim et al., 2002) and several studies reported rare mutations in the gene encoding AR in patients with hypospadias (Hiort et al., 1994; Alléra et al., 1995; Sutherland et al., 1996; Nordenskjöld et al., 1999; Wang et al., 2004; Thai et al., 2005) (Table I). In addition, polymorphisms in AR have been investigated for associations with the anomaly and may increase hypospadias risk. For example, expansion of the polyglutamine (CAG) repeat in the N-terminus of AR, shown to decrease AR transactivation function (Chamberlain et al., 1994), was found to be associated with undermasculinisation (Lim et al., 2000). Two studies reported that longer GGN repeat length increased the risk of penile hypospadias (Aschim et al., 2004b; Radpour et al., 2007) but these two (and one other) studies did not find an association between CAG repeat length and hypospadias (Muroya et al., 2001; Aschim et al., 2004b; Radpour et al., 2007) (Table II). DHT binding capacity of the AR in genital skin fibroblasts was reported to be decreased in some patients with hypospadias (Schweikert et al., 1989; Alléra et al., 1995), whereas normal binding capacity was found in others (Gearhart et al., 1988; Terakawa et al., 1990). In addition, AR levels were similar in foreskin samples of hypospadias cases and controls (Bentvelsen et al., 1995).

Several proteins are needed for AR function. FK506 binding protein 4, 59kDa (FKBP4, also known as FKBP52), for example, is a component of AR complexes, enhancing AR-mediated transactivation (Cheung-Flynn et al., 2005). However, no differences in FKBP4 expression were noted between patients with hypospadias and controls and no mutations in FKBP4 were observed (Beleza-Meireles et al., 2007a).

As normal male urethral development requires testosterone and DHT, defects in steroidogenesis could also account for hypospadias. One article stated that up to 50% of patients with hypospadias have a testosterone biosynthesis defect (Aaronson et al., 1997), a conclusion that could not be confirmed in
two other studies that found no enzymatic defects (Feyaerts et al., 2002; Holmes et al., 2004).

Nevertheless, mutations have been found in hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 (HSD3B2) (Codner et al., 2004) and SRD5A type II (SRD5A2) (Silver and Russell, 1999; Wang et al., 2004; Thai et al., 2005).

The gene encoding SRD5A2 is particularly interesting because this enzyme is expressed during male genital development around the ventral part of the remodelling urethra and it converts testosterone to the more potent androgen DHT, which induces formation of the external genitalia (Kim et al., 2002).

Two single nucleotide polymorphisms (SNPs) in this gene seemed to be associated with hypospadias in some but not all studies (Silver and Russell, 1999; Wang et al., 2004; Thai et al., 2005; Sata et al., 2010; van der Zanden et al., 2010b) (Table II). One of these SNPs (rs523349) causes a valine to leucine substitution (V89L), resulting in a decrease in enzyme activity by approximately 30% (Makridakis et al., 1997; Makridakis et al., 2000), whereas the other SNP (rs9282858) results in an alanine to threonine replacement (A49T), which causes an increase in enzyme function (Makridakis et al., 2000). Another SNP that seems to be associated with hypospadias and to have functional consequences is rs2066479 in HSD17B3. The glycine to serine substitution (G289S) caused by this SNP results in reduced HSD17B3 mRNA expression levels in utero (Sata et al., 2010).

**Other genes**

Not only steroidogenesis but also the balance between androgens and estrogens appears to be important in development of the male external genitalia. The estrogen receptors ESR1 and ESR2 are expressed in the developing human male GT (Crescioli et al., 2003) and associations have been reported between hypospadias and several SNPs in the genes encoding these receptors, as well as with the CA-repeat in ESR2 (Beleza-Meireles et al., 2006; Watanabe et al., 2007; Beleza-Meireles et al., 2007b; Ban et al., 2008; van der Zanden et al., 2010b) (Table II). One of the SNPs in ESR1, rs9340799, was shown to increase enhancer activity of ESR1 (Maruyama et al., 2000).
Some additional genes are also suggested to be involved in development of hypospadias. Activating transcription factor 3 (ATF3) is an estrogen-responsive gene showing strong up-regulation in hypospadias (Liu et al., 2005; Wang et al., 2007; Kalfa et al., 2008a; Gurbuz et al., 2010). Studies focusing on the relation between this gene and hypospadias found mutations and associations with several SNPs (Beleza-Meireles et al., 2008; Kalfa et al., 2008a) but not all associations could be replicated (van der Zanden et al., 2010b) (Tables I and II). Recently, mastermind-like domain containing 1 (MAMLD1, previously known as CXorf6) was identified as a causal gene for hypospadias. MAMLD1 contains the SF1 target sequence (Fukami et al., 2008) and mutations and polymorphisms in MAMLD1 have been found in patients with hypospadias (Fukami et al., 2006; Kalfa et al., 2008b; Chen et al., 2010) (Tables I and II). A recent genome-wide association study using pooled DNA samples identified diacylglycerol kinase, kappa (DGKK) as a major risk gene for hypospadias (van der Zanden et al., 2010a). An intronic SNP was associated with a 2.5 times increased hypospadias risk, while DGKK expression in preputial skin was shown to be lower in boys carrying the risk allele. In the van der Zanden et al. (2010a) study, additional candidate genes i.e. peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PPARGC1B), glutamate receptor, ionotropic, delta 1 (GRID1) and KIAA2022 were also identified but these still need to be confirmed. One study investigated MIDI in relation to hypospadias and found mutations in patients with hypospadias as well as a SNP in this gene to be associated with the disorder (Zhang et al., 2011) (Tables I and II). Insulin-like 3 (INSL3) mutations have been found in patients with cryptorchidism but no alterations were detected in 94 hypospadias cases (El Houate et al., 2007) (Table I).

Expression studies have also identified some candidate genes. Using prepuce samples of patients with hypospadias and controls, Wang et al. (2007) not only found ATF3 to be upregulated in patients but also connective tissue growth factor (CTGF) and cysteine-rich, angiogenic inducer, 61 (Cyr61), two other estrogen-responsive genes. In addition, epidermal growth factor (EGF) staining in prepuce showed lower expression of EGF within the penile skin adjacent to the urethra in patients with hypospadias compared to controls (el-Galley et al., 1997).
A balanced translocation in a man with hypospadias and other congenital anomalies indicated basoonuclin 2 (BNC2) as a candidate gene. This gene is expressed in developing human periurethral tissue and mutations were found in 6 out of 48 patients with hypospadias but also in 2 out of 23 controls (Bhoj et al., 2011) (Table I).

As exposure to environmental toxicants has also been suggested to cause hypospadias, and cytochrome P4501A1 (CYP1A1) and glutathione S-transferases (GSTM1 and GSTT1) are involved in the metabolism of various toxicants, two studies evaluated the effect on hypospadias risk of polymorphisms in the genes encoding these enzymes. One study found an association with hypospadias for concomitant deletion of GSTM1 and GSTT1 (Yadav et al., 2011) (Table II). The other study investigated associations between maternal smoking, maternal SNPs in the genes and the risk of hypospadias in offspring. They found an association between a SNP in CYP1A1 and hypospadias, which was not modified by smoking behaviour (Kurahashi et al., 2005).

One genome-wide linkage analysis in 69 families with at least 2 members with hypospadias found suggestive linkage at 9q22, 2p11, 10p15 and 10q21 (Frisen et al., 2004), while another linkage study in a three-generational family showing autosomal dominant inheritance of hypospadias found a peak on 7q32.2-q36.1 (Thai et al., 2008). Mutation analysis of two genes in this region, AKRID1 and PTN, failed to reveal any mutations (Thai et al., 2008).

Screening 17 isolated patients with hypospadias and 12 patients with associated anomalies for copy number variants (CNVs) revealed clinically significant CNVs in 3 patients with isolated hypospadias (5p15, 12p13 and Xq28) and in 2 patients with an associated anomaly, which were cryptorchidism (2q22) and cleft palate (16p11) (Tannour-Louet et al., 2010).

The role of environmental factors in the aetiology of hypospadias
Introduction

While genes involved in the aetiology of hypospadias have received a considerable amount of attention, research on environmental factors has been even more extensive. Despite the large number of studies, however, clear evidence for causal environmental factors is still lacking, although some consistent associations have been reported. Table III gives a summary of environmental factors investigated in relation to hypospadias.

Testicular dysgenesis syndrome

In 2001, Skakkebæk et al. suggested that poor sperm quality, testicular cancer, undescended testes and hypospadias are symptoms of one underlying entity, the Testicular Dysgenesis Syndrome (TDS) (Skakkebæk et al., 2001). They were convinced of its existence because countries with high incidences of testicular cancer also had high prevalence rates of hypospadias, cryptorchidism and poor sperm quality (Virtanen et al., 2005). Other researchers question whether TDS actually exists as there is little evidence of shared causes (Akre and Richardi, 2009), only a few patients display all features, and incidences of the four components of the syndrome did not increase over time at the same rate (Thorup et al., 2010). Although testicular germ cell cancer risk was increased in patients with hypospadias or undescended testis, risk was not increased in their family members. This does not support the hypothesis of shared heritability (Schnack et al., 2010). Recently, Skakkebæk et al. concluded that TDS does exist but that it encompasses only a fraction of hypospadias and impaired spermatogenesis cases (Jørgensen et al., 2010).

Estrogen hypothesis

In 1993, Sharpe and Skakkebæk hypothesized that the increasing incidence of reproductive abnormalities in males may have a common cause, namely increased estrogen exposure in utero, leading to disturbances in AMH secretion or impairment of Leydig cell development (Sharpe and Skakkebæk, 1993). Ten years after the introduction of this hypothesis, Sharpe concluded that evidence...
for foetal estrogen exposure inducing TDS had strengthened (Sharpe, 2003). New pathways were identified through which estrogens could induce TDS, including suppression of testosterone production, AR expression and insulin-like 3 secretion. Whether increased estrogen exposure will turn out to be an important aetiologic factor for TDS is not so certain, however.

The initial ‘estrogen hypothesis’ was superseded by a more refined definition of endocrine disrupting chemicals (EDCs), suggesting that chemicals may act on the endocrine systems in a plethora of ways (Fisher, 2004). In 2008, Sharpe and Skakkebæk highlighted the central role of deficient androgen production or action during foetal testis development in the origin of the downstream disorders of TDS (Sharpe and Skakkebæk, 2008). However, the question remains whether levels of exposure to EDCs are sufficient to influence male reproductive health (Fisher, 2004) and several reviews concluded that there is little evidence for a role of environmental EDCs (Raman-Wilms et al., 1995; Safe, 2000; Chia, 2000; Vidaeff and Sever, 2005; Storgaard et al., 2006; Martin et al., 2008).

**Exogenous exposure to estrogens**

**Oral contraceptives**

Although oral contraceptives probably provide the strongest estrogen exposure that humans can experience, an association between hypospadias and use of oral contraceptives for some time during pregnancy was not found in most studies (Morera et al., 2006; Wogelius et al., 2006; Brouwers et al., 2007; Akre et al., 2008; Nørgaard et al., 2009; Brouwers et al., 2010).

**Assisted reproductive technology**

Assisted reproductive technologies (ART) frequently involve hormonal stimulation and some studies showed an increased risk of hypospadias with ART (Carmichael et al., 2007; Brouwers et al., 2007; Brouwers et al., 2010). More specifically, ICSI increased hypospadias risk in most (Wennerholm et al., 2000; Ericson and Källén, 2001; Pinborg et al., 2004; Källén et al., 2005; Fedder et al., 2007; Funke et al., 2010) but not all studies (Bonduelle et al., 2002; Källén et al., 2010), whereas studies on IVF did
not report increased risks or were inconclusive (Ericson and Källén, 2001; Bonduelle et al., 2002; Morera et al., 2006; Funke et al., 2010; Källén et al., 2010), except for one study that did not report whether ICSI was excluded (Silver et al., 1999). In one study, increased hypospadias risk was associated with hormonal stimulation (Carmichael et al., 2005a) but this was not confirmed in other studies (Källén et al., 2002; Sørensen et al., 2005b; Morera et al., 2006; Meijer et al., 2006).

Other authors assumed that the increased hypospadias risk may be explained by reduced maternal or paternal fertility. Fathers of hypospadias cases were reported to have lower sperm concentration, sperm count (Asklund et al., 2007) and sperm motility, as well as a higher proportion of abnormal sperm morphology (Fritz and Czeizel, 1996). In addition, several studies reported a prolonged time-to-pregnancy (TTP) for parents of patients with hypospadias (Källén, 2002; Pierik et al., 2004; Asklund et al., 2007; Brouwers et al., 2010) and only one study did not confirm these results (Akre et al., 1999).

The fact that ICSI, rather than IVF, and sperm quality are associated with hypospadias supports the idea that paternal fertility problems in particular play a role in hypospadias (Brouwers et al., 2007; Brouwers et al., 2010).

ART may be associated with genomic imprinting disorders (Laprise, 2009). This possible interference with epigenetic regulation is another mechanism by which ART could increase hypospadias risk. A very recent study indicated that alterations in the methylation pattern of AR, leading to abnormal expression of the gene in foreskin tissue from patients, may contribute to the development of hypospadias (Vottero et al., 2011).

**Endogenous hormone levels**

**Endogenous estradiol levels**

Endogenous levels of free estradiol increase with increasing BMI and are elevated in women with an early age at menarche (Apter and Vihko, 1983; Emaus et al., 2008). Several studies found associations between hypospadias and mothers being overweight (25 ≤ BMI < 30 kg/m²) (Waller et al., 2007) or
severely overweight or obese (BMI > 29 or 30 kg/m²) (Waller et al., 2007; Akre et al., 2008; Giordano et al., 2010; Blomberg and Källén, 2010) but one study did not (Brouwers et al., 2010). Another study found increased risks for underweight but not for overweight or obese women (Rankin et al., 2010).

The results for early age at menarche were inconsistent (Morera et al., 2006; Giordano et al., 2010).

Estradiol levels are also higher in first pregnancies and twin pregnancies (Kappel et al., 1985; Bernstein et al., 1986), which were both repeatedly investigated for their association with hypospadias. Most studies showed that women in their first pregnancy (Akre et al., 1999; Weidner et al., 1999; Hussain et al., 2002; Källén, 2002; Carmichael et al., 2003; Aschim et al., 2004a; Sørensen et al., 2005a; Meyer et al., 2006; Morera et al., 2006; Carmichael et al., 2007; Nassar et al., 2009; Jin et al., 2010) or with a twin or triplet pregnancy (Akre et al., 1999; Fredell et al., 2002b; Morera et al., 2006; Brouwers et al., 2007; Carmichael et al., 2007; Sun et al., 2009; Nassar et al., 2009; Brouwers et al., 2010; Funke et al., 2010; Jin et al., 2010) were at increased risk of having a son with hypospadias but a few studies could not replicate the findings for primiparity or for multiple pregnancies (Weidner et al., 1999; Carmichael et al., 2003; Aschim et al., 2004a; Sørensen et al., 2005a; Ghirri et al., 2009). The latter may be caused by overadjustment for birthweight in some studies. As only early-onset intrauterine growth restriction (IUGR) could be a risk factor for hypospadias, it is more likely that low birthweight and hypospadias share an underlying cause rather than low birthweight being a risk factor for hypospadias.

**Foetal hCG provision**

Placental hCG stimulates foetal testicular steroidogenesis before the foetus’s own pituitary-gonadal axis is established. Placental insufficiency may result in inadequate foetal hCG provision and IUGR, possibly explaining the association between hypospadias and low birthweight or being small for gestational age (SGA) that was consistently reported, although not always statistically significant (Weidner et al., 1999; Akre et al., 1999; Gatti et al., 2001; Hughes et al., 2002; Hussain et al., 2002; Fredell et al., 2002b; Carmichael et al., 2003; Pierik et al., 2004; Aschim et al., 2004a; Boisen et al., 2005; Chong et al., 2006; Morera et al., 2006; Brouwers et al., 2007; Akre et al., 2008; Giordano et al., 2008; Sun et al., 2009; Nassar et al., 2009; Ghirri et al., 2009; Brouwers et al., 2010; Funke et al., 2010).
al., 2010; Jin et al., 2010; Giordano et al., 2010). However, because hCG levels were similar in maternal
serum samples of hypospadias cases and controls, this is unlikely to be related to decreased maternal
hCG production (Kiely et al., 1995). IUGR was also found more often in the affected twin of same-sex
twin pairs discordant for hypospadias (Fredell et al., 1998; Chambers et al., 2006). Direct proof of a
link between placental insufficiency and hypospadias was provided by research showing an
association between hypospadias and low placental weight (Stoll et al., 1990), an increased frequency
of placental infarction among extremely low birthweight boys with hypospadias (Fujimoto et al., 2008)
and a high rate of early-onset IUGR related to placental insufficiency among SGA newborns with
hypospadias, with the more posterior cases having more severe IUGR (Yinon et al., 2010). The
association with low birthweight also seems to be stronger for more posterior forms of hypospadias
(Carmichael et al., 2003; Carlson et al., 2009; Ghirri et al., 2009; Brouwers et al., 2010).
Nausea in early pregnancy may be caused by the early surge of hCG (Furneaux et al., 2001),
suggesting that placental insufficiency may cause absence of nausea. Indeed, vomiting and nausea
during early pregnancy were shown to decrease hypospadias risk (Carmichael et al., 2007; Akre et
al., 2008). Maternal hypertension during pregnancy (Morera et al., 2006; Akre et al., 2008; Caton et
al., 2008; Sun et al., 2009; Brouwers et al., 2010) and preeclampsia (Akre et al., 1999; Aschim et
al., 2004a; Sorensen et al., 2005a; Chong et al., 2006; Morera et al., 2006; Sun et al., 2009; Brouwers et
al., 2010) were consistently associated with hypospadias, and both factors may be associated with
placental dysfunction, possibly by compromising uteroplacental perfusion (Caton et al., 2008). Preterm
delivery may be associated with late placental dysfunction and several studies demonstrated an
association with hypospadias (Pierik et al., 2004; Meyer et al., 2006; Akre et al., 2008; Sun et al., 2009;
Nassar et al., 2009; Funke et al., 2010; Jin et al., 2010; Giordano et al., 2010; Akin et al., 2011) while
others could not confirm this (Akre et al., 1999; Weidner et al., 1999; Carmichael et al., 2003; Aschim
et al., 2004a; Chong et al., 2006; Ghirri et al., 2009), again possibly because of overadjustment for
birthweight in some studies.

Clinical factors
Pregnancy complications

In a few studies, associations were investigated between hypospadias and complications during pregnancy, such as maternal bleeding, which seemed to be more prevalent among cases (Aschim et al., 2004a; Jin et al., 2010). The amount of weight gain was not associated with hypospadias (Morera et al., 2006; Meyer et al., 2006). Complications during labour, such as labour induction and Caesarean section, occurred more frequently among mothers of hypospadias cases (Aschim et al., 2004a; Meyer et al., 2006), indicating that pregnancies affected by hypospadias are associated with other difficulties that make them prone to these complications. Diabetes has been another focus of research, but most studies were too small to draw conclusions (Hussain et al., 2002; Sørensen et al., 2005a; Morera et al., 2006; Sun et al., 2009; Brouwers et al., 2010). One study found maternal gestational and pre-existing diabetes not to be associated with occurrence of hypospadias (Aschim et al., 2004a), whereas others reported an increased risk for pre-existing but not for gestational diabetes (Åberg et al., 2001; Porter et al., 2005). Results were inconsistent for thyroid disease (Aschim et al., 2004a; Browne et al., 2009) and fever during pregnancy (Stoll et al., 1990; Jin et al., 2010). Women with gynaecological diseases (ovarian cysts or benign uterine tumours) (Giordano et al., 2008), those who are carriers of hepatitis B antigen (Sun et al., 2009) and women experiencing a viral infection or influenza in the first trimester of pregnancy (North and Golding, 2000; Morera et al., 2006) seem to be at increased risk of giving birth to a son with hypospadias but evidence was derived from only one study. Urinary infections and anaemia do not seem to increase hypospadias risk (Aschim et al., 2004a).

Maternal drug use

Most therapeutic drugs, such as corticosteroids, antibiotics, antipsychotics, antifungal and anti-asthmatic drugs, do not seem to be associated with hypospadias, although some studies may suffer from under reporting (Czeizel and Rockenbauer, 1997; Czeizel et al., 2001; Brouwers et al., 2007; Källén and Otterblad, 2007; Carter et al., 2008; Carmichael et al., 2009a; Brouwers et al., 2010). Based on data from the Swedish Medical Birth Register 1995-2001, Källén et al. reported 15 hypospadias cases in 2780 infants born after maternal use of loratadine, an antihistamine, during pregnancy (Källén...
and Olausson, 2001) but in 2001-2004 only two cases were identified among 1911 infants exposed to 
loratadine, indicating that the primary finding occurred by chance (Källén and Olausson, 2006). Other 
studies also failed to find an association between loratadine and hypospadias (CDC 2004; Pedersen et 
al., 2008). Results for progestogens/progestins used for threatened abortion vary (Katz et al., 1985; 
Calzolari et al., 1986). Use of loperamide (Källén et al., 2008), antiretroviral therapy (Watts et 
al., 2007), antihypertensive drugs (Caton et al., 2008; Brouwers et al., 2010), nystatin (Czeizel et 
al., 2003) or paroxetine (Reis and Källén, 2010) during early pregnancy may increase hypospadias risk, 
while codeine (North and Golding, 2000) may decrease the risk but most of these associations were 
reported only once. In contrast, use of anti-epileptic drugs was linked to hypospadias several times 
(Arpino et al., 2000; Hunt et al., 2008; Rodriguez-Pinilla et al., 2008; Bánhidy et al., 2010; Jentink et 
al., 2010). Most studies showed no effects of folate (Källén, 2007; Carmichael et al., 2009b; Brouwers 
et al., 2010) or iron supplementation (Morera et al., 2006; Brouwers et al., 2010) on hypospadias risk, 
although one study showed a reduced risk of folate (Ormond et al., 2009) and two others an increased 
risk of iron supplementation (North and Golding, 2000; Brouwers et al., 2007).

Maternal intrauterine DES exposure

In 2002, Klip et al. reported a 21 times increased hypospadias risk among sons of women exposed to 
diethylstilbestrol (DES) in utero in a cohort of women with fertility problems (Klip et al., 2002). 
Thereafter, other studies were consistent in showing an increased risk for sons of DES-daughters, 
although less strong (Palmer et al., 2005; Pons et al., 2005; Brouwers et al., 2006; Brouwers et 
al., 2010). This transgenerational effect may have been related to genetic or epigenetic changes in 
primordial oocytes, which were transmitted to the next generation, or in somatic cells of the DES-
daughter, resulting in disturbed hormonal balance in adult life (Klip et al., 2002). Another explanation 
would be that pathology of the DES-daughter’s reproductive structures interferes with normal foetal 
development (Brouwers et al., 2006).

Behavioural factors
Parental age

Women become pregnant at different ages but, overall, maternal and paternal age at time of conception did not seem to increase the risk of having a son with hypospadias (Akre et al., 1999; Weidner et al., 1999; Källén, 2002; Hussain et al., 2002; Aschim et al., 2004a; Sørensen et al., 2005a; Morera et al., 2006; Meyer et al., 2006; Brouwers et al., 2007; Akre et al., 2008; Materna-Kiryluk et al., 2009; Sun et al., 2009; Nassar et al., 2009; Ghirri et al., 2009; Lund et al., 2009; Brouwers et al., 2010). However, some studies reported a higher maternal age (Fisch et al., 2001; Hussain et al., 2002; Carmichael et al., 2003; Reefhuis and Honein, 2004; Porter et al., 2005; Carmichael et al., 2007; Fisch et al., 2009; Akin et al., 2011) or lower or higher paternal age (McIntosh et al., 1995; Materna-Kiryluk et al., 2009) to increase hypospadias risk.

Maternal diet

In 2000, North and Golding reported a five times increased risk of a hypospadias-affected son for women with a vegetarian diet (North and Golding, 2000), a finding that was confirmed in one study (Akre et al., 2008) but not in others (Brouwers et al., 2007; Ormond et al., 2009; Brouwers et al., 2010). However, all of these results were based on case-control studies with relatively few exposed cases and controls (<15) except for a study in England reporting no association in more than 75 cases and controls who were vegetarian (Ormond et al., 2009). The suggestion that an increased risk might be related to intake of phytoestrogens was refuted by a small study involving phytoestrogen-specific questionnaires that did not find an association (Pierik et al., 2004). Another dietary factor found to be associated with hypospadias in two small studies is the frequent consumption of fish, possibly associated with the bioaccumulation of contaminants in fish (Giordano et al., 2008; Giordano et al., 2010). However, a larger case-control study found a decreased hypospadias risk for frequent fish consumption (Akre et al., 2008).

Other lifestyle factors
Alcohol consumption during pregnancy was consistently found not to be associated with hypospadias (Hussain et al., 2002; Meyer et al., 2006; Brouwers et al., 2007). For maternal smoking, most studies showed no association (Akre et al., 1999; Källén, 2002; Hussain et al., 2002; Carmichael et al., 2005b; Morera et al., 2006; Meyer et al., 2006; Brouwers et al., 2007; Akre et al., 2008; Brouwers et al., 2010).

One small study found maternal cocaine use to be associated with hypospadias (Battin et al., 1995).

**Occupational factors**

**Exposure to pesticides**

Occupational exposures have been a major focus in hypospadias research, especially exposure to pesticides, with contradicting results. Paternal exposure to pesticides before pregnancy does not seem to be associated with hypospadias (Weidner et al., 1998; Brouwers et al., 2007; Nassar et al., 2009; Brouwers et al., 2010), although one small study reported a possibly increased risk (Giordano et al., 2008). In addition, an increased risk was found among farmers who were indicated as exposed to pesticides in a register-based study (Kristensen et al., 1997). Most studies showed no association with maternal occupational exposure to pesticides (Weidner et al., 1998; Vrijheid et al., 2003; Brouwers et al., 2007; Nassar et al., 2009; Brouwers et al., 2010; Morales-Suarez-Varela et al., 2011) but being involved in agricultural activities (Sun et al., 2009) or using insect repellents (Dugas et al., 2010) seemed to increase hypospadias risk in two studies. Maternal serum levels of dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethane (DDE) during pregnancy were not associated with hypospadias (Longnecker et al., 2002; Bhatia et al., 2005) but maternal serum hexachlorobenzene (HCB) concentrations approximately one year after birth were more often above the median of all subjects among hypospadias cases than among controls (Giordano et al., 2010).

**Other occupational exposures**

Boys conceived to mothers employed in the leather industry (García and Fletcher, 1998) and post-war to mothers who served in the Gulf war (Araneta et al., 2003) seemed to have a higher prevalence of hypospadias. Most other maternal occupational exposures were not associated with hypospadias,
although results for EDCs, heavy metals and phthalates vary, while exposure to hairspray increased the risk in one study (Vrijheid et al., 2003; Brouwers et al., 2007; Ormond et al., 2009; Nassar et al., 2009; Brouwers et al., 2010; Giordano et al., 2010; Morales-Suarez-Varela et al., 2011). For fathers, being a vehicle mechanic or manufacturer (Schnitzer et al., 1995; Irgens et al., 2000), police officer or fire fighter (Schnitzer et al., 1995), and occupational exposure to dusts from grinding metals (Brouwers et al., 2010) seemed to increase the risk of having a son with hypospadias. Results on heavy metals vary (Nassar et al., 2009; Morales-Suarez-Varela et al., 2011) but most other paternal occupational exposures were not associated with hypospadias (Brouwers et al., 2007; Nassar et al., 2009; Brouwers et al., 2010; Morales-Suarez-Varela et al., 2011).

Living environment

Results on living in rural or (sub)urban areas are contradictory (Sun et al., 2009; Nassar et al., 2009), whereas living close to a landfill site seemed to be associated with an increased hypospadias risk (Dolk et al., 1998). Maternal serum levels of polychlorinated biphenyls (PCBs) were elevated during pregnancies affected by hypospadias in two small studies but these results were not statistically significant (Carmichael et al., 2010; Giordano et al., 2010). Another study found marginally increased PCB levels in serum samples of women pregnant with a hypospadias-affected son, but the study samples were collected in the 1960s, when PCB exposure was substantially higher than nowadays (McGlynn et al., 2009). Maternal exposure to water disinfection by-products was also suggested to increase hypospadias risk but most studies provided little evidence for this association (Källén and Robert, 2000; Luben et al., 2008; Iszatt et al., 2011).

In one study, the prevalence of hypospadias seemed to be higher in areas of intensive pesticide use or in agricultural areas (Morera et al., 2006). Another study showed an increased risk of hypospadias for living in an area where diclofopmethyl was applied but a decreased risk for alachlor and permethrin, or for pesticide application in aggregate (Meyer et al., 2006).
In some older studies, a seasonal trend for hypospadias was identified (Wehrung and Hay, 1970; Roberts and Lloyd, 1973; Avellan, 1977), which was attributed to factors such as hours of daylight, climate or temperature, whereas more recent studies did not find seasonal variation (Skriver et al., 2004; Morera et al., 2006; Jin et al., 2010).
CONCLUSION

Most hypospadias cases have an unknown aetiology, which is likely to be a mix of monogenic and multifactorial forms, implicating both genes and environmental factors. Several mutations have been found that might cause hypospadias but most investigators are convinced that single mutations are not likely to be the cause for the majority of isolated hypospadias cases. Nevertheless, studies screening patients with hypospadias for single-gene defects found mutations in the genes WT1, SF1, BMP4, BMP7, HOXA4, HOXB6, FGF8, FGFR2, AR, HSD3B2, SRD5A2, ATF3, MAML1, MID1 and BNC2. Association studies found polymorphisms in FGF8, FGFR2, AR, HSD17B3, SRD5A2, ESR1, ESR2, ATF3, MAML1, DGKK, MID1, CYP1A1, GSTM1 and GSTT1 to be risk factors for hypospadias. In addition, gene expression studies identified CTGF, CYR61 and EGF as candidate genes.

Additional evidence for the involvement of genes can be derived from syndromes commonly associated with hypospadias, which were not reviewed in this article. For example, additional evidence for the involvement of WT1 comes from the fact that WT1 mutations cause syndromes such as Denys-Drash and Frasier syndromes, characterized by progressive nephropathy, intersex and predisposition to develop genitourinary tumours (Morrison et al., 2008). Male cases having hypospadias were reported for both syndromes (Sherbotie et al., 2000; Melo et al., 2002; Kaltenis et al., 2004). Syndromes which are commonly associated with hypospadias can also help in the identification of new candidate genes. One example is hand-foot-genital syndrome, which is caused by mutations in HOXA13 (Mortlock and Innis, 1997; Goodman and Scambler, 2001). HOxa13 mutant mice also exhibited hypospadias (Morgan et al., 2003) and expansion of a polyalanine tract in HOXD13 found in synpolydactyly families also seems to be associated with hypospadias (Goodman et al., 1997; Tüzel et al., 2007). Mutations in zinc finger E-box binding homeobox 2 (ZEB2) cause Mowat-Wilson syndrome, which is associated with hypospadias in more than 50% of affected males (Mowat et al., 2003; Zweier et al., 2005; Adam et al., 2006; Garavelli and Mainardi, 2007; Garavelli et al., 2009).
Additional candidate genes for hypospadias aetiology include genes for which mutations were described in case reports, such as \textit{CYP11A1} (Rubtsov \textit{et al.}, 2009), \textit{CYP17A1} (Sherbet \textit{et al.}, 2003) and \textit{HSD17B3} (Lee \textit{et al.}, 2007).

Animal studies also provide some additional candidate genes, such as the genes encoding the cell-surface molecules ephrins and their receptors, EPH receptor B2 (EphB2) and Ephrin-B2 (Efnb2) (Lorenzo \textit{et al.}, 2003; Dravis \textit{et al.}, 2004). \textit{EFNB2} has been suggested as the gene underlying genital malformations in patients with a 13q33-34 deletion (Garcia \textit{et al.}, 2006; Walczak-Sztulpa \textit{et al.}, 2008; Andresen \textit{et al.}, 2010).

In conclusion, many candidate genes have been suggested for hypospadias. Although some associations with hypospadias were found, none of these associations were replicated consistently, with the possible exception of \textit{DGKK}. Therefore, we suggest that a genome-wide association study using individual genotyping of a large group of cases and controls is the way forward to generate more knowledge about the genetic factors underlying isolated hypospadias. In addition, the novel exome or even whole-genome sequencing techniques generate new opportunities. Currently, the high costs make these techniques only suitable for identification of causes of monogenic forms of hypospadias but with falling prices, the techniques may also be applied to large cohorts of patients with isolated hypospadias in the future.

As for environmental factors, development of the male external genitalia is dependent on the balance between androgens and estrogens. The fact that maternal exposure to synthetic estrogens can induce hypospadias in murine models (Kim \textit{et al.}, 2004) and that antiandrogens acting as inhibitors of steroid hormone synthesis or AR antagonists can induce male reproductive abnormalities in animal models (Gray \textit{et al.}, 2001) suggests that EDCs have the potential to induce hypospadias. However, because of considerable species differences and markedly different estrogen levels in humans compared to rodent pregnancy, it is debatable whether EDCs also induce hypospadias in humans. Phthalates inhibit
steroidogenesis in the foetal rat testis but this does not occur \textit{in vitro} with human foetal Leydig cells (van Gelder \textit{et al}., 2010). The question remains as to whether exposure levels in humans are high enough to exert an effect on the occurrence of hypospadias. Given that even exposures to high levels of exogenous hormones, such as in case of hormonal stimulation used to induce pregnancy and use of oral contraceptives while pregnant, do not show consistent associations with hypospadias, we suggest that exogenous hormones and EDCs may not be as important in the aetiology of hypospadias as has previously been assumed.

The consistent association of hypospadias with low birthweight, maternal hypertension and preeclampsia suggests that placental insufficiency may be a major risk factor for hypospadias, possibly through inadequate provision of hCG to the foetus. A role for endogenous hormones is suggested by free estradiol levels linked to high maternal BMI, primiparity and multiple pregnancies that appear to contribute to susceptibility to hypospadias.

In addition, maternal intrauterine DES exposure, use of anti-epileptic drugs, pre-existing diabetes, prolonged TTP and pregnancies resulting from ICSI have been associated with hypospadias in most studies. Other potential environmental risk factors were not, or not consistently, associated with hypospadias or studied too infrequently to draw conclusions.

In our opinion, the lack of replication of results for both genetic and environmental factors associated with hypospadias may be related to subtle isolated effects of factors that may have larger influences in combination with other factors (e.g. gene-gene or gene-environment interactions). While a different genetic background of a population may affect its vulnerability to an environmental exposure, different environmental exposures may influence the effect of a genotype. Therefore, we think that the challenges for future research in disentangling the pathogenesis of hypospadias mainly lie in studies focussing on gene-gene or gene-environment interactions.
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### TABLE I. Mutations found in studies screening candidate genes in groups of patients with hypospadias

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<tr>
<th>Gene</th>
<th>Locus</th>
<th>N cases</th>
<th>N controls</th>
<th>Ethnicity</th>
<th>Mutation</th>
<th>Phenotype</th>
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<td><strong>WT1</strong></td>
<td>11p13</td>
<td>35 - different ethnicities</td>
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<td>N130N A131T S159S</td>
<td>• penoscrotal, micopenis</td>
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<td>• showed that variant impairs transcriptional activity</td>
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<td>90</td>
<td>276</td>
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<td>• penile</td>
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<td>(Wang et al., 2004)</td>
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<td><strong>WTAP</strong></td>
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<td>60a</td>
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<td>Q107X c.103-3C&gt;A E11X</td>
<td>• penoscrotal, bilateral cryptorchidism</td>
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<td>• penoscrotal, micropenis, bilateral cryptorchidism</td>
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<td>H207D R223H H251Y</td>
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<td>R305C Q199Q c.1465T&gt;A c.1567A&gt;G</td>
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<td>G129C S290C</td>
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<td>M186T&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>• Swedish patient</td>
<td>(Beleza-Meireles et al., 2007c)</td>
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**Masculinisation stage of development**

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<td>G566V</td>
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<td>hemi</td>
<td>• family history suggested familial component</td>
<td>(Alléra et al., 1994)</td>
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<td>P546S</td>
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<td>(Sutherland et al., 1996)</td>
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<td>(Nordenskjöld et al., 1999)</td>
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<td></td>
<td>• severe hypospadias, cryptorchidism, bifid scrotum</td>
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<td>21</td>
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TABLE I. (continued) Mutations found in studies screening candidate genes in groups of patients with hypospadias

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<tr>
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<th>Heterozygosity</th>
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<td>(Wang et al., 2004)</td>
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<td>R840H</td>
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<td>I842T</td>
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<td>R855H</td>
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<td>• mother heterozygous, uncle has same mutation and phenotype, variant previously described in 2 brothers with perineal hypospadias, bilateral cryptorchidism and micropenis</td>
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<td>L859L</td>
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<td>hetero</td>
<td>• mother and brother heterozygous, showed that variant reduces enzyme activity</td>
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<td>S284R</td>
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<td>A238A</td>
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<td>T259T</td>
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<td>N controls</td>
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<td>T320T</td>
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<td>• father heterozygous, has bifid preputium and a wide meatus</td>
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<td>90 ²</td>
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<td>• variant previously described in patient with scrotal hypospadias, bifid scrotum and micropenis and shown by others to inhibit NADPH binding, reduce testosterone binding, and reduce enzyme half-life</td>
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<td>• variant previously described in 2 patients with perineoscrotal hypospadias, micropenis and cryptorchidism and shown by others to reduce enzyme activity</td>
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<td>Q6X</td>
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<td>• father heterozygous, 2 brothers of patient have same genotype and phenotype as patient</td>
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<td>• Japanese patient</td>
<td>(Fukami et al., 2006)</td>
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<td>hemi</td>
<td>• Japanese patient</td>
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<td>99o</td>
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<td>?</td>
<td>Q529KD686D</td>
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<td>hemi</td>
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<td>(Chen et al., 2010)</td>
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### TABLE I. (continued) Mutations found in studies screening candidate genes in groups of patients with hypospadias

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<th>N controls</th>
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<td>• penoscrotal, hypertelorism</td>
<td>hemi</td>
<td>• mother heterozygous, brother has same mutation and phenotype, variant previously described in Opitz syndrome</td>
<td>(Zhang et al., 2011)</td>
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<td>K560R</td>
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<td>hemi</td>
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<td>(El Houate et al., 2007)</td>
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<td>(Bhoj et al., 2011)</td>
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</table>

All studies included in this table screened patients with hypospadias for mutations in specific genes. Most studies checked whether mutations were present in healthy controls. The table includes only exonic (including 3'-UTR and splice acceptor sites) mutations that were not found in healthy controls, were not previously reported polymorphisms, and were not described as a polymorphism by the authors of the article. Results from functional analyses, either performed by the study reporting the mutation or performed by earlier studies and referred to by the study reporting the mutation, are included in the table. Most studies included patients with different degrees of hypospadias or information about phenotype was not reported. Most studies excluded syndromal patients, but did not exclude patients with cryptorchidism, micropenis, bifid scrotum, or other associated anomalies, or information about associated anomalies was not reported. Most studies did not exclude patients with affected relatives or information about affected relatives was not reported. Family members carrying the same mutation were unaffected, unless indicated differently.

N, number; hetero, heterozygous; homo, homozygous; hemi, hemizygous; PAIS, partial androgen insensitivity syndrome; *only DSD (disorders of sex development) patients with severe penile to penoscrotal hypospadias included; †splice acceptor site; ‡only patients with at least one affected relative included; §3'-UTR; ‖synonymous variant, not mentioned in which amino acid; ‡variant is known as rs755793, but with allele frequency of 0% in Caucasians; §only patients with severe hypospadias included; †only patients without other genitourinary abnormalities included; ‡only patients with severe hypospadias or a familial form included; †only patients from families contributing most to a linkage peak in the vicinity of HSD17B3 included; †patients with cryptorchidism, intersex condition, or endocrine abnormalities excluded; †only patients with elevated testosterone/DHT ratios without mutations in AR or SRD5A2 included;
variant was later found in 2 more patients and in 2 controls (Chen et al., 2010); variant was later found in 3 more patients and in 1 control (Chen et al., 2010); only sporadic patients included; only patients with distal hypospadias included.
**TABLE II.** Genetic association results for hypospadias

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>SNP</th>
<th>N cases</th>
<th>N controls</th>
<th>Controls</th>
<th>Ethnicity</th>
<th>Genotypes / alleles associated with increased risk (P &lt; 0.05)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FGF8</strong></td>
<td>10q24</td>
<td>rs3218238 or rs3218233&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>A allele&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(Beleza-Meireles et al., 2007c)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>FGFR2</strong></td>
<td>10q26</td>
<td>c.382+52→G&lt;sup&gt;e&lt;/sup&gt; c.550+27T&gt;C&lt;sup&gt;e&lt;/sup&gt; c.727+180T&gt;G</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>G allele of c.382+52→G&lt;sup&gt;g&lt;/sup&gt; C / T allele of c.550+27T&gt;C&lt;sup&gt;g&lt;/sup&gt; G allele of c.727+180T&gt;G&lt;sup&gt;g&lt;/sup&gt;</td>
<td>(Beleza-Meireles et al., 2007c)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td>Xq12</td>
<td>CAG repeat</td>
<td>78&lt;sup&gt;f&lt;/sup&gt;</td>
<td>425</td>
<td>anonymous females</td>
<td>?</td>
<td>longer repeat</td>
<td>(Lim et al., 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>100</td>
<td>boys with short stature and normal external genitalia and fertile males</td>
<td>Japanese</td>
<td>no association</td>
<td>(Muroya et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>210</td>
<td>males from military service, no history of hypospadias or cryptorchidism</td>
<td>cases are Caucasian, controls have Swedish mothers</td>
<td>no association</td>
<td>(Aschim et al., 2004b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GGN repeat</td>
<td>92</td>
<td>190</td>
<td>fertile males</td>
<td>Iranian</td>
<td>no association</td>
<td>(Radpour et al., 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>210</td>
<td>males from military service, no history of hypospadias or cryptorchidism</td>
<td>cases are Caucasian, controls have Swedish mothers</td>
<td>longer repeat&lt;sup&gt;g&lt;/sup&gt;</td>
<td>(Aschim et al., 2004b)</td>
</tr>
<tr>
<td><strong>FKBP4</strong></td>
<td>12p13.33</td>
<td>rs1062478 rs3021522</td>
<td>333</td>
<td>380</td>
<td>voluntary blood donors</td>
<td>different ethnicities</td>
<td>no association</td>
<td>(Beleza-Meireles et al., 2007a)</td>
</tr>
<tr>
<td><strong>HSD17B3</strong></td>
<td>9q22</td>
<td>rs4743709 rs2066476 rs2066474 rs2066480 rs2066479</td>
<td>89&lt;sup&gt;h&lt;/sup&gt;</td>
<td>291</td>
<td>male newborns without malformations</td>
<td>Japanese</td>
<td>A allele of rs2066479 AA genotype of rs2066479</td>
<td>(Sata et al., 2010)</td>
</tr>
<tr>
<td><strong>SRD5A2</strong></td>
<td>2p23</td>
<td>rs9282858</td>
<td>81&lt;sup&gt;i&lt;/sup&gt;</td>
<td>100+</td>
<td>normal controls</td>
<td>different ethnicities</td>
<td>T allele</td>
<td>(Silver and Russell, 1999)&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
TABLE II. (continued) Genetic association results for hypospadias

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>SNP</th>
<th>N cases</th>
<th>N controls</th>
<th>Controls</th>
<th>Ethnicity</th>
<th>Genotypes / alleles associated with increased risk ($P &lt; 0.05$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRD5A2</td>
<td>2p23</td>
<td>rs523349</td>
<td>90</td>
<td>87</td>
<td>normal males</td>
<td>Chinese</td>
<td>G allele CG and GG genotypes</td>
<td>(Wang et al., 2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>158</td>
<td>96</td>
<td>unaffected persons</td>
<td>cases have different ethnicities, controls are Caucasian</td>
<td>G allele CG and GG genotypes</td>
<td>(Thai et al., 2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>89</td>
<td>281</td>
<td>male newborns without malformations</td>
<td>Japanese</td>
<td>CG genotype *</td>
<td>(Sata et al., 2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>620</td>
<td>596</td>
<td>unaffected males</td>
<td>Caucasian</td>
<td>no association</td>
<td>(van der Zanden et al., 2010b)</td>
</tr>
</tbody>
</table>

**Other genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>SNP</th>
<th>N cases</th>
<th>N controls</th>
<th>Controls</th>
<th>Ethnicity</th>
<th>Genotypes / alleles associated with increased risk ($P &lt; 0.05$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>6q25.1</td>
<td>rs6932902</td>
<td>43</td>
<td>135</td>
<td>boys with short stature and normal external genitalia and fertile males</td>
<td>Japanese</td>
<td>A allele AA genotype</td>
<td>(Watanabe et al., 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>620</td>
<td>596</td>
<td>unaffected males</td>
<td>Caucasian</td>
<td>A allele</td>
<td>(van der Zanden et al., 2010b)</td>
</tr>
<tr>
<td></td>
<td>TA repeat</td>
<td>rs1801132</td>
<td>90</td>
<td>94</td>
<td>voluntary blood donors</td>
<td>different ethnicities</td>
<td>no association</td>
<td>(Beleza-Meireles et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>rs2234693</td>
<td>rs9340799</td>
<td>59</td>
<td>286</td>
<td>boys without malformations</td>
<td>Japanese</td>
<td>A allele of rs9340799</td>
<td>(Ban et al., 2008)</td>
</tr>
<tr>
<td>ESR2</td>
<td>14q23.2</td>
<td>CA repeat</td>
<td>90</td>
<td>94</td>
<td>voluntary blood donors</td>
<td>different ethnicities</td>
<td>longer repeat</td>
<td>(Beleza-Meireles et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>354</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>longer repeat</td>
<td>(Beleza-Meireles et al., 2007b)</td>
</tr>
<tr>
<td></td>
<td>rs1887994</td>
<td>rs1256040</td>
<td>rs1256062</td>
<td>rs10483774</td>
<td>rs1271572</td>
<td>354</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
</tr>
<tr>
<td></td>
<td>rs944050</td>
<td></td>
<td>90</td>
<td>94</td>
<td>voluntary blood donors</td>
<td>different ethnicities</td>
<td>AG genotype</td>
<td>(Beleza-Meireles et al., 2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59</td>
<td>286</td>
<td>boys without malformations</td>
<td>Japanese</td>
<td>AG genotype</td>
<td>(Ban et al., 2008)</td>
</tr>
</tbody>
</table>
**TABLE II. (continued) Genetic association results for hypospadias**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>SNP</th>
<th>N cases</th>
<th>N controls</th>
<th>Controls</th>
<th>Ethnicity</th>
<th>Genotypes / alleles associated with increased risk ((P &lt; 0.05))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESR2</strong></td>
<td>14q23.2</td>
<td>rs2987983</td>
<td>354</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>G allele GG genotype</td>
<td>(Beleza-Meireles et al.,2007b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1256049</td>
<td>620</td>
<td>596</td>
<td>unaffected males</td>
<td>Caucasian</td>
<td>AG genotype</td>
<td>(van der Zanden et al.,2010b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4986938</td>
<td>51</td>
<td>186</td>
<td>control males from military service without genital anomalies and with sperm concentrations &gt;5(\times)10⁶ spermatozoa/ml cases are Caucasian, controls have Swedish parents</td>
<td>no association</td>
<td></td>
<td>(Aschim et al.,2005)</td>
</tr>
<tr>
<td><strong>ATF3</strong></td>
<td>1q32.3</td>
<td>rs11119982</td>
<td>330</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>C allele CC genotype</td>
<td>(Beleza-Meireles et al.,2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2137424</td>
<td>620</td>
<td>596</td>
<td>unaffected males</td>
<td>Caucasian</td>
<td>T allele TT and CT genotypes</td>
<td>(van der Zanden et al.,2010b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3125289 rs1877474</td>
<td>330</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
<td>different ethnicities</td>
<td>T allele of rs3125289 TT genotype of rs3125289 T allele of rs1877474 TT genotype of rs1877474 strongest association for combination of risk alleles: rs3125289 (T), rs1877474 (T) and rs11119982 (C)</td>
<td>(Beleza-Meireles et al.,2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10735510 rs9429889 rs12070345 rs10475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAML1</strong></td>
<td>Xq28</td>
<td>rs61740566</td>
<td>370</td>
<td>380</td>
<td>healthy voluntary blood donors</td>
<td>?</td>
<td>no association</td>
<td>(Chen et al.,2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs41313406 rs2073043</td>
<td>370</td>
<td>418</td>
<td>male healthy voluntary blood donors</td>
<td>?</td>
<td>T allele of rs41313406 G allele of rs2073043</td>
<td>(Chen et al.,2010)</td>
</tr>
<tr>
<td><strong>DGKK</strong></td>
<td>Xp11.22</td>
<td>rs1934179 rs7063116</td>
<td>436</td>
<td>449</td>
<td>healthy control males</td>
<td>Caucasian</td>
<td>A allele of rs1934179 A allele of rs7063116</td>
<td>(van der Zanden et al.,2010a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1934179 rs7063116</td>
<td>133</td>
<td>133</td>
<td>mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2073043</td>
<td>266</td>
<td>402</td>
<td>male healthy voluntary blood donors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MID1</strong></td>
<td>Xp22</td>
<td>rs16986145</td>
<td>366</td>
<td>405</td>
<td>male controls</td>
<td>?</td>
<td>A allele</td>
<td>(Zhang et al.,2011)</td>
</tr>
<tr>
<td><strong>CYP1A1</strong></td>
<td>15q24.1</td>
<td>? gene deletion gene deletion</td>
<td>31</td>
<td>64</td>
<td>mothers of boys without any malformation</td>
<td>Japanese</td>
<td>heterozygous CYP1A1 genotype</td>
<td>(Kurahashi et al.,2005)</td>
</tr>
<tr>
<td><strong>GSTM1</strong></td>
<td>1p13.3</td>
<td>? gene deletion gene deletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTT1</strong></td>
<td>22q11.23</td>
<td>? gene deletion gene deletion</td>
<td>80</td>
<td>120</td>
<td>age-matched boys</td>
<td>?</td>
<td>concomitant deletion of GSTM1 and GSTT1</td>
<td>(Yadav et al.,2011)</td>
</tr>
</tbody>
</table>

#### Additional Notes
- **Ethnicity**: Genetic association results are provided for different ethnicities where applicable.
- **Controls**: Details on controls include number of cases and controls, and specific criteria for inclusion (e.g., healthy voluntary blood donors).
- **References**: Literature references are cited for the genetic association results, including gene names and specific SNP identifiers.

### Definitions
- **Genotypes / alleles associated with increased risk (\(P < 0.05\))**: Indicate the genotypes or alleles that were found to be associated with an increased risk of hypospadias.
- **Control criteria**: Detailed criteria for control groups, such as military service without genital anomalies and sperm concentrations greater than 5\(\times\)10⁶ spermatozoa/ml.
Most studies were association studies with a case-control design. Most studies included patients with different degrees of hypospadias. Most studies excluded syndromal patients, but did not exclude patients with cryptorchidism, micropenis, bifid scrotum, or other associated anomalies or information about associated anomalies was not reported. Most studies did not exclude patients with affected relatives or information about affected relatives was not reported. Deviations from these statements are included in the specified footnotes.

N, number;  the SNP reported in the text was different from the SNP reported in the table; all patients have at least one affected relative; this SNP was found in heterozygous form in 3 patients, while it was not found in controls; this was not an association study, but a study screening FGF8 and FGFR2 for mutations; these SNPs were found in heterozygous form in 1 patient, while they were not found in controls. For c.550+27T>C it is not clear whether T or C is the risk allele because the SNP reported in the text was different from the SNP reported in the table (c.550+27T>C and c.550+27C>T); undermasculinized patients, most of them with perineoscrotal openings and unfused or partially fused scrotum; only penile patients have longer repeats; patients with affected family members excluded; patients with cryptorchidism, intersex condition, or endocrine abnormalities excluded; this was not an association study, but a study screening SRD5A2 for mutations. This SNP was found in homozygous form in 2 patients and in heterozygous form in 3 patients, while it was not found in controls. In another study, this SNP was found in 1 out of 37 patients, but as that study did not genotype controls to perform an association analysis, it was not included in the table (Thai et al., 2005); only associated with severe hypospadias; this was an association study with a case-parent triad design analyzed using the transmission disequilibrium test; SNP tagged the ‘AGATA’ haplotype of rs926779, rs3020364, rs6932902, rs3020371 and rs3020375; all six patients with this genotype had affected family members, and the SNP was inherited from the affected line twice; associated with decreased risk; only patients with anterior and middle hypospadias included; this part of the study was an association study with a case-parent triad design analyzed using the transmission disequilibrium test, but as this in an X-chromosomal SNP, only mothers were taken into account; this was a genome wide association study with a case-control design, suggesting more associations with hypospadias than reported in this table; four cases were familial. Two affected relatives carried the variant and one did not. Five of the nine cases with the variant had at least one parent born in North Africa, where the A allele is more prevalent; mothers of patients with hypospadias.
Table III. Clinical, behavioural, occupational and environmental factors investigated for their association with hypospadias in more than one study.

<table>
<thead>
<tr>
<th>FACTORS FREQUENTLY INVESTIGATED</th>
<th>FACTORS NOT FREQUENTLY INVESTIGATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors consistently associated with hypospadias</td>
<td>Factors consistently not associated with hypospadias</td>
</tr>
<tr>
<td>Low birthweight / being small for gestational age</td>
<td>Gestational diabetes</td>
</tr>
<tr>
<td>Placental insufficiency</td>
<td>Maternal alcohol consumption</td>
</tr>
<tr>
<td>Maternal hypertension</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td></td>
</tr>
<tr>
<td>Maternal intrauterine diethylstilbestrol exposure</td>
<td></td>
</tr>
</tbody>
</table>

Factors with consistent results in all studies

Factors with consistent results in most studies

Factors showing inconsistent results

Factors associated with hypospadias in most studies

Factors not associated with hypospadias in most studies

Factors that seem to be associated with hypospadias

Factors that do not seem to be associated with hypospadias

Factors showing inconsistent results

<table>
<thead>
<tr>
<th>Factors associated with hypospadias</th>
<th>Factors not associated with hypospadias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of ICSI</td>
<td>Use of oral contraceptives during pregnancy</td>
</tr>
<tr>
<td>Prolonged time-to-pregnancy</td>
<td>Use of IVF</td>
</tr>
<tr>
<td>High maternal BMI</td>
<td>Use of hormonal stimulation to induce pregnancy</td>
</tr>
<tr>
<td>Primiparity</td>
<td>Maternal medication use:</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>Loratadine</td>
</tr>
<tr>
<td>Pre-existing maternal diabetes</td>
<td>Maternal folate supplementation</td>
</tr>
<tr>
<td>Maternal medication use:</td>
<td>Paternal age</td>
</tr>
<tr>
<td>Anti-epileptic drugs</td>
<td>Maternal smoking</td>
</tr>
<tr>
<td>Maternal iron supplementation</td>
<td>Maternal exposure to water disinfection by-products</td>
</tr>
<tr>
<td>Maternal age</td>
<td></td>
</tr>
<tr>
<td>Maternal vegetarian diet</td>
<td></td>
</tr>
<tr>
<td>Maternal fish consumption</td>
<td></td>
</tr>
<tr>
<td>Maternal and paternal exposure to pesticides</td>
<td></td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>Maternal occupational exposure to:</td>
</tr>
<tr>
<td>Maternal age</td>
<td>Endocrine disruptors</td>
</tr>
<tr>
<td>Maternal iron supplementation</td>
<td>Heavy metals</td>
</tr>
<tr>
<td>Maternal and paternal occupational exposures</td>
<td>Phthalates</td>
</tr>
<tr>
<td>Maternal thyroid disease</td>
<td>Maternal serum levels of polychlorinated biphenyls</td>
</tr>
<tr>
<td>Fever during first trimester of pregnancy</td>
<td>Seasonal trend</td>
</tr>
<tr>
<td>Father being a vehicle mechanic or manufacturer</td>
<td></td>
</tr>
<tr>
<td>Early age at menarche</td>
<td>Use of progestogens / progestins for threatened abortion</td>
</tr>
<tr>
<td>Maternal thyroid disease</td>
<td>Paternal occupational exposure to heavy metals</td>
</tr>
<tr>
<td>Fever during first trimester of pregnancy</td>
<td>Living in rural or urban areas</td>
</tr>
</tbody>
</table>
FIGURES

Figure I  
Hypospadias subgroups

Figure II  
Simple schematic drawing of the normal embryology of the human male external genitalia, which is disturbed in case of hypospadias development.
Figure III  Steroidogenesis in the mitochondrion (top) and smooth endoplasmic reticulum (bottom) of the foetal Leydig cell
REFERENCE LIST


24. Bánhidy F, Puhó EH, and Czeizel AE. Efficacy of medical care of epileptic pregnant women based on the rate of congenital abnormalities in their offspring. *Congenit Anom (Kyoto)* 2011;51;34-42.


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