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REVIEW ARTICLE

Is hypospadias a genetic, endocrine or environmental disease, or still an unexplained malformation?

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Summary

Hypospadias is one of the most frequent genital malformations in the male newborn and results from an abnormal penile and urethral development. This process requires a correct genetic programme, time- and space-adapted cellular differentiation, complex tissue interactions, and hormonal mediation through enzymatic activities and hormonal transduction signals. Any disturbance in these regulations may induce a defect in the virilization of the external genitalia and hypospadias. This malformation thus appears to be at the crossroads of various mechanisms implicating genetic and environmental factors. The genes of penile development (HOX, FGF, Shh) and testicular determination (WT1, SRY) and those regulating the synthesis [luteinizing hormone (LH) receptor] and action of androgen (5α reductase, androgen receptor) can cause hypospadias if altered. Several chromosomal abnormalities and malformative syndromes include hypospadias, from anterior to penoscrotal forms. More recently, CXorf6 and ATF3 have been reported to be involved. Besides these genomic and hormonal factors, multiple substances found in the environment can also potentially interfere with male genital development because of their similarity to hormones. The proportion of hypospadias cases for which an aetiology is detected varies with the authors but it nevertheless remains low, especially for less severe cases. An interaction between genetic background and environment is likely.

Introduction

Hypospadias is the most frequent genital malformation in the male newborn and estimates of its prevalence range from three to eight cases per 1000 male births (Paulozzi *et al.*, 1997; Carmichael *et al.*, 2003; CDCP, 2004). Hypospadias is defined as a midline fusion defect of the male urethra which results in a misplaced urethral meatus. This malformation is usually corrected surgically when the infant is between 6 and 24 months, depending on the team and country. It may require endocrine management, as well, especially for the most severe forms and for patients with other genital malformations.

Normal penile and urethral development begins in the sixth week of gestation with the formation of the urogenital sinus. By the end of the 16th week, the penile urethra has tubularized and the glanular urethra has started to form. This process of differentiation is initially directed under the guidance of maternal human chorionic gonadotropin (HCG) stimulation of the foetal gonads to produce testosterone and its 5α-reduced form, dihydrotestosterone (DHT) (Baskin, 2000). The process requires a correct genetic programme, time- and space-adapted cellular differentiation, complex tissue interactions, and hormonal mediation through enzymatic activities and hormonal transduction signals.

The aetiology of this frequent malformation has not been elucidated despite intensive investigation. Several authors reported increasing trends in its birth prevalence from the 1960s to the 1990s (Czeizel *et al.*, 1986; Paulozzi *et al.*, 1997; Canning, 1999), with exogenous factors (environmental) as suspected causes. In addition, hypospadias can be considered as an incomplete virilization of the genital tubercle related to insufficient development of the tissues forming the ventral aspect of the penis (Mouriquand & Mure, 2001). The role of foetal

androgens is crucial, especially during the first trimester of pregnancy, but any environmental factor with anti-androgenic activity can alter the complex regulation of male sex differentiation during foetal life.

Hypospadias thus appears at the crossroads of genetic, endocrine and environmental mechanisms. We here propose to review these mechanisms separately, as they may interact or remain independent (Table 1).

Genetics of hypospadias

Genetic factors play a crucial role in the occurrence of this early developmental defect, in both the isolated (non-syndromic) and syndromic forms. Mutations in the genes affecting penile development and those implicated in the determination of male gonad and the biosynthesis or cell action of androgens have been identified in various forms of hypospadias.

Table 1 Aetiologies of hypospadias

Abnormality of testicular determination
Pure
Duplication of Dax 1
Duplication of WnT4
Mutation of gene DMRT1 and 2
Chromosomal deletion (2q32…)
Associated with other abnormalities
Mutation of SF1 gene (±no adrenal development)
Mutation of WT1 gene (abnormality of renal morphogenesis and function)
Denys-Drash syndrome (Wilms' tumor)
WAGR syndrome (Wilms' tumor, aniridia, mental retardation)
Frasier syndrome (female phenotype)
Mutation of SOX9 gene (bone malformation, IUGR)
'Mixed' with karyotype 46,XY, 46,X0
Disorder of sex development (often 46,XX)
Disorder of androgen biosynthesis
Abnormality in cholesterol biosynthesis ('deficient in' 7 dehydro-cholesterol reductase = SLO syndrome)
Abnormality in testosterone biosynthesis
Mutation of LH receptor, Leydig cell aplasia
Mutation of LH (micropenis)
Adrenal hyperplasia (mutation of STAR gene)
'Deficient in' 3β HSD deshydrogenase (17 hydroxy pregnenolone increased)
'Deficient in' 17α alpha hydroxylase (17–20 Desmolase) = mutation of CYP 17
'Deficient in' 17β HSD deshydrogenase, type 3 (increased Δ4 androstenedione)
Androgen resistance
Abnormality of cellular and molecular action of ('deficient in' 5 α reductase type 2)
Androgen insensitivity (increased or normal plasma androgen levels)
With AR mutation
Partial insensitivity: hypospadias, micropenis
Without AR mutation
Isolated
Associated with malformations
Associated with IUGR
Environment (endocrine disruptor/chemical pollutants)
Idiopathic

Arguments for a genetic aetiology of hypospadias

Familial clustering, defined as patients with one or more first-, second- or third-degree relatives also affected with hypospadias, is seen in about 10% of cases (Chen & Woolley, 1971; Czeizel *et al.*, 1979; Kallen *et al.*, 1986; Fredell *et al.*, 2002). The recurrence risk in the male siblings of an affected patient is about 15% and, conversely, the incidence in fathers of a child with hypospadias is 7% (Bauer *et al.*, 1979; Stoll *et al.*, 1990; Asklund *et al.*, 2007). The risk of recurrence depends on the severity of the hypospadias and the more proximal the malformation, the higher the risk is for the next male sibling (Bauer *et al.*, 1979). Segregation analyses have suggested that hypospadias might be due to monogenic effects in a small proportion of families, whereas a multifactorial mode of inheritance is assumed to be more likely in the majority of families (Harris & Beaty, 1993; Fredell *et al.*, 2002).

Genes coding for non-endocrine-related morphogenetic factors

These genes are in fact implicated in the development of the phallus:

1 *Homeobox genes (HOX)*. HOXA and HOXD genes are expressed in the foetal urogenital structures. Knock-out of these genes in mice induces a malformation in the external genitalia: loss of function in both HOXA13 genes induces an agenesis of the genital tubercle, and heterozygosity is associated with a defect in penile development and patterning (Morgan *et al.*, 2003). Similarly, mutations of HomeoboxA3 (HOXA13) have been reported in humans with hand–foot-genital syndrome (HFGS), in which small hands, malformed thumbs with flat thenar eminence, small big toe and short first metacarpal and phalanx are associated with genital abnormalities, including hypospadias in males (Mortlock & Innis, 1997; Frisen *et al.*, 2003). HOXA13 is essential for the normal expression of fibroblast growth factor (FGF) 8 and bone morphogenetic protein 7 in the developing urethral epithelium in mice. It also acts in androgen receptor expression and mediates glans vascularization (Mouriquand & Mure, 2001).

2 FGF genes also participate in the development of genital structures in mice (Petiot *et al.*, 2005) and knock-out of FGF10 is associated with hypospadias (Yucel *et al.*, 2004). In humans, the FGF family, especially FGF8,

FGF10 and FGFR2, is suspected to increase the risk of hypospadias (Beleza-Meireles *et al.*, 2007).

3 Other genes are implicated in the interactions between mesenchyme and urothelium. Sonic Hedgehog (Shh) in mice is expressed in the endodermally derived urethral plate epithelium situated along the ventral side of the genital tubercle and is required for outgrowth and patterning of the genital tubercle (Digilio *et al.*, 2003; Yucel *et al.*, 2004). Mice with a targeted deletion of Shh have penile and clitoral agenesis, consistent with the crucial role of Shh in genital development (Haraguchi *et al.*, 2001; Perriton *et al.*, 2002). No mutations have yet been reported in children with hypospadias.

Genes or chromosomal aberrations leading to testicular dysgenesis

Severe abnormalities in testis development classically cause complete (pure) gonadal dysgenesis (Swyer syndrome) with marked underandrogenization and persistent Mullerian structures. However, gonadal dysgenesis can be viewed as a spectrum of disorders, with partial forms associated with normal Mullerian regression and varying degrees of testicular descent and external malformation, such as hypospadias. Thus, milder loss of function mutations in established testis determining/promoting factors can all present with hypospadias.

Heterozygous mutations of WT1 (Wilms Tumour 1 gene) are associated with severe hypospadias and other genital abnormalities. In humans and mice, WT1 is implicated in male gonadal determination and its knock-out in mice induces bilateral renal agenesis, anorchia and defective genital tubercle development (van Heyningen *et al.*, 1990; Pritchard-Jones *et al.*, 1990; Pelletier *et al.*, 1991a,b; Shimamura *et al.*, 1997; Gao *et al.*, 2006; Kohler *et al.*, 2007). In humans, its mutations are associated with the syndromes described below (Kaltenis *et al.*, 2004).

Mutations in steroidogenic factor 1 (SF1) have yet to be identified as causes of isolated hypospadias.

SOX9, DMRT1 and GATA4 encode transcription factors acting immediately before the differentiation of the gonad into testis. Mutations of these genes may be associated with male disorders of sex differentiation (DSD), including severe hypospadias, often associated with testicular dysgenesis (Huang *et al.*, 1999; Wang *et al.*, 2004; Leipoldt *et al.*, 2007; Maciel-Guerra *et al.*, 2008). SOX9 may also be duplicated on a rearranged chromosome 17, which could explain the occurrence of penoscrotal hypospadias in patients with mosaicisms 46,XX and 46,XX d17 (Huang *et al.*, 1999). Last, the observation of 46,XX male hypospadiac patients with no detectable SRY or SOX9 suggests the existence of other virilizing genes.

Gonosomal abnormalities are also detected in about 7% of patients with hypospadias (Moreno-Garcia & Miranda, 2002). They include Klinefelter's syndrome, 47,XXY (Moriyama *et al.*, 1988), 48,XXYY (Neugebauer *et al.*, 1991) and various mosaicisms, e.g. 45,X/46,XY, which is a relatively common chromosomal abnormality known as mixed gonadal dysgenesis (Telvi *et al.*, 1999), 45,X/46,XYq- (Mailhes *et al.*, 1979), 45,X/46,X,idic(Yp) (Raff *et al.*, 2000), 45,X/69,XXY (Quigley *et al.*, 2005). Abnormal genital development in these patients may be related to a dosage effect of the SRY gene (Sinisi *et al.*, 2003).

Genes driving to isolated androgen synthesis or action defects

Genes driving to androgen synthesis defects

Whereas early genital development is controlled by a genetic program that operates prior to the production of steroid hormones, the second phase of penile development requires exposure to an androgen, either testosterone or DHT (Abney, 1999). Androgenic steroids, synthesized by the Leydig cells of the testes, are first seen just prior to the onset of androgen-induced genital differentiation. 5α-reductase type 2, an enzyme that converts testosterone to 5α-DHT, is highly expressed in the mesenchymal stroma surrounding the urethra (Kim *et al.*, 2002). Mutations of 5α-reductase have been identified in severe variants of hypospadias in combination with other genital abnormalities (Ocal *et al.*, 2002; Wang *et al.*, 2004; Nicoletti *et al.*, 2005). Conversely, the V89 allele in the SRD5A2 gene reduces the risk of hypospadias (Thai *et al.*, 2005).

Other defects in the androgen synthesis pathway are secondary to an abnormality in Leydig cell development or an enzymatic defect in testosterone synthesis. These defects are characterized by low concentrations of plasma testosterone in the neonatal period.

- Mutations of the LH receptor (Leydig cells hypoplasia) are associated with hypospadias and micropenis. Testosterone secretion is dramatically low and contrasts with higher values of LH in early life (Huhtaniemi & Alevizaki, 2006).
- A deficit in 3β-hydroxysteroid-deshydrogenase induces a testicular and adrenal block which is autosomal and recessive. Diagnosis is based on the association of hypospadias and adrenal insufficiency and an increase in dehydroepiandrosterone (DHEA) and 17-hydroxypregnenolone (Perrone *et al.*, 1985; Codner *et al.*, 2004).
- A defect in 17-hydroxysteroid-reductase induces a testicular block (autosomal and recessive) by altering the final step in testosterone synthesis. A marked increase in Δ4 androstenedione with low testosterone despite an HCG test allows the diagnosis. If the diagnosis is missed in the neonatal period, the patient presents with viriliza-

tion at the time of puberty (Mendonca *et al.*, 2000; Lee *et al.*, 2007).

- Rare defects are also described in steroidogenic acute regulatory protein (STAR) and CYP11A1 (P450scc), which usually cause a salt-losing adrenal phenotype and more severe underandrogenization, although in rare cases hypospadias may theoretically be the presenting feature of these conditions. Combined 17a-hydroxylase/17,20-lyase deficiency (or isolated 17,20-lyase deficiency) or P450 oxidoreductase deficiency can present with varying degrees of hypospadias or micropenis.

Overall, endocrine investigation confirms the aetiology of hypospadias as a defect in androgen synthesis in 20% of cases (Rey *et al.*, 2005).

Genes driving to androgen action defects

Mutations in the androgen receptor gene (AR) have also been found in patients with severe forms of hypospadias (Sultan *et al.*, 2001), e.g. perineo-scrotal hypospadias (Kaspar *et al.*, 1993; Holterhus *et al.*, 2005), hypospadias associated with cryptorchidism (Hiort *et al.*, 1994), and micropenis (Sultan *et al.*, 1993; Li *et al.*, 2004). The phenotype is variable in partial androgen insensitivity syndrome (Sultan *et al.*, 1993; Deeb *et al.*, 2005), and a mutation in one of the eight exons is found in less than 10% of cases. Similarly, AR is expressed in the epithelium of the urethra (Kim *et al.*, 2002), as is the FGF receptor 2 gene (FGFr2), a transcriptional target of AR (Petiot *et al.*, 2005).

New genes of hypospadias

The ATF3 gene is a suspected aetiology of hypospadias for several reasons. First, microarray analysis of tissues from normal and hypospadiac patients revealed upregulation of this gene in hypospadias (Wang *et al.*, 2007). Second, using a mouse model of steroid hormone-dependent genital tubercle development, ATF3 messenger RNA levels were found to be elevated in all oestrogen-exposed foetal genital tubercles compared with controls (Liu *et al.*, 2006). Third, immunohistochemical analysis on human foreskin showed 86% of the hypospadias samples to be positive for expression of ATF3 whereas only 13% of those from normal penises were positive (Liu *et al.*, 2005). In addition, ATF3 expression and promoter activity in human foreskin fibroblasts were responsive to in vitro exposure to ethinyl oestradiol (Liu *et al.*, 2005). Finally, ATF3 is implicated in cell cycle suppression and its upregulation may interfere with urethra formation (Willingham & Baskin, 2007).

Another of the most recently identified candidate genes in the development of the male genitalia is CXorf6 (formerly F18). This gene, discovered in the course of identifying the gene responsible for X-linked myotubular myopathy, MTM1, maps to proximal Xq28 (Laporte *et al.*, 1997a,b). CXorf6 is expressed ubiquitously, but its expression is especially high in skeletal muscle, brain and heart. It is also hypothesized to be implicated in male genital development. Indeed, myopathic individuals with intragenic mutations of MTM1 have normal sexual development whereas those with microdeletions of MTM1 extending to the CXorf6 locus have abnormal genitalia (Hu *et al.*, 1996; Bartsch *et al.*, 1999; Biancalana *et al.*, 2003). Subsequent studies have demonstrated that CXorf6 is mutated in 46,XY disorders of sexual development (46,XY DSD): Fukami *et al.* (2006) recently identified three nonsense mutations in four individuals with 46,XY DSD including micropenis, bifid scrotum and penoscrotal hypospadias. The exact mechanism by which CXorf6 induces hypospadias remains to be established but CXorf6 augments testosterone production and contains the SF1 target sequence (Fukami *et al.*, 2008).

Overall, a genetic basis of hypospadias is likely when the defect is associated with an inactivating mutation of the genes involved in penile development or the hypothalamo–pituitary–testicular axis, including testicular dysgenesis, defect in the synthesis or the molecular action of testosterone (5αR, AR), and a chromosomal abnormality.

Environmental factors affecting gene expression or endocrine pathways

A 'web of arguments' for an environmental contribution

1 Hypospadias, whether associated or not with micropenis, has been reported in numerous wildlife species when the habitat is particularly contaminated by pesticides (Hayes *et al.*, 2002).

2 Male rat pups exposed to DES during gestation (at concentrations similar to those measured in first-trimester human foetal tissues) developed hypospadias. Hypospadias in male rodents was found after maternal treatment with vinclozolin (dose–response effect) (Gray *et al.*, 2001), and similar findings were recorded for prenatal exposure to polychlorinated biphenyls (PCB), phthalates and dioxin (Baskin *et al.*, 2001; Gray *et al.*, 2001; Fisher *et al.*, 2003).

3 Over the last 30 years, male reproductive health has been marked by a deterioration in sperm count and an increasing number of undescended testes, testicular cancers and hypospadias (Czeizel *et al.*, 1986; Paulozzi *et al.*, 1997; Canning, 1999). This phenomenon has raised some concerns regarding environmental chemicals, such as industrial and agricultural by-products.

4 In a recent epidemiologic study, we observed a 4% incidence of hypospadias in neonates whose mothers were treated with DES during pregnancy. This incidence was

8.4% in the neonates of the second generation and suggests a transgenerational effect [Sultan C., personal data; (Klip *et al.*, 2002)].

Endocrine environmental disruptors

Multiple substances found in the environment can potentially interfere with male genital development because of their similarity to hormones. Humans are in constant contact with these substances (Brock *et al.*, 1998; Gray *et al.*, 2001) as they are found in water, soil, food and air (Restrepo *et al.*, 1990a,b). Although there is a long list of suspicious substances contained in herbicides, fungicides, insecticides, and industrial by-products and end-products (plasticizers, cosmetics, paints, etc), none of them has been clearly identified as causing the hypospadiac penis (Restrepo *et al.*, 1990a,b; Brock *et al.*, 1998; Zumbado *et al.*, 2005). These pollutants enter the body by ingestion, inhalation, or absorption or they may be conveyed through the placenta. Individual exposure varies with diet, lifestyle and workplace. As 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 2. Xenooestrogens have both oestrogenic and anti-androgenic actions and compete with natural androgens for the ligand-binding domain (LBD) of the AR (Paris *et al.*, 2002). The conformation of the LBD is therefore changed and the nuclear transfer of AR is altered, as are the transcriptional co-activators and the expression of the androgen-specific gene.

To date, three epidemiologic studies have reported the possible relationship between exposure to pesticides and hypospadias. Kristensen found a moderate increase in the odds ratio (OR) for hypospadias in individuals exposed to farm chemicals (OR = 1.5%). Weidner observed that maternal farming or gardening led to a low risk of hypospadias (OR = 1.27), and Longnecker found no significant risk of hypospadias (OR = 1.2) when mothers were exposed to DTT. The critical level of exposure to EDCs was not assessed in any of these epidemiologic studies (Kristensen *et al.*, 1997; Weidner *et al.*, 1999; Longnecker *et al.*, 2002). Residence in the vicinity of hazardous waste-disposal sites has been associated with a high incidence of hypospadias (Dolk *et al.*, 1998). Similarly, an increased rate of hypospadias was reported in boys from parents exposed to dioxin after the Seveso industrial accident (Mastroiacovo *et al.*, 1988). A vegetarian diet in pregnant women is reported to carry a significant risk of hypospadias (Fig. 1, OR = 4.99) (North & Golding, 2000).

Table 2 Molecular actions of xenooestrogens

Binding of ERα/ERβ nuclear receptor and transcription of activation (or repression) of specific gene expression
Non-genomic actions mediated by a plasma membrane oestrogen receptor
Induction of more potent oestrogenic metabolites
Reduced binding of endogenous oestrogens to sex hormone-binding globulin
Inhibition of transcription of androgen-dependent genes
Potential additive effects
Oncogenic effects

According to Sultan *et al.* (2007) Environment and hypospadias. *Dialogues in Pediatric Urology* **28**, 8–9.

Multifactorial aetiology involving the interaction of environmental factors and genetic polymorphisms

Three risk factors of hypospadias illustrate the multifactorial aspect of this malformation

Low birth weight, small head circumference and birth length are also associated with increased risk of hypospadias. Studies that controlled for length of gestation found that the association remained, indicating that at least in part it may be related to growth retardation [×10 according some authors (Hussain *et al.*, 2002)]. This intra-uterine growth retardation may be related to a dysfunction of the placenta, which is at the crossroads of maternal and foetal genetics and environmental influences (Brouwers *et al.*, 2007).

A number of studies have shown an association between hypospadias and a prolonged time to pregnancy (mother older than 35 years: 50% increase in the risk of hypospadias) or subfertility (Sweet *et al.*, 1974; Czeizel, 1985) (Czeizel & Toth, 1990). Several authors have even hypothesized a central role of subfertility in the aetiology of this defect (Wennerholm *et al.*, 2000). Low sperm motility was noted in fathers of boys with hypospadias in one study (Fritz & Czeizel, 1996) but not in two others (Sweet *et al.*, 1974). However, an association with assisted reproductive technology has been found, particularly with intracytoplasmic sperm injection (ICSI) (Wennerholm *et al.*, 2000; Lie *et al.*, 2005). The subfertility of parents and an alteration in the spermogram, both of which are associated with hypospadias, may themselves be dependent on genetic, endocrine and environmental factors (Wennerholm *et al.*, 2000; Ericson & Kallen, 2001).

Interactions between genetics and environment

Beleza-Meireles *et al.* (2006) reported that polymorphisms of ERβ2 may increase susceptibility to xenooestrogens and increase the risk of hypospadias (10%). Similarly, Baskin (Liu *et al.*, 2005, 2007; Wang *et al.*, 2007) demonstrated that the expression of ATF3 (a CREB family

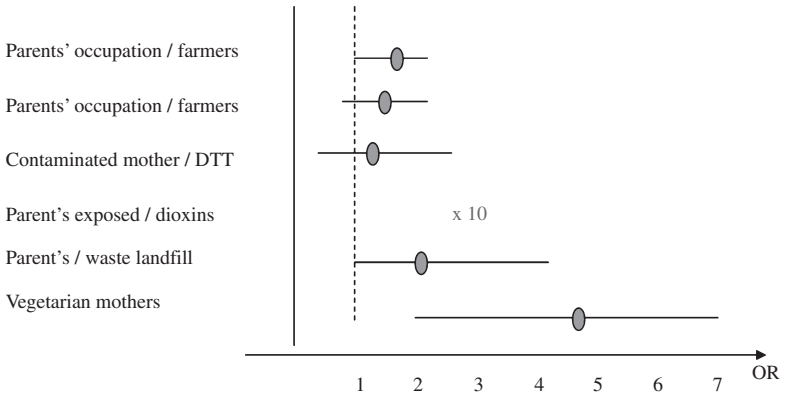

Figure 1 Relative risk of hypospadias according to EDC exposure during maternal gestation and parents' occupation, environmental contamination and maternal diet [according to Sultan *et al.* (2007) Genetics of hypospadias. *Dialogues in Pediatric Urology* 28, 8–9].

transcription factor) was oestrogen-dependent in human and animal models. Thus, susceptibility to environmental factors might depend not only on the endocrine disruptor itself, but also on individual sensitivity, which is modulated by genetic background, including polymorphisms.

Isolated hypospadias vs. syndromic hypospadias

Autosomal dominant forms of syndromic hypospadias are caused by mutations in genes involved in early genital development. Hypospadias may also be associated with various chromosomal abnormalities, including gonosomal mosaicisms (exposed in previous sections) and autosomal deletions.

Almost 200 syndromes have been associated with hypospadias. For example, Smith-Lemli-Opitz (SLO) syndrome, which includes mental retardation, microcephaly, facial dysmorphism, 2–3 syndactily of the toes and, in males, hypospadias and a hypoplastic scrotum, is caused by a defect in steroid biosynthesis. SLO syndrome is because of recessive mutations of the DHCR7 gene coding for 7-dehydrocholesterol reductase, localized on chromosome 11q13 (Ryan *et al.*, 1998; Mnayer *et al.*, 2006). Wilms' tumour, aniridia, genital abnormalities, and growth and mental retardation (WAGR) syndrome is considered to be a contiguous gene syndrome because of a deletion involving band 11p13 (Kaltenis *et al.*, 2004). 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 abnormalities observed in this syndrome (Bickmore *et al.*, 1989; van Heyningen *et al.*, 1990). WT1 point mutations may also result in urogenital abnormalities, depending on the nature and location of the mutation: Denys-Drash syndrome (mesangial sclerosis, gonadal dysgenesis and high risk of Wilms' tumours) (Pelletier *et al.*, 1991a,b; Ogawa *et al.*, 1993), Frasier syndrome (focal glomerular sclerosis, gonadal dysgenesis (Klamt *et al.*, 1998), or severe hypospadias and Wilms' tumour (Kohler *et al.*, 1999).

Other autosomal abnormalities have also been reported with syndromic hypospadias. For example, deletion syndromes with hypospadias have been observed on chromosomes 3q29 (Willatt *et al.*, 2005), 4p (Balci *et al.*, 2006), 9p23 (Ogata *et al.*, 1997), 9q34.3 (Iwakoshi *et al.*, 2004), 10q26 (Ogata *et al.*, 2000) and 13q32-q34 (Bartsch *et al.*, 1996).

Hypospadias of unknown origin

The proportion of hypospadias cases for which aetiology is detected varies according to the authors but it remains low, especially for less severe cases. For example, McPhaul (Allera *et al.*, 1995) identified an AR mutation in only one case of nine isolated hypospadias, and Marcelli in one of 40 cases (Sutherland *et al.*, 1996). In a series of 90 patients, Wang *et al.* (2004) described a mutation of AR in no more than two cases, a mutation of 5αR2 in two cases and three mutations of WT1. The proportion of hypospadias with an identified endocrine disorder, even if significant, remains low: Cassorla (Rey *et al.*, 2005) identified hormonal abnormalities in 13 cases of 61 isolated hypospadias (20% of patients). The occurrence of hypospadias thus remains unexplained in most cases. A multifactorial explanation and the implication of unknown genes or unidentified environmental factors remain possible.

Conclusion

Is hypospadias a genetic disease?

Yes, especially in familial and syndromic forms, and hypospadias due to abnormal genital development (phallus or testicular dysgenesis) or associated with a defect of the androgens pathway (20% of the cases).

Is hypospadias an environmental disease?

Probably yes, especially when the hormonal work-up is normal or the parents are known to live or work in an at-risk environment. But a definitive demonstration remains to be made!

Is hypospadias still an unexplained malformation?

Yes, in most cases, especially the less severe ones…

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