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Role of Hormones, Genes, and Environment in Human Cryptorchidism

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Cryptorchidism is the most frequent congenital birth defect in male children (2–4% in full-term male births), and it has the potential to impact the health of the human male. In fact, although it is often considered a mild malformation, it represents the best-characterized risk factor for reduced fertility and testicular cancer. Furthermore, some reports have highlighted a significant increase in the prevalence of cryptorchidism over the last few decades. Etiology of cryptorchidism remains for the most part unknown, and cryptorchidism itself might be considered a complex disease. Major regulators of testicular descent from intraabdominal location into the bottom of the scrotum are the Leydig-cell-derived hormones testosterone and insulin-like factor 3. Research on possible genetic causes of cryptorchidism has increased recently. Abundant animal evidence supports a genetic cause, whereas the genetic contribution to human cryptorchidism is being elucidated only recently. Mutations in the gene for insulin-like factor 3 and its receptor and in the androgen receptor gene have been recognized as causes of cryptorchidism in some cases, but some chromosomal alterations, above all the Klinefelter syndrome, are also frequently involved. Environmental factors acting as endocrine disruptors of testicular descent might also contribute to the etiology of cryptorchidism and its increased incidence in recent years. Furthermore, polymorphisms in different genes have recently been investigated as contributing risk factors for cryptorchidism, alone or by influencing susceptibility to endocrine disruptors. Obviously, the interaction of environmental and genetic factors is fundamental, and many aspects have been clarified only recently. (*Endocrine Reviews* 29: 560–580, 2008)

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Abbreviations: AIS, Androgen insensitivity syndrome; AMH, anti-Mullerian hormone; AR, androgen receptor; AZF, azoospermia factor; CAIS, complete AIS; CGRP, calcitonin gene-related peptide; CSL, cranial suspensory ligament; DES, diethylstilbestrol; GFN, genitofemoral nerve; hCG, human chorionic gonadotropin; HH, hypogonadotropic hypogonadism; hpg, natural hypogonadal; INSL3, insulin-like factor 3; LuRKO, LH receptor knockout; PAIS, partial AIS; TDS, testicular dysgenesis syndrome; *tfm*, testicular feminization.

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I. Introduction

THE IMPORTANCE OF having both testes normally present in the scrotum was recognized centuries ago in the Middle Ages when "*testiculos habet, et bene pendentes*" (he has testicles, and they dangle nicely) was proclaimed to confirm, from a hole in the chair, that the cardinal elected as future Pope was a man. Cryptorchidism (from the Greek "hidden testicle") or undescended testis is the failure of one or both testes to descend into the scrotal sac and is the most frequent congenital birth defect in male children. Although cryptorchidism is often considered a mild malformation, it represents the best-characterized risk factor for infertility and testicular cancer in adulthood. Failure of the testes to normally descend occurs bilaterally in one third of cases and unilaterally in two thirds of cases. Cryptorchid testes are classified on the basis of their position along the normal route of descent (high/low abdominal, inguinal, suprascrotal, high scrotal) or as ectopic (1). In the clinical setting, however, a simple distinction between palpable and nonpalpable and between unilateral and bilateral is most often used (1). In some cases, cryptorchid patients are found to have an absence of one or both testes, a condition better defined as anorchia or vanishing testis syndrome.

Although the frequency of cryptorchidism may vary among different countries (2), a figure of 2–4% in full-term male births is generally accepted (3). It is notable that the definition of cryptorchidism is still not uniform, and this might influence epidemiological studies reporting its prevalence. For example, a high prevalence of cryptorchidism

(9%) has been reported in Denmark (2) using the definition of Scorer from 1964 (4) that includes testis in a high scrotal position (7% of all cases) among the cryptorchid testis. On the contrary, a more recent study from another group in Denmark reported a frequency of 2.4% (5) using the classification of the John Radcliffe group that includes high scrotal testes among the normally descended testes (6). A large proportion (about 50%) of cryptorchid testes at birth may spontaneously descend in the first months after birth, so that the prevalence of true cryptorchidism at 1 yr of age is 1–2%. Low birth weight, prematurity, and small size for gestational age are associated with substantial increase in the incidence of cryptorchidism, which may reach 20–25% in infants with birth weight less than 2.5 kg (4, 6). Comparison of the studies performed during the last two decades suggests that there are geographical differences in the birth rate of cryptorchidism, varying between 1.8 and 8.4% in boys with birth weight of at least 2.5 kg (7). Some reports have highlighted a significant increase in the prevalence of cryptorchidism over the last few decades. Prospective cohort studies in boys at term and/or with a birth weight of at least 2.5 kg and infant boys indicated an increase in the prevalence of cryptorchidism in England between the 1950s and 1980s (6, 8, 9) and in Denmark between the 1960s and 2000 (2). However, the increased incidence in Denmark should be regarded with caution because other reports from the same country did not observe variations from the 1950s (5). Nevertheless, the increased prevalence of hypospadias, male infertility, and testicular cancer observed in Western countries suggests a possible influence of environmental factors acting as endocrine disruptors of testicular descent and leads to the theory of the testicular dysgenesis syndrome (TDS) (10). In Italy, the prevalence of cryptorchidism at birth in full-term boys seems to be reduced from 4.3% in 1978–1987 to 2.7% in 1988–1997 (11). However, at 1 yr of age the prevalence was similar, being 1.5% in the first decade and 1.2% in the second decade (11).

Cryptorchidism can occur as an isolated disorder or may be associated with other congenital anomalies. Etiology of cryptorchidism remains for the most part unknown, and cryptorchidism itself might be considered a complex disease. In the last few years, a large amount of research focused on the possible genetic contribution to human cryptorchidism and on environmental factors acting as endocrine disruptors of testicular descent that might also contribute to the increased incidence of cryptorchidism in last few decades. Furthermore, polymorphisms in different genes have recently been investigated as contributing risk factors for cryptorchidism, alone or by influencing susceptibility to endocrine disruptors. Obviously, the interaction of environmental and genetic factors is fundamental, and many aspects have only recently been clarified.

II. Hormonal Regulation of Testicular Descent

A. Normal descent of the testis

Testicular position is highly variable within the Mammalia, and three anatomical character states of the scrotum and testicular descent are observed: 1) testicles descended and scrotal; 2) testicles descended but ascrotal (testes within the abdominal cavity or sc); and 3) testicles not descended (testicondy) (12). Evolution has generally proceeded from a scrotal condition to progressively more ascrotal, and descended scrotal testicles are the primitive condition for all the Mammalia. Evolutionarily speaking, testicular descent is a costly process, and many difficulties, developmental and physiological, might be encountered during this process (12).

The descent of the testis is a complex, multistage process requiring the interaction of anatomical and hormonal factors. The most accepted theory describes the descent from an intraabdominal location into the bottom of the scrotum in two major phases, the transabdominal and the inguinoscrotal descent (13). This two-stage process is guided by two mesenteric ligaments: the cranial suspensory ligament (CSL), and the caudal genitoinguinal ligament or gubernaculum (Fig. 1). These two ligaments evolve as sexually dimorphic structures. Initially, the undifferentiated gonads are attached to the abdominal wall in a pararenal position. CSL attaches the gonad to the posterior abdominal wall, whereas gubernaculum connects the testis via the epididymis to the future intraabdominal inner ring of the inguinal canal (Fig. 1A). During the first phase, the testes remain close to the future inguinal region during enlargement of the abdominal cavity and under the pressure of the abdominal visceral growth. Under the effects of hormones, CSL regresses, whereas gubernaculum develops its caudal segment into the so-called gubernacular bulb, a reaction called the "swelling reaction" or "gubernacular outgrowth," protruding into the forming scrotal sac. The swelling reaction of the gubernaculum holds the testis very close to the future internal inguinal ring, and this causes the transabdominal migration of the testes into the inguinal region (Fig. 1B). Therefore, the transabdominal phase of testicular descent is the result of the vector sum of traction by the CSL and the gubernaculum (13). During the second, inguinoscrotal phase the testes move from the inguinal region to the scrotum. This phase is due to the shortening of the gubernacular cord and the outgrowth of the gubernacular bulb (Fig. 1, C and D). The transabdominal stage occurs between 10 and 23 wk gestation in human embryos, and the inguinoscrotal phase starts at around 26 gestational weeks and ends between 28 wk gestation and birth.

A critical role in testicular descent has been attributed to hormones (13–15), in particular testosterone, anti-Mullerian hormone (AMH), and insulin-like factor 3 (INSL3) (Fig. 1). Schematically, testosterone acts on the CSL and gubernaculum and stimulates the development of Wollfian derivatives, AMH causes involution of the Mullerian ducts, and INSL3 controls gubernaculum differentiation. The two phases of testicular descent are differently regulated, and the first, transabdominal phase is essentially INSL3-dependent, whereas the second, inguinoscrotal phase is essentially androgen-dependent.

B. Role of insulin-like factor 3 (INSL3)

INSL3, initially named Leydig insulin-like peptide (Ley-I-L) (16) and also known as relaxin-like factor (RLF), is a member of the relaxin-like hormone family. INSL3 is a peptide produced as an immature preprohormone, composed of A and B chains connected by a C-peptide. A signal peptide

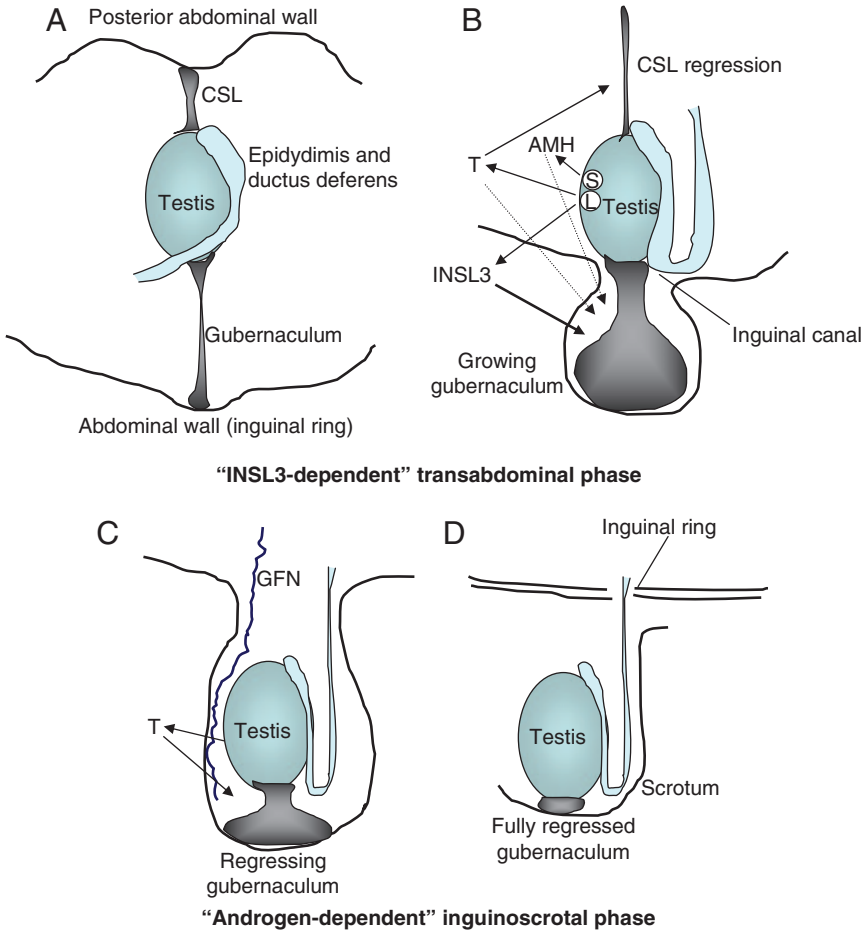

FIG. 1. Model of testicular descent in humans, showing the INSL3-dependent transabdominal phase and the androgen-dependent inguinoscrotal phase. The major structures and the roles of hormones are shown. Testicular differentiation from the ambisexual gonad in the presence of the Y chromosome (A) led to the production of AMH from the developing Sertoli cells (S) and production of testosterone (T) and INSL3 from the Leydig cells (L) (B). The direct and indirect (via the GFN and CGRP) effects of these two hormones principally on the CSL and gubernaculum cause the two-step process of testicular descent. Regression of CSL is mainly under the control of testosterone (B). Masculinization of gubernaculum is under the major control of INSL3 (B) whereas minor roles seem to be exerted by AMH and androgens, possibly via the GFN and CGRP (C).

at the N terminus and the connecting C-peptide are then removed during the processing of the inactive hormone, and two interchain and one intrachain disulfide bond are formed in the active hormone. Research on INSL3 in humans has expanded in the last few years after the identification, in rodents, of a role for this peptide in the transabdominal phase of testicular descent by acting on gubernaculum (17, 18). Another aspect of this research derived from the identification of the INSL3 receptor, RXFP2 (relaxin family peptide 2), also known as LGR8 (leucine-rich repeat-containing G protein-coupled receptor 8) (19–22). As a consequence, numerous human mutation analyses have sought to elucidate the possible involvement of INSL3 and RXFP2 in human cryptorchidism (see *Section III.B*).

Like testosterone, INSL3 is produced by the Leydig cells of the testis during the fetal life and adulthood. It is produced under the Leydig cell differentiation action of LH, and it is regarded as a sensitive marker of Leydig cell function and differentiation status (23–26). INSL3 is highly expressed in the fetal testis but is down-regulated after birth before expression is again up-regulated at puberty (27). Aside from the role in testicular descent and cryptorchidism, INSL3 has possible important yet unidentified endocrine and paracrine actions in adults, and deficiency of this hormone may represent an important sign of hypogonadism (26).

The role of INSL3 in testicular descent is mainly related to its effect on gubernaculum differentiation during the transabdominal phase. Evidence of this role is abundant. *Insl3*$^{-/-}$ and *Rfxp2*$^{-/-}$ male mice exhibit bilateral cryptorchidism due to impaired development of gubernaculum, which shows absence of a central core of mesenchyme (17, 18, 28, 29). Thus, testes remain located high in the abdominal cavity close to the kidney (17, 18) but are loosely connected in the peritoneal cavity because CSL involution occurs normally under the effect of androgens. Furthermore, transgenic *Insl3*$^{-/-}$ male mice overexpressing *Insl3* in pancreatic β-cells showed normal transabdominal descent of the testes (30). Interestingly, in male mice heterozygotes for the *Insl3* deletion there was delayed gubernacular regression and delayed testicular descent, suggesting that the descent of the testis is dependent on the dosage of *Insl3* (31). Gubernaculum shows the highest expression of Rxfp2 (19, 20), and functional Rxfp2 receptor

has been demonstrated in rat gubernacula explants (21). Synthetic or testes-derived Insl3 was shown to induce cell proliferation and gubernacular growth in culture (32–35) and to potentiate androgen and/or AMH-induced cell proliferation in fetal rat gubernacular organ culture (33, 34). However, testosterone and AMH appear to be of relatively minor importance (32), and overexpression of Insl3 in female mice is sufficient to cause ovarian descent (30). INSL3 has a direct stimulatory effect on the swelling reaction of the gubernaculum, whereas testosterone and AMH may have roles in augmenting this growth (34). The fetal expression of Insl3, however, does not seem to be completely dependent on LH, and human chorionic gonadotropin (hCG) probably has a stimulatory effect because hypogonadal mice express Insl3 (31) and these mice and LH receptor knockout mice show normal transabdominal phase of testicular descent (36). In these latter mice, testosterone treatment induces the inguinoscrotal descent (37). Interestingly, transgenic overexpression on *Insl3* in mice failed to reverse the inguinoscrotal cryptorchidism observed in *Gnrhr*-deficient males, confirming that Insl3 is sufficient to direct the first transabdominal phase of testicular descent in the absence of hypothalamic-pituitary-gonadal axis signaling (38).

In male mice, Insl3 expression is developmentally regulated. Insl3 is expressed starting from d 14 of embryonic development, shortly before the induction of gubernacular mesenchymal cell development and gubernaculum outgrowth necessary for the transabdominal descent that occurs between 15.5 and 17.5 d post coitum (35, 39, 40). The Insl3 concentration declines in the juvenile period and then reaches the maximum expression in adulthood (39, 41, 42). In rats, males show maximum Insl3 expression before birth, and both serum concentration of Insl3 and Insl3 mRNA fall significantly in the first days of life (43, 44). All these data clarified the major role of Insl3 in the transabdominal phase of testicular descent mediated by enlargement of the gubernaculum.

In humans, apart from mutations in *INSL3* and *RXFP2* genes found in cryptorchid boys and men (discussed in *Section III.B*), further evidence of the role of INSL3 in testicular descent arose from the demonstration that INSL3 concentrations are high in cord blood and 3-month-old boys and that INSL3 concentrations are reduced in cord blood in persistently cryptorchid boys (45). Studies of the last 3 yr have therefore clarified the dynamic of INSL3 production. INSL3 is produced from the developing testis under the influence of maternal hCG and/or fetal LH to control the first phase of testicular descent; it increases early postnatally, presumably stimulated by the transient postnatal LH peak (45); it declines until puberty, when it is again produced under the influence of LH secretion typical of pubertal development (27, 46); its concentrations maintain during adulthood for yet unknown endocrine and paracrine roles (23, 24); and finally, it reduces in advanced age (47).

Some decades ago, pregnant mothers were treated with diethylstilbestrol (DES) as a hormonal support to pregnancy. This treatment was abandoned when it was found that it led to a high rate of cryptorchidism and other genital defects (48). Interestingly, similar to observations in Insl3-deficient mice, DES-induced cryptorchidism was later shown to be associated with the inhibition of gubernaculum development, and it was demonstrated that DES suppresses Insl3 expression in male mice fetuses (49, 50). Other than DES, 17α- and 17β-estradiol were shown to down-regulate Insl3 expression in fetal mouse Leydig cells (50). These data suggested that environmental factors acting as endocrine disruptors by mimicking estrogen activity and *in utero* exposure to estrogens may cause cryptorchidism by suppressing INSL3 production (see *Section IV*).

C. Role of androgens

It is generally accepted that androgens act on both the gubernaculum and the CSL and are the major mediators of the inguinoscrotal phase of testis descent. During the transabdominal descent, androgens at least contribute to the regression of CSL. However, gubernacular development in this phase seems to be independent of androgens, which are on the contrary necessary for gubernaculum development during the second inguinoscrotal descent. In humans, an intact hypothalamo-pituitary-testicular axis is considered a fundamental prerequisite for normal testicular descent (13, 51). For example, a large percentage of cryptorchidism resolves spontaneously during the postnatal surge of high serum gonadotropin and steroid hormone levels at the age of 1–3 months. Cryptorchidism is a common feature of hypogonadotropic hypogonadism (HH) (51, 52), and mutations in the androgen receptor (AR) gene, causing the androgen insensitivity syndrome (AIS), are known to be associated with variable development of the Wolffian ducts and with micropenis, hypospadias, and cryptorchidism (53–56). Finally, patients with inactivating mutations in the LH receptor gene exhibit cryptorchidism among other phenotypes (57).

Different natural animal models and transgenic mouse models support the role of androgens in testis descent. For example, the natural *tfm* (testicular feminization) mutant mouse, which has no functional AR, has undescended testes due to retention of the CSL (40, 58, 59). The phenotype of the natural *tfm* mouse is similar to that observed in humans with mutations in the AIS, and in both cases the transabdominal phase is normal but the inguinoscrotal phase is deficient (58). However, these mice have external female phenotype without a scrotum, and thus testicular descent cannot be adequately assessed. More importantly, the *LuRKO* (LH receptor knockout) mouse, where the LH receptor has been ablated, and the *hpg* (natural hypogonadal) and *Gnrhr*$^{-/-}$ mouse, where the GnRH gene or GnRH receptor gene are mutated, respectively, and therefore both FSH and LH are lacking, have very low levels of testosterone and cryptorchidism due to alteration of the inguinoscrotal phase (60–63). In LH receptor-null mice, the gubernacular development is indistinguishable from wild-type animals until 7 d of age, but it was severely damaged subsequently, due to a reduction in mesenchimal cell division, differentiation, and maturation (37). Testosterone replacement therapy completes testicular descent and corrects the morphological and gene expression changes. Furthermore, the prenatal exposure of pigs to the androgen antagonist flutamide causes failed gubernacular regression and cryptorchidism (64).

The effects of androgens on the CSL and gubernaculum are

mediated by the AR. Mesenchimal cells of gubernaculum express the AR in a ligand-dependent manner (65), so that AR expression or AR stabilization increases in males and declines in females. In rats, during testis descent the gubernaculum shows a dramatic increase in the number of AR-positive cells. These cells are located in the connective tissue among smooth muscle cells in the gubernacular cord and between striated muscle fibers in the bulb. In both regions, the AR-positive cells were identified as fibroblasts (66). In rats and pigs, administration of flutamide inhibits gubernaculum bulb development, and treatment with testosterone or dihydrotestosterone reverses these effects (64, 67–70). However, the effect of testosterone on the gubernaculum has also been proposed to be indirect and mediated by the calcitonin gene-related peptide (CGRP) produced by the genitofemoral nerve (GFN), as discussed below.

D. Role of anti-Mullerian hormone (AMH)

The essential role of INSL3 and testosterone in the transabdominal and inguinoscrotal phase, respectively, highlights the fact that normally functioning Leydig cells are fundamental in the mechanisms regulating testicular descent. However, the developing Sertoli cells may also have a role. In particular, Sertoli cells produce AMH that is responsible for the involution of the Mullerian tract in male embryos (71).

AMH, or Mullerian inhibiting substance (MIS), is a circulating glycoprotein hormone member of the TGFβ multigene family. AMH is produced as a 140-kDa homodimer precursor and undergoes posttranslational processing for activation, requiring cleavage and dissociation to release bioactive C-terminal fragments (72). When the two-phases theory was proposed, AMH was considered the hormone responsible for the first, transabdominal phase. This was derived from observations that in humans with genetic defects in the *AMH* gene or its receptor with the so-called persisting Mullerian duct syndrome, the testes are undescended and the gubernaculum is thin and elongated (13, 73). The latter defect suggested that the gubernacular swelling reaction fails to occur in persisting Mullerian duct syndrome, leading to cryptorchidism. More recently, a possible effect on the growth of the gubernaculum has been suggested by action on the AMH type 2 receptor (34). However, comparative analysis of the effects of INSL3 and AMH on gubernacular growth indicates that INSL3 has a much marked role (34). Numerous arguments against a role of AMH in the gubernaculum development exist (13). For example, transgenic mice with AMH deficiency have retained Mullerian ducts but fully descended testes (74). In these mice, cryptorchidism is observed when androgens are also genetically eliminated (*tfm*/AMH double mutants) highlighting the fact that the variable gonadal position depends on the androgenic status: those with normal androgen level and AR have normally descended testes (74). Furthermore, testicular descent is normal in AMH receptor-deficient mice (75, 76), and male offspring of rabbits immunized against AMH during early gestation have persistent Mullerian ducts but normal testicular descent (77). Therefore the role of this hormone other than on the regression of the Mullerian ducts is still controversial.

E. Role of calcitonin gene-related peptide (CGRP) and genitofemoral nerve

Besides these testicular hormones, there appears to be a very important function that can be attributed to the GFN and its principal neurotransmitter CGRP, a neuropeptide produced by alternative splicing of the mRNA transcript of the calcitonin gene (78). The GFN undergoes a sex-dependent masculinization and releases CGRP into the gubernaculum, causing rhythmic contractions in rodents (79). Receptors for CGRP are found in the gubernaculum (80). Indeed, the effect of androgens on the gubernaculum during the inguinoscrotal phase might be mediated by CGRP (81). In fact, androgens secreted from the testis have a masculinizing effect on the sensory nuclei of the GFN (L1 and L2 ganglia) (82), with synthesis and release of CGRP from the sensory nerve terminals (83). The importance of GFN is highlighted by the evidence that transection of the GFN prevents gubernacular migration and testicular descent (84). Also prenatal antiandrogen (flutamide) treatment caused a significant decrease in CGRP immunoreactive cells and overall cell number in the sensory nucleus in male mice. In rodents, exogenous CGRP elicits rhythmic gubernacular contractions in organ culture that may have an important role in the process of gubernacular migration *in vivo* (85, 86). Moreover, gubernacular contractility is virtually absent in congenitally cryptorchid, mutant trans-scrotal rats (87). Trans-scrotal rats are known to have a decreased number of CGRP binding sites in the gubernacula. In this strain, gubernacular contractions to exogenous CGRP may be restored by previous GFN transection or by selective chemical ablation of the sensory branch of the GFN (88).

In conclusion, the current model of testicular descent (Fig. 1) involves testicular differentiation from the ambisexual gonad in the presence of the Y chromosome, with production of AMH from the developing Sertoli cells and production of testosterone and INSL3 from the Leydig cells. The direct and indirect (via the GFN and CGRP) effects of these two hormones principally on the CSL and gubernaculum cause a two-step process of testicular descent called the transabdominal and inguinoscrotal phases. Regression of CLS is mainly under the control of testosterone, whose production is dependent on a normally functioning hypothalamus-pituitary-testicular axis. Masculinization of gubernaculum is under the major control of INSL3, whereas minor roles seem to be exerted by androgens, possibly via the GFN and CGRP, and AMH.

III. Genetic Causes and Polymorphisms Associated with Cryptorchidism

A. Introduction

Evidence supporting genetic causes of cryptorchidism is abundant. In some cases of unilateral cryptorchidism, the contralateral, normally descended testis may be altered, too (89), and testicular cancer may originate from the contralateral, not retained testis (90). These findings suggest that cryptorchidism might be considered a sign of an underlying congenital alteration of the testes and contributed to the

development of the TDS theory, which includes cryptorchidism, testicular cancer, spermatogenic impairment, and hypospadias (10). Familial cases have been described, and a family history for cryptorchidism represents a risk factor for undescended testes (91); initial studies suggested that 6.2% of the brothers and 1.5–4.0% of the fathers of patients with cryptorchidism have undescended testes with a heritability value of 0.67 ± 0.16 (92). More recent data highlighted the fact that 22.7% of patients with undescended testis have a positive family history *vs.* 7.5% in controls (93), with a calculated risk for cryptorchidism in newborn males of 6.9 if a brother is affected and 4.6 if the father is affected.

Numerous animal models suggested candidate genes for cryptorchidism and allowed better comprehension of the mechanisms regulating testicular descent (Table 1). Some of them have been cited above, as mice with deficiency in INSL3 (17, 18) and INSL3 receptor (19), which have intraabdominal testes due to absence INSL3 action, and mice deficient in the hypothalamus-pituitary-testicular axis, as the *hpg* (60), Gnrhr$^{-/-}$ (63), *LuRKO* (62), and *tfm* (58), which have disturbed inguinoscrotal phase for absent androgen action. Also, mice deficient for two transcription factors of the *AbdB* homeobox (HOX) gene family (Hoxa10$^{-/-}$ and Hoxa11$^{-/-}$) have intraabdominal testes (94, 95) probably due to alterations in gubernacular development, whereas mice deficient for Desrt (developmentally and sexually retarded with transient immune abnormalities) (99), a transcription factor of the AT-rich interaction domain (ARID) class or mice deficient for Hmgi (98), which encodes for a member of the high mobility group (HMG) DNA-binding protein family, have alterations in the inguinoscrotal phase. The natural mutant trans-scrotal (TS) rat strain also has disturbed inguinoscrotal phase of testicular descent (100) caused by a decreased number of CGRP binding sites in gubernaculum. Furthermore, other than these animal models in which cryptorchidism is the major phenotype, additional transgenic mice, such as those knockout for *WT1*, *Dax1*, or *Dhh*, may present with alterations in testicular descent, but in these cases cryptorchidism is part of a more complex syndrome involving disturbances of sexual development and external genitalia (101).

TABLE 1. Animal models with cryptorchidism (modified from Ref. 101)

Animal models	Testicular position	Ref.
Insl3$^{-/-}$ (INSL3 deficiency)	Intraabdominal	17,18
crsp (Great$^{-/-}$, Rxfp2$^{-/-}$) (INSL3 receptor deficiency)	Intraabdominal	19
Hoxa10$^{-/-}$ (HOXA 10 deficiency)	Intraabdominal	94
Hoxa11$^{-/-}$ (HOXA 11 deficiency)	Intraabdominal	95
p450arom^{+} transgenic (aromatase overexpression)	Intraabdominal	96
hpg (GnRH deficiency)	Inguinoscrotal	60
Gnrhr$^{-/-}$ (GnRH receptor deficiency)	Inguinoscrotal	63
Gnrh-promoter driven SV40-T mutants (LH receptor/FSH receptor deficiency)	Inguinoscrotal	97
LuRKO (LHR deficiency)	Inguinoscrotal	62
Tfm (androgen receptor deficiency)	Inguinoscrotal	58
Pygmy insertional mutants [HMGI protein(s) insertional inactivation]	Inguinoscrotal	98
Desrt$^{-/-}$ (ARID class transcription factor deficiency)	Inguinoscrotal	99
TS-rat (CGRP receptor down-regulation)	Inguinoscrotal	100

B. INSL3 and INSL3 receptor genes

The identification of the fundamental role of INSL3 in gubernaculum development, testicular descent, and cryptorchidism in rodents and other animals led to an extensive search for mutations in the *INSL3* and its receptor *RXFP2* genes in human patients with cryptorchidism.

Mutations in these genes have been analyzed in 15 and seven studies, respectively, including more than 1500 and almost 1000 subjects with cryptorchidism, respectively, and similar numbers of controls (29, 102–118). Some mutations have been found in both controls and patients and are therefore considered as normal variants, whereas other mutations are detected only in cases. The cumulative frequency of such mutations is 1.8% (30 of 1650 cases) and 2.9% (28 of 979 cases) for *INSL3* and *RXFP2*, respectively, giving a combined frequency of 4.7%. The phenotypes of men with *INSL3* and *RXFP2* mutations vary from bilateral cryptorchidism, unilateral cryptorchidism, and failure of the testes to descend normally in the scrotum at birth with spontaneous descent during the first years of life. Although all these phenotypes agree with an alteration of gubernaculum development, the abnormality detected in humans seems to be less severe than in the mouse mutants. However, location of the cryptorchid testes (abdominal or inguinal) has not been reported in many studies, and therefore a direct comparison with knockout mice is not evident. Furthermore, all mutations detected in humans are heterozygous, a condition obviously different from the mouse model in which both genes are ablated. Hypotheses to explain how these heterozygote mutations might be responsible for causing cryptorchidism are different, including a dominant negative effect, the possible presence of another unidentified mutation elsewhere in the gene, and haploinsufficiency. However, it should be noted that heterozygous *Insl3*$^{-/+}$ mice had partial cryptorchidism at birth and fully descended testes in adulthood (17), indicating that, as in humans, cryptorchidism can correct itself after birth.

The phenotype of subjects with *INSL3* or *RXFP2* mutations varies not only in terms of severity of cryptorchidism, but also in terms of testicular damage in adulthood, suggesting that other genetic, endocrine, and/or environmental factors might affect the severity of the undescended testes phenotype. In fact, *INSL3* and *RXFP2* mutations found in adult men with a history of cryptorchidism could be associated with different seminal patterns, including normozoospermia, and are compatible with fertility, and the eventual damage of the spermatogenic function (up to complete absence of germ cells—Sertoli cell-only syndrome) seems to be secondary to the abnormal position of the testis or other causes (surgical trauma, associated varicocele, *etc.*) (111, 114). Therefore, these data suggest that the timing of orchidopexy is crucial for the phenotype of cryptorchid testes in adult. This is in agreement with that found in the rodent model. In fact, although *Insl3* and *Rxfp2* knockout mice show bilateral cryptorchidism at birth and absence of spermatogenesis in adulthood (17, 18, 29, 119), normal spermatogenesis is seen when

cryptorchidism is surgically corrected soon after birth (120). This finding suggests that the clinical consequence of alterations of the INSL3-RXFP2 system seems to be failure of the testis to descend correctly in the scrotum, without apparently affecting the spermatogenic and endocrine components of the testis itself.

With regard to cryptorchidism-specific mutations in the *INSL3* gene, the vast majority of them lead to a single amino acid substitution (missense mutations) (Fig. 2). Analysis of the literature suggests that the mutations A24G, V43L, and A60T in *INSL3* and I604V in *RXFP2* represent common polymorphisms, whereas 12 mutations (C-19G, V18M, P49S, W69R, R73X, P93L, R102C, R102H, R105H, and N110K in *INSL3*, and T222P and R223K in *RXFP2*) are found exclusively in cryptorchid boys or adults with a history of cryptorchidism. The most variable part of *INSL3* is represented by C-peptide, where 6 of 10 mutations are found (Fig. 2). *RXFP2* mutations are almost exclusively represented by the T222P mutation, the R223K being observed only in one single case (118). A limitation in assigning conclusive a pathogenetic role

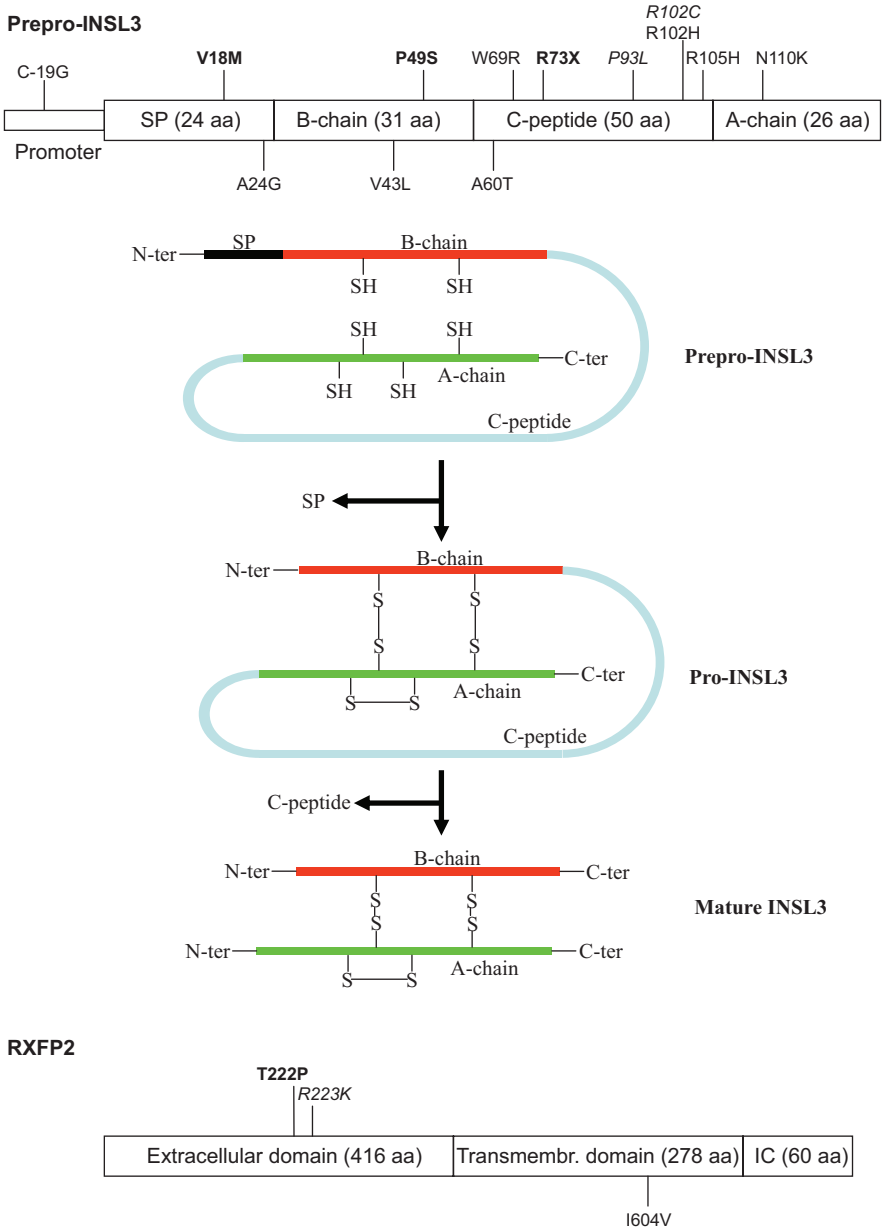

FIG. 2. Structure and mutations in the *INSL3* and *RXFP2* genes. In the upper part the prepro-*INSL3* is shown, including the promoter, signal peptide (SP), B-chain, C-peptide, and A-chain; *below*, the maturation process is schematized from prepro-INSL3 to pro-INSL3 and mature INSL3, showing the release of C-peptide and formation of inter- and intrachain disulfide bonds. Mutations are shown as amino acids (R73X indicates a mutation introducing a stop codon), whereas the C-19G refers to nucleotide. In the *lower part* the RXFP2 is shown, including the extracellular domain, transmembrane domain, and intracellular domain (IC). Mutations found only in the cryptorchid subjects are listed *above* the genes, whereas mutations found in cryptorchid and control subjects (normal variants) are listed *below* the genes. Mutations in *bold* indicate those with strong evidence of causality by *in vitro* studies and/or protein modeling, mutations in *italics* indicate those with weak evidence, and mutations in *normal character* indicate those for which no clear evidence exists.

for many *INSL3* and *RXFP2* mutations is due to the fact that *in vitro* functional studies showed a detrimental effect for only a few of them. In particular, evidence of the inability or reduced ability of the mutated peptide to activate the RXFP2 receptor exists only for the V18M and P49S mutations in *INSL3* (22, 116). Strong evidence also exists for the R73X mutation that results in the termination of translation. Weaker evidence exists for the R102C (22), whereas normal activation of the receptor was observed with the W69R (115), P93L (22), R102H (22), R105H (116), and N110K (22). However, it has to be considered that many of these mutations are located in the C-peptide that is normally excised during processing of the preprohormone, and molecular modeling and comparative analyses of protein sequences in most cases revealed the importance of the mutated amino acids. The novel substitution C-19G in the promoter region could be interesting, but expression analysis of promoter activity did not reveal any difference with respect to wild-type INSL3 (116).

On the contrary, there is no doubt that the T222P mutation in *RXFP2*, which represents the far most frequent mutation of this gene, severely compromises the INSL3 signaling *in vitro*. In fact, the INSL3/RXFP2-mediated cAMP production in cells transfected with a T222P mutant receptor is strongly decreased due to a reduction of receptor surface expression that renders the protein functionally inactive (118). Furthermore, the T222P mutation has not been detected in almost 1000 noncryptorchid controls. The mutation R223K, found only in one case of bilateral cryptorchidism and spontaneous postnatal testicular descent, has little effect on the receptor functions *in vitro* (reduction of 20% in maximal cAMP response) (118), and therefore a causative role for the abnormal phenotype is not obvious.

C. The androgen receptor (AR) gene

Based on the fundamental role of androgens in testicular descent and animal models with absent androgen activity, mutations in the AR gene have obviously been hypothesized as a possible cause of human cryptorchidism. The AR is a member of the nuclear receptor superfamily and mediates physiological effects of androgens [testosterone (T) and 5α-dihydrotestosterone (DHT)] as a ligand-dependent transcription factor. AR is characterized by four different domains: an N-terminal transactivation domain, a DNA-binding domain, a hinge region, and a C-terminal ligand-binding domain. Binding of androgens (T and DHT) to the AR initiates receptor dimerization and translocation to the nucleus where the dimer binds to specific DNA sequences in several gene promoters or regulatory regions, leading to transcription regulation of androgen-responsive genes.

More than 300 different mutations have been described in the *AR* gene (http://www.androgendb.mcgill.ca), and they cause varying degrees of the AIS. At one end, the complete form (CAIS) has complete female phenotype; at the other end, the mild form (MAIS) exhibits only spermatogenic impairment, and the partial form (PAIS) has different phenotypes including variable development of the Wolffian ducts, micropenis, hypospadias, and cryptorchidism (53–56). In these cases therefore cryptorchidism represents one sign of a complex syndrome, and it is usually associated with failure of the inguinoscrotal descent (121, 122). In particular, more severe androgen insensitivity is associated with abdominal testes, whereas inguinal testes are found in milder insensitivity syndrome. In fact, analysis of the literature on more than 100 cases of CAIS and PAIS demonstrated abdominal testes in up to 86% of CAIS subjects and 3% of PAIS subjects (122). The abdominal phenotype of more severe forms of androgen insensitivity is most likely due to the absent production also of INSL3 from a dysgenetic gonad, whereas the inguinal phenotype of less severe forms is only the consequence of androgen insensitivity. Conversely, screening for mutations in the *AR* gene in patients with isolated cryptorchidism failed to find any abnormality (123, 124). However, no conclusion can be made from these two small studies involving a total of 69 cryptorchid subjects. We recently reported a large screening for *AR* mutations in more than 1500 unselected infertile men (55) and found 26 mutations (1.7%). Mutations localized in different regions of the *AR* gene, and seven of them represented novel findings. The others have been described already and shown to be associated with different degrees of androgen insensitivity. No mutations were found in more that 300 controls. The frequency of mutations in men with a history of cryptorchidism was 1.6% (2 of 123). Of 26 men with *AR* gene mutations, two presented cryptorchidism, one cryptorchidism and hypospadia, and one gynaecomastia, whereas 22 did not show signs of androgen insensitivity other than impairment of spermatogenesis. These data might therefore suggest that the mildest phenotype associated with *AR* mutations is represented by spermatogenic impairment, whereas *AR* mutations are not a frequent cause of isolated cryptorchidism. However, additional studies are needed to better clarify the eventual role of *AR* mutations in disorders of testicular descent in humans.

The contribution of polymorphisms in the *AR* gene in human cryptorchidism has been better studied. The *AR* gene is located on the X chromosome, and it exhibits two polymorphic sites in exon 1, characterized by different numbers of CAG and GGC repeats, resulting in variable lengths of polyglutamine and polyglycine stretches in the N-terminal region of the AR protein. The normal range for the number of CAG and GGC is 10–35 (with a mean of 21–23) and 4–24 (with a mean of 16–17), respectively. Longer CAG repeats result in reduced transcriptional activity (125, 126), and there is evidence that an inverse correlation between CAG number and androgenicity exists. Consistent with this, expansion of the tract to more than 40 CAG repeats results in Kennedy's syndrome, a rare motoneuron disorder also characterized by low masculinization, testicular atrophy, reduced sperm production, and infertility (127–129). However, cryptorchidism is not a major feature of Kennedy's syndrome. Although polymorphisms in CAG tract length correlate with sperm concentration in normal men (130), numerous studies examining CAG repeats in infertile men have reported conflicting results justifiable only in part by ethnicity, some showing no expansion and others reporting increased length (but still in the normal range) with respect to fertile control men (131).

Initial studies on isolated cryptorchidism failed to find any association with the length of CAG repeats (132–134). How-

ever, later analyses on both CAG and GGC repeats suggested an association between particular CAG/GGC combinations (135), longer GGC repeats (134, 136) or longer CAG repeats (137) and cryptorchidism, especially in the bilateral form. Therefore, although further studies are needed, expansion of these triplets is emerging as a risk factor for human cryptorchidism, possibly by interacting with other genetic or environmental factors (138).

D. The Y chromosome

The familial occurrence of cryptorchidism described in some studies (see *Section III.A*) may suggest the involvement of the Y chromosome in its pathogenesis. In recent years, a great body of research focused on the role of the Y chromosome in testicular functions, particularly spermatogenesis. These studies clearly showed that microdeletions of the Y chromosome long arm (Yq) represent the most frequent molecular genetic cause of severe male infertility, observed with a prevalence of 10–15% in nonobstructive azoospermia and 5–10% of severe oligozoospermia (139, 140). Three regions, referred to as "azoospermia factors" (AZFa, b and c from proximal to distal), have been defined as spermatogenesis loci (141) and may be deleted in infertile men. However, the completion of the sequencing of the Y chromosome revealed the real structure and organization of this chromosome. In particular, it was shown that most of the AZF microdeletions are generated by intrachromosomal homologous recombination between repeated sequence blocks organized into palindromic structures showing nearly identical sequences (142–144). The original classification in the three AZF regions was therefore modified according to the mechanism of deletion in: AZFa, P5/Proximal-P1 (previous AZFb), P5/Distal-P1, P4/distal P1, and b2/b4-AZFc. Furthermore, a new type of deletion called "gr/gr" removing half of the AZFc region has been described (145) and has been associated with increased risk for spermatogenic impairment (131, 146).

Several studies analyzed the possible contribution of Y chromosome microdeletions to human cryptorchidism. Initial studies performed by our group in men with a history of unilateral cryptorchidism associated with variable spermatogenic status of the contralateral normally descended testis showed deletions only in men with a bilateral severe testicular damage and absence of deletions in men with a normally functioning contralateral testis (147). The frequency of Yq microdeletions was not different from that observed in idiopathic severe testiculopathies. We therefore concluded that such deletions were probably responsible for a bilateral testicular damage, a consequence of which could be cryptorchidism, rather than suggesting a role for the Y chromosome in controlling the testicular descent. We further confirmed these data showing that the frequency of Yq deletions was similar in infertile men with and without a history of cryptorchidism (148). Other studies corroborated this conclusion (149–152) and showed that Yq microdeletions are found in both idiopathic infertile men and infertile men with a history of cryptorchidism, but not in cryptorchid subjects with normal seminal parameters. More recently we reported a comprehensive analysis of clinical features of 99 men with Yq microdeletions detected out of 3073 screened subjects over a period of 10 yr (140). Comparisons between men with and without Yq microdeletions showed that the prevalence of cryptorchidism was similar (9.3% in men with Yq microdeletions and 10.1% in men without Yq microdeletions) and that the prevalence of Yq microdeletions was not statistically different between men with idiopathic severe infertility and men with nonidiopathic infertility associated with cryptorchidism.

Only a few studies analyzed the possible contribution of partial AZFc deletions to human cryptorchidism. By using a simple method to distinguish between presence or absence of *DAZ* gene deletions, we initially reported that they were not present in infertile men with history of cryptorchidism (148). More recently, a deeper analysis of gr/gr deletions in infertile men with and without a history of cryptorchidism found a similar frequency of deletions (4.2% in cryptorchid and 5% in noncryptorchid patients) (153). Therefore, there is a similar effect of partial AZFc deletions on spermatogenesis regardless of the presence or absence of cryptorchidism, and partial AZFc deletions, as classical AZF deletions, seem not to play any role in the pathogenesis of human cryptorchidism. However, the presence of Yq microdeletions in boys with cryptorchidism may predict spermatogenic impairment later in adulthood.

E. The estrogen receptor (ER) genes

The role of estrogens in testicular descent is unclear, but the identification of environmental factors with estrogenic activity able to affect the development of the male urogenital tract (154, 155) led to increased interest on their possible involvement on human cryptorchidism. The two genes encoding for ERα (*ESR1*) and ERβ (*ESR2*) are the most obvious candidates for such a modulating effect because the estrogenic effect of environmental endocrine disruptors is primarily modulated by ER (154, 156). However, *ESR1* has received more attention because ERα seems to play some role in male genital and reproductive health, whereas the role of ERβ is less apparent. The only man with a homozygous *ESR1* mutation has descended testes (157, 158). However, although *Esr1* knockout male mice also have descended testes, defects in cremaster muscle development were noted in these animals, indicating a role for ERα in some aspects of male reproductive tract development and testicular descent (159). On the contrary, *Esr2* knockout male mice have an apparently normal fertile phenotype (158).

Only two studies analyzed the possible involvement of polymorphisms in the *ESR1* gene in human cryptorchidism (160, 161), and they detected an opposite effect. In the Japanese study (160), five DNA markers (single nucleotide polymorphisms) in the 3′ region of the gene (the AGATA allele) were found to be overrepresented in cryptorchid patients in comparison with controls (34.0 *vs.* 21.3%), and the homozygosity for this variant was found only among patients with undescended testes. On the contrary, in the Italian study (161) the AGATA haplotype was found to be associated with a reduced risk of cryptorchidism. The observed differences could be due to the different genetic backgrounds of the Japanese and Italian populations or low number of cases studied (63 in the Japanese study and 118 in the Italian

study); however, these preliminary studies encourage future investigation of these aspects. The possible role of *ESR2* gene polymorphisms in cryptorchidism is even less clear because only one study analyzed it in 23 cases (162). No difference was noted in the *Rsa*I and *Alu*I polymorphism distribution.

F. Other genes

Other genetic factors might have a role in the etiology of human cryptorchidism, but available data either are not conclusive or represent rare genetic defects. Numerous data from animal models suggesting a role for some genes in testicular descent and cryptorchidism have not been confirmed in humans.

For example, because animal models have suggested a role for CGRP in the mechanisms regulating the testicular descent, we screened for mutations four candidate genes (*αCGRP*, *βCGRP*, *CGRPR*, and *CGRP-RCP*) in 90 cryptorchid patients, but no pathogenic sequence changes were detected (163). Although mice deficient for Hoxa10 have intraabdominal testes (94), two studies in human cryptorchidism led to contrasting results (164, 165). In fact, the initial study performed on 45 cryptorchid found a significantly high frequency of *HOXA10* exon 1 variants with respect to controls (164), but our study detected only polymorphisms in both cases and controls (165).

Cryptorchidism is a clinical manifestation of HH that may be caused by mutations in different genes, the most common being represented by *KAL1* on the X chromosome, responsible for the Kallmann syndrome; *FGFR1* (*KAL2*), responsible of an autosomal dominant form of HH; and the receptor for GnRH (*GNRHR*), responsible of an autosomal recessive form of normosmic HH (166). Although mutations in other genes have been reported in HH, they are currently thought to be rare. These include mutations in the leptin (*LEP*) and leptin receptor (*LEPR*), which have been detected in a minority of obese patients with HH, and mutations in the *GPR54* gene detected in a few families with normosmic HH (166).

Inactivating mutations of LH receptor gene, although rare, cause different developmental defects depending on the grade of receptor insensitivity (57, 167). Complete inactivating mutations cause male pseudohermaphroditism similarly to complete AIS, whereas milder forms of *LHR* inactivation are associated with some testicular androgen and INSL3 production, and therefore the male phenotype is partially induced, ranging from cryptorchidism and micropenis to severe perineoscrotal hypospadias and resembling PAIS. Three men with inactivating *LHβ* gene mutations have been described; however, they are normally masculinized at birth but totally lack pubertal sexual differentiation. The reason for the difference is that placental hCG is able to stimulate fetal androgen and INSL3 production (57, 167).

G. Chromosomal alterations

The reports on the incidence of chromosomal anomalies in patients with cryptorchidism indicate a prevalence of 3–4%. In particular, the frequency of chromosomal abnormalities observed in more than 1000 subjects with isolated cryptorchidism is 2.4%, whereas it increases to 7.6% in almost 400 subjects with cryptorchidism associated with other anomalies, as hypospadias. Bilateral cryptorchidism is more frequently associated with chromosomal alterations (5%) than unilateral forms (3%) (168–176).

The most frequent chromosome anomalies are those involving the sex chromosomes, such as 47,XXY (Klinefelter syndrome), 46,XX male, and 46,XY,inv(Y)(p11q11), and sex chromosome mosaicisms (46,XY/47,XXY; 46,XX/46XY; and 46,XX/47,XXY), but several other chromosome structure aberrations, such as inversions (involving especially chromosome Y), translocations, deletions, and duplications, concerning both autosomal and sex chromosomes may be found. In general, Klinefelter syndrome is the most frequent observed sex chromosomal anomaly.

Klinefelter syndrome describes a group of chromosomal disorders in which there is at least one extra X chromosome added to a normal male karyotype, 46,XY. The classic form is the most common chromosomal disorder, characterized by an extra X chromosome resulting in the 47,XXY karyotype (177). The extra X chromosome in 47,XXY results from either maternal or paternal meiotic nondisjunction (failing of a chromosome to separate during the first or second division of gametogenesis) or from mitotic nondisjunction in the developing zygote.

XXY aneuploidy is the most common disorder of sex chromosomes in humans, with a prevalence of one in 500 males (178). Recent findings suggest that the prevalence of Klinefelter syndrome has risen in the last few decades (179), without a concomitant increase in the prevalence of 47,XXX. This may suggest that the increase in Klinefelter syndrome seems to be related to increased paternal meiotic alterations.

Boys with 47,XXY have variable phenotypic characteristics and do not have obvious facial dysmorphology; thus, they are indistinguishable from boys with normal karyotypes (180). Many 47,XXY boys appear to enter puberty normally, but later they present a progressive testicular failure with hypergonadotropic hypogonadism, and therefore have frequently reduced testosterone concentrations at late adolescence and early adulthood. With a decrease in androgen production, secondary sexual characteristics do not completely develop, and features of eunuchoidism and gynecomastia can develop. The testicular phenotype is progressive with life, and adults have a classic form of hypogonadism (181).

The exact prevalence of cryptorchidism at birth in patients with Klinefelter syndrome is unclear, and few studies examined this aspect. The largest, cross-sectional study undertaken in an andrological clinic reported that 27% of patients with Klinefelter syndrome had a history of undescended testes, compared with 8% of the total number of patients who attended the same clinic (182). Nevertheless, Klinefelter syndrome is an underdiagnosed condition; only 25% of the expected number of patients are diagnosed (183), and of these only a minority are diagnosed before puberty. Indeed, a major effort should be made to clearly identify patients. In fact, Klinefelter subjects, other than being affected by a severe primary testiculopathy, represent the paradigm of genetically determined TDS (10), with frequent cryptorchidism at birth, infertility, and increased risk of testicular cancer in adulthood. Furthermore, Klinefelter patients are at increased

risk of mental retardation, breast cancer, metabolic syndrome, diabetes mellitus, hypothyroidism, and autoimmune and other systemic diseases (183).

The adult phenotype of males with 48,XXYY (1:17,000) is similar to that of Klinefelter syndrome, with eunuchoid habitus with long legs, sparse body hair, small testicles and penis, hypergonadotropic hypogonadism, and gynecomastia (184). On the contrary, males with 48,XXXY chromosome karyotype (1:50,000) can have average or tall stature with ocular hypertelorism, flat nasal bridge, radioulnar synostosis, fifth-finger clinodactyly, and small penis and testicles with hypergonadotropic hypogonadism. Their IQ is usually between 40 and 60, with severely delayed speech (184). Also, males with 49,XXXXY (1:85,000–100,000) are severely affected, with many congenital disorders including small genitalia with hypergonadotropic hypogonadism. Their IQ ranges between 20 and 60 (184).

The 46,XX male syndrome occurs in about one in 20,000 newborn males (185). Phenotypically there are three groups of sex-reversed 46,XX individuals. The first classical group includes phenotypically normal XX males, the second group consists of males with genital ambiguities, and the third group describes true hermaphrodites (186). Ninety percent of 46,XX patients have Y chromosomal material including the SRY gene located on the distal tip of the short arm of the X chromosome, whereas 10% of XX males are SRY negative, with different degrees of masculinization. Two major mechanisms are involved in this phenomenon. A dominant autosomal or X-chromosomal inheritance of XX maleness has been described in several cases (187). It is suggested that a mutation in these unknown genes can trigger testis determination. As a second mechanism, a mosaicism with a prevalent XX-lineage and a hidden or scarce lineage containing a Y chromosome has been proposed (188). Males with 46,XX typically have external genitalia that range from normal to ambiguous (penoscrotal hypospadias with or without chordee), and cryptorchidism is frequent (189).

H. Syndromes and complex malformations

More than 250 entries for cryptorchidism are present in OMIM, the Online database of Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM), which catalogs all human genes and genetic disorders. This result points out the complexity of the pathogenesis of cryptorchidism, having existed as an isolated symptom, or been associated with other anomalies, or included in complex syndromes, too.

There is, of course, a considerable involvement of genetic factors in cryptorchidism, as demonstrated in inherited X chromosome-linked anomalies and sex chromosome aneuploidies (Table 2) and in autosomal syndromes associated frequently (Table 3) or occasionally (Table 4) with cryptorchidism. Many of these disorders are very rare (with few cases reported worldwide). However, some of them are quite frequent. These include the deletion 22q11.2 (1/5,000), also known as velocardiofacial syndrome (190), the deletion 1p36 (1/5,000–1/10,000) (191), the AIS (1/7,000–1/20,000, discussed above), the Beckwith-Wiedemann syndrome (1/13,000) (192), the Smith-Lemli-Opitz syndrome (1/10,000–1/30,000) (193), the De Lange syndrome (1/19,000) (194), and the Prader-Willi syndrome (1/25,000) (195).

Furthermore, particular attention should be given to the five more frequent syndromes in the general population, represented by the Klinefelter syndrome (1/500, discussed above), the FG syndrome (1/1000), the Noonan syndrome (1/1000–1/2500), the Down syndrome (1/800–1/2000), and the 47,XYY syndrome (1/2000), which may present cryptorchidism at birth.

FG syndrome, initially considered a rare, completely recessive X-linked disorder occurring only in males and constituting a unique disorder, is now increasingly documented as a common disorder that may also affect carriers with a wide range of manifestations that extensively overlap those of the G/BBB (Opitz) syndrome. Genitourinary abnormalities are frequent in this syndrome, including hypospadias (14%), cryptorchidism (24%), and herniae or hydrocele (13%)

TABLE 2. X-Linked syndromes and sex chromosome aneuploidies associated with cryptorchidism in humans, shown along with the corresponding candidate gene and OMIM number

Syndromes	Chromosomal location	Gene	OMIM
Klinefelter (47,XXY) and its variants			
47,XYY			
Aarskog– Scott	Xp11.21	FGD1	300546
FG	Xp11.4-p11.3	//	
Prieto	Xp11-q21	//	
Opitz G/BBB	Xp22	MID1	300552
FG	Xp22.3	//	
Kallmann syndrome, type 1	Xp22.31	KAL1	308700
Ichthyosis	Xp22.32	ARSC1	308100
Androgen insensitivity	Xq11 –q12	AR	313700
FG	Xq12-q21.31	MED12	300188
FG	Xq22.3	//	
Fanconi pancytopenia	Xp22.31	FANCB	300515
Simpson-Golabi-Behmel	Xq26	GPC3	300037
Lowe oculocerebrorenal syndrome	Xq26.1	OCRL1	300535
Borjeson-Forssman-Lehmann	Xq26.3	PHF6	300414
FG	Xq28	//	
Oto-palato-digital sindrome, type 2	Xq28	FLNA	300017

The syndromes are listed in p-telomere/q-telomere order. //, Unidentified gene.

TABLE 3. Autosomic syndromes frequently associated with cryptorchidism, shown along with the corresponding candidate gene and OMIM number

Syndrome	Inheritance	Chromosomal location	Gene	OMIM
Poplietal pterygium	AD	1q32-q41	IRF6	607199
Noonan	AD	2p22-p21	SOS1	182530
Mowat-Wilson	AD	2q22	ZEB2	605802
Escobar	AR	2q33-q34	CHRNG	100730
LEOPARD	AD	3p25	RAF1	164760
Noonan	AD	3p25	RAF1	164760
Seckel	AR	3q22-q24	ATR	601215
EEC	AD	3q27	TP73 L	603273
Fraser	AR	4q21	FREM2	608947
De Lange	AD	5p13.1	NIPBL	608667
Weaver	AD	5q35	NSD1	606681
Carpenter	AR	6p11	RAB23	606144
EEC	AD	7q11.2-q21.3	//	
Roberts-SC phocomelia	AR	8p21.1	ESCO2	609353
Meckel-Gruber	AR	8q21.13-q22.1	TMEM67	609884
Robinow	AR	9q22	ROR2	602337
Pena-Shokeir	AR	10q11	ERCC6	609413
WAGR	AD	11p13	Deletion	
Beckwith-Wiedemann	AD	11p15.5	KIP2,NSD1	600856,606681
Smith-Lemli-Opitz	AR	11q12-q13	DHCR7	602858
Meckel-Gruber	AR	11q13	//	
Noonan	AD	12p12.1	KRAS	190070
LEOPARD	AD	12q24.1	PTPN11	176876
Noonan	AD	12q24.1	PTPN11	176876
Ulnar-mammary	AD	12q24.1	TBX3	601621
KID	AD	13q11-q12	GJB2	121011
Peters'-plus	AR	13q12.3	B3GALTL	610308
Fraser	AR	13q13.3	FRAS1	607830
Seckel	AR	14q21-q22	//	
Prader-Willi	Sporadic	15q11-q13	Deletion	
Johanson-Blizzard	AR	15q15-q21.1	UBR1	605981
Shprintzen-Goldberg	AD	15q21.1	FBN1	134797
Rubinstein-Taybi	AD	16p13.3	CREBBP	600140
Miller-Diker	AD	17p13.3	Deletion	
Meckel-Gruber	AR	17q23	MKS1	609883
Seckel	AR	18p11.31-q11.2	//	
Opitz G/BBB	AD	22q11.2	//	
Rubinstein-Taybi	AD	22q13	EP300	602700
Femoral-facial	Sporadic	//	//	
Fryns	AR	//	//	
Meier-Gorlin	AR	//	//	
Toriello-Carey	AR	//	//	
Acrodysostosis	AD	//	//	
Lenz-Majewski hyperostosis	AD	//	//	
Robinow	AD	//	//	
Deletion 4p, 5p, 9p, 11q, 13q, 18q				
Duplication 3q, 10q				
Trisomy 13, 18				

The syndromes are listed from chromosome 1 to chromosome 22 in p-telomere/q-telomere order. AD, Autosomal dominant; AR, autosomal recessive; //, unidentified.

(196). This syndrome is due to mutations of one of the three FGS genes present on the X chromosome (Table 2).

Noonan syndrome is an autosomal dominant syndrome characterized by short stature, typical facial dysmorphology, and congenital heart defects. Cryptorchidism is a typical feature in Noonan syndrome (197). In approximately 50% of cases, the disease is caused by missense mutations in the *PTPN11* gene on chromosome 12, resulting in a gain of function of the nonreceptor protein tyrosine phosphatase SHP-2 protein. Recently, mutations in other genes from the RAS MAPK pathway (*KRAS*, *SOS1*) have been identified in a small proportion of patients with Noonan syndrome.

Trisomy 21, or Down syndrome, is a chromosomal abnormality characterized by the presence of a third (partial or total) copy of chromosome 21. Variable and often light intellectual deficiency, almost constant muscular hypotonia, and joint laxity are usual consequences, often associated with morphological signs and risks of complications (198). Cryptorchidism has been reported to be present in 6.5% of patients with trisomy 21 (199).

47,XYY males have no unusual physical features or medical problems. 47,XYY boys and men are usually taller than average and several centimeters taller than their parents and siblings. Testosterone levels (prenatally and postnatally) are normal in 47,XYY males. Most 47,XYY males have normal sexual development and usually have normal fertility. Because XYY is not characterized by distinct physical features, the condition is usually detected only during genetic analysis for other reasons (200, 201). 47,XYY is not inherited but

TABLE 4. Autosomic syndromes occasionally associated with cryptorchidism and for which a chromosomal location and/or a candidate gene have been identified

Syndrome	Inheritance	Chromosomal location	Gene	OMIM
Marshall	AD	1p21	COL11A1	120280
Zellweger	AR	1p36.2	PEX14	601791
Chondroplasia punctata type 2	AR	1q42	RCDP2	222765
Fanconi pancytopenia	AR	2p16.1	FANCL	300515
Larsen	AD	3p14.3	FLNB	603381
Fanconi pancytopenia	AR	3p25.3	FANCD2	227646
Ellis-van Creveld	AR	4p16	EVC,EVC2	604831,607261
Cockayne	AR	5q12	ERCC8	609412
Diastrophic dysplasia	AR	5q32-q33.1	SLC26A2	606718
Treacher-Collins	AD	5q32-q33.1	TCOF1	606847
Zellweger	AR	6p21.1	PEX6	601498
Fanconi pancytopenia	AR	6p22-p21	FANCE	600901
Chondroplasia punctata type 1	AR	6q22-q24	PEX7	601757
Zellweger	AR	6q23-q24	PEX3	603164
Acrocallosal	AR	7p13	GLI3	165240
Greig cephalopolysyndactyly	AD	7p13	GLI3	165240
Saethre-Chotzen	AD	7p21	TWIST1	601622
Zellweger	AR	7q21-q22	PEX1	602136
Cardio-facio-cutaneous	AD	7q32	MEK2	601263
Cardio-facio-cutaneous	AD	7q34	BRAF	164757
Pfeiffer	AD	8p11.2-p11.1	FGFR1	136350
Zellweger	AR	8q21.1	PEX2	170993
Langer-Giedion	AD	8q24.11-q24.13	TRPS1,EXT1	604386,608177
Rothmund-Thomsom	AR	8q24.3	RECQL4	603780
Fanconi pancytopenia	AR	9p13	FANCG	602956
Distal arthrogryposis, type 1	AD	9p13.2-p13.1	TPM2	190990
Fanconi pancytopenia	AR	9q22.3	FANCC	227645
Gorlin	AD	9q22.3	PTCH1	601309
Cockayne	AR	10q11	ERCC6	609413
Apert	AD	10q26	FGFR2	176943
Pfeiffer	AD	10q26	FGFR2	176943
Fanconi pancytopenia	AR	11p15	FANCF	603467
Cardio-facio-cutaneous	AD	12p12.1	KRAS	190070
Zellweger	AR	12p13.3	PEX5	600414
Fanconi pancytopenia	AR	13q12.3	FANCD1	605724
Fanconi pancytopenia	AR	14q21.3	FANCM	609644
Cardio-facio-cutaneous	AD	15q21	MEK1	176872
Fanconi pancytopenia	AR	15q25-q26	FANCI	611360
Fanconi pancytopenia	AR	16p12	FANCN	610832
Distichasis-lymphoedema	AD	16q24.3	FOXC2	602402
Fanconi pancytopenia	AR	16q24.3	FANCA	607139
Smith-Magenis	Sporadic	17p11.2	Deletion	
Fanconi pancytopenia	AR	17q22	FANCJ	609054
Jarcho-Levin	AR	19q13	DLL3	602768
Myotonic dystrophy	AD	19q13.2-q13.3	DMPK	605377
McKusick-Kaufmann	AR	20p12	MKS	604896
Zellweger	AR	22q11.21	PEX26	608666
Deletion 5p				
Duplication 15q				
Trisomy 9 mosaic				
Deletion 3p, 22q11.2				
Down (trisomy 21)				
Duplication 9p				
1p36 deletion				
Trisomy 8				

The syndromes are listed from chromosome 1 to chromosome 22 in p-telomere/q-telomere order. AD, Autosomal dominant; AR, autosomal recessive; //, unidentified.

usually occurs as a random event during the formation of sperm cells. A nondisjunction in metaphase II can result in sperm cells with an extra copy of the Y chromosome.

IV. Endocrine Disruptors and Cryptorchidism

Environmental factors acting as endocrine disruptors of testicular descent have been suggested to contribute to cryptorchidism and its increased incidence in recent years. Endocrine disruptors are defined as exogenous substances with an ability to disrupt normal endocrine homeostasis and reproduction, and they include xenoestrogens (industrial chemicals), synthetic and natural hormones, phyto- and mycoestrogens, and other substances affecting endocrine signaling (202). Obviously, the interaction of environmental and genetic factors is fundamental, and some individuals may be

more susceptible or, in contrast, more resistant to endocrine disruptors than others (203).

Environmental compounds that are supposed to play a role in cryptorchidism mimic the action of hormones involved in testicular descent, and they act mainly as estrogens or antiandrogens. Among endocrine disruptors are persistent organohalogen pollutants, which accumulate in the food chain and are assumed by humans through diet of animal origin, and phthalates, which are used in industrial products and are considered to be ubiquitous environmental contaminants. Other endocrine disruptor candidates include different herbicides and fungicides and other industrial chemicals such as bisphenol A (203).

According to the TDS theory presented by Skakkebaek *et al*. (10), cryptorchidism, hypospadias, testicular cancer, and spermatogenic impairment share the same risk factors and have a fetal origin, caused by a combination of genetic and environmental factors, including endocrine disrupting chemicals. This hypothesis is supported by evidence that exogenous estrogens and antiandrogens cause disorders of genital development in animals (155). In particular, the "estrogen hypothesis" postulated that the apparent increase in TDS incidence may be related to increased estrogen exposure of the human fetus (204). One of the main facts that prompted the formulation of this theory was the evidence that boys born to women who had been treated with DES in early pregnancy had an increased incidence of cryptorchidism and other genital defects (48). The mechanisms by which altered estrogen exposure could induce cryptorchidism and other male reproductive disorders were initially considered to be related to suppression of gonadotropin secretion and impairment of Leydig cell development leading to inadequate testosterone production (204). However, later studies highlighted the fact that additional, and perhaps more plausible, mechanisms could be related to suppression of androgen production, suppression of AR expression, and suppression of INSL3 production (205). Therefore, estrogenic, antiandrogenic, and anti-INSL3 effects are linked together to determine the phenotypic expression. Of these routes, the suppression of INSL3 production is probably the best characterized in relation to cryptorchidism. The exact mechanism by which estrogens regulate fetal Leydig cell Insl3 transcription is unclear. Initial studies suggested an estrogen-mediated alteration in steroidogenic factor-1, which is one of the most important regulators of Insl3 expression, but more recent data did not confirm this hypothesis (49, 50, 206). Anyway, estrogens affect fetal Leydig cell endocrine functions, more precisely the steroidogenic function, Insl3 expression, and testicular descent, via an ERα-dependent mechanism (206). Another mechanism could be related to a direct effect of estrogens on CSL and gubernaculum, which express ERα (207).

All these experimental data therefore highlight the fact that any estrogenic and antiandrogenic substance may have deleterious effects on testicular descent. However, *in vitro* studies generally use much higher concentrations of substances than those to which a pregnant woman and her fetus may be exposed, and the routes and levels of human exposure to such compounds are largely unknown (205, 208). Furthermore, although the number of environmental chemicals with intrinsic estrogenic or antiandrogenic activity is very high (203, 209), all of them possess weak or very weak endocrine activity when compared with the potency of DES (205). Therefore, it seems unlikely that any of the identified environmental compounds could induce cryptorchidism or other signs of TDS in humans (205). Even human exposure to phthalates, which are the class of environmental compounds to which human exposure is highest, is at concentrations without adverse effect when used *in vitro* (205). Nevertheless, one should bear in mind that synergies between several different low-dose xenobiotic compounds, which is a more realistic situation, may exist (208). In this situation, low levels of environmental estrogens may be capable of inducing adverse effects if androgen production or action is also reduced by antiandrogens.

Substances with estrogenic and/or antiandrogenic effects that might be involved in human cryptorchidism include some environmental chemicals such as phthalates, which act primarily by impairing production of testosterone by fetal Leydig cells (210), and bisphenol-A, which has intrinsic estrogenic effect but probably acts also via and estrogen-independent mechanism (211). However, the increased bioavailability and transplacental transfer of maternal estradiol during pregnancy could also be involved. This is suggested by the increased risk of cryptorchidism in first pregnancies and twin pregnancies (205) and the higher risk for boys born from diabetic and obese insulin-resistant women due to reduced SHBG levels induced by insulin (212). Furthermore, polychlorinated biphenyls and polyhalogenated hydrocarbons can suppress the activity of sulfotransferase (SULT1E1), which is the primary mechanism via which estradiol is inactivated and excreted (213). Other routes of estrogen exposure have been suggested (205). These include milk, meat, and other dietary products that might contain higher amounts of estrogens used as growth promoters and exposure to ethinyl estradiol via recycled drinking water. However, even if data on animals are quite alarming, demonstrating for example feminization of fathead minnow males exposed at low concentrations of 17α-ethinyl estradiol (214), it seems highly unlikely that human exposure to these substances would be sufficient to induce male reproductive tract abnormalities such as cryptorchidism (201).

V. Consequences of Cryptorchidism

A. Infertility

Cryptorchidism is a heterogeneous disorder, and testicular function in adulthood may be altered at different degrees, with seminal quality ranging from normozoospermia to complete azoospermia (13, 89). Men with a history of cryptorchidism are frequently subfertile in adulthood due to an alteration of spermatogenesis. More serious damage is usually observed in bilateral forms, even if in some cases of unilateral cryptorchidism the normally descended testis might be altered, too (89). Commonly, excryptorchid subjects show reduced sperm concentration, increased FSH, and reduced inhibin B plasma levels that correlate to the reduced testicular volume, indicating the deterioration of fertility potential (215, 216). Abnormal sperm count has been reported

in about 50% of unilateral and about 80% of bilateral ex-cryptorchid males, respectively. In the former group, when the contralateral descended testis is normal, the younger the cryptorchid boys are at surgery the higher their sperm concentration (217). Actually, cryptorchidism is one of the most common causes of secretory azoospermia and is accountable for 20% of all azoospermic patients (218).

It is well accepted that testicular malposition leads to early and progressive histological worsening. On this basis, the age of surgical correction of cryptorchidism (orchidopexy) has decreased, and it is currently recommended by age 2 (215). However, despite surgery for cryptorchidism, it has been reported that infertility is probable in one third (44 of 135) of the patients (219). Hadziselimovic and Herzog (220) reported that 89% of bilateral cryptorchid patients who remain untreated become azoospermic, whereas this occurs in only 32% of subjects who respond successfully to hormonal administration of hCG and in 46% of those treated by orchidopexy. On the contrary, in unilateral cryptorchidism the incidence of azoospermia seems independent from treatment modalities, and the same authors reported an incidence of 13% (217).

Also, the paternity rate is different when comparing unilateral and bilateral cryptorchidism. In fact, regardless of the age of orchidopexy, it is compromised in bilateral forms compared with the general population, whereas it seems unchanged in cases of unilateral undescended testes (221, 222). In the latter cases, causes other than testicular malposition are probably involved in inducing testicular damage, including testicular intrinsic (congenital) and testicular extrinsic (anatomical) factors (89). As discussed in *Section III* , a variety of congenital syndromes, due to either chromosomal alterations or monogenic diseases, may present with cryptorchidism. For example, in cases of karyotypic aberration or Y chromosome microdeletions, the tubular impairment is congenital, and it is already present at birth; thus early surgery may have very little effect on fertility. Another possible pathogenesis of tubular damage in cryptorchid testes could be related to a gonadotropin and testosterone deficiency during the first postnatal maturational step (223). It has been shown that after 5 months of life, the surge of gonadotropins and testosterone is necessary for transformation of the germ cells (217). This time is considered a crucial step for the development of male fertility potential, because during this period a pool of Ad spermatogonia that are fundamental for next maturation of germ cells develop from gonocytes (220). The impairment of this process, leading to early developmental arrest of Ad spermatogonia, seems strictly related to damaged spermatogenesis. In these clinical situations, an early surgery probably has little or no effect because of the scarce number or absence of spermatogenesis precursors at that time. Furthermore, this hypothesis would explain the reported benefit of treatment with a low dose of LHRH analog for fertility of cryptorchid boys (223).

On the other hand, there are congenital alterations inducing specific failure of the testis to normally descend in the scrotum during embryonic development, as exemplified by mutations in *INSL3* and *RXFP2* genes, discussed in *Section III.B*. In these cases, early orchidopexy may rescue the cryptorchid testes from their fate of Sertoli cell-only syndrome, and surgically descended testes have normal spermatogenesis (29). Also in humans, mutations in *INSL3* and *RXFP2* genes interfere with normal testicular descent without affecting the spermatogenic and endocrine components of the testis itself (111).

B. Testicular cancer

Although the cause of testicular cancer is still unclear, a strong association with cryptorchidism is widely accepted. In fact, cryptorchid boys are at high risk of testicular cancer, and their lifetime risk of testicular neoplasia (carcinoma *in situ* testis and invasive tumors) is 2–3%, which is about four times higher than in the general population (224). It has been reported that the risk of testicular cancer is reduced among men successfully treated for cryptorchidism (225), whereas a 32-fold increased risk has been reported among men whose cryptorchidism persisted after 11 yr of age (226). However, the mechanism by which cryptorchidism contributes to carcinogenesis is largely unknown. Although much evidence suggested a genetic role for both testicular cancer (227–230) and cryptorchidism, no genetic links between these two conditions have been reported yet.

In the last few years, two theories have been proposed to explain the association between undescended testes and testicular cancer. The most widely accepted is the TDS hypothesis that describes the relation between genetic factors, endocrine disruptors, and the subsequent development of cryptorchidism, infertility, hypospadias, and testicular cancer (10). With this scenario, cancer would be the result of disturbed gonadal development in which altered gonocytes fail to differentiate and progressively degenerate up to carcinoma *in situ*, which precedes germ cell tumor and invasive progression (231). This theory is supported by the evidence that the increased incidence of cryptorchidism has paralleled that in testicular cancer, hypospadias, and male infertility (226). Although this theory suggests a common cause of cryptorchidism and testis neoplasia, other authors suggested a cause and effect association between these two conditions (232). On this basis, the testicular malposition in an abnormal site would lead to the development of cancer, suggesting that cryptorchidism is a possible factor involved in carcinogenesis. This hypothesis is reinforced by the observation that early surgical correction is able to reduce the risk of cancer (233). However, surgery of cryptorchidism, although it enables self-examination for early detection of cancer, does not completely remove the risk of testis cancer. Furthermore, even when the contralateral testis in cases of unilateral cryptorchidism is normally descended, the cancer risk in that testis is higher compared with noncryptorchid subjects (232).

In conclusion, it seems that testicular carcinogenesis may be the final effect of combined factors comprising environmental endocrine insults on genetically abnormal testicles predisposed to cryptorchidism and cancer (234). Although the relationship between testicular cancer and cryptorchidism is much more complex than expected, nevertheless early surgery is mandatory because prepubertal orchidopexy may decrease the risk of testicular cancer.

VI. Conclusions

In recent years, major advances in the clinical approach to human cryptorchidism have been the discovery of INSL3 as the crucial hormonal regulator of the gubernacular differentiation, the identification of its specific receptor RXFP2, and mutation analyses of *INSL3*/*RXFP2* genes. Furthermore, studies on INSL3 regulation and action have led to increasing evidence suggesting that disruption of the INSL3/RXFP2 pathway, other than gene mutations, may be responsible for testicular maldescent. This is particularly important because an increased incidence of cryptorchidism has been reported in recent decades and geographical differences in its prevalence are reported. Several risk factors including environmental factors acting as endocrine disruptors have been evocated for this trend. One of the major mechanisms by which they act actually seems to be a suppressed or reduced expression of INSL3. The interrelations between INSL3, androgens, and estrogens suggest that research on genetic polymorphisms (for example in the AR and ER genes) influencing susceptibility to endocrine disruptors might shed light on this field.

Primary basic research and clinical studies should look at cryptorchidism as a serious anomaly with potentially severe consequences, such as infertility and testicular cancer, and major research efforts should be made with the aim of better management of cryptorchid boys and adult men with a history of cryptorchidism.

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