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Genetics of cryptorchidism and testicular regression

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Cryptorchidism, i.e., undescended testis, is one of the most common genital malformations in newborn male babies. The birth rate of cryptorchidism varies from 1.6 to 9.0 %. Etiology of disrupted testicular descent is complex and predisposing causes include genetic, hormonal, environmental, lifestyle and maternal factors. Testicular descent occurs in two major steps and testicular hormones and normal function of hypothalamic–pituitary–testicular axis are important for normal descent. Several gene mutations are associated with syndromic cryptorchidism but they are rarely found in boys with isolated undescended testis.

Testicular regression can also cause an empty scrotum. Normal male genital phenotype indicates that the boy has had functioning testis during development. Torsion of the testis can cause testicular regression but in many cases the reason for vanishing testis remains elusive.

In this narrative review we discuss genetics of cryptorchidism and testicular regression.

Abbreviation: INSL3, insulin-like peptide 3.

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Introduction

Cryptorchidism i.e., undescended testis (UDT) or retentio testis refers to a testis that is not normally located in the bottom of the scrotum. Cryptorchidism may occur on one side or on both sides. Testis may locate in any part of its normal route of descent from the abdomen to the scrotum (i.e., it may have an abdominal, inguinal, suprascrotal or high scrotal location) [1]. About 80 % of all undescended testes are palpable [2]. Testis can also be ectopic which means that it has deviated from its normal route of descent. The most common location of an ectopic testis is in the inguinal area [3].

Cryptorchidism may be congenital or acquired. Testes that were normally descended at birth sometimes ascend later on (this is called acquired cryptorchidism or ascending testis). This happens usually in prepubertal boys and spontaneous descent of such a testis is common when testosterone surges at puberty [4]. A shared etiology between congenital and acquired cryptorchidism has been suggested, and minimal differences between these conditions further support this hypothesis [5].

Regression of the testicular tissue can also be the reason for an empty scrotum. Regression can happen already in the abdomen or later after testicular descent to the scrotum. Testicular regression is also known as TRS (testicular regression syndrome), vanishing testes or bilateral anorchia [6].

Prevalence of cryptorchidism

In prospective studies on cryptorchidism, the prevalence of congenital cryptorchidism has varied between 1.1 and 8.4% among boys with birth weight $\geq$2500 g [1,7–15]. Furthermore, an increasing trend in the prevalence of congenital cryptorchidism has been reported based on repeated studies in some countries [1,8–10,15]. Cryptorchidism is more common among preterm boys, and boys who are born small for gestational age, and boys whose birth weight is under 2500 g [1,11–17]. The rate of undescended testis in premature boys or boys whose birth weight was <2.5 kg has varied between 1.1 and 45.3 % [18]. Due to spontaneous testicular descent, lower rates of congenital cryptorchidism have been reported at the age of 3 months, i.e., between 0.9 and 1.8% among boys with birth weight $\geq$2500 g [1,8,10,11]. Spontaneous testicular descent is likely due to transient activation of the hypothalamus–pituitary–gonadal (HPG) axis, which causes an increase in reproductive hormone levels [7,19–22].

Increasing rates of cryptorchidism have been described after the age of 3 months, because of testicular ascent and recurrent cryptorchidism (re-ascent of spontaneously descended cryptorchid testes) [15,23]. In the prospective Cambridge Baby Growth Study, the cumulative incidence of acquired cryptorchidism by the age of 2 years was 7.0% [15]. In a Danish prospective study on cryptorchidism the prevalence of testicular ascent was 0.6% at 18 and 36 months [23]. In a Dutch study the prevalence of acquired cryptorchidism was 1.1–2.2 % among boys aged 6–13 years [24]. Retractile testes have a 32 % risk of becoming an acquired UDT [25]. Retractile testis means that a testis can be pulled to the scrotum but jumps back to a higher position after releasing the grip.

Although genetics may have a role in the observed geographical differences in the prevalence of congenital cryptorchidism, environmental factors are thought to be more likely reasons for the observed increasing rates of cryptorchidism [1]. Exposures to environmental chemicals have been associated with cryptorchidism in some epidemiological studies, but the results are inconsistent [26]. Chemicals with endocrine disrupting properties (anti-androgenic or estrogenic properties) may disrupt testicular descent. In addition to environmental factors, also fetal exposure to pharmaceuticals like mild analgesics has been associated with cryptorchidism [27,28]. Also, maternal lifestyle factors, such as alcohol consumption and smoking during pregnancy have been associated with an increased risk of UDT. According to a recent meta-analysis based on 20 original studies, the risk of cryptorchidism is increased among sons whose mothers had smoked during pregnancy (pooled crude OR 1.18, 95% CI 1.12–1.24) [29]. Gestational diabetes and maternal alcohol intake during pregnancy have been associated with increased risk of cryptorchidism in some studies [30–32] but in a meta-analysis only marginal association or no statistically significant association, respectively, was observed [33].

Familial occurrence of cryptorchidism

In a Danish study, the concordance rate of cryptorchidism in brothers of cryptorchid boys and in sons of fathers with a history of cryptorchidism were as follows: Boys with no relation 3.2 %, paternal half-brothers 3.4 %, maternal half-brothers 6.0 %, full brothers 8.8 %, dizygotic twin brothers 24.1 % and monozygotic twin brothers 27.3 % [34]. When compared with brothers with the same parents, paternal half-brothers had significantly lower cryptorchidism concordance rate than maternal half-brothers [34]. Thus, maternal inheritance and exposures seem to have more influence than paternal factors. The findings suggest that research on the etiological factors of UDT should include maternal factors like maternal genes, intrauterine conditions and maternal gene–environmental interaction [34]. In Elert's study the risk of UDT for a newborn boy with overall family history of UDT was increased to 3.6-fold. Risk increased to 6.9-fold if a brother was affected and 4.6-fold if the father was affected [35]. Also, other studies have described higher occurrence of cryptorchidism among brothers of cryptorchid boys than among sons of fathers with a history of cryptorchidism [36,37].

Process of testicular descent

Testicular descent has traditionally been described as a biphasic process with a transabdominal and an inguinoscrotal phase [38,39]. The first phase is completed by gestational week (GW) 15 and the second one is completed by the end of week 35 [38]. First the human male and female gonads are located high in abdomen near the kidneys and during the gonadal development there is so called inner descent which is passive and hormone independent and it is closely related to the descent of the diaphragm [40,41]. Bipotential gonads are located on the dorsal wall of the body cavity and developing testis is anchored by two ligaments – upper part is anchored to the diaphragm via cranial the suspensory ligament (CSL) and lower part to the inguinal area via the gubernaculum [38].

In the transabdominal phase of testicular descent, between GW 8–15, the gubernaculums enlarge so that the testes remain anchored close to the inguinal canal [38,42]. This process was discovered by mouse studies, and it was confirmed that Insulin-like peptide 3 (Insl3) secreted by the Leydig cells is the primary hormone regulating the male like development of the gubernaculum (swelling of the bulb and shortening of the cord) [43–45]. The gubernaculum is feminized in *Insl3*-deficient mice [43–45]. INSL3 has been detected only in amniotic fluid of male fetuses, which supports its role also in testicular descent in man [46,47]. Furthermore, reduced cord blood INSL3 levels have been reported in boys with congenital cryptorchidism [48,49]. Testosterone in turn causes the regression of the CSL during the transabdominal phase in mice [38,44]. However, the role of this ligament in testicular descent is less clear in humans and studies on human fetuses have suggested that both genders have a transitory CSL, which is then replaced in girls by an ovarian suspensory ligament [40]. Androgen and INSL3 secretion in Leydig cells are stimulated by human chorionic gonadotropin (hCG) from the placenta during the first trimester of pregnancy and later also by luteinizing hormone (LH) from the anterior pituitary gland of the fetus [50].

Anti-Müllerian hormone (AMH) is secreted by Sertoli cells in testes. The main role of AMH is to induce regression of the Müllerian ducts and therefore uterus, fallopian tubes and proximal vagina do not develop. Infantile uterus and fallopian tubes, cryptorchidism and abnormally long gubernaculums have been reported in boys with mutations of the *AMH* gene or its receptor (Persistent Müllerian Duct Syndrome (PMDS)) [38,51]. One role of AMH in testicular descent might be shortening the gubernaculum, although AMH is not indispensable for testicular descent in mice [51,52].

According to human studies, when the Müllerian ducts have regressed and the cranial mesonephric ligament has regressed, the testes glide across the Wolffian ducts and dive into swollen gubernaculums [40]. However, it has also been suggested that gubernaculum is not directly attached to testis, but only to ductus deferens and epididymis [53,54]. In man, the inguinal canal forms around the gubernaculum during early fetal development [53] and swelling of the gubernaculum widens the inguinal canal before the testis descends through it to the scrotum [40,55].

The second phase of testicular descent occurs between GW 25–35 when the epididymes and testes migrate through the inguinal canal and follow the gubernaculums into the scrotum [38,40]. Androgens have an important role in this inguinoscrotal phase of testicular descent, and accordingly

inguinoscrotal testicular descent is usually disrupted both in subjects with an inactivating androgen receptor mutation (androgen insensitivity syndrome AIS and testicular feminization syndrome in boys and mice, respectively) [56,57]. Animal studies suggest that androgens influence the migration of the gubernaculum during the inguinoscrotal phase indirectly via genitofemoral nerve and its neurotransmitter Calcitonin-gene related peptide (CGRP) [38]. However, there are differences between men and mice in the timing and process of inguinoscrotal phase of testicular descent [58,59]. Intra-abdominal pressure is also believed to aid the transinguinal descent of the testes in man [55]. Failure of testicular descent leads to congenital cryptorchidism and failure in the closure and fusion of processus vaginalis may lead to inguinal hernia or hydrocele and failure in the complete involution of the processus vaginalis may be the cause for acquired cryptorchidism [38]. Cryptorchidism is usually due to abnormalities in the inguinoscrotal phase of testicular descent and the transabdominal phase is more seldomly disrupted [1,60]. Besides androgens, also INSL3 and its receptor have been proposed to affect the inguinoscrotal descent of the testis [41,61].

Known genetic reasons of cryptorchidism

A large majority of cryptorchidism is non-syndromic, i.e. there are no comorbidities or other deformities. Etiology of non-syndromic cryptorchidism remains elusive [41]. However, several conditions have been associated with cryptorchidism. These include diseases or syndromes associated with decreased androgen levels [different forms of disorders of sex development (DSD) (including disorders of testicular development and disorders in androgen synthesis or action), congenital hypogonadotropic hypogonadism, congenital hypergonadotropic hypogonadism, syndromes associated with both primary and secondary hypogonadism], conditions associated with decreased INSL3 or AMH levels or actions, and several other syndromes [62]. Cryptorchidism is present in 150 comorbid conditions, including several syndromes, reproductive, cardiovascular, ophthalmologic, dermatologic, mental, and bone disorders, deafness and cancer [63]. The most frequently reported comorbid feature of cryptorchidism is hypospadias [63]. Thus, several genomic variations have been associated with cryptorchidism.

Decreased androgen levels

Disorders of testicular development

Patients with partial gonadal dysgenesis have variable internal and external genital phenotypes and the gonads vary from streak gonads to dysgenetic or regressed testes and the gonadal function is decreased [62]. Described mutations include mutations in *ARX, ATRX, CBX2, DAX1, DHH, DHX37, DMRT1, EMX2, ESR2, FGFR2, GATA4, HHAT, MAP3K1, NR5A1, SF1, SOX9, SRY, TSPYL1, WNT4, WT1, ZFPM2*, and *ZNRF3* genes [62].

Disorders in androgen synthesis or action

Male external genitalia are dependent on androgen binding to its receptor and subsequent activation of gene expression [64]. Differentiation to male fails if there are too little androgens, as in normal female fetus, or without a functioning AR (androgen insensitivity syndrome, AIS) [64]. In normal male fetus androgen directs the formation of male phenotype during early fetal development [64]. In complete androgen insensitivity, Wolffian structures will disappear, and the external genitalia are female type except for a short vagina, while the female internal genitalia are missing [65]. Partial androgen insensitivity syndrome refers to a phenotype with varying degrees of masculinization of the external genitalia due to partial androgen responsiveness [65]. The phenotype can include micropenis, hypospadias and bifid scrotum that might contain gonads [66]. Firm diagnosis of PAIS is based on identification of an abnormal AR by sequencing the *AR* gene [65]. Over 300 different mutations have been described in the *AR* gene and they cause variable degrees of AIS [66].

As mentioned earlier, androgens have a pivotal role in the descent of the testes in the inguinoscrotal phase [66]. Any reason reducing Leydig cell function and reducing production of testosterone may cause cryptorchidism [62]. In a study on 600 singleton boys with isolated nonfamilial cryptorchidism, less than 1% of boys were found to have a mutation in the *AR* gene and thus *AR* gene mutations seem to

be rare in nonsyndromic cryptorchidism [67]. However, the numbers of CAG and GGN nucleic acid repeats in the *AR* gene have been shown to vary. Multiple studies have investigated the association between CAG and GGN repeat lengths within the normal range and the risk of isolated cryptorchidism (reviewed in [41]). GGN repeat length of 24 has been found to be more prevalent in cryptorchid men than among controls [41]. In Wang's meta-analysis UDT was divided into unilateral and bilateral subgroups and longer CAG repeat region was significantly associated with cryptorchidism in the bilateral group [68]. In the same meta-analysis, the results indicated that GGN repeat length was significantly higher in patients compared with controls. Longer GGN and CAG repeat length may cause impairment of *AR* transcriptional activity and AR function, and thus affect testicular descent [68]. Genes encoding cytoskeletal proteins may also contribute to AR signaling and subsequent migration of the testis [69]. Mutations in *LH* receptor gene and also enzyme deficiencies can be behind androgen production defects [65]. WT1 is likely involved in gubernaculum differentiation and testis migration, and it could also regulate the expression of *AR* gene [69,70]. Nonsyndromic cryptorchidism in boys has also been associated with reduced expressions of *AR* and slow twitch specific myosin heavy chain *MYH7* mRNA in the cremaster muscle [71].

Congenital hypogonadotropic hypogonadism

A normal function of the hypothalamic–pituitary–testicular (HPG) axis is required for normal testicular descent [72]. This axis is most active in midgestational fetus, but it silences toward term because of placental function [72]. Reactivation of HPG happens in mini-puberty during the first 3 months of life and next time in puberty [72]. Cryptorchidism can be one manifestation of congenital hypogonadism [62]. Boys who have congenital hypogonadotropic hypogonadism frequently present with cryptorchidism and/or micropenis [62]. Hypogonadotropic hypogonadism and cryptorchidism belong to the findings of the CHARGE syndrome, which is connected to *CHD7* gene mutation [62]. Lot of mutations of different genes underly the isolated hypogonadotropic hypogonadism [73]. There are claims that up to 70% of cryptorchid boys would suffer of hypogonadotropic hypogonadism [54,74]. However, a majority of the studies refute such claim [reviewed in [62]]. We observed that LH levels were elevated in the cryptorchid group when compared with healthy boys at mini puberty [20]. High gonadotropin drive may compensate for mild Leydig cell dysfunction in cryptorchidism and suggests that cryptorchidism is a primary sign of testicular dysgenesis [75]. In our Finnish patients with isolated cryptorchidism, we found no mutations in *FGFR1*, *PROK2*, *PROKR2*, *TAC3* or *TACR3* (genes underlying isolated hypogonadotropic hypogonadism) [76]. A heterozygous missense mutation, Q106R or R262Q in *GNRHR* gene was observed in two subjects, but the frequency was similar also among male controls [76]. Thus, isolated cryptorchidism is not commonly caused by genetic defects underlying isolated hypogonadotropic hypogonadism [76].

Hypergonadotropic hypogonadism

In hypergonadotropic hypogonadism LH and FSH levels are elevated, and the androgen levels are low. Testes cannot produce enough testosterone. The underlying reasons can be genetic or environmental insults [77]. In Klinefelter's syndrome, boys with a karyotype 47, XXY can have a malfunction of Leydig cells causing deficiency of INSL3 and testosterone [78].

Decreased INSL3 levels or actions

Numerous human mutation analyses have sought to elucidate the possible involvement of *INSL3* and its specific receptor *RXFP2* in human cryptorchidism. However, according to a recent review the prevalence of mutations in *INSL3* is 1–2 % [69,79], and in *RXFP2* 4% [69], although several polymorphisms have also been described [79]. It has been suggested that mutations are more frequent in bilateral cases [69,80]. In Finnish children we found only polymorphisms in *INSL3* and *RXFP2* that were not associated with cryptorchidism [81,82]. However, in a recent study on Egyptian children an association between G178A-*INSL3* polymorphism and undescended testis was reported [83]. In an Indian study four brothers had isolated bilateral cryptorchidism and whole exome sequencing found a homozygous missense variant in the *RXFP2* gene in all of them (heterozygous in both parents). Poor cell surface expression and failure to bind INSL3 or respond to the ligand with cAMP signaling was

observed in functional analysis of the variant protein. Conclusion was that recessive inheritance of variants in the *RXFP2* gene can underlie familial cryptorchidism [84]. The phenotype of men with *INSL3*/*RXFP2* mutations is not uniform, ranging from severe bilateral abdominal cryptorchidism to delayed testicular descent [85]. In conclusion, mutations of *INSL3* or its receptor *RXFP2* are not usual in cryptorchid cases.

Decreased AMH levels or actions

Children with mutations of the *AMH* gene or its receptor may have undescended testis and they also have infantile uterus and fallopian tubes, and this is called PMDS [38]. The long gubernaculum allows the testes to migrate to a wrong position, even into the contralateral processus vaginalis (transverse testicular ectopia) [38]. Also, there is a high frequency of intraabdominal torsion causing vanishing testis [86].

miRNAs

Increased expression of microRNA-210 (miR-210) in murine cryptorchidism models as well as in human cryptorchid testes suggests that miR-210 can be considered a biomarker of cryptorchidism in man [69,87].

Other conditions associated with cryptorchidism

In midline disorders there is quite often also cryptorchidism. Midline disorders can be associated with central nervous system disorders, there can be abdominal wall defects, cardiac anomalies, cleft lip and palate anomalies and gastrointestinal and chromosomal anomalies [88,89]. Cryptorchidism can be found sometimes in association with gastroschisis, in omphalocele, in bladder exstrophy, in anorectal malformations, in Prune belly and in spina bifida [41,62,90,91].

Cryptorchidism can occur as a part of a genetic syndrome and the mechanisms can be unclear, including Down and Noonan syndrome [62]. A catalog of genetic loci of syndromic cryptorchidism consists of 60 loci associated with 44 syndromes that include cryptorchidism in the clinical picture – of them 38 are protein-coding genes and 22 of those loci include structural variations like microdeletions and microduplications [92]. The highest number of loci associated with syndromic cryptorchidism was found in chromosome X and 1 [92].

Other candidate genes

Findings from animal models and small clinical studies have suggested that the posterior homebox (HOX) genes could be potential candidate genes for UDT [93]. The variants found in *HOXA10* gene in the study by Wang et al was not associated with a risk for UDT, and no variant was found in *HOXA11*, but *HOXD13* variant was associated with the risk of UDT [93]. Inconsistency between animal and human studies could be explained by different phenotypes – most human cases were mild and UDT cases were nonsyndromic [93]. Mutations in *HOXA10* gene seem to be uncommon among cryptorchid patients also according to other studies [94–96].

Axin1 is a central component of the Wnt signaling pathway and *AXIN1* polymorphisms have been associated with the risk of cryptorchidism in man [97]. Animal studies suggest that Insl3 and Rfxp2 affect myogenic differentiation of the gubernaculum possibly via Wnt/β-catenin pathways [98]. The role of CGRP and genitofemoral nerve in testicular descent in man is unclear, but it has been suggested that RBFOX proteins may regulate neuro-hormonal signaling in testicular descent and contribute to risk of cryptorchidism [99].

A genome-wide copy number variation (CNV) association analysis suggested that CNV is not a major cause of nonsyndromic cryptorchidism [100]. Genome-wide association studies (GWAS) have recently identified several genetic loci that predispose to testicular dysgenesis syndrome (TDS): genomic variants in *TGFBR3* and *BMP7* were associated with TDS, especially with testicular cancer and possibly with cryptorchidism [101]. *TGFBR3* gene encodes the TGFβ receptor type III. *TGFBR3* and its co-receptors and

ligands are expressed in most endocrine tissues, also the testis [101]. Another study suggested complex or phenotype specific association of nonsyndromic cryptorchidism with *TGFBR3* and the gubernaculum as a potential target of TGFβ signaling [102]. A more recent GWAS concluded that nonsyndromic cryptorchidism has apparently heterogeneous multilocus susceptibility and multifactorial backgound [103]. Table 1 summarizes the above discussed genes and conditions that have been linked to cryptorchidism.

Testicular regression

Testicular degeneration is a process where loss of testicular function happens after the tissue has formed. Terms testicular regression syndrome (TRS) or bilateral anorchia or vanishing testes have also been used. A boy who has normal external genitals and karyotype 46,XY but both testes are missing has had testicular regression. Sometimes only one testis is missing. Testicular regression occurs in about 1:20 000 baby boys and 0.5–4.5 % of cryptorchid boys belong to this category [6]. The presence of spermatic cord structure is evidence supporting the presence of testis in early pregnancy [104]. Pathological features of TRS include discrete vascularized fibrosis, dystrophic calcification, and presence of hemosiderin and especially then, when also spermatid cord structures are included [104]. Typical male genitalia develop during the first 16 weeks of gestation. The reason for testicular regression is still unclear but some kind of vascular accident is believed to cause it. Testis torsion is the most common reason. Thrombosis is also one possible option. If regression occurs during the second half of gestation, it causes deficiency of testosterone and subsequently impaired penile growth, leading to micropenis in about 50 % of TRS males, while penile length is typically normal if the incident occurs closer to term or soon after birth [105]. These patients will need testosterone replacement starting from puberty and psychosocial support, and maybe also testis prosthesis and treatment of infertility are needed in adulthood. Methods for diagnosing TRS include measurement of gonadotropin and testosterone levels, human chorionic gonadotropin stimulation test, measurement of AMH, inhibin B, and INSL3 levels, karyotype analysis, imaging, and surgical exploration. In bilateral TRS testosterone, inhibin B and INSL3 are undetectable, gonadotropins are elevated, and karyotype is 46,XY. Scrotal and

Table 1
List of discussed conditions and genes that have been associated with cryptorchidism.

Decreased androgen levels
Disorders of testicular development
Mutations in *ARX, ATRX, CBX2, DAX1, DHH, DHX37, DMRT1, EMX2, ESR2, FGFR2, GATA4, HHAT, MAP3K1, NR5A1, SF1, SOX9, SRY, TSPYL1, WNT4, WT1, ZFPM2* and *ZNRF3*
Disorders in androgen synthesis or action
Mutations in *AR, LHR, MYH7*
Enzyme deficiencies affecting biosynthesis of androgens (please see Ref. [62])
Congenital hypogonadotropic hypogonadism
e.g. Mutations in genes underlying isolated hypogonadotropic hypogonadism (such as *FGFR1, PROK2, PROKR2, TAC3* or *TACR3, GNRHR*), mutation in *CHD7*. For more reasons please see Ref. [62]
Hypergonadotropic hypogonadism
Klinefelter's syndrome 47,XXY
Decreased INSL3 levels or actions
Mutations in *INSL3, RXFP2*
Decreased AMH levels or actions
Mutations in *AMH, AMHR*
miRNAs
microRNA-210
Other conditions associated with cryptorchidism
Midline disorders
Gastroschisis, omphalocele, bladder exstrophy, anorectal malformations, Prune belly and spina bifida
Genetic syndromes
e.g., Down and Noonan syndrome, for more genetic syndromes please see Ref. [92]
For more conditions please see Ref. [62]
Other candidate genes
HOXD13, HOXA10, AXIN1, RBFOX, TGFBR3

abdominal US imaging are usually performed. In infancy, testes are also searched for to exclude congenital adrenal hyperplasia (CAH). There is no clear evidence to support surgical exploration, and if that is considered necessary, it should be delayed until hormonal evaluation is complete [6]. Longitudinal care with endocrinologist at least every 2–3 years during childhood is recommended [6].

There is no clear consensus of the operation need in TRS. Quite conservative approach is prevailing, but there is also a hypothetical potential risk of future malignancy of dysgenetic remnant of the testis [106]. The incidence of germ cells (GCs) within testicular nubbins varies between 0 and 16 % [106]. In 1 out of 20 resected testicular remnants viable GCs were identified and 1 out of 10 had seminiferous tubules left and this is considered evidence for future malignant potential [106]. If nubbin is intra-abdominal it might contain more elements favoring decision to operate (limited evidence) [106]. Theoretically testicular remnants without germ cells are not at risk for later malignant development [107]. TRS is said to be part of the clinical spectrum of 46,XY gonadal dysgenesis and it may share a common genetic etiology but identification of the genes involved has proven elusive [108]. In one study 25 % of children with clinically well-defined TRS carried pathogenic variants in the *DHX37* gene and it may have a critical role not only in early human testis determination but also in the maintenance of testicular tissue during an early phase of testis development [108]. In TRS, the gonads may have been functionally abnormal before regression, suggesting an underlying pathological condition [105]. Some evidence suggests that the risk of testis torsion can be inherited, particularly in cases of bilateral torsion [109]. Also, a meta-analysis suggested inheritance of testicular torsion [110]. *INSL3* gene may be involved in this mechanism predisposing to testicular torsion [109].

In summary cryptorchidism may present as a non-syndromic form or it may be part of other congenital anomalies. Necessity of normal hormonal function of the hypothalamus–pituitary–testicular axis has been known for years but the understanding of genetic determinants has increased only recently. Mutations in genes encoding for reproductive hormones, including INSL3, AMH, testosterone, and gonadotropins, or their receptors can cause cryptorchidism. Multiple genetic loci have been found to associate with cryptorchidism. However, genetic defects can be rarely identified in non-syndromic cryptorchidism. Maternal non-genetic factors seem to have an important role. Testicular regression is uncommon, and its genetic predisposition is largely unknown. Testicular torsion is a well-known reason for regression of the testes, but in many cases the etiology of regression remains unknown.

Practice points

- Cryptorchidism is one of the most common birth defects in newborn baby boys.
- Etiology of cryptorchidism is multifactorial including genetic, hormonal, environmental, lifestyle, and maternal factors.
- Testicular torsion is the most common reason of testicular regression.

Research agenda

- Continuous survey of the incidence of cryptorchidism is necessary for monitoring male reproductive health, because it may serve as a sentinel for other reproductive problems.
- Methodology of genetics keeps improving and may help studies on cryptorchidism and testicular regression.
- Epigenetic studies with environmental aspects will be an important step forward in studies on cryptorchidism and testicular regression.

Declaration of competing interest

The authors have no conflict of interest to declare.

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References

[1] Boisen KA, Kaleva M, Main KM, et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 2004;363:1264–9.
[2] Kollin C, Ritzen M. Cryptorchidism: a clinical perspective. Pediatr Endocrinol Rev 2014;11:240–50.
[3] Radmayr C, Bogaert G, Dogan HS, et al. EAU-guidelines: paediatric urology. https://uroweb.org/guideline/paediatric-urology/; 2021.
[4] Hack WWM, Goede J, van der Voort-Doedens LM, et al. Acquired undescended testis: putting the pieces together. Int J Androl 2012;35:41–5.
[5] van Brakel J, de Muinck Kaizer-Schrama MPF, van Castren NJ, et al. Scrotal ultrasound findings in previously congenital and acquired unilateral undescended testes and their contralateral normally descended testis. Andrology 2015;3:888–94.
*[6] Heksch RA, Matheson MA, Tishelman AC, et al. Testicular regression syndrome: practice variation in diagnosis and management. Endocr Pract 2019;25:779–86.
*[7] Virtanen HE, Toppari J. Epidemiology and pathogenesis of cryptorchidism. Hum Reprod Update 2008;14:49–58.
[8] Scorer CG. The descent of the testis. Arch Dis Child 1964;39:605–9.
[9] Buemann B, Henriksen H, Villumsen AL, et al. Incidence of undescended testis in the newborn. Acta Chir Scand Suppl 1961;283:289–93.
[10] John Radcliffe Hospital Cryptorchidism Study Group. Cryptorchidism: a prospective study of 7500 consecutive male births, 1984-8. Arch Dis Child 1992;67:892–9.
[11] Berkowitz G, Lapinski R, Dolgin S, et al. Prevalence and natural history of cryptorchidism. Pediatrics 1993;92:44–9.
[12] Thong M-K, Lim C-T, Fatimah H. Undescended testes: incidence in 1,002 consecutive male infants and outcome at 1 year of age. Pediatr Surg Int 1998;13:37–41.
[13] Ghirri P, Ciulli C, Vuerich M, et al. Incidence at birth and natural history of cryptorchidism: a study of 10,730 consecutive male infants. J Endocrinol Investig 2002;25:709–15.
[14] Kübarsepp V, Varik K, Varendi H, et al. Prevalence of congenital cryptorchidism in Estonia. Andrology 2021. https://doi.org/10.1111/andr.13121.
[15] Acerini CL, Miles HL, Dunger DB, et al. The descriptive epidemiology of congenital and acquired cryptorchidism in a UK infant cohort. Arch Dis Child 2009;94:868–72.
[16] Berkowitz GS, Lapinski RH, Godbold JH, et al. Maternal and neonatal risk factors for cryptorchidism. Epidemiology 1995;6:127–31.
[17] Preiksa RT, Zilaitiene B, Matulevicius V, et al. Higher than expected prevalence of congenital cryptorchidism in Lithuania: a study of 1204 boys at birth and 1 year follow-up. Hum Reprod 2005;20:1928–32.
[18] Sijstermans K, Hack WWM, Meijer RW, et al. The frequency of undescended testis from birth to adulthood: a review. Int J Androl 2008;31:1–11.
[19] Raivio T, Toppari J, Kaleva M, et al. Serum androgen bioactivity in cryptorchid and noncryptorchid boys during the postnatal reproductive hormone surge. J Clin Endocrinol Metab 2003;88:2597–9.
[20] Suomi A-M, Main KM, Kaleva M, et al. Hormonal changes in 3-month-old cryptorchid boys. J Clin Endocrinol Metab 2006;91:953–8.
[21] Kuiri-Hänninen T, Koskenniemi JJ, Dunkel L, et al. Postnatal testicular activity in healthy boys and boys with cryptorchidism. Front Endocrinol 2019;10:489. https://doi.org/10.3389/fendo.2019.00489.
[22] Koskenniemi JJ, Virtanen HE, Wohlfahrt-Veje C, et al. Postnatal changes in testicular position are associated with IGF-I and function of sertoli and leydig cells. J Clin Endocrinol Metab 2018;103:1429–37.
[23] Wohlfahrt-Veje C, Boisen KA, Boas M, et al. Acquired cryptorchidism is frequent in infancy and childhood. Int J Androl 2009;32:423–8.
[24] Hack WWM, Sijstermans K, van Dijk J, et al. Prevalence of acquired undescended testis in 6-year, 9-year and 13-year-old Dutch schoolboys. Arch Dis Child 2007;92:17–20.
[25] Agarwal PK, Diaz M, Elder JS. Retractile testis – is it really a normal variant? J Urol 2006;175:1496–9.
*[26] Rodprasert W, Toppari J, Virtanen HE. Endocrine disrupting chemicals and reproductive health in boys and men. Front Endocrinol 2021;12:706532.
[27] Kristensen DM, Hass U, Lesné L, et al. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Hum Reprod 2011;26:235–44.
[28] Kilcoyne KR, Mitchell RT. Effect of environmental and pharmaceutical exposures on fetal testis development and function: a systematic review of human experimental data. Hum Reprod Update 2019;25:397–421.
[29] Yu C, Wei Y, Tang X, et al. Maternal smoking during pregnancy and risk of cryptorchidism: a systematic review and meta-analysis. Eur J Pediatr 2019;178:287–97.
[30] Damgaard IN, Jensen TK, Petersen JH, et al. Cryptorchidism and maternal alcohol consumption during pregnancy. Environ Health Perspect 2007;115:272–7.

[31] Virtanen HE, Tapanainen AE, Kaleva MM, et al. Mild gestational diabetes as a risk factor for congenital cryptorchidism. J Clin Endocrinol Metab 2006;91:4862–5.
[32] Arendt L, Lindhard M, Henriksen T, et al. Maternal diabetes mellitus and genital anomalies in male offspring: a nationwide cohort study in 2 Nordic countries. Epidemiology 2018;29:280–9.
[33] Zhang L, Wang X-H, Zheng X-M, et al. Maternal gestational smoking, diabetes, alcohol drinking, pre-pregnancy obesity and the risk of cryptorchidism: a systematic review and meta-analysis of observational studies. PLoS One 2015;10: e0119006.
*[34] Jensen MS, Toft G, Thulstrup AM, et al. Cryptorchidism concordance in monozygotic and dizygotic twin brothers, full brothers, and half-brothers. Fertil Steril 2010;93:124–9.
[35] Elert A, Jahn K, Heidenreich A, et al. Population-based investigation of familial undescended testis and its association with other urogenital anomalies. J Pediatr Urol 2005;1:403–7.
[36] Czeizel A, Erödi E, Tóth J. Genetics of undescended testis. J Urol 1981;126:528–9.
[37] Schnack TH, Zdravkovic S, Myrup C, et al. Familial aggregation of cryptorchidism – a nationwide cohort study. Am J Epidemiol 2008;167:1453–7.
*[38] Hutson JM, Li R, Southwell BR, et al. Regulation of testicular descent. Pediatr Surg Int 2015;31:317–25.
[39] Hutson JM. A biphasic model for the hormonal control of testicular descent. Lancet 1985;2:419–21.
[40] Barteczko KJ, Jacob MI. The testicular descent in human. Origin, development and fate of the gubernaculum Hunteri, processus vaginalis peritonei, and gonadal ligaments. Adv Anat Embryol Cell Biol 2000;156:1–98.
[41] Bay K, Main KM, Toppari J, et al. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. Nat Rev Urol 2011;8:187–96.
[42] Shono T, Ramm-Anderson S, Hutson JM. Transabdominal testicular descent is really ovarian ascent. J Urol 1994;152: 781–4.
[43] Nef S, Parada LF. Cryptorchidism in mice mutant for Insl3. Nat Genet 1999;22:295–9.
[44] Zimmermann S, Steding G, Emmen J, et al. Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. Mol Endocrinol 1999;13:681–91.
[45] Gorlov I, Kamat A, Bogatcheva N, et al. Mutations of the GREAT gene cause cryptorchidism. Hum Mol Genet 2002;11: 2309–18.
[46] Anand-Ivell R, Ivell R, Driscoll D, et al. Insulin-like factor 3 levels in amniotic fluid of human male fetuses. Hum Reprod 2008;23:1180–6.
[47] Bay K, Cohen AS, Jørgensen FS, et al. Insulin-like factor 3 levels in second-trimester amniotic fluid. J Clin Endocrinol Metab 2008;93:4048–51.
[48] Bay K, Virtanen H, Hartung S, et al. Insulin-like factor 3 levels in cord blood and serum from children: effects of age, postnatal hypothalamic-pituitary-gonadal axis activation, and cryptorchidism. J Clin Endocrinol Metab 2007;92: 4020–7.
[49] Fenichel P, Lahlou N, Coquillard P, et al. Cord blood insulin-like peptide 3 (INSL3) but not testosterone is reduced in idiopathic cryptorchidism. Clin Endocrinol 2015;82:242–7.
[50] Scott HM, Mason JI, Sharpe RM. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev 2009;30:883–925.
[51] Hutson JM, Lopez-Marambio FA. The possible role of AMH in shortening the gubernacular cord in testicular descent: a reappraisal of the evidence. J Pediatr Surg 2017;52:1656–60.
[52] Behringer RR, Finegold MJ, Cate RL. Müllerian-inhibiting substance function during mammalian sexual development. Cell 1994;79:415–45.
[53] Bouzada J, Vazquez T, Duran M, et al. New insights into the morphogenesis of the gubernaculum testis and the inguinal canal. Clin Anat 2017;30:599–607.
[54] Hadziselimovic F. On the descent of the epididymo-testicular unit, cryptorchidism, and prevention of infertility. Basic Clin Androl 2017;27:21.
[55] Heyns CF. The gubernaculum during testicular descent in the human fetus. J Anat 1987;153:93–112.
[56] Hutson JM. Testicular feminization: a model for testicular descent in mice and men. J Pediatr Surg 1986;21:195–8.
[57] Barthold JS, Kumasi-Rivers K, Upadhyay J, et al. Testicular position in the androgen insensitivity syndrome: implications for the role of androgens in testicular descent. J Urol 2000;164:497–501.
[58] Hutson JM, Baskin LS, Risbridger G, et al. The power and perils of animal models with urogenital anomalies: handle with care. J Pediatr Urol 2014;10:699–705.
[59] Hutson JM, Li R, Vikraman J, et al. What animal models of testicular descent and germ cell maturation tell us about the mechanism in humans. Eur J Pediatr Surg 2016;26:390–8.
[60] Cendron M, Huff DS, Keating MA, et al. Anatomical, morphological and volumetric analysis: a review of 759 cases of testicular maldescent. J Urol 1993;149:570–3.
[61] Yuan FP, Li X, Lin J, Schwabe C, et al. The role of RXFP2 in mediating androgen-induced inguinoscrotal testis descent in LH receptor knockout mice. Reproduction 2010;139:759–69.
*[62] Rodprasert W, Virtanen HE, Mäkelä J-A, et al. Hypogonadism and cryptorchidism. Front Endocrinol 2020;10:906.
[63] Urh K, Kunej T. Molecular mechanisms of cryptorchidism development: update of the database, disease comorbidity, and initiative for standardization of reporting in scientific literature. Andrology 2016;4:894–902.
[64] Lubahn DB, Joseph DR, Sullivan PM, et al. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 1988;240:327–30.
[65] Hughes IA, Davies JD, Bunch TI, et al. Androgen insensitivity syndrome. Lancet 2012;380:1419–28.
[66] Foresta C, Zuccarello D, Garolla A, et al. Role of hormones, genes, and environment in human cryptorchidism. Endocr Rev 2008;29:560–80.
*[67] Ferlin A, Zuccarello D, Zuccarello B, et al. Genetic alterations associated with cryptorchidism. JAMA 2008;300:2271–6.
[68] Wang Q, Ge X, Wang H-X, et al. Association of androgen receptor gene CAG and GGN repeat polymorphism with cryptorchidism: a meta-analysis. Andrologia 2017;50:1–6.

*[69] Kalfa N, Gaspari L, Olliver M, et al. Molecular genetics of hypospadias and cryptorchidism recent developments. Clin Genet 2019;95:122–31.
[70] Köhler B, Delezoide A-L, Boizet-Bonhoure B, et al. Coexpression of Wilms' tumor suppressor 1 (WT1) and androgen receptor (AR) in the genital tract of human male embryos and regulation of AR promoter activity by WT1. J Mol Endocrinol 2007;38:547–54.
[71] Barthold JS, Wang Y, Reilly A, et al. Reduced expression of androgen receptor and myosin heavy chain mRNA in cremaster muscle of boys with nonsyndromic cryptorchidism. J Urol 2012;188:1411–6.
[72] Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. Horm Res Paediatr 2014;82:73–80.
[73] Young J, Xu C, Papadakis GE, Acierno JS, et al. Clinical management of congenital hypogonadotropic hypogonadism. Endocr Rev 2019;40:669–710.
[74] Hadziselimovic F, Hoecht B. Testicular histology related to fertility outcome and postpubertal hormone status in cryptorchidism. Klin Pädiatr 2008;220:302–7.
[75] Toppari J, Kaleva M, Virtanen HE, et al. Luteinizing hormone in testicular descent. Mol Cell Endocrinol 2007;269:34–7.
[76] Laitinen E-M, Tommiska J, Virtanen HE, et al. Isolated cryptorchidism: no evidence for involvement of genes underlying isolated hypogonadotropic hypogonadism. Mol Cell Endocrinol 2011;341:35–8.
[77] Sobel V, Imperato-McGinley J. Hypergonadotropic hypogonadism. In: Martini L, editor. Encyclopedia of endocrine diseases. 1st ed. Academic Press; 2004. p. 549–54.
[78] Zitzmann M, Aksglaede L, Corona G, et al. European academy of andrology guidelines on Klinefelter syndrome endorsing organization: European society of endocrinology. Andrology 2021;9:145–67.
[79] Mamoulakis C, Georgiou I, Dimitriadis F, et al. Genetic analysis of the human insulin-like 3 gene: absence of mutations in a Greek paediatric cohort with testicular maldescent. Andrologia 2014;46:986–96.
[80] Ferlin A, Zuccarello D, Garolla A, et al. Mutations in INSL3 and RXFP2 genes in cryptorchid boys. Ann N Y Acad Sci 2009;1160:213–4.
[81] Koskimies P, Virtanen H, Lindström M, et al. A common polymorphism in the human relaxin-like factor (RLF) gene: no relationship with cryptorchidism. Pediatr Res 2000;47:538–41.
[82] Roh J, Virtanen H, Kumagai J, et al. Lack of LGR8 gene mutation in Finnish patients with a family history of cryptorchidism. Reprod BioMed Online 2003;7:400–6.
[83] EL-Ella SSA, Tawfik MA, El-Aziz TFA, et al. The G178A polymorphic variant of INSL3 may be linked to cryptorchidism among Egyptian pediatric cohort. Pediatr Surg Int 2020;36:1387–93.
[84] Ayers K, Kumar R, Robevska G, et al. Familial bilateral cryptorchidism is caused by recessive variants in RXFP2. J Med Genet 2019;56:727–33.
[85] Ferlin A, Bogatcheva NV, Gianesello L, et al. Insulin-like factor 3 gene mutations in testicular dysgenesis syndrome: clinical and functional characterization. Mol Hum Reprod 2006;12:401–6.
[86] Imbeaud S, Rey R, Berta P, et al. Testicular degeneration in three patients with the persistent Müllerian duct syndrome. Eur J Pediatr 1995;154:187–90.
[87] Duan Z, Huang H, Sun F. The functional and predictive roles of miR-210 in cryptorchidism. Sci Rep 2016;6:32265.
[88] Koivusalo A, Taskinen S, Rintala R. Cryptorchidism in boys with congenital abdominal wall defects. Pediatr Surg Int 1998;13:143–5.
[89] Raitio A, Syvänen J, Tauriainen A, et al. Congenital abdominal wall defects and cryptorchidism: a population-based study. Pediatr Surg Int 2021;37:837–41.
[90] Cheng W, Mya GH, Saing H. Associated anomalies in patients with undescended testes. J Trop Pediatr 1996;42:204–6.
[91] Virtanen HE, Bjerknes R, Cortes D, et al. Cryptorchidism: classification, prevalence and long-term consequences. Acta Paediatr 2007;96:611–6.
*[92] Urh K, Kolenc Z, Hrovat M, et al. Molecular mechanisms of syndromic cryptorchidism: data synthesis of 50 studies and visualization of gene-disease network. Front Endocrinol 2018;9:1–9.
[93] Wang Y, Barthold J, Kanetsky PA, et al. Allelic variants in HOX genes in cryptorchidism. Birth Defects Res A Clin Mol Teratol 2007;79:269–75.
[94] Kolon TF, Wiener JS, Lewitton M, et al. Analysis of homeobox gene HOXA10 mutations in cryptorchidism. J Urol 1999;161:275–80.
[95] Bertini V, Bertelloni S, Valetto A, et al. Homeobox HOXA10 gene analysis in cryptorchidism. J Pediatr Endocrinol Metab 2004;17:41–5.
[96] Cheng Z, Wang M, Xu C, et al. Mutational analysis of *HOXA10* gene in Chinese patients with cryptorchidism. Andrologia 2017;49. https://doi.org/10.1111/and.12592.
[97] Zhou B, Tang T, Chen P, et al. The variations in the AXIN1 gene and susceptibility to cryptorchidism. J Pediatr Urol 2015;11:132.e1–5.
[98] Kaftanovskaya EM, Feng S, Huang Z, et al. Suppression of insulin-like3 receptor reveals the role of β-catenin and Notch signaling in gubernaculum development. Mol Endocrinol 2011;25:170–83.
[99] Barthold JS, Ivell R. Perspective: a neuro-hormonal systems approach to understanding the complexity of cryptorchidism susceptibility. Front Endocrinol 2018;9:401.
[100] Wang Y, Li J, Kolon TF, et al. Genomic copy number variation association study in Caucasian patients with nonsyndromic cryptorchidism. BMC Urol 2016;16:62.
[101] Dalgaard MD, Weinhold N, Edsgärd D, et al. A genome-wide association study of men with symptoms of testicular dysgenesis syndrome and its network biology interpretation. J Med Genet 2012;49:58–65.
[102] Barthold JS, Wang Y, Kolon TF, et al. Phenotype specific association of the TGFBR3 locus with nonsyndromic cryptorchidism. J Urol 2014;193:1637–45.
*[103] Barthold JS, Wang Y, Kolon TF, et al. Pathway analysis supports association of nonsyndromic cryptorchidism with genetic loci linked to cytoskeleton-dependent functions. Hum Reprod 2015;30:2439–51.
[104] Spires SE, Woolums S, Pulito AR, Spires SM. Testicular regression syndrome: a clinical and pathologic study of 11 cases. Arch Pathol Lab Med 2000;124:694–8.

[105] Zenaty D, Dijoud F, Morel Y, et al. Bilateral anorchia in infancy: occurence of micropenis and the effect of testosterone treatment. J Pediatr 2006;149:687–91.
[106] Nataraja RM, Yeap E, Healy CJ, et al. Presence of viable germ cells in testicular regression syndrome remnants: Is routine excision indicated? A systematic review. Pediatr Surg Int 2018;34:353–61.
[107] Wood HM, Elder JS. Cryptorchidism and testicular cancer: separating fact from fiction. J Urol 2009;181:452–61.
[108] McElreavey K, Jorgensen A, Eozenou C, et al. Pathogenic variants in the DEAH-box RNA helicase DHX37 are a frequent cause of 46,XY gonadal dysgenesis and 46,XY testicular regression syndrome. Genet Med 2020;22:150–9.
[109] Capra AP, Ferro E, La Rosa MA, et al. Genetic analysis of the human insulin-like 3 gene in pediatric patients with testicular torsion. Pediatr Surg Int 2018;34:807–12.
[110] Shteynshlyuger A, Yu J. Familial testicular torsion: a meta analysis suggests inheritance. J Pediatr Urol 2013;9:683–90.