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REVIEW ARTICLE

Regulation of testicular descent

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Abstract Testicular descent occurs in two morphologically distinct phases, each under different hormonal control from the testis itself. The first phase occurs between 8 and 15 weeks when insulin-like hormone 3 (Insl3) from the Leydig cells stimulates the gubernaculum to swell, thereby anchoring the testis near the future inguinal canal as the foetus grows. Testosterone causes regression of the cranial suspensory ligament to augment the transabdominal phase. The second, or inguinoscrotal phase, occurs between 25 and 35 weeks, when the gubernaculum bulges out of the external ring and migrates to the scrotum, all under control of testosterone. However, androgen acts mostly indirectly via the genitofemoral nerve (GFN), which produces calcitonin gene-related peptide (CGRP) to control the direction of migration. In animal models the androgen receptors are in the inguinoscrotal fat pad, which probably produces a neurotrophin to masculinise the GFN sensory fibres that regulate gubernacular migration. There is little direct evidence that this same process occurs in humans, but CGRP can regulate closure of the processus vaginalis in inguinal hernia, confirming that the GFN probably mediates human testicular descent by a similar mechanism as seen in rodent models. Despite increased understanding about normal testicular descent, the common causes of cryptorchidism remain elusive.

Keywords Testicular descent · Gubernaculum · Testosterone · INSL3 · CGRP · Genitofemoral nerve

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Introduction

In the 18th and 19th centuries, the anatomy of descent of the testis was the primary impetus of research, while throughout the 20th century, the hormonal regulation of testicular descent became the main focus. By the 1980s, attempts were being made to integrate the disparate anatomical and regulatory evidence into a unifying schema, culminating in the proposal of the two-stage model, with the recognition that different hormones were regulating the early and later stages of descent [1].

It is now generally accepted that testicular descent occurs in two discrete anatomical and hormonal stages. In the human both phases occur prenatally, with the transabdominal phase between 10 and 15 weeks' gestation and the inguinoscrotal phase between 25 and 35 weeks of gestation. By contrast, in rodents the transabdominal phase occurs in the third trimester while the inguinoscrotal phase occurs in the first week to 10 days after birth. Apart from these differences in timing, however, the anatomy and hormonal regulation of the two stages of testicular descent are remarkably similar between rodent models and humans [2] (Fig. 1).

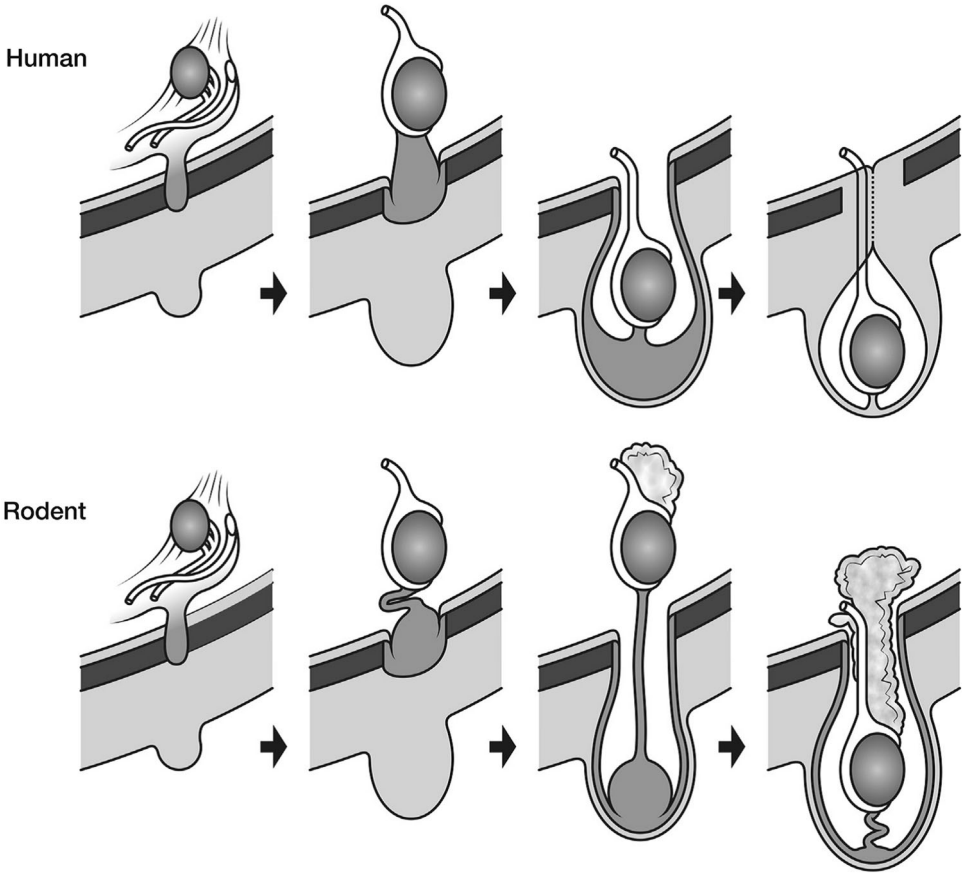

Fig. 1 Testicular descent in the human foetus vs that in a rodent. In both species, the process occurs in two separate phases: the transabdominal and inguinoscrotal stages. Migration of the gubernaculum is similar, except that in rodents the extracellular matrix in the gubernaculum regresses before migration begins at birth, while in humans this occurs after the gubernaculum reaches the scrotum, and the entire process is prenatal. Also in humans, the last step after descent is closure of the processus vaginalis (to prevent inguinal hernia), while in rodents the processus remains open and a fat pad on the epididymis, which plugs the inguinal canal, prevents herniation (reproduced with permission from J Pediatr Urol [2])

Early embryology

The gonads develop on the anteromedial surface of the mesonephros in urogenital ridge, which is attached to the posterior coelomic wall and by thickenings of the attachment cranially (cranial suspensory ligament) and caudally by the genitoinguinal ligament, or gubernaculum. About the time of sexual differentiation in the human (7–8 weeks' gestation) the mesonephros regresses, leaving the developing ovary or testis on a mesentery, now called the mesovarium or mesorchium. In the free edge of the urogenital ridge, the mesonephric (Wolffian) duct and the paramesonephric (Müllerian) duct develop. The Wolffian duct initially drains the mesonephros, but after regression of the latter structure the duct becomes attached directly to the testis to form the rete testis. Under the action of androgen from the newly formed Leydig cells in the developing testis, the Wolffian duct continues to differentiate into the epididymis and vas deferens. At the caudal end of the Wolffian duct the ureteric bud forms, and cranially to this a second, hormone-dependent bud forms the seminal vesicle. The Müllerian duct in the male regresses under the influence of the hormone secreted by the newly differentiated Sertoli cells, anti-Müllerian hormone (AMH) (also known as Müllerian inhibiting substance (MIS)) [3, 4].

Transabdominal phase

Shortly after sexual differentiation at 7–8 weeks' gestation, the transabdominal phase of testicular descent occurs between 10 and 15 weeks. In male, the cranial suspensory ligament regresses and the gubernaculum enlarges; while in the female both ligaments persist without obvious changes as the foetus grows. By a combination of gonadal enlargement and the 'swelling reaction' in the gubernaculum, the testis remains close to the future inguinal canal, where the gubernaculum is attached to the inguinal abdominal wall (Fig. 2a). By contrast the developing ovary moves relatively further from the inguinal region as the female foetus enlarges.

The 'swelling reaction' in the gubernaculum is caused by cell division in the primitive mesenchymal cells of the distal gubernaculum along with a sudden increase in extracellular matrix molecules, especially glycosaminoglycan and hyaluronic acid [5]. This 'swelling reaction' leads to the caudal end of the gubernaculum enlarging to a similar size as the testis where the former is embedded in the abdominal wall [6]. The swollen distal gubernaculum is known as the bulb, and the inguinal abdominal wall muscles differentiate around it to produce the inguinal canal. The proximal attachment of the gubernaculum to the testis

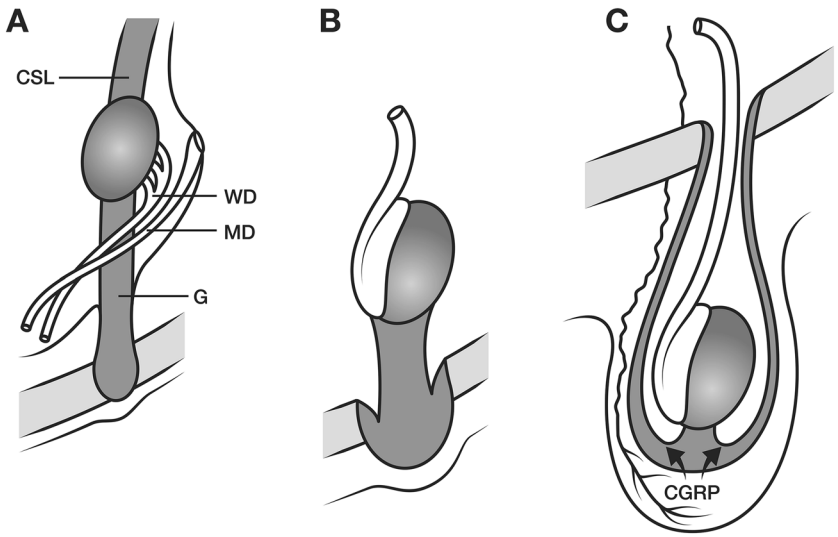

Fig. 2 The two stages of testicular descent. **a** Before descent the developing testis is held in the urogenital ridge by the cranial suspensory ligament (CSL) cranially and the gubernaculum (G) caudally. The adjacent Wolffian duct (WD) forms the epididymis and vas deferens in a male while the Müllerian duct (MD) forms the uterus and tubes in a female. **b** At the end of the transabdominal phase (~15 weeks) the testis is held near the future inguinal ring by the swelling reaction in the gubernaculum. The skin just beyond the gubernaculum is over the future external inguinal ring, as the scrotum is remote in the perineum of a mammal. **c** Inguinoscrotal phase requires the gubernaculum to elongate to the scrotum, under control of androgens and calcitonin gene-related peptide (CGRP) released from the genitofemoral nerve (GFN). After migration is complete, the peritoneum of the processus vaginalis (PV) closes and then completely involutes and disappears (see Fig. 4)

and developing epididymis is known as the gubernacular cord, and this also remains short in the male (Fig. 2b), unlike the female where the entire gubernaculum remains long and forms the round ligament.

The peritoneum over the intraabdominal surface of the gubernacular bulb forms an annular diverticulum that grows into the gubernaculum, dividing the gubernaculum into 3 distinct anatomical parts. The infravaginal part (-caudal to the peritoneal diverticulum) is the bulb of the gubernaculum, which contains undifferentiated mesenchymal cells. Inside the annular diverticulum, the central column of gubernacular cells differentiates into fibroblasts to form the gubernacular cord, which anchors the intraperitoneal gonad and epididymis to the bulb, which is embedded in the inguinal muscles. Just outside the peritoneum in the gubernaculum the cremaster muscle develops, and with elongation of the diverticulum later the muscle comes to lie around the outside of the diverticulum (which will form the processus vaginalis) in the extravaginal part (outside the processus vaginalis).

In 1999, it was discovered that knockout of the insulin-like hormone 3 gene in mice had undescended intraabdominal testes [7, 8], and it has now been confirmed that insulin-like hormone 3 (INSL3) is the primary hormone regulating the swelling reaction. INSL3 secreted from the Leydig cells of the testis stimulates the swelling reaction, and in conjunction with the short gubernacular cord, this provides traction on the testis to keep it close to the inguinal abdominal wall as the foetal abdomen enlarges. INSL3 is secreted in mid gestation which is at the right time to control the swelling reaction, and its receptor (the relaxin family receptor 2, RXFP2) also is located in the gubernaculum at the right time.

INSL3 is a member of the insulin family of related hormones and growth factors, and is synthesised as a 131-amino acid preprotein, which contains a 24-amino acid signalling peptide [9]. In vitro studies of the foetal rat gubernaculum showed that INSL3 stimulated gubernacular growth, with both AMH and testosterone providing some augmented stimulus [10]. Moreover, INSL3 knockout in mice prevented the swelling reaction, so that the gubernacular bulb lacked a central core of undifferentiated mesenchyme at embryonic day 16.5 [7]. When the INSL3 gene was activated in female mice, the ovaries descended to the lower abdomen beside the bladder neck, analogous to transabdominal descent in male mice [11]. INSL3 appears to act through the RXFP2 receptor and then the downstream intracellular signalling involves both NOTCH and Wnt/β-catenin pathways [12–14].

Mutations of INSL3 or its receptor are uncommon in humans with cryptorchidism, which is in keeping with the fact that intraabdominal testes with deficient transabdominal descent are uncommon [15, 16]. However, when considered in proportion to children with impalpable testis

only, defects in INSL3 signalling are likely to be common [17].

Although INSL3 is now accepted as the primary hormone controlling the gubernacular swelling reaction and transabdominal descent, there are some pieces of evidence that suggest a role for AMH, particularly in the human. First, in children with mutations of the AMH gene or its receptor, they have undescended testes with persistence of an infantile uterus and tubes, known as the persistent Müllerian duct syndrome (PMDS). The testes are intraabdominal (~70 %) or prolapsed into a hernial sac (~20 %, 'hernia uteri inguinalis'), or where both testes (along with uterus and tubes) are prolapsed into the same patent processus vaginalis (~10 %, transverse testicular ectopia) [18]. The gubernacular cord in these patients is abnormally long (i.e., >10 cm rather than <½ cm), which prevents the testis being held near the internal inguinal ring as in normal transabdominal descent. By contrast, the swelling reaction is presumed to be normal so that the gubernacular bulb creates an inguinal canal and migration to the scrotum in the inguinoscrotal phase is also normal. The elongated gubernacular cord mirrors a very long round ligament, and allows the testes to flop about in the pelvis and prolapse into the ipsilateral processus vaginalis (to create 'hernia uteri inguinalis'), or even into the contralateral processus vaginalis (to cause transverse testicular ectopia) [18, 19]. In addition, the extreme mobility of the testis in PMDS may predispose to the reported high frequency of intraabdominal torsion causing vanishing testis in this anomaly [20].

Inguinoscrotal phase

The second phase of testicular descent occurs about 25–35 weeks of gestation in the human foetus and in the first week postnatally in a mouse. The overall process is quite similar in most mammals, allowing for some differences [2]. In human, the gubernaculum which originally ended in the inguinal abdominal wall bulges out to create a future external inguinal ring. The gubernaculum then migrates to the scrotum by remodelling from an inert ligament into an actively migrating and elongating structure, with many analogies to an embryonic limb bud [21–23] (Fig. 2c). What triggers this sudden change in the biology of the gubernaculum is incompletely understood, but there is some evidence that the mammary line may be involved [24].

The possible role of the mammary line in the inguinoscrotal phase is suggested by the anatomy of the marsupial, which separated in evolution from eutherian mammals about 200 million years ago. The mammary line persists over the inguinal ring in both modern marsupials, such as the kangaroo, as well eutherian mammals such as rodents and humans. In the kangaroo and wallaby, the homologous muscle to the cremaster is known as the ilio-marsupialis, and it is supplied by the genitofemoral nerve (GFN) in both sexes (the same as in mammals) [25]. In male, it has a suspensory function similar to the retractile reflex; while in female, the muscle is attached to the breast and is the suspensory muscle of the nipple [26] (Fig. 3).

Once the close association between the cremaster muscle and GFN and the breast was appreciated in the marsupial, we went back and looked specifically in the foetal rodent, and to our surprise we found that the GFN supplies not only the gubernaculum itself but also the breast bud. Moreover, the breast bud is located just superficial to where the gubernaculum ends in the abdominal wall [24, 27]. The mammary line has inductive properties that control the underlying mesenchyme similar to the apical ectodermal ridge of an embryonic limb bud, as it arises on the side of the embryo precisely between the upper and lower limb buds

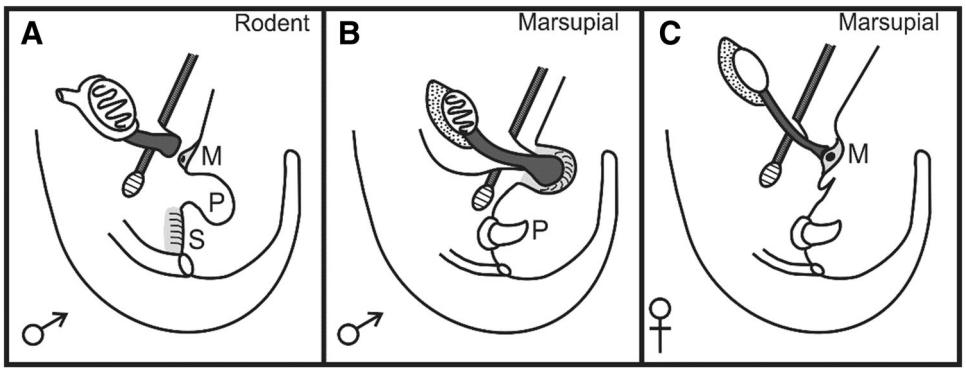

Fig. 3 **a** Foetal male rodent showing close relationship between gubernaculum and mammary bud (M) before inguinoscrotal phase begins (*P* penis, *S* scrotum). **b** Marsupial male pouch young showing gubernaculum extending into scrotum, which is above the pubis, in the same site as the mammary bud in rodent (**a**) and female marsupial (**c**). **c** Marsupial female pouch young showing muscle of gubernaculum (ilio-marsupialis, homologue of cremaster muscle) attaching to the developing mammary bud

[28]. Although it is not completely established, we suspect that the mammary line over the end of the gubernaculum provides the initial signalling to enable remodelling and outgrowth of the gubernaculum like an embryonic limb bud at the start of inguinoscrotal descent [29].

During inguinoscrotal descent, the gubernaculum elongates towards the scrotum while the processus vaginalis inside it also elongates, enabling the intraperitoneal testis to leave the abdomen while still remaining inside an extension of the peritoneum. It is likely that extracellular matrix enzymes, which are produced by the gubernaculum or the inguinoscrotal fat pad itself, dissolve the matrix to enable the gubernaculum to elongate in a free tissue plane without obstruction [30]. Once the gubernaculum has reached the scrotum in human several important changes occur. The bulky, gelatinous extracellular matrix of the gubernacular bulb resorbs, leaving a small fibrous remnant. In addition, the fibrous remnant becomes adherent to the inside of the scrotum. In this short time interval, between arrival of the gubernaculum in the scrotum and development of its fibrous connection to the surrounding tissues, is when perinatal torsion of the testis is likely to occur, because of the extreme mobility of the gubernaculum and its contained testis at that time [31].

The final event after the gubernaculum and testis reach the scrotum is closure of the proximal processus vaginalis in the human, thereby preventing inguinal hernia and/or hydrocele. This final stage of testicular descent is seen in humans and primates, but many other mammals have a processus vaginalis that remains patent. In mouse and rat, for example, the inguinal canal remains patent throughout life, and inguinal hernia is prevented by the presence of a large fat pad attached to the head of the epididymis, which effectively plugs the inguinal canal. Patency of the processus vaginalis is demonstrated by the fact that the testis can retract back into the peritoneal cavity even in an adult rat when the retractile reflex is stimulated.

When all the stages of inguinoscrotal descent are seen together, it can be appreciated that there are actually three steps: (1) migration of the gubernaculum and elongation of the processus vaginalis inside it to enable the intraperitoneal testis to reach the scrotum while still inside the processus vaginalis; (2) closure of the proximal processus but not the distal tunica vaginalis, leaving the testis inside a satellite peritoneal cavity within the hemiscrotum; (3) obliteration of the remnant of the processus vaginalis, which enables the spermatic cord to elongate normally after birth (Fig. 4). Failure of migration leads to congenital cryptorchidism, while failure of the processus closure leads to inguinal hernia or hydrocele. Failure of the last step, i.e., complete involution of the PV, is likely to be the cause of acquired cryptorchidism.

Androgens control the inguinoscrotal phase, as in both humans and animals with complete androgen insensitivity, the testis remains in the inguinal canal or groin, demonstrating normal transabdominal descent but completely deficient inguinoscrotal descent [1]. It was not appreciated until recently that androgens act in a narrow time window, which in the rat is embryonic days 15–19 [32]. It was assumed for many years that androgens would act directly on the gubernaculum, and hence androgen receptors (AR) should be present in the gubernaculum itself. However, on quantitative analysis of AR localisation using immunohistochemistry we found that AR only appeared in the gubernaculum perinatally, just after the window of androgenic sensitivity controlling postnatal inguinoscrotal descent [33]. Not only were AR absent in the gubernaculum during the foetal ‘programming window’, but they were also absent from the sensory cell bodies of the GFN in the L1-2 dorsal root ganglia until after birth [33]. As the GFN has been proposed to not only supply the gubernaculum and its contained structures (processus vaginalis and cremaster muscle), but also direct gubernacular migration to the scrotum, the mechanism by which androgens control descent via the GFN remains unknown, and it is the subject of current research (see below).

The role of the genitofemoral nerve (GFN)

In 1948, Lewis reported that transection of the GFN in a neonatal rat, just before inguinoscrotal migration occurs, caused cryptorchidism [34]. After repeating Lewis’ study in the 1980’s, Beasley and Hutson proposed that the effect of androgen on gubernacular migration may be via the nerve itself, which may release a neuropeptide [35] to control descent. Calcitonin gene-related peptide (CGRP) was subsequently identified in the sensory nucleus of the GFN after initial proposals that the neuropeptide may be in the motor nucleus proved incorrect [36].

There is now abundant evidence that CGRP synthesised in the GFN sensory branches modulates inguinoscrotal migration of the gubernaculum in rodent models. Both transection of the nerve and ablation of sensory nerves with a specific neurotoxin, capsaicin, not only interfere with gubernacular migration but also sensitise the gubernaculum to CGRP by upregulation of CGRP receptors with the gubernaculum to exogenous CGRP in vitro [37–39]. CGRP causes rhythmic contractibility of the developing cremaster muscle in the gubernaculum [40–42], which is thought to orientate the gubernaculum towards the scrotum by chemotaxis [43]. CGRP stimulates mitosis in the gubernacular bulb in vitro, although androgens are required for gubernaculum to respond [44, 45].

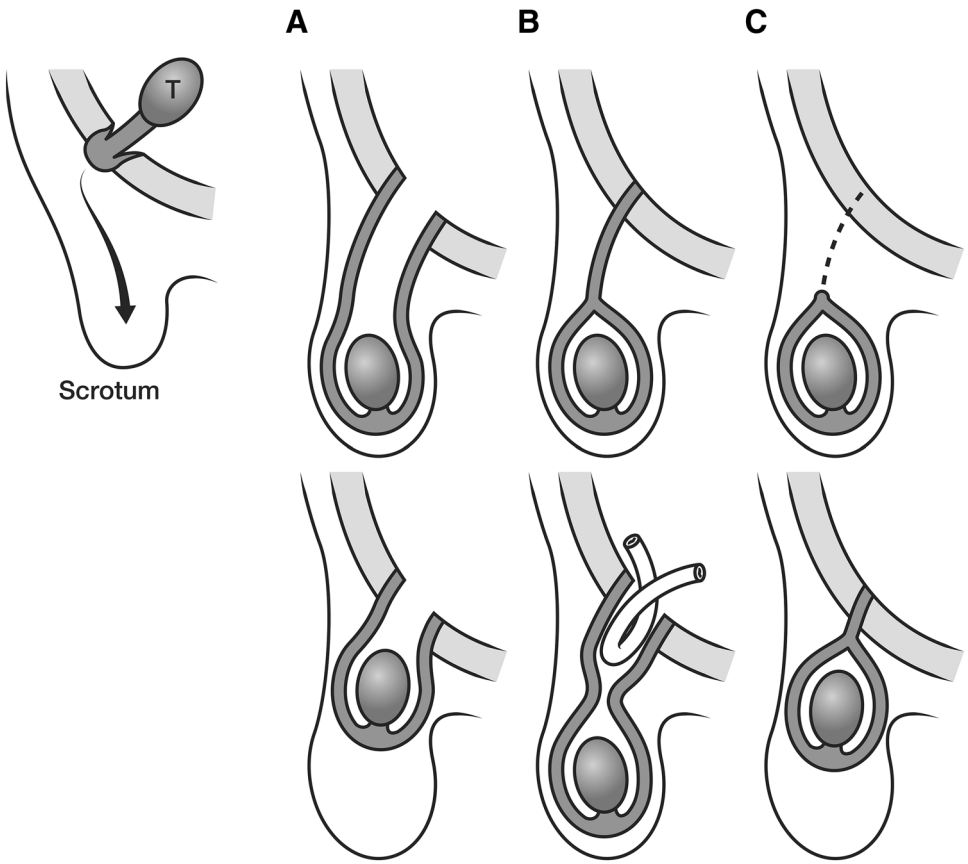

Fig. 4 Schema showing the sequential processes comprising the inguinoscrotal phase of testicular descent. At the end of the transabdominal phase, the enlarged gubernaculum occupies the future inguinal canal, and must migrate 3–5 cm to the scrotum (**a** step 1), taking the testis inside the processus vaginalis, which elongates inside the gubernaculum. Failure of this first step causes congenital cryptorchidism. After migration is complete, the processus vaginalis (PV) closes (**b** step 2), and failure of this causes inguinal hernia or hydrocele. The final (**c** step 3) process is complete involution of the PV remnant, allowing the spermatic cord to elongate after birth. Failure of this step is the likely cause of acquired cryptorchidism, as the fibrous remnant of the PV prevents the spermatic cord growing normally

CGRP receptors are present in the rodent gubernaculum and in models of androgen blockade they are upregulated, consistent with androgen controlling CGRP release from the GFN [46], like the flutamide-treated rat and the TFM mouse. In a rat model of congenital cryptorchidism (transcrotal or TS rat), we found that there was no abnormality of androgenic action, but the GFN in this model contained too many sensory nerves and an excess of CGRP [47]. Cryptorchidism in the TS rat appears to be caused by an excess of CGRP in the GFN sensory branches causing downregulation of the gubernacular response to exogenous CGRP, as this can be reversed by transection or capsaicin, the sensory nerve toxin [39, 47].

With clear evidence that the sensory branches of the GFN control gubernacular development via CGRP, and knowing that this is androgen dependent, the failure of AR to be present in either the GFN or the gubernaculum itself in the foetal 'programming window' suggested that androgens must masculinise the GFN indirectly [48]. One possibility is that target organs of the GFN other than the GFN itself contains AR and responds to androgenic stimulation by synthesising a neurotrophin that is taken up by the nerve endings to modify their function (Fig. 5). This is already been shown to be the case in an adjacent perineal structure, the bulbocavernosus muscle, a muscle that is important in ejaculation, and that is sexually dimorphic. The bulbocavernosus muscle is thought to masculinise its own nerve supply in response to androgens, by producing neurotrophins to modify the nerve. Both brain-derived neurotrophin factor (BDNF) and ciliary neurotrophic factor (CNTF) have been linked to the bulbocavernosus providing peripheral neurotrophic regulation of the perineal branch of the pudendal nerve [49–54]. On the principle that important signalling systems are likely to be preserved in evolution, we are currently investigating the role of BDNF and CNTF in the GFN [25, 55] (Fig. 5).

Current issues

To understand how androgens act on the GFN to produce CGRP for gubernacular migration in inguinoscrotal descent is an important step needed at present. Once we have finally unravelled the complex mechanism, we will be able to assess what are the likely steps that might be abnormal in cryptorchidism. Another issue still to be resolved is regulation of the remodelling that occurs in the inguinoscrotal fat pad to allow gubernacular migration to the scrotum. We need to know which extracellular matrix

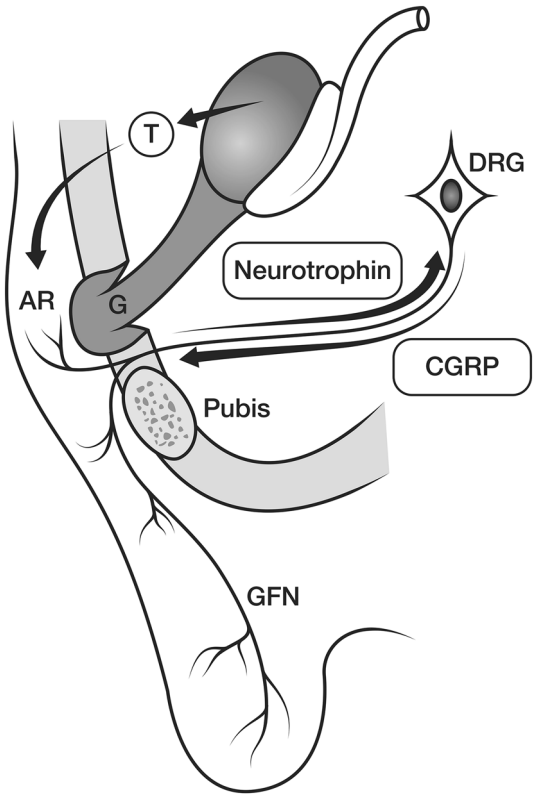

Fig. 5 Current hypothesis on how testosterone (T) masculinises the genitofemoral nerve (GFN) sensory fibres with cell bodies in the dorsal root ganglion (DRG) at L1, L2. The androgen receptors (AR) during the critical narrow time window are located in the inguinoscrotal fat pad which is supplied by the GFN, and through which the gubernaculum (G) must migrate to reach the scrotum. It is possible that the inguinoscrotal fat pad produces neurotrophins in response to androgen that regulate GFN function, so that it can produce CGRP to modulate the migration

enzymes are produced and how they are regulated. At present, we have just begun such a study [30].

Another area still to be explored is how the gubernaculum involutes at the completion of migration, and what controls its fibrous adherence to the inside of the scrotum to prevent perinatal torsion of the testis.

An important current issue is how all the research we have done on the inguinoscrotal phase of descent in animal models relates to the human situation. Certainly there are some who have suggested that the GFN and its neuropeptide, CGRP, may be important in animal models but may not be relevant for testicular descent in humans [56]. It is a truism that extrapolation of biological findings in animal models to the human must be done with care, but the commonality of testicular descent in nearly all modern mammals implies that the important parts of the mechanism are likely to be the same or very similar, once we allow for some minor anatomical differences [2].

The key evidence supporting a role for the GFN in children comes from study of the processus vaginalis, and what controls its perinatal closure to prevent inguinal hernia [57]. As mentioned above, the processus vaginalis (PV) is derived from the specialised peritoneum covering the urogenital ridge [58], and it forms inside the gubernaculum to allow the intraperitoneal testis to exit from the abdomen while remaining inside the peritoneal cavity [59]. We reasoned that the development of the PV and inguinoscrotal descent must be integrated so that testicular descent and PV obliteration could be precisely coordinated in timing. PV closure usually occurs just before birth, when foetal androgens are present, or shortly after birth when the transient postnatal surge of androgen occurs, known as 'minipuberty' [60, 61]. As androgens were postulated to act via the GFN in rodents, we looked at inguinal hernia in children and found CGRP-immunoreactive nerve fibres and CGRP receptors in the human processus vaginalis [62]. More importantly, we found in an in vitro system that exogenous CGRP could induce fusion PV excised during inguinal herniotomy by epithelial transformation [63]. The CGRP receptors in the PV are not in the mesothelium itself, but in the underlying mesenchyme, and fusion of the PV can be induced by both CGRP and hepatocyte growth factor (HGF) which may be released by the mesenchyme to trigger the adjacent epithelium to transform into motile fibroblasts [64]. Taken together, all these studies suggest that the GFN is controlling both testicular descent and subsequent PV closure in humans, and that inguinal hernia is likely to respond to medical treatment, such as a local, slow-release injection of CGRP into the inguinal region [65].

The transient postnatal surge in gonadotropins and androgen known as 'minipuberty' occurs about 2–6 months of age in humans. It is thought to have a role in male gender identity, by regulating changes in brain function [66]. In addition, it is likely to have an important function in closing the PV (to prevent inguinal hernia), and also to remodel any remaining fibroblasts so that the PV has completely disappeared. This process is likely to be crucial to prevent acquired cryptorchidism, which is often caused by failure of the fibrous remnant of the PV to completely disappear [67]. Spontaneous descent of many acquired undescended testes at puberty suggests that PV involution is under androgenic control, probably indirectly via CGRP release from the GFN. Taken together these observations suggest that the GFN (via CGRP) regulates not only gubernacular migration prenatally, but also PV closure and subsequent obliteration postnatally. A possible cause for congenital cryptorchidism, therefore, is insufficient CGRP release from the GFN prenatally. If minipuberty is deficient, there might also be a deficiency of CGRP release

postnatally, predisposing the infant to inguinal hernia, hydrocele and acquired UDT.

Conclusion

There is still much to learn about the regulation of testicular descent, but the evidence suggests that cryptorchidism is likely to be caused by a large number of defects interfering with the very complex mechanism whereby the previously 'inert' foetal gubernaculum is triggered to remodel and migrate 3–4 cm from the external inguinal ring to the scrotum [68]. The link between congenital and acquired UDT and inguinal hernia raises the possibility that all three conditions may respond to a local medical treatment, such as a depot injection of CGRP itself or a synthetic analogue.

References

1. Hutson J (1985) A biphasic model for the hormonal control of testicular descent. Lancet 2(8452):419–420
2. Hutson JM et al (2014) The power and perils of animal models with urogenital anomalies: handle with care. J Pediatr Urol 10(4):699–705
3. Lee MM, Donahoe PK (1993) Mullerian inhibiting substance: a gonadal hormone with multiple functions. Endocr Rev 14(2): 152–164
4. Lane AH, Donahoe PK (1998) New insights into mullerian inhibiting substance and its mechanism of action. J Endocrinol 158(1):1–6
5. Heyns CF (1990) Exstrophy of the testis. J Urol 144:724–725
6. Backhouse KM (1964) The gubernaculum testis hunteri: testicular descent and maldescent. Ann Royal Colleg Surg Engl 35:15–33
7. Nef S, Parada LF (1999) Cryptorchidism in mice mutant for Insl3. Nat Genet 22(3):295–299
8. Zimmermann S et al (1999) Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. Mol Endocrinol 13(5):681–691
9. Ivell R, Hartung S, Anand-Ivell R (2005) Insulin-like factor 3: where are we now? Ann N Y Acad Sci 1041(1):486–496
10. Kubota Y et al (2001) Leydig insulin-like hormone, gubernacular development and testicular descent. J Urol 165(5):1673–1675
11. Adham IM et al (2002) The overexpression of the insl3 in female mice causes descent of the ovaries. Mol Endocrinol 16(2): 244–252
12. Chen N et al (2011) Gone with the Wnt: the canonical Wnt signaling axis is present and androgen dependent in the rodent gubernaculum. J Pediatr Surg 46(12):2363–2369
13. Kaftanovskaya EM et al (2011) Suppression of insulin-like3 receptor reveals the role of beta-catenin and Notch signaling in gubernaculum development. Mol Endocrinol 25(1):170–183
14. Bay K et al (2011) Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. Nat Rev Urol 8(4):187–196
15. Virtanen HE, Toppari J (2008) Epidemiology and pathogenesis of cryptorchidism. Hum Reprod Update 14(1):49–58
16. Ferlin A et al (2007) Hormonal and genetic control of testicular descent. Reprod Biomed Online 15(6):659–665
17. Virtanen HE, Toppari J (2014) Embryology and physiology of testicular development and descent. Pediatric Endocrinol Rev PER 11(Suppl 2):206–213
18. Hutson JM, Baker ML (1994) A hypothesis to explain abnormal gonadal descent in persistent mullerian duct syndrome. Pediatr Surg Int 9:542–543
19. Hutson JM, Chow CW, Ng W-D (1987) Persistent mullerian duct syndrome with transverse testicular ectopia. Pediatr Surg Int 2:191–194
20. Imbeaud S et al (1995) Testicular degeneration in three patients with the persistent mullerian duct syndrome. Eur J Pediatr 154(3):187–190
21. Huynh J et al (2007) Signalling molecules: clues from development of the limb bud for cryptorchidism? Pediatr Surg Int 23(7):617–624
22. Wolpert L (1999) Vertebrate limb development and malformations. Pediatr Res 46(3):247–254
23. Dudley AT, Ros MA, Tabin CJ (2002) A re-examination of proximodistal patterning during vertebrate limb development. Nature 418(6897):539–544
24. Nation T et al (2009) The antiandrogen flutamide perturbs inguinoscrotal testicular descent in the rat and suggests a link with mammary development. J Pediatr Surg 44(12):2330–2334
25. Su S et al (2012) Regression of the mammary branch of the genitofemoral nerve may be necessary for testicular descent in rats. J Urol 188(4 Suppl):1443–1448
26. Coveney D et al (2002) The development of the gubernaculum and inguinal closure in the marsupial Macropus eugenii. J Anat 201:239–256
27. Nightingale SS, Western P, Hutson JM (2008) The migrating gubernaculum grows like a "limb bud". J Pediatr Surg 43(2): 387–390
28. Krajnc-Franken MA et al (2004) Impaired nipple development and parturition in LGR7 knockout mice. Mol Cell Biol 24(2): 687–696
29. Na AF et al (2007) Cell membrane and mitotic markers show that the neonatal rat gubernaculum grows in a similar way to an embryonic limb bud. J Pediatr Surg 42(9):1566–1573
30. Churchill JA et al (2011) Gubernaculum as icebreaker: do matrix metalloproteinases in rodent gubernaculum and inguinal fat pad permit testicular descent? J Pediatr Surg 46(12):2353–2357
31. Das S, Singer A (1990) Controversies of perinatal torsion of the spermatic cord: a review, survery and recommendations. J Urol 143:231–233
32. Welsh M et al (2008) Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest 118(4):1479–1490
33. Nation TR et al (2011) The effect of flutamide on expression of androgen and estrogen receptors in the gubernaculum and surrounding structures during testicular descent. J Pediatr Surg 46(12):2358–2362
34. Lewis LG (1948) Cryptorchism. J Urol 60:345–356
35. Beasley SW, Hutson JM (1987) Effect of division of genitofemoral nerve on testicular descent in the rat. ANZ J Surg 57:49–51
36. Schwindt B et al (1999) Localization of calcitonin gene-related peptide within the genitofemoral nerve in immature rats. J Pediatr Surg 34(6):986–991
37. Yamanaka J, Metcalfe SA, Hutson JM (1992) Demonstration of calcitonin gene-related peptide receptors in the gubernaculum by computerized densitometry. J Pediatr Surg 27:876–878
38. Yamanaka J et al (1993) Testicular descent. II. Ontogeny and response to denervation of calcitonin gene-related peptide receptors in neonatal rat gubernaculum. Endocrinology 132(1):280–284

39. Hrabovszky Z, Hutson JM (1999) Capsaicin restores gubernacular contractility in TS rats. J Pediatr Surg 34(12): 1769–1772
40. Park W-H, Hutson JM (1991) The gubernaculum shows rhythmic contractility and active movement during testicular descent. J Pediatr Surg 26(5):615–617
41. Momose Y, Griffiths AL, Hutson JM (1992) Testicular descent III. The neonatal gubernaculum shows rhythmic contraction in organ culture in response to calcitonin gene-related peptide. Endocrinology 131:2881–2884
42. Terada M et al (1994) Ontogeny of Gubernacular Contraction and Effect of Calcitonin Gene-Related Peptide in the Mouse. J Pediatr Surg 29(5):609–611
43. Yong EXZ et al (2008) Calcitonin gene-related peptide stimulates mitosis in the tip of the rat gubernaculum in vitro and provides the chemotactic signals to control gubernacular migration during testicular descent. J Pediatr Surg 43(8):1533–1539
44. Shenker NS et al (2006) A new role for androgen in testicular descent: permitting gubernacular cell proliferation in response to the neuropeptide, calcitonin gene-related peptide. J Pediatr Surg 41(2):407–412
45. Ng YH et al (2009) Growth of the rat gubernaculum in vitro and localisation of its growth centre. J Pediatr Surg 44(2):422–426
46. Terada M et al (1994) Calcitonin gene-related peptide receptors in the gubernaculum of normal rat and 2 models of cryptorchidism. J Urol 152(2 Pt 2):759–762
47. Terada M et al (1995) The role of the genitofemoral nerve and CGRP in congenitally cryptorchid mutant TS rats. J Urol 154:734–737
48. Nation T et al (2011) Androgen and estrogen receptor expression in the spinal segments of the genitofemoral nerve during testicular descent. J Pediatr Surg 46(8):1539–1543
49. Sengelaub DR et al (1989) Hormonal control of neuron number in sexually dimorphic spinal nuclei of the rat: III. differential effects of the androgen dihydrotestosterone. J Comp Neurol 280(4): 637–644
50. Popper P, Micevych PE (1990) Steroid regulation of calcitonin gene-related peptide mRNA expression in motoneurons of the spinal nucleus of the bulbocavernosus. Brain Res Mol Brain Res 8(2):159–166
51. Yang LY, Verhovshek T, Sengelaub DR (2004) Brain-derived neurotrophic factor and androgen interact in the maintenance of dendritic morphology in a sexually dimorphic rat spinal nucleus. Endocrinology 145(1):161–168
52. Forger NG et al (1998) Ciliary neurotrophic factor receptor alpha in spinal motoneurons is regulated by gonadal hormones. J Neurosci 18(21):8720–8729
53. Bartoletti A et al (2002) Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation. J Neurosci 22(23):10072–10077
54. DeChiara TM et al (1995) Mice lacking the CNTF receptor, unlike mice lacking CNTF, exhibit profound motor neuron deficits at birth. Cell 83(2):313–322
55. Liu Y et al (2012) Sexually dimorphic BDNF signaling directs sensory innervation of the mammary gland. Science 338(6112): 1357–1360
56. Husmann DA (2009) Testicular descent: a hypothesis and review of current controversies. Pediatr Endocrinol Rev 6(4):491–495
57. Clarnette TD, Hutson JM (1996) The genitofemoral nerve may link testicular inguinoscrotal descent with congenital inguinal hernia. Aust NZ J Surg 66(9):612–617
58. Buraundi S et al (2011) Gubernacular development in the mouse is similar to the rat and suggests that the processus vaginalis is derived from the urogenital ridge and is different from the parietal peritoneum. J Pediatr Surg 46(9):1804–1812
59. Clarnette TD, Hutson JM, Beasley SW (1996) Factors affecting the development of the processus vaginalis in the rat. J Urol 156(4):1463–1466
60. Clarnette TD, Lam SKL, Hutson JM (1998) Ventriculo-Peritoneal shunts in children reveal the natural history of closure of the processus vaginalis. J Pediatr Surg 33:413–416
61. Hadziselimovic F et al (2007) Infertility in cryptorchidism is linked to the stage of germ cell development at orchidopexy. Horm Res 68(1):46–52
62. Sugita Y et al (1999) Calcitonin gene-related peptide (CGRP)-immunoreactive nerve fibres and receptors in the human processus vaginalis. Hernia 3:113–116
63. Hutson JM et al (2000) In vitro fusion of human inguinal hernia with associated epithelial transformation. Cells Tissues Organs 166(3):249–258
64. Cook BJ, Hasthorpe S, Hutson JM (2000) Fusion of childhood inguinal hernia induced by HGF and CGRP via an epithelial transition. J Pediatr Surg 35(1):77–81
65. Hutson JM, Temelcos C (2005) Could inguinal hernia be treated medically? Med Hypotheses 64(1):37–40
66. Hrabovszky Z, Hutson JM (2002) Androgen imprinting of the brain in animal models and humans with intersex disorders: review and recommendations. J Urol 168:2142–2148
67. Clarnette TD et al (1997) Incomplete disappearance of the processus vaginalis as a cause of ascending testis. J Urol 157:1889–1891
68. Kollin C, Ritzen EM (2014) Cryptorchidism: a clinical perspective. Pediatric Endocrinol Rev PER 11(Suppl 2):240–250