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INVITED REVIEW

Male Health

The embryology of persistent cloaca and urogenital sinus malformations

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Cloacal malformations are characterized by the confluence of the lower urinary tract, the female reproductive tract, and the rectum to create a common channel with a single opening on the perineum. The presence of a cloaca is a normal phase of early human embryological development. Between the 4th and 7th weeks of gestation, the cloaca undergoes subdivision to form the hindgut and urogenital sinus. Failure of this process results in the congenital anomaly termed persistent cloaca (PC). The term urorectal septum malformation sequence (URSMS) is also used to describe this anomaly. The classic description of this process which is still cited in many standard textbooks dates from the 19th century. However, this has been increasingly called into question by the findings of studies using modern scientific methodology. Urogenital sinus anomalies are defined by the confluence of the urethra and vagina to form a common channel of varying length with a single perineal opening. In this condition, the anorectal canal opens separately on the perineum. The presence of a urogenital sinus represents a transient phase of the normal development of the lower genital tract in the female fetus. However, the form of urogenital sinus most commonly encountered in the developed world is a feature of disordered sexual differentiation and does not arise simply from the persistence of the anatomical structure which is a feature of normal fetal development.

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PERSISTENT CLOACA: INTRODUCTION

The term "cloaca" is derived from the Latin word for a sewer. The defining anatomical feature of persistent cloacal anomalies (PC) is the confluence of the urethra, vagina, and rectum to form a common channel which opens via a single opening on the perineum at a site usually corresponding to the region of the external urethral meatus (**Figure 1**).

The incidence of this anomaly has been historically cited in the literature as 1: 50 000.[1] An epidemiological study of over 4 million births in the United Kingdom recorded a prevalence of 2.8 cases per 100 000 live births.[2] However, the number of liveborn cases was matched by a comparable number of cases in which elective termination of pregnancy had been undertaken following prenatal detection of the abnormality. A study of risk factors undertaken by the National Birth Defects Prevention Study in the United States identified positive associations with maternal obesity, maternal use of fertility medication, and periconceptual exposure to X-rays.[3]

Persistent cloacal malformations can be considered as the female equivalent of high anorectal malformations which correspond to rectal atresia accompanied by a congenital recto urethral fistula in males.[4]

Persistent cloacal malformations do not constitute a single homogeneous entity. The most obvious variation relates to the level of the confluence and length of the common channel. Accordingly, PC can be categorized into "high," "low," or "intermediate" anatomical variants. High-confluence variants are associated with a greater incidence of coexisting spinal anomalies and have poorer outcomes from reconstructive surgery than "low-" confluence variants with a short common channel.[5] Distension of the vagina due to obstruction at the point of confluence with the common channel (termed hydrocolpos) is relatively common – occurring in around a third of cases.[6]

PERSISTENT CLOACA: EMBRYOLOGY

At the start of the 4th week, the primitive hindgut and the caudal component of the allantois remain merged as a single cavity constituting the cloaca. Subdivision (or "compartmentalization") occurs between the 4th and 7th weeks to create a separate anorectal canal on the dorsal aspect and urogenital sinus on the ventral aspect.

The standard account of this process dates from the 19th century with the description by Rathke (1832) of lateral longitudinal folds (Rathke folds) on opposite walls of the cloaca which extend toward each other and fuse in the midline to subdivide the cloaca into two cavities. Tourneaux (1888) then postulated a mechanism of subdivision whereby a cranial fold (Tourneaux fold or "urorectal septum") descends in a caudal direction toward the cloacal membrane. These two mechanisms are not mutually exclusive and a postulated model of cloacal subdivision was developed in which descent of the urorectal septum acts in synergy with the ingrowth of lateral folds.

Over the last three decades, this model has been increasingly called into question by the introduction of modern scientific methodologies and the findings of experimental studies in animal models, notably by the use of targeted gene ablation to create knockout mutant embryos in mice.[7–9]

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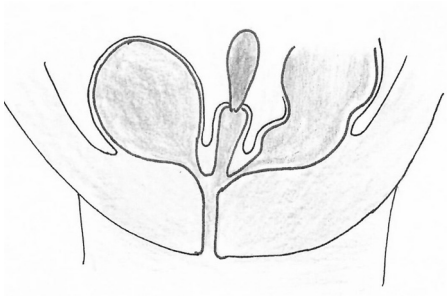

Figure 1: Diagram illustrating the anatomical features of persistent cloaca. These are characterized by a confluence of the bladder, vagina, and rectum to form a common channel with a single opening on the perineum.

The weight of published evidence now supports the concept of subdivision of the cloaca occurring as a more passive process consequent on spatial realignment and differential dorsoventral growth of the cloaca associated with unfolding (or straightening) of the caudal body axis.

A detailed review is beyond the scope of this article, but some of the studies which have contributed to the evolution of the "passive concept" can be briefly summarized as follows.

In 1986, van der Putte[7] questioned the validity of the classic account of cloacal subdivision, noting that it had been derived from drawings rather than photographs and relied on subjective interpretation of transverse histological sections. Having previously studied pig embryos obtained from a herd of pigs with a high incidence of hereditary anorectal malformations, van der Putte went on to study patterns of normal anorectal development in a series of human embryos, fetuses, and newborn infants. Refuting the "classic" model, van der Putte concluded that the principal event in anorectal development is the "*….. shift of the dorsal part of the cloaca and the adjacent gut to the body surface of the tail groove. Regression of the dorsal part of the cloacal membrane forms an essential part of this process.*"

Using electron microscopy, Kluth and associates (1995)[9] studied hindgut development in normal rat embryos and in the embryos of a Sprague–Dawley mutant strain of mouse embryos with a high incidence of anorectal anomalies. These authors identified a structure corresponding to the urorectal septum, but were unable to confirm the existence of lateral folds. They observed that while the ventral part of the cloacal membrane and surrounding structures underwent a significant shift in their relative position, the dorsal part of the cloacal membrane and dorsal wall of the cloaca remained in a relatively fixed position in contact with the tail region. Penington and Hutson (2003)[10] studied cloacal development in human and rat embryos with scanning electron microscopy and immunostaining of serial sections using the T–uridine nick end labeling (TUNEL) method to identify markers of apoptosis. These authors also found no evidence to substantiate the existence of lateral (Rathke) folds. Moreover, they postulated that what has classically been described as the urorectal septum is not a discrete structure (septum) in the conventional sense, but a confluence of proliferative mesenchyme at the interface between the genitourinary sinus and hindgut.

In recent years, digitized three-dimensional (3D) imaging has proved to be a powerful additional tool for visualizing the dynamic spatial changes occurring at the crucial stages of normal and abnormal cloacal morphogenesis in human and mouse embryos. Huang and associates[11] used high-resolution episcopic microscopy (HREM) technology combined with 3D visualization software to create detailed three-dimensional morphological images at different stages of cloacal development in normal and knockout mutant mouse embryos. These authors' findings indicate that, in mouse embryos, cloacal morphogenesis and separation of the urogenital tract from the anorectal canal occurs as the consequence of dynamic spatial realignment of tissues surrounding the dorsal pericloacal mesenchyme, rather than active subdivision caused by descent of a urorectal septum. The use of similar methodology (3D reconstruction and 4D remodeling software) has also been reported by Kruepunga and associates[12] to study cloacal morphogenesis in digitized sections of 4–10-week human embryos. The authors observed that differential growth between ventral and dorsal regions of the cloaca with "near absent" growth of the dorsal cloaca are the normal characteristics of development of the cloaca.

It is important to recognize the potential limitations of extrapolating the findings in mouse embryos to human cloacal development. Nevertheless, there are obvious similarities because cloacal morphogenesis appears to be closely linked to a pattern of differential growth and spatial realignment which occurs in conjunction with the straightening or "unfolding" of the caudal body axis common to mammalian embryos.

In summary, the weight of evidence now supports the concept of subdivision of the cloaca as a predominantly passive process. Insofar as a septum or lateral folds are represented during this process, they are probably best regarded as features of differential growth and spatial realignment of surrounding tissues, rather than being responsible for initiating or regulating the subdivision of the cloaca themselves.

Tourneaux's original concept of active subdivision of the cloaca by the descent of a urorectal septum has been largely refuted. Nevertheless, for clinicians, it provides a simple conceptual model with which to envisage the normal pattern of development and the origins of the spectrum of persistent cloacal malformations.

PERSISTENT CLOACA: GENETIC AND MOLECULAR FACTORS

The genes and gene products involved in the regulation of normal differentiation and development of the hindgut and lower urinary tract have been extensively studied. Sonic hedgehog (*Shh*) signaling genes play a particularly important role.[13,14] Seifert *et al.*[15] reported that cellular proliferation in the urorectal septum mesoderm and subdivision of the cloaca in mice is dependent on Shh signaling from the cloacal endoderm and targeted ablation of the *Shh* gene causes cloacal malformations in knockout mice. Using high-resolution episcopic microscopy and 3D visualization software, Huang and associates[11] characterized the patterns of morphological disruption of cloacal development observed in *Shh* and *Dkk1* mutant mouse embryos. Persistent cloaca, in common with other anorectal malformations, is encompassed within the VACTERL association – comprising vertebral, anorectal, cardiovascular, tracheoesophageal, renal, and limb abnormalities. Although diverse, these anomalies are believed to originate from defects of gastrulation – the formation of the three primary germ layers within the embryonic plate around the 3rd week.[16] Many genes have been shown to be involved in the regulation of gastrulation and the critical early stages of organogenesis. Unsurprisingly, therefore, a correspondingly wide range of genetic mutations have been identified in individuals with VACTERL malformations.

PERSISTENT CLOACA: CLINICAL ASPECTS

Abnormalities of the lower spine date from the same stage in gestation – as reflected in the high incidence of sacral agenesis (>40%) associated

with cloacal malformations. Depending on the severity of the sacral agenesis (number of missing sacral segments), patients with persistent cloaca may suffer from neuropathic bladder dysfunction in addition to any functional impairment arising from the abnormal anatomy of their lower urinary tract.[17] Upper urinary tract abnormalities are also very common, with a reported overall incidence approaching 90%.[18] Upper tract obstruction is often secondary to the abnormal anatomy of the lower urinary tract, but other abnormalities such as renal agenesis and renal dysplasia are clearly intrinsic primary defects arising from aberrant mesodermal differentiation in the first few weeks of gestation. Renal insufficiency of varying severity occurs in around 50% of individuals with persistent cloaca.[19]

To a considerable degree, the extent and complexity of reconstructive surgery is determined by the severity of the cloacal malformation – as evidenced by the level of the confluence and length of the common channel and by the presence of coexisting abnormalities. The range of techniques currently in use in specialist centers includes total urogenital mobilization (TUM), posterior sagittal anorecto-vagino-urethroplasty, and vaginal substitution. Several operations may be required and the incidence of complications is relatively high, even in major specialist centers. In their series of 490 patients, Levitt and Peña[5] reported that the best functional outcomes were achieved in patients in whom the length of the common channel was less than or equal to 3 cm.

UROGENITAL SINUS: INTRODUCTION

The defining anatomical feature of this condition is the confluence of the urethra and vagina to form a common channel (urogenital sinus) with a single external opening (**Figure 2**). The presence of a common urogenital sinus is a transient feature of normal development, and urogenital sinus can rarely occur as a variant of persistent cloaca. In such cases, the anorectal canal has a separate perineal opening (which may be ectopic), but in other respects, the anatomy of the lower urinary and female genital tracts resembles persistent cloaca. Hydrocolpos is relatively common and affected infants may demonstrate other syndromic features.

In the Western world, however, most cases of urogenital sinus are not solely attributable to the abnormal persistence of fetal anatomy, but occur within the context of disordered sexual differentiation (DSD). Of these, the most common is congenital adrenal hyperplasia (CAH), which is estimated to account for approximately 80% of DSDs.[20]

UROGENITAL SINUS: EMBRYOLOGY

The undifferentiated precursors of the internal and external genital tracts are identical in males and females at around 6 weeks of gestation. Male differentiation occurs in response to the influence of the *SrY* gene and the relevant downstream genes and gene products, notably anti-Müllerian hormone and testosterone. Normal male differentiation is also dependent on the enzyme 5-alpha reductase for conversion of testosterone into dihydrotestosterone and the expression of relevant receptor genes in the target tissues.

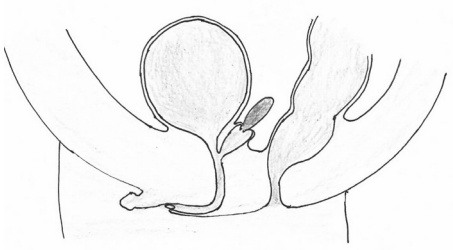

Figure 2: Diagram illustrating the anatomy of a urogenital sinus in a virilized female infant with congenital adrenal hyperplasia. High confluence of the vagina and urethra with a long urogenital sinus which opens onto the ventral aspect of a hypertrophied clitoris.

In the absence of the *SrY* gene, the genital tract differentiates down a female pathway.[21] The paramesonephric ducts fuse at their distal ends to form the ureterovaginal canal. This has a single lumen which is destined to form the uterine cavity and much of the vagina. At around 10 weeks, the ureterovaginal canal attaches to the urogenital sinus to form a condensation of solid tissue which is conventionally described as the sinuvaginal bulb. However, the existence of this structure and indeed the conventional account of the formation of the vagina have been called into question by recent studies. In the conventional description, the solid tissue of the sinuvaginal bulb extends in a caudal direction toward the perineum as a solid "vaginal plate." In the conventional model, the section of vaginal plate derived from the urogenital sinus then canalizes to form the lower part of the vagina, with most of the remaining length of the vagina being derived from the (paramesonephric) ureterovaginal canal. This process occurs between the 10th and 20th weeks. However, it has become increasingly clear that development of the human vagina is more complicated than was previously thought. Using immunostaining for PAX2 (which is reactive for paramesonephric epithelium) and FOXA1 (which is reactive for urogenital sinus epithelium), Robboy *et al.*[22] studied the location of the junction between these two types of epithelia at different stages between 10 and 21 weeks. At 12 weeks, almost the entire vaginal plate expressed PAX2, with only the most caudal part expressing FOXA1. However, subsequent growth of the vaginal plate occurred through proliferation of cells expressing FOXA1 (that is to say urogenital sinus), with the effect that the boundary between urogenital sinus and paramesonephric epithelia moved steadily in a cranial direction. By 21 weeks, the epithelium of the entire vagina from the introitus to the cervix stained for FOXA1. It is unclear to what extent the origin of vaginal epithelium is representative of the origin of the vagina itself. Moreover, the possibility of inductive stromal signaling and epithelial transformation in response to oestrogenic stimulation has also been acknowledged by these authors as possible explanations for their findings.

In this respect, vaginal development in humans appears to differ from the mouse, in which adult vaginal epithelium has been reported by Kurita[23] to be derived solely from Müllerian duct (paramesonephric duct) epithelium.

The findings of some earlier studies have also indicated that the human vagina is derived entirely from the fused caudal ends of the paramesonephric ducts. Ongoing studies can be expected to resolve some of the controversies surrounding the development of the human vagina.

As with the internal genitalia, the precursors of the external genitalia are present in an identical undifferentiated form in both sexes at 6 weeks and it is not until the 12th week that obvious differences begin to emerge.[24] Following the formation of the urogenital sinus by subdivision of the cloaca, the cloacal membrane ruptures to create the urogenital plate on the perineal surface. Anterior to the urogenital plate lies a condensation of mesenchyme which develops to become the genital tubercle. It is from this that the penis and clitoris are derived. The urogenital plate advances anteriorly from the opening of the urogenital sinus along the under surface of the phallus toward the glans located on the tip of the genital tubercle. Urogenital folds then appear on either side of the urogenital plate to convert it into the urethral groove. In the female, the urogenital folds give rise to the labial folds (labia majora

and labia minora) while the urogenital plate remains open to create the vaginal introitus.

The use of three-dimensional reconstruction techniques such as optical projection tomography (OPT), scanning electron microscopy (SEM), and light sheet fluorescence microscopy (LSFM) has greatly advanced understanding of the mechanisms and timescale of events involved in normal penile and clitoral development.[25]

The formation of the male urethra has been likened by the San Francisco group to the action of a "double zipper."[26] The "opening zipper" mechanism is observed between 9.5 and 15 weeks of gestation and consists of canalization and opening out of the solid urethral plate, which, combined with extension of its lateral margins, creates a wide urethral groove lying between the urethral folds. At a cellular level, this process appears to be driven by a centrally placed proliferative zone on the dorsal aspect of the urethral groove from where cells migrate laterally to widen the groove prior to its subsequent closure. This appears to be a predominantly proliferative process because apoptotic markers are not expressed. The term "closing zipper" has been applied to the process of adhesion of the lateral margins of the urethral groove to create the tubular penile urethra. The action of the "closing zipper" commences proximally and extends along the length of the penis in a distal direction toward the glans. Using identical methodology, these authors then studied external genital development in second trimester female fetuses. As in the male, the solid urethral plate opens out by a process of cellular proliferation and extends laterally to create a vestibular groove by a mode of action comparable to the "opening zipper."[27] By contrast to the male, however, there is no evidence of "closing zipper" activity in females, in whom the vestibular groove remains open to form the vestibule and introitus.

This difference between male and female fetuses does not seem to be attributable to sex – related differences in either the expression or distribution of androgen receptors or 5-alpha reductase – because these are broadly comparable in the genital tissues of male and female fetuses. It appears, therefore, that formation of the urethral groove (the action of the "opening zipper" in both sexes) is not dependent on androgenic stimulation. By contrast, the fusion of the lateral urethral folds which results in the formation of the tubularized male urethra by the "closing zipper" mechanism is an androgenic-dependent process.

In Western countries, urogenital sinus abnormalities are most often encountered in girls with virilized genitalia – of which CAH is the most common cause, accounting for more than 80% of cases of ambiguous genitalia. Other, much rarer causes, include exposure to androgens of maternal origin (*e.g.*, androgen-secreting tumors) during pregnancy.

Congenital adrenal hyperplasia is an enzyme deficiency which can either be inherited as an autosomal recessive or occurs as the result of a random mutation. The most common form is associated with deficiency of the enzyme 21-hydroxylase which plays a critical role in the biosynthetic pathway of the production of cortisol and aldosterone. Deficiency of this enzyme leads to a block in the synthetic pathway of these hormones, which, in turn, leads to an accumulation of steroid precursors. These precursors are then converted via 4-androstenedione to testosterone which enters the circulation. In female fetuses, exposure of the developing external genitalia to circulating testosterone secreted by the adrenal glands results in varying degrees of virilization.[19] The genital phenotype of females with CAH at the time of birth is characterized by clitoral hypertrophy (due to androgenic stimulation of the phallus). This is accompanied by the presence of a variable length of tubularized male urethra formed by the partial or complete closure of the urethral groove, reflecting the androgen-dependent action of the "closing zipper." However, there is considerable individual phenotypic variation – to the extent that siblings may exhibit considerably different degrees of virilization despite sharing the same genetic defect.

Because differentiation of the internal genitalia is unaffected by exposure to androgens of adrenal origin, females with CAH are born with normal internal reproductive structures, *i.e.*, ovaries, fallopian tubes, and uterus. The vagina is also represented because this anatomical structure is a paramesonephric duct derivative. Schoenwolf *et al.*[21] stated that regardless of the origins of the vagina, the lower end of the developing vagina lengthens between the 3rd and 4th month and its junction with the urogenital sinus is translocated caudally until it comes to rest on the posterior wall of the urogenital sinus and opens separately from the urethra within the vestibule. It is not difficult to envisage how this process is seriously disrupted when the urethral groove which would normally remain open to constitute the vestibule undergoes closure in response to androgenic stimulation to form a tubularized male urethra.

UROGENITAL SINUS: CLINICAL ASPECTS

The severity of virilization in females with CAH is reflected in a broad correlation between the degree of clitoral hypertrophy and the length of the tubular urethra. Accordingly, a marked degree of phallic enlargement is likely to be accompanied by a high confluence between the vagina and urethra and a correspondingly long common urogenital sinus.

By contrast, when clitoral hypertrophy is mild, the vagina and urethra are usually present as separate structures for most of their length with only a short distal section of common channel (urogenital sinus) just above the level of the perineum. Less severe forms of urogenital sinus are usually amenable to correction by use of a perineal-based skin flap incorporated into an incision in the posterior vaginal wall.[28] However, exteriorization of a high confluence vagina is a much more challenging surgical undertaking. In such cases, the options include total urogenital mobilization (in which the vagina is mobilized separately and the urogenital sinus becomes the urethra) and variants of the vaginal pull-through technique. Partial, rather than total, urogenital mobilization has been advocated to minimize the possible risk of urinary incontinence. Perineal skin flaps are usually employed in conjunction with urogenital sinus mobilization to facilitate adequate exteriorization of the vagina and reduce the risk of introital stenosis. Using modern techniques, even very-high-confluence variants can usually be successfully corrected. As a consequence, techniques of vaginal replacement now play little or no role in female genital reconstruction surgery for CAH.

SUMMARY

The introduction of new research methodology is yielding important new insights into the development of the genitourinary tract and the mechanisms involved in regulating this process. However, considerable caution is required when attempting to extrapolate findings in experimental animal models to human development, particularly in the context of the genital tract.[29] Some of these limitations are now being overcome by the increasing number of morphological studies undertaken with comparable methodology in human embryos and fetuses. Advances in the field of developmental biology are providing clinicians with a greater understanding of the origins of congenital abnormalities such as persistent cloaca and urogenital sinus.

COMPETING INTERESTS

The author declared no competing interests.

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