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Complex epithelial remodeling underlie the fusion event in early fetal development of the human penile urethra

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ABSTRACT

We recently described a two-step process of urethral plate canalization and urethral fold fusion to form the human penile urethra. Canalization ("opening zipper") opens the solid urethral plate into a groove, and fusion ("closing zipper") closes the urethral groove to form the penile urethra. We hypothesize that failure of canalization and/or fusion during human urethral formation can lead to hypospadias. Herein, we use scanning electron microscopy (SEM) and analysis of transverse serial sections to better characterize development of the human fetal penile urethra as contrasted to the development of the human fetal clitoris.

Eighteen 7–13 week human fetal external genitalia specimens were analyzed by SEM, and fifteen additional human fetal specimens were sectioned for histologic analysis. SEM images demonstrate canalization of the urethral/vestibular plate in the developing male and female external genitalia, respectively, followed by proximal to distal fusion of the urethral folds in males only. The fusion process during penile development occurs sequentially in multiple layers and through the interlacing of epidermal "cords". Complex epithelial organization is also noted at the site of active canalization. The demarcation between the epidermis of the shaft and the glans becomes distinct during development, and the epithelial tag at the distal tip of the penile and clitoral glans regresses as development progresses.

In summary, SEM analysis of human fetal specimens supports the two-zipper hypothesis of formation of the penile urethra. The opening zipper progresses from proximal to distal along the shaft of the penis and clitoris into the glans in identical fashion in both sexes. The closing zipper mechanism is active only in males and is not a single process but rather a series of layered fusion events, uniquely different from the simple fusion of two epithelial surfaces as occurs in formation of the palate and neural tube.

1. Introduction

We recently described a two-zipper hypothesis for formation of the human penile urethra. The "opening zipper" refers to the proximal to distal canalization of the urethral plate to form the urethral groove in males and the opening of the vestibular plate into the vestibular groove in females, and is likely to be an androgen-independent event (Li et al., 2015; Overland et al., 2016). The "closing zipper" refers to the proximal to distal closure of the urethral groove in males to form the penile urethra, and thus is presumed to be androgen dependent (Li et al., 2015). This fusion of the urethral folds is the key event that distinguishes the development of male from female human external genitalia in that the closing zipper mechanism is not evident during normal female development (Overland et al., 2016).

Fusion events occur throughout development, for example in neural tube closure and fusion of the palate and lip (Kim et al., 2015; Pai et al., 2012). Failure of the fusion process results in neural tube defects, cleft palate and lip, and, presumably in the case of urethral fold fusion, hypospadias. In comparison to the extensive bodies of work characterizing neural tube closure and palatal shelf fusion, (Kim et al., 2015; Pai et al., 2012) very little has been published directly describing the formation of the human penile urethra (Li et al., 2015; Kurzrock et al., 1999; van der Werff et al., 2000; van der Werff, 2002; Penington and Hutson, 2002; Altemus and Hutchins, 1991). In contrast, development of most (the proximal portion) of the mouse penile urethra occurs by direct canalization of the urethral plate to generate a lumen within the

Abbreviations: SEM, scanning electron microscopy

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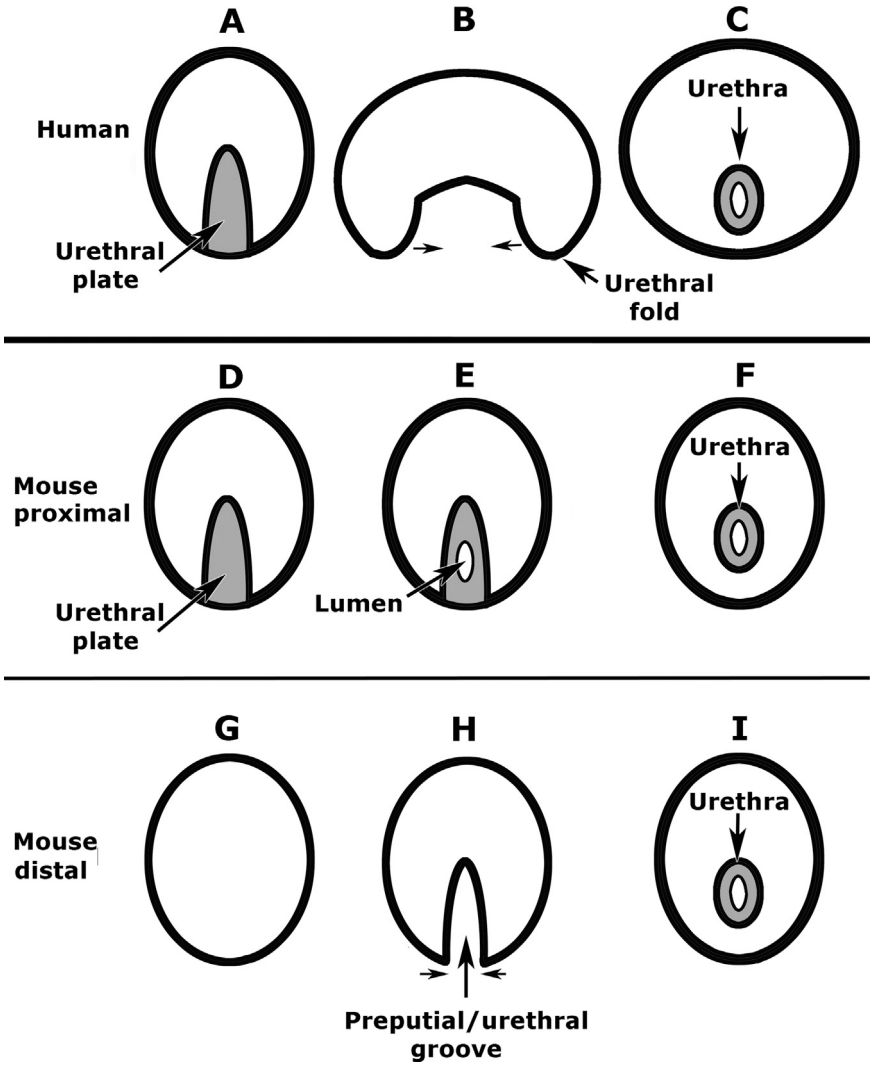

Fig. 1. Diagrammatic representation of penile urethral development in mouse and human. In human penile urethral development A-C the solid urethral plate canalizes to form an open urethral groove whose edges (urethral folds) fuse in the midline to form the penile urethra. Most of the mouse penile urethra (proximal portion) forms via direct canalization of the urethral plate to form the urethral lumen (D–F). The distal portion of the mouse penile urethra (including the urethral meatus) forms as a result of formation of the preputial/urethral groove, which subsequently fuses to complete the distal aspect of the penile urethra (G–I). Opposed arrows in (B and H) indicate fusion of the human urethral folds (B) or the mouse preputial/urethral folds (H). The urethral plate does not extend to the distal aspect of the mouse genital tubercle (G).

urethral plate (Fig. 1) but without creating an open urethral groove, a process radically different from penile urethral development in humans in which urethral plate canalization results in an open urethral groove, which subsequently closes to form the penile urethra (Hynes and Fraher, 2004; Seifert et al., 2008, Sinclair et al., 2016a). Only the most distal aspect of the mouse penile urethra forms as a result of formation of the preputial-urethral groove and its subsequent fusion (Fig. 1), a process analogous to that of human penile development (Sinclair et al., 2016c). By better visualizing the morphologic and histologic transitions that underlie the canalization and fusion processes during human penile and clitoral development, we aim to gain a better understanding of normal penile urethral development and thereby be better poised to dissect the pathophysiology underlying abnormal development of the male urethra, in particular hypospadias.

Previous studies of the human urethra have been limited by insufficient specimens and age ranges and by low-resolution imaging. Using optical projection tomography, we were previously able to accurately visualize the internal epithelial structures in developing human male and female external genitalia (Li et al., 2015; Overland et al., 2016). However, the detail in these 3D reconstructions is limited by the ability of labeled antibodies to penetrate fixed human fetal tissue and by the physical resolution limits of light microscopy. Based on immunostained sections from developing human fetal genitalia, we recognized that the mechanistic details of urethral plate canalization and urethral fold