

EARLY DEVELOPMENT OF THE URETHRAL SPHINCTER COMPLEX IN THE PELVIC FLOOR: A MOUSE MODEL

Hypothesis / aims of study

Knowledge about the embryonic development of the pelvic floor, urethral sphincter, and bladder detrusor is very limited in the available literature. Systematic sampling of a human developmental series is very difficult because of paucity of available human material, although a recent study reported on early fetal development of the male urethra {1}. To see if a suitable animal model is available for developmental urethral sphincter complex studies we have attempted to study the urethral sphincter complex of wild type (WT) mice from embryonic to neonatal stages. We have attempted to study a urethral sphincter complex development in wild type (WT) mice from the embryo to the neonate stage to see if a suitable animal model is available for urethral sphincter complex studies

Study design, materials and methods

Investigations were undertaken by serial sections of mouse embryos, fetuses, and neonates [n=15, embryonic day (ED) 13.5 to neonate day (ND) 7]. Histological sections were stained histochemically for haematoxylin and either azophloxine or eosine, or with azan. Additionally, serial sections were stained immunohistochemically for the presence of striated muscle [mouse monoclonal anti-myosin heavy chain (MHC): Upstate, clone A4.1025] and smooth muscle [mouse monoclonal anti-alpha smooth muscle actin (SMA): Sigma, A-2547]. To visualize a possible overlap in SMA and MHC expression, a fluorescent triple-staining method was used, including cell-nucleus staining (Dapi), MHC (mentioned earlier), and SMA (rabbit polyclonal). For both primary and secondary antibodies negative controls were processed. 3D reconstructions were made from two ND7 mouse embryos: male and female. To better understand the basic underlying morphology of the sphincter complex, a comparison with material from an earlier published human fetal pelvis study (with available 3D reconstructions), was employed {2}.

Results

In WT mice, as well as in humans, the urethral sphincter complex is composed of 2 muscular layers: an internal smooth muscle and an external rhabdosphincter. In the earliest development stage of our research, in mouse ED13.5 the SMA positive layer can be clearly identified as an unitary layer (Fig.1 A). The MHC positive layer (representing the future rhabdosphincter layer) can only be identified from stage ED15.5 onward (Fig.1 B, not staining visible, and 2; MHC, ED15.5, poor staining visible). At stage ED13.5 and 15.5, SMA positive tissue can also be identified at a more cranial "vesical" position, as the primordial detrusor (Fig.1 C,D). Interestingly, the two SMA positive masses develop as two independent muscle masses (Fig.1 C,D). Early in development (ED 15.5) the relative amount of SMA positive tissue is large, while only a few strands of MHC positive muscle fibers can be identified. At this stage of development the MHC positive precursor of the rhabdosphincter fibers are embedded within the circular SMA positive sphincter tissue (Fig.2). These precursor rhabdosphincter fibers also express SMA at early stages. However, SMA expression in these fibers is lower than in the SMA positive part located at the genuine internal smooth muscle of the developing sphincter complex. A gradual increase in the amount of MHC positive muscle fibers occurs during further development and eventually the MHC positive cells of the rhabdosphincter are much more prominently present than the SMA positive smooth muscle cells of the sphincter complex (Fig.2 and 3 A,B). This phenomenon also occurs in human fetuses (Fig.3 C,D). Due to relative growth changes, the SMA positive fibers of the genuine internal smooth muscle seems to decrease (Fig.2). Also the SMA positive parts of the precursor rhabdosphincter fibers seem to decrease while MHC positive fibers are increasing (Fig.2). In both, the human fetus and WT mice, the urethral sphincter muscle complex appears similar, and perhaps the development origins are very similar too. In both species the female external urethral sphincter muscle has a superior part surrounding only the urethra, and an inferior part surrounding both the urethra and the vagina (urethrovaginal sphincter complex) (Fig.4 and ref.2).

Interpretation of results

Obtaining satisfactory early stage human embryos for histochemical studies is a big problem under current legal requirements. From our study in the WT mice it appears that during embryonic development, the detrusor and the smooth muscle of the sphincter complex have separate origins. The sphincter complex originates in an early stage of embryonic development as a mass of tissue within the urogenital sinus with SMA positive expression only. In later embryonic development it gradually begins to show MHC positive expression in its outer layer which over time becomes the predominant layer and persists as the later rhabdosphincter. The exact phenomenon underlying this change from smooth muscle expression to myosin heavy chain expression needs further study.

Concluding message

The WT mouse seems to be a good animal model for further developmental studies of the urethral sphincter complex in the absence of early staged human embryos. The detrusor muscle of the bladder and the urethral sphincter complex have separate embryological origins. From the 3D reconstructions of WT mice and human foetuses it is obvious that the models correspond well with each other.

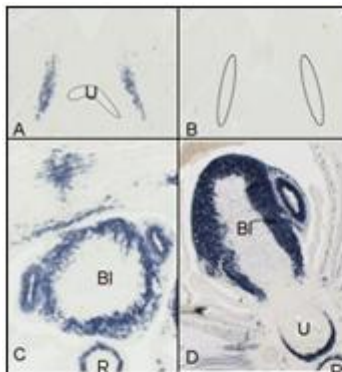


Figure 1: Development of the urethral sphincter complex and detrusor muscle. Transverse sections. Ventral is up, dorsal is down. A - C. ED13.5 mouse embryo stained against SMA (A and C) and MHC (B). A, B: Sections are taken through the level of the "urethral" portion of the "urogenital sinus". A: SMA staining is visible. The lumen of the urethra has been schematically illustrated. B: the MHC stains negative at the level of the urogenital sinus. The dashed lines show the position of SMA staining from A. C: The section is taken through the level of the "vesical" portion (i.e. detrusor muscle) of the "urogenital sinus". The smooth muscle mass of the urethral portion is not in contact with the smooth muscle of the bladder at this stage, since the smooth muscle of the urethra is not developed at this anatomical level yet. D: shows an ED15.5 mouse stained against SMA. Also here the SMA positive mass of the urethra is not in contact with SMA positive mass of the bladder. U = Urethra; Bl = bladder; R = rectum.

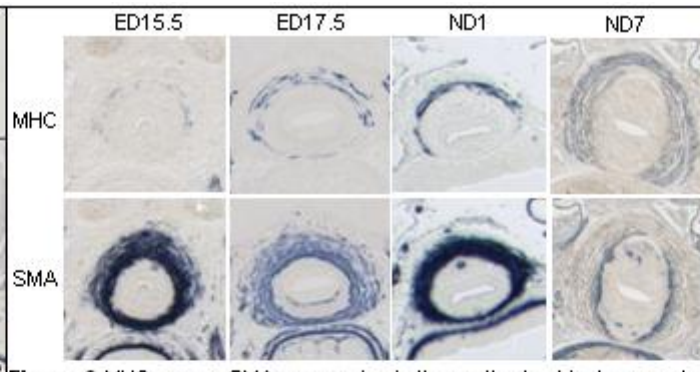


Figure 2: MHC versus SMA expression in the urethral sphincter complex through mouse development. Dorsal down, ventral up. These transverse sections were taken at the level just distal to the bladder neck. ED15.5, 17.5, and ND7 are males, ND1 = female.

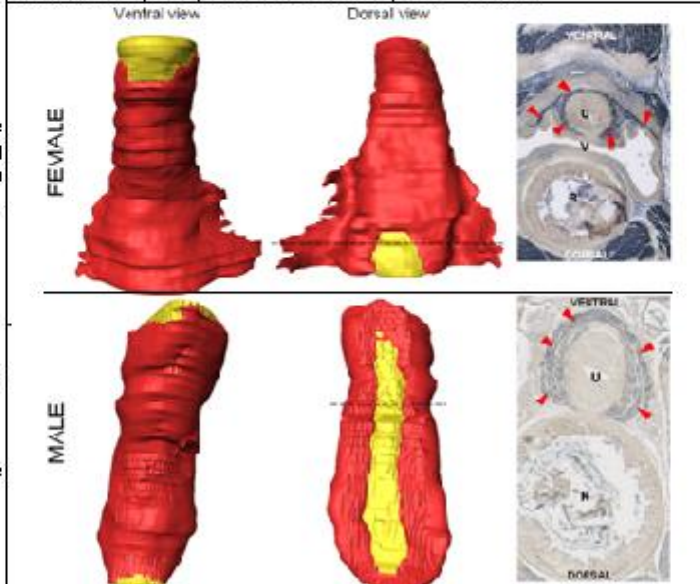


Figure 4: 3D reconstructions of MHC positive structures in the urethral sphincter complex of a female and a male mouse (ND 7). Dashed lines indicate the level of the transverse sections (shown on the right) through the urethral sphincter complex stained against MHC.

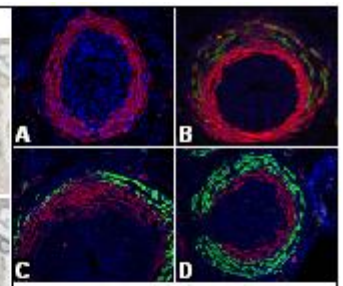


Figure 3: Fluorescent double stain in urethra sphincter complex. A-C: Dorsal down, ventral up. D: Dorsal left, ventral right. MHC = green, SMA = red, nuclei (dapi) = blue. A: mouse ED15.5, MHC not visible in urethra. B: mouse ED16.5, MHC starts to express. C, D = human fetuses. C = female, D = male.

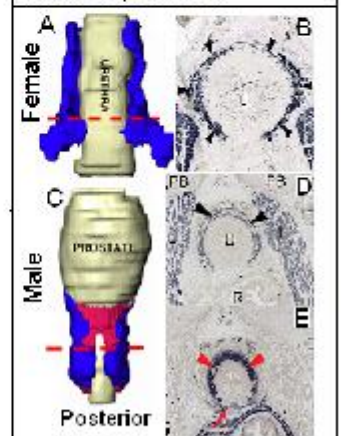


Figure 5: 3D images of fetuses (12wk). Blue = MHC pos. Pink in C: SMA pos. B, D, E: transverse sections at the position of the dotted line in the 3D images. B, D: MHC stained. E: SMA stained².

References

1. Sebe et al., 2005; Fetal development of striated and smooth muscle sphincters of the male urethra from a common primordium and modifications due to the development of the prostate: an anatomic and histologic study
2. Wallner et al., 2009; The anatomical components of urinary continence

Specify source of funding or grant

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Is this a clinical trial?

No

What were the subjects in the study?

ANIMAL

Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?

Yes

Name of ethics committee

DEC
DEC number = DAE101610