Morphology of Mouse External Genitalia: Implications for a Role of Estrogen in Sexual Dimorphism of the Mouse Genital Tubercle

Jennifer H. Yang, Julia Menshenina, Gerald R. Cunha, Ned Place and Laurence S. Baskin*

From the Division of Pediatric Urology, University of California, San Francisco Children's Hospital, San Francisco, California, and Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University (NP), Ithaca, New York

Abbreviations and Acronyms

αERKO = ERα knockout
AR = androgen receptor
ER = estrogen receptor
ERKO = ER knockout
ExG = external genitalia
MUMP = male urogenital mating protuberance
T = testosterone
X^{Tfm}/Y = androgen receptor knockout

Study received University of California-San Francisco institutional animal care and use committee approval.

Supported by National Science Foundation Grant IOS-0920793.

* Correspondence: Department of Urology, University of California-San Francisco, 400 Parnassus Ave., Box 0738, San Francisco, California 94143 (telephone: 415-353-2200; e-mail: lbaskin@urology.ucsf.edu).

Purpose: We examined the role of androgens and estrogens in mammalian sexual differentiation by morphological characterization of adult wt and mutant mouse external genitalia. We tested the hypothesis that external genitalia development depends on androgen and estrogen action.

Materials and Methods: We studied serial sections of the external genitalia of the CD-1 and C57BL6 wt strains of adult mice (Charles River Laboratories, Wilmington, Massachusetts). We recorded linear measurements of key structures in each specimen, including the urethra, erectile tissue, bone and cartilage. We used similar methodology to analyze mice mutant for estrogen receptor α (αERKO) and androgen receptor (X^{Tfm}/Y) (Jackson Laboratory, Bar Harbor, Maine).

Results: Morphology in X^{Tfm}/Y adult murine external genitalia was remarkably similar to that in wt females. Bone and clitoral length was similar in wt females and X^{Tfm}/Y mice. Conversely the αERKO clitoris was 59% longer and bone length in αERKO females was many-fold longer than that in female wt mice or X^{Tfm}/Y mutants. The αERKO clitoris contained cartilage, which is typical of the wt penis but never observed in the wt clitoris. Serum testosterone was not increased in female αERKO mice 10 days postnatally when sex differentiation occurs, suggesting that masculinization of the αERKO clitoris is not a function of androgen.

Conclusions: Masculinization of the αERKO clitoris suggests a role for estrogen in the development of female external genitalia. We propose that normal external genital development requires androgen and estrogen action.

Key Words: penis, clitoris, androgens, estrogens, sex differentiation

THE conceptual framework of our contemporary understanding of mammalian sexual differentiation was described by Jost and is based on patterns of sex differentiation after fetal castration.[1,2] According to Jost ExG masculine development involves androgen action. In the absence of androgens the default female pattern of sex differentiation occurs. In laboratory rodents a unique role for dihydroT[3] was noted, especially in regard to the development of skeletal elements.[4,5] Since the Jost description of the morphological effects of fetal castration, AR has been cloned and shown to have an essential role in sex differentiation.[5–7] While the androgen theory of Jost is correct, is it the whole story?

A possible role of estrogen in normal ExG development is currently debated, stemming from observations of aromatase, and ERα and ERβ in rat developing ExG.[8,9] Unfortunately these

0022-5347/10/1844-1604/0
THE JOURNAL OF UROLOGY®

Vol. 184, 1604-1609, October 2010
Printed in U.S.A.
DOI:10.1016/j.juro.2010.03.079

studies do not identify a single morphogenetic event in normal ExG development that depends on estrogen. Preliminary observations in the spotted hyena on the morphogenetic effects of the aromatase inhibitor letrozole on developing ExG (Cunha and Glickman, unpublished data) stimulated us to examine the role of estrogen in a more conventional animal, the mouse, for which many relevant transgenic strains exist.

The literature on morphology of the penis and clitoris in the adult mouse is inadequate and to some extent inaccurate, especially regarding the exact size, shape and position of certain key internal structures. Adult ExG morphology is by definition the culmination of normal development and, thus, adult morphology was used as the end point of the developmental process. Our goal was to characterize the morphology of the adult wt penis and clitoris by modern morphological technique (3-dimensional reconstruction and morphometrics) to identify specific epithelial and stromal structures, and attribute developmental events to androgen or estrogen action. Previous studies of the sex differentiation of rodent ExG focused on androgenic induction of the os penis and os clitoris in rats and mice.[8,9] We propose that the precise morphological organization in adult mouse ExG result from signaling via AR and ERα. By analyzing αERKO and AR mutant (X^{Tfm}/Y) mice we suggest a role for estrogen as well as androgens in normal mammalian sexual differentiation.

MATERIALS AND METHODS

All animal care and protocols used were approved by the University of California-San Francisco institutional animal care and use committee. ExG of adult wt CD-1 and C57BL6, and mutant αERKO mice and X^{Tfm}/y mice were fixed in formalin and serially sectioned at 7 μm for histological staining. We examined the ventral cleft, urethra, clitoris and bone in wt female mice, and the ventral cleft, urethra, bone and cartilage in wt male mice. We examined 5 wt C57BL6 mice of each gender as well as 4 wt male CD-1 mice and 3 wt female CD-1 mice. The C57BL6 strain was chosen because the ERKO mutant colony used in this study also originated from the same strain. The CD-1 strain was used because it is a commonly used, multipurpose outbred strain. Linear measurements of key structures were made by counting sections containing the object of interest. Statistical analysis was done using Student's t test with $p < 0.05$ considered significant. We created 3-dimensional computer reconstructions from serial sections using Surf-driver® 3.5, as described previously.[10] We performed scanning electron microscopy by routine methods.[11] Serum T was measured using a solid phase ^{125}I radioimmunoassay kit, as previously described.[12]

RESULTS

wt Morphology

Male. The surface elevation in the perineum of wt males is the prepuce, which is bifid (fig. 1, *B*). The penis is located internally in the preputial space. The wt penis is adorned by a bifid distal structure which to our knowledge has not been reported previously. It contains a central core of cartilage. This distal bifid projection is called MUMP (figs. 1, *C* and *D*, and 2, *A*). Cartilage begins distal in MUMP and extends proximal to dorsally overlap the os penis. We made linear measurements of penile structures in wt male mice (fig. 3, *A*). All parameters were remarkably similar in each wt mouse strain. From a qualitative perspective the adult wt mouse penis has 8 diagnostic morphological features (Appendix 1).

Female. The surface elevation in the perineum of the female mouse is not the clitoris but represents the prepuce, which in wt females is also bifid (fig. 1, *A*). The clitoris is deeply placed in the perineum, mostly defined by a U-shaped solid epithelial lamina and contains a small os clitoris (fig. 2, *B*). The adult mouse clitoris is tethered to ventral stroma and, thus, is immobile. Figure 2, *B* shows the anatomy of the distal portion of the adult wt clitoris. Unlike the penis, the adult wt mouse clitoris does not contain cartilage or epithelial spines and is not located in an epithelium lined space. Likewise, the urethra lies completely or partly outside the wt clitoris. Figure 3, *B* shows linear measurements of structures in the adult wt clitoris. As in the male, all parameters were not significantly different in CD-1 vs C57/6bl wt mice. Qualitatively the adult wt mouse clitoris has 8 characteristic morphological features (Appendix 1).

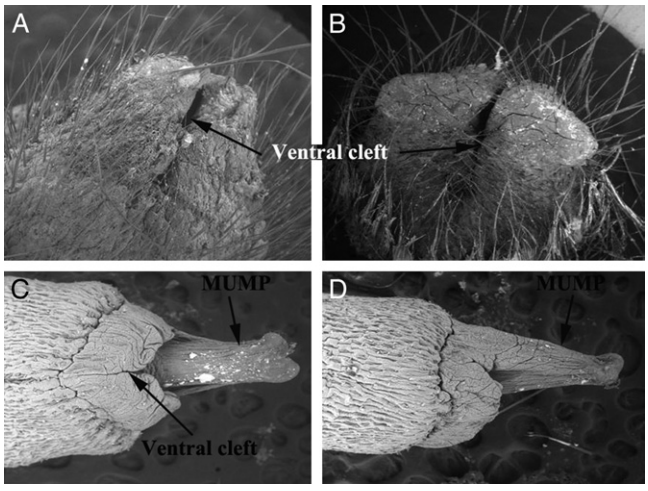

Figure 1. Scanning electron microscopy shows mouse ExG. *A*, wt female ExG. Reduced from ×50. *B*, wt male ExG. Reduced from ×50. *C*, ventral view of wt penis. Reduced from ×100. *D*, lateral view of wt penis. Reduced from ×100.