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

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Abstract

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.

Keywords

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

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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 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).

*a*ERKO Clitoral Morphology

Figure 2, *C* shows detailed anatomy of the adult *a*ERKO clitoris. In female *a*ERKO mice the bifid prepuce is followed proximal by a ventrally cleft region and even more proximal by a tubular structure (antrum) formed by fusion of the edges of the ventral cleft. More proximal the antrum divides into the clitoral pouch dorsally and the urethra ventrally (fig. 4). The *a*ERKO clitoris is radically different from the wt clitoris in several respects. The *a*ERKO clitoral body is 1.7-fold longer than the wt clitoris. The *a*ERKO os clitoris is many times longer than that in wt mice ($p < 0.001$, fig. 5). Also, the *a*ERKO clitoris contains cartilage, which is absent in the wt clitoris. Morphometrics of the *a*ERKO male mutant are not significantly different from those of the wt male (data not shown).

Tfm Morphology

ExG in X^{Tfm}/Y mice are remarkably similar to those in wt females (fig. 2, *D*). Internal structures, including the length of the X^{Tfm}/Y clitoris and bone, were not significantly different from those in wt females (fig. 5).

From a purely qualitative perspective ExG in all wt CD-1 and C57/6Bl mice, and in X^{Tfm}/Y mice showed an identical female phenotype, in contrast to the wt male phenotype. The phenotype of the *a*ERKO clitoris has several masculine features and, thus, the *a*ERKO clitoris has an intermediate phenotype (Appendix 2).

Serum T

Serum T in 10-day-old *a*ERKO female mice was lower than in wt female mice of the same age (fig. 6), although the difference was not statistically significant. Serum T in 10-day-old *a*ERKO and wt female mice was many-fold lower than that reported previously in adult male mice.[13]

DISCUSSION

The current paradigm of sexual differentiation states that in the presence of androgen masculine ExG develop and in the absence of androgen the default female pattern develops. Sparse data exist on internal morphological features of the adult penis, such as the position of the urethral meatus and especially the morphology of the distal penile tip. Morphology of the mouse clitoris is poorly described. Our study shows in detail the external and internal architecture of the wt adult murine male and female ExG, which are used as end points of sexually dimorphic development. Sexually dimorphic structures are remarkably uniform in wt male and female mice. MUMP in males, the amount of bone, and cartilage and erectile tissue appear to be influenced by androgen and estrogen action, based on our data on *a*ERKO and X^{Tfm}/Y mutant animals, and those reported previously.[5] Initial development of the os penis is androgen independent based on its presence in androgen insensitive of X^{Tfm}/

Y mice,[5] although subsequent bone growth is androgen dependent.[14,15] The absolute length of the os clitoris was considerably greater in αERKO vs wt females ($p < 0.001$). These differences may be explained by the effects of estrogen on osteogenesis.[16,17] Also, studies of bone metabolism in aromatase KO mice implicate a role for androgen and estrogen in bone metabolism.[17] Thus, estrogen insensitivity or androgen acting unopposed by estrogen may contribute to the increased amount of bone in αERKO female ExG.

A substantial cartilaginous element was consistently noted in the αERKO clitoris while cartilage is never observed in the wt clitoris. Neonatal administration of androgen augments bone growth in the mouse clitoris but does not induce cartilage formation.[4] Our study suggests that signaling via ER may influence chondrogenesis. In this regard neonatally administered tamoxifen inhibits postnatal differentiation of skeletal elements in the mouse penis, resulting in a decrease in or absence of the os penis and distal cartilaginous segment.[18] These changes may be inherent to tamoxifen since treatment with other antiestrogens did not result in cartilage loss. In mice tamoxifen can act as an estrogen agonist or antagonist.

Our findings of a potential role of estrogen in sexual dimorphic development are consistent with previous studies showing ERα, ERβ and aromatase in the developing rat penis,[8,9] and ERα in the developing human male fetal ExG.[19] Consistent with the ER in the developing ExG is the presence of T and estradiol in the serum of female rodents during the neonatal period, when ExG sex differentiation occurs.[20] To our knowledge the biological significance of low T in newborn females is unclear and, thus, especially in αERKO females, must be considered since in this situation androgen can act unopposed by estrogen.

ExG sensitivity was previously established in studies by Goyal et al, in which exogenous estrogen at pharmacological levels was administered during the perinatal period.[21] Such treatment elicited penile dysmorphogenesis, including malformations such as hypospadias, abnormal penile muscles and bone formation, and decreased penile length, diameter and weight.[21] From these studies Pang et al suggested that ER and AR based mechanisms estrogen induce penile deformity.[20] Unfortunately these findings represent nonphysiological conditions and, thus, are not relevant to normal development. Our findings document various developmental effects of the ERα mutant state on normal clitoral development.

A potential explanation for the partial masculinization of the αERKO clitoris may be serum T, which is increased 40-fold in adult αERKO mice.[22] However, serum T fails to explain the phenotype of the αERKO clitoris for several reasons. Adult serum T does not necessarily have any relation to sex differentiation in the neonatal period. Serum T is not increased in female αERKO mice 21 days postnatally[23] or at day 10, when sex differentiation occurs. Given that masculinization in the αERKO clitoris appears to be independent of systemic androgen, the masculinized phenotype of the αERKO clitoris may be due to absent ERα signaling in developing ExG tissue.

CONCLUSIONS

To our knowledge we describe precise morphometrics of the adult male and female ExG for the first time. Overall key structures are uniform in wt male and female mice while X^{Tfm}/Y mutant ExG are almost identical to those of wt females. In contrast, the αERKO clitoris contains significantly more bone as well as cartilage, which is typical of the wt male penis. We suggest that the masculinized phenotype of αERKO mice is due to absent estrogen action since serum T is not increased during ExG sex differentiation. We propose that disruption of the balance between androgen and estrogen action alters normal development and leads to abnormal ExG morphology.

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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

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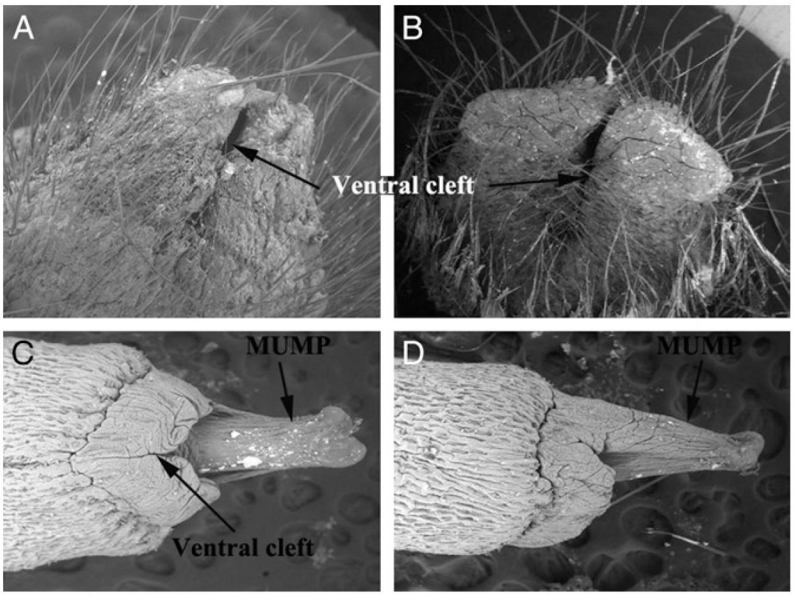

Figure 1.
Scanning electron microscopy shows mouse ExG. *A*, wt female ExG. Reduced from ×50. *B*, wt male ExG. Reduced from ×50. *C*, ventral view of wt penis. Reduced from ×100. *D*, lateral view of wt penis. Reduced from ×100.

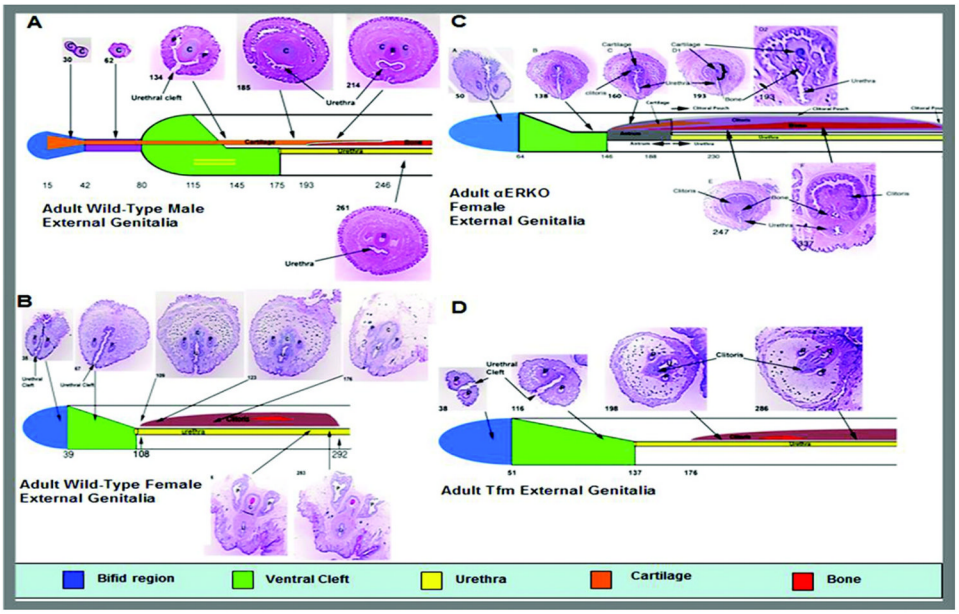

Figure 2.

Detailed anatomy of key ExG structures in adult mouse. Numbers indicate section number from distal tip. *A*, MUMP, cartilage (*C*), process (*P*) and bone (*B*) in adult wt male ExG. *B*, bifid (blue area), ventrally cleft (green area) distal prepuce, ventrally tethered clitoris (*C*), bone, preputial duct (*P*) and urethra (*U*) in adult wt female ExG. *C*, ventrally tethered clitoris containing bone and cartilage in adult *α*ERKO female ExG. *D*, bifid distal portion and clitoris with bone but no cartilage in adult Tfm male ExG.

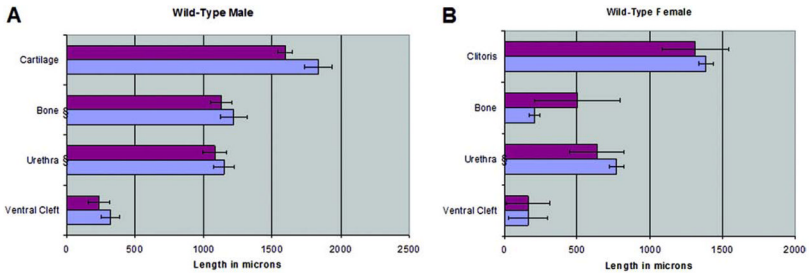

Figure 3.

Morphometric measurements of key structures were similar in CD-1 (blue bars) and C57BL6 (red bars) adult wt mice. *A*, 4 CD-1 and 5 C57BL5 wt males. *B*, 3 CD-1 and 5 C57BL5 wt females. Bars with curley represent distance from ExG tip to beginning of structure. Other bars represent length.

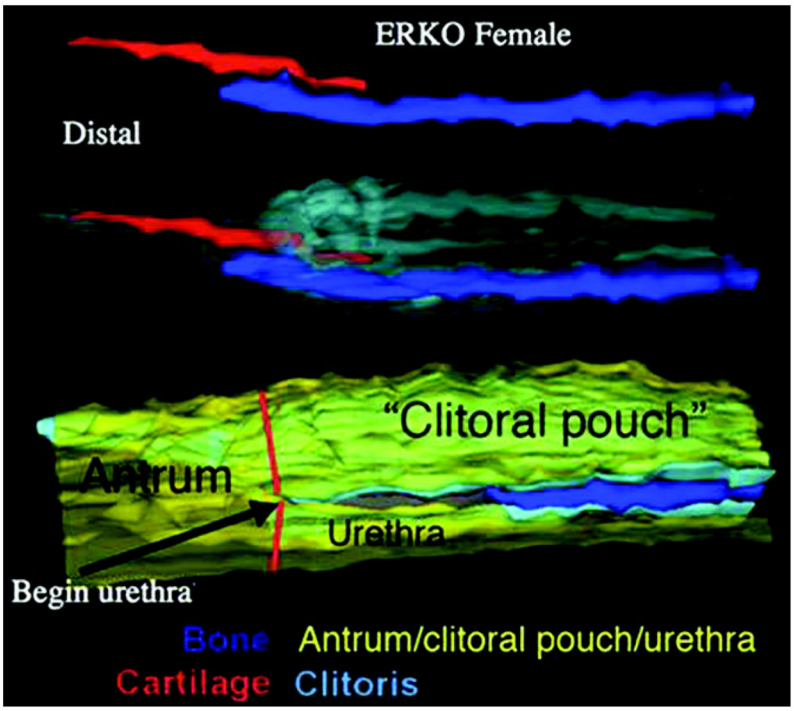

Figure 4.
Three-dimensional reconstruction of adult αERKO female ExG reveals cartilage overlapping bone dorsal and antrum divided into clitoral pouch and urethra.

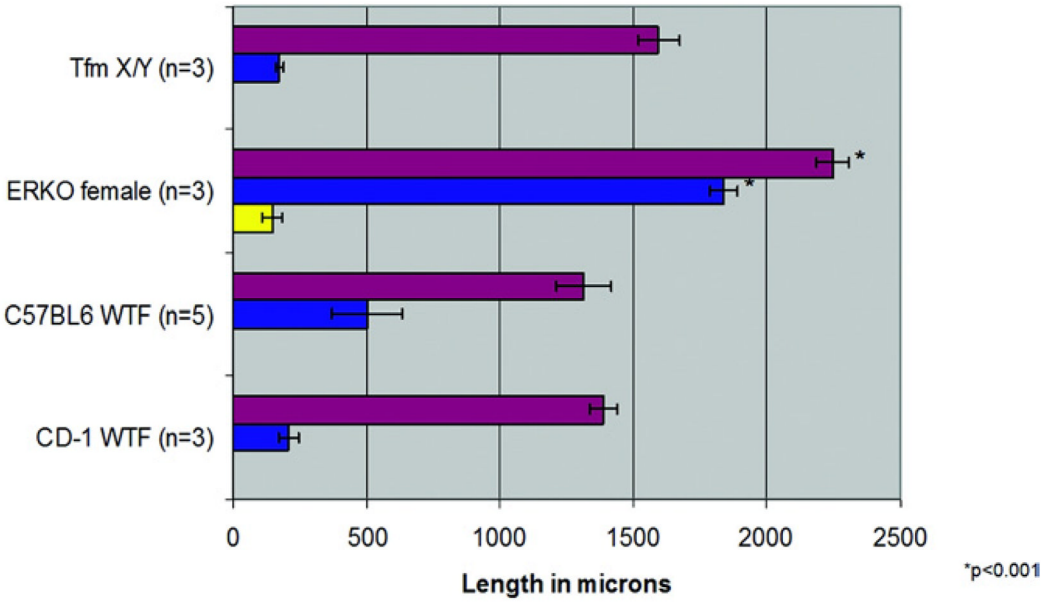

Figure 5.

Clitoral (red bars), bone (blue bars) and cartilage (yellow bars) length in CD-1 and C57BL6 wt females, and αERKO and Tfm mutants. Bone and clitoris were significantly longer in αERKO female vs wt mice and Tfm mutants ($p < 0.001$).

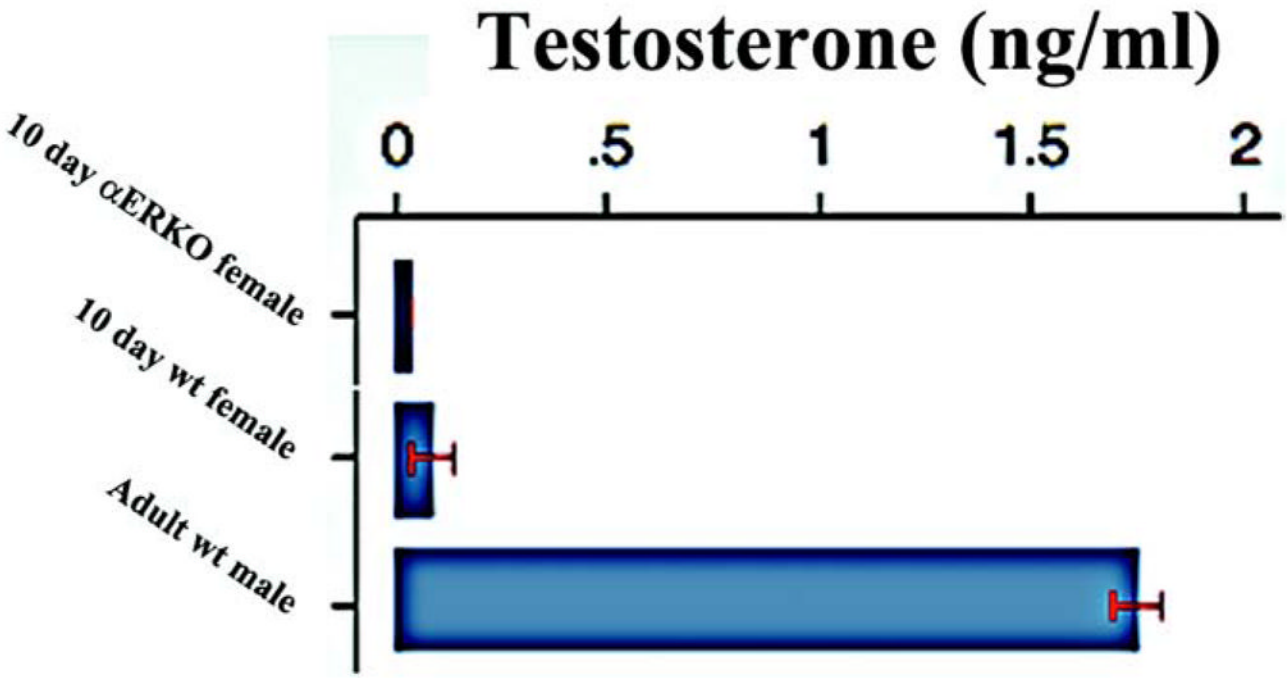

Figure 6.
Serum T in WT and αERKO mice with adult wt male value as reported by DePaolo and Masoro.[13]

APPENDIX 1

Qualitative morphological features of adult mouse penis and clitoris

Penis
Circular transverse profile
No tethering, freely moving organ
Urethra completely within the penis
Large os penis
Contains cartilage
Located in epithelium lined space
Surface (penile) spines
Large organ size
Clitoris
U-shaped epithelial lamina
Ventral tethering/immobile
Urethra never completely within clitoris
Miniscule os clitoris
No cartilage
Never located in epithelium lined space
No epithelial spines
Small organ size

APPENDIX 2

External genitalia phenotypes of adult wt and mutant female and male mice

	WT (feature)	X^{Tim}/Y (feature)	*α*ERKO (feature)	WT Male (feature)
Shape	Female	Female	Intermediate	Male
Ventral tethering	Yes (female)	Yes (female)	Yes (female)	No (male)
Urethral site	Female	Female	Female	Male
Bone	Small (female)	Small (female)	Large (male)	Large (male)
Cartilage	Absent (female)	Absent (female)	Present (male)	Present (male)
Epithelial space	Absent (female)	Absent (female)	Present (male)	Present (male)
Spines	Absent (female)	Absent (female)	Present (male)	Present (male)
Size	Small (female)	Small (female)	Large (male)	Large (male)