Canalization of the Vestibular Plate in the Absence of Urethral Fusion Characterizes Development of the Human Clitoris: The Single Zipper Hypothesis

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Purpose: We characterized the early gestation development of the female external genitalia using optical projection tomography to visualize anatomical structures at high resolution.

Materials and Methods: First and early second trimester human female fetal external genitalia were collected with consent after voluntary termination. Specimens labeled with anti-E-Cadherin antibody underwent analysis with optical projection tomography. Histological sections were immunostained for androgen receptor, 5α-reductase, Ki67 for proliferation and Caspase 3 for apoptosis.

Results: Three-dimensional reconstructions demonstrated proximal to distal canalization of the epithelial vestibular plate and formation of a vestibular groove, which remained open. Ki67 was observed throughout with greatest density in the dorsal vestibular plate at the level of the opening groove. Staining for Caspase 3 was minimal in all sections. Androgen receptor staining was seen throughout the mesenchyme and in the apical epithelium of the dorsal vestibular groove. Throughout the epithelium and epidermis 5α-reductase staining was observed.

Conclusions: Early development of the external genitalia in the female is analogous to that in the male, demonstrating a similar opening zipper driving canalization of the vestibular plate with localized epithelial proliferation in the absence of significant apoptosis. Thus we hypothesize that the mechanism underlying the opening zipper must be androgen independent and the absence of androgen driven urethral fusion characterizes the normal development of the human clitoris.

Key Words: genitalia, female; clitoris; embryonic and fetal development; tomography, optical; androgens

Abbreviations and Acronyms

AR = androgen receptor

OPT = optical projection tomography

PCR = polymerase chain reaction

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While a large body of work has been published describing fetal development of the human penis,[1–5] far less is known about the development of the female external genitalia in humans or even laboratory animals. The few studies of the development of the human clitoris were based on the study of a small number of gross fetal specimens and serial sections of the specimens.[1,6,7] What little is known about the molecular mechanisms underlying patterning of the external genitalia comes primarily from animal studies. For example, expression of the morphogen Shh

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(sonic hedgehog) is required during distinct temporal windows for the early outgrowth and ambisexual development of the genital tubercle in mice.[8] However, to our knowledge analogous mechanisms have not yet been found in human development.

We recently described the development of the human male genital tubercle and penile urethra using OPT.[9] Two key observations were made, including 1) canalization of the urethral plate or the opening zipper followed by 2) fusion of the urethral folds or the closing zipper. We hypothesized that androgen independent canalization of the vestibular plate (opening zipper) occurs in the same fashion in female development as it does in male development. In contrast, the androgen dependent fusion process (closing zipper) remains quiescent in normal females with the potential for closure under the influence of abnormal exogenous or endogenous androgen exposure.

We performed OPT to determine early fetal development of the human external female genitalia. OPT allows for visualization of internal structures in whole mount specimens.[9] Specimens are labeled with fluorescent antibodies and unlabeled tissues are rendered optically clear. Hundreds of projection images are integrated into a 3-dimensional reconstruction, which can be virtually rotated and sectioned to elucidate relationships among intact internal structures at a level of detail previously not possible. After OPT the same specimens can be sectioned for standard immunohistochemical staining. Thus, 3-dimensional structures visualized by OPT can be correlated with subcellular patterns of protein expression.

METHODS

First and early second trimester human fetal specimens were collected after elective termination of pregnancy with approval from the committee for human research at University of California-San Francisco (IRB 12-08813). Fetal age was estimated using heel-toe length.[10] We report fetal age from the time of fertilization and not from the last menstrual period (ie gestational age minus 2 weeks).

Gender was determined using PCR to detect X and Y chromosomal sequences. When available, gender was confirmed by identifying wolffian and müllerian duct morphology (fig. 1). External genitalia were identified under a dissecting microscope. A total of 18 female genital tubercle specimens were processed for OPT as previously described.[9]

Briefly, genital tubercles were fixed in 10% formalin, bleached with hydrogen peroxide, stained using a whole mount immunofluorescence protocol with EP700Y anti-E-Cadherin monoclonal antibody (Abcam®) and Alexa Fluor® 488 anti-rabbit secondary antibody, optically cleared in benzyl alcohol and benzyl benzoate, embedded in agarose and imaged with the 3001M OPT scanner (Bioptonics, Edinburgh, Scotland). Similar to the principles of x-ray computerized tomography 800 projected images from each channel were constructed into 3-dimensional voxel data sets with in-house software. They were then visualized with the Volocity software suite (PerkinElmer®). Measurements were made using the caliper function in Volocity.

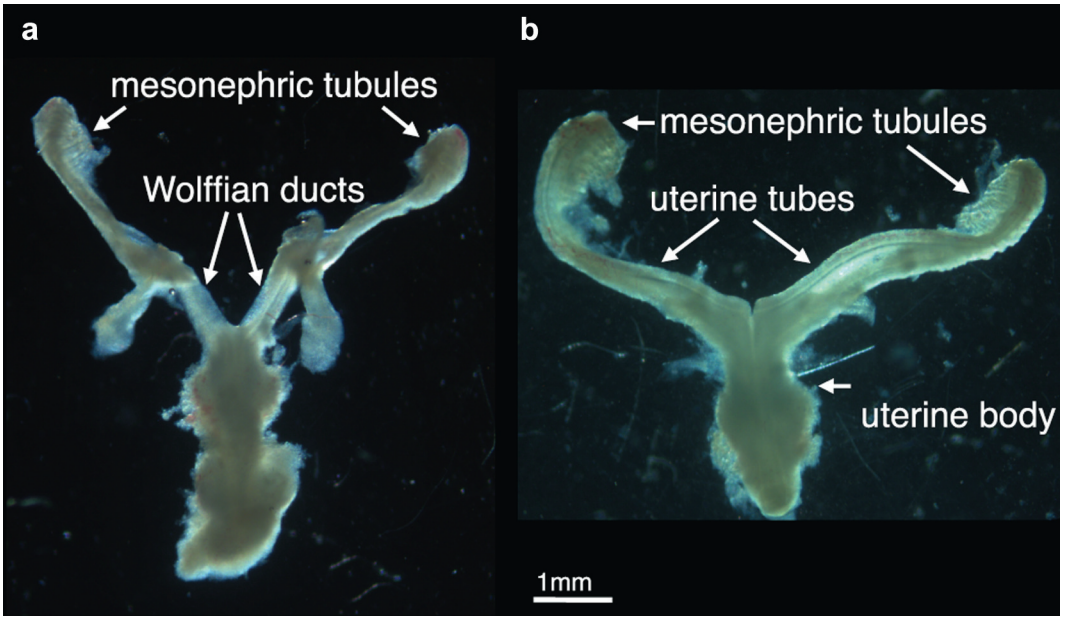

Figure 1. Images of reproductive tract show subtle morphology differences between genders. Morphology of male and female specimens is distinctive and diagnostic of gender, which was further verified by PCR. *a*, 9-week male human fetus. *b*, 9-week female human fetus.

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