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Neonatal Phytoestrogen Exposure Causes Hypospadias in Female Mice

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We use a mouse model of neonatal exposure to the phytoestrogen genistein as a model of human infant exposure to soy formula (Jefferson and Williams 2011). Female CD-1 mice given 50 mg/kg/day genistein subcutaneously on postnatal days 1–5 have serum genistein levels of 1–5 μ M, which closely approximates serum genistein levels measured in human infants fed soy formula. The genistein-treated mice are completely infertile due to functional abnormalities of the female reproductive tract. While performing these studies, we noted that the mice formed vaginal plugs that were softer and more yellow in appearance than in controls, and their external genitalia appeared abnormal. At 3 weeks of age, the clitoris was abnormally widened and erythematous, and the urethral opening was located at varying positions on the ventral aspect rather than at the distal tip of the clitoris as observed in controls (Fig. 1A, B). At 6 weeks of age, the clitoris was less erythematous, but the urethral opening remained abnormally positioned (Fig 1C, D). We confirmed that the observed opening was the urethra by euthanizing the mice and passing a suture from the bladder through the urethra to its exit point on the clitoris (Fig. 1E, F). This female hypospadias phenotype was completely penetrant as 10/10 Gen-treated females had hypospadias compared to 0/10 controls.

The mammalian urethra forms from medial fusion of the urethral folds that arise from urogenital sinus mesenchyme. In humans of both sexes and in male mice, urethral development is completed prenatally. In males, this is an androgen-dependent process and can be disrupted by prenatal exposure to steroid hormones. In female mice, development of the distal vagina and urethra is incomplete until after birth. Neonatal exposure of female mice to diethylstilbestrol (DES) causes hypospadias as a result of dorsal urethral and sinus cord apoptosis, combined with failure of both urethral-fold fusion and distal urethral migration (Miyagawa et al. 2002). Excretion of urine through this abnormal location likely explains the accumulation of vaginal crystals observed in female mice exposed to DES (Takasugi and Bern 1962).

Endocrine disrupting chemicals are suspected of contributing to the increased incidence of hypospadias in human males observed over the past three decades. Indeed, there is a 5-fold

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increased risk of hypospadias in boys whose mothers consumed a vegetarian diet during pregnancy as compared to omnivores, possibly as a result of prenatal phytoestrogen exposure (North and Golding 2000). Hypospadias occurs in human females, but is rare and often goes undiagnosed until adulthood. Urethrocele and anterior vaginal prolapse, however, are common gynecological pathologies that we hypothesize could result from inhibition of urethral-fold fusion or increased apoptosis of urogenital sinus-derived tissues during development. Our finding of female hypospadias in mice after neonatal genistein exposure suggests that prenatal exposure of human female fetuses to environmental estrogens could promote hypospadias or gynecological pelvic support pathologies that only become apparent later in life. Combined with the association of vegetarian diet with male hypospadias, these findings support recommendations for women to avoid exposure to high phytoestrogen levels during pregnancy.

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Figure 1. Photographs of external genitalia of neonatal genistein-treated female mice. (A,B) 3-wk old; (C–F) 6-wk old. (E,F) Prolene suture (blue) placed from bladder through urethral opening. (A, C, E) Controls; (B, D, F) Genistein-treated.