Loss of Wnt5a impairs upper Müllerian duct differentiation in mice.

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Müllerian anomalies (MAs) are anatomical variations of the female reproductive tract resulting from the incomplete development of the Müllerian ducts. MAs are complex gynecological birth defects occurring in 5.5% of the general female population and 8% of infertile women. In addition, MAs are diagnosed in 13.3% of women with miscarriages and 24.5% of those with miscarriages and infertility. Association with other several congenital malformations including renal, skeletal, and cardiovascular anomalies are common. Despite the immense impact on a woman's health, the etiology of MAs remains largely unexplained.

WNT5A is a critical regulator of cell migration and polarity through the noncanonical Wnt pathway. Mutations of *WNT5A* have been associated with Robinow syndrome (OMIM 180700), which is characterized by skeletal and genital anomalies. The most commonly reported reproductive tract malformations in individuals with *WNT5A* mutations are genital hypoplasia and vaginal/cervical atresia. Studies in the mouse confirmed the requirement of Wnt5a for vaginal formation but analyses involving the upper reproductive tract are lacking.

Ablation of *Wnt5a* in the mouse results in severely hypoplastic uterine horns, suggesting that Wnt5a may have a broader involvement in Müllerian duct development. *Wnt5a*-/- uterine horns were more than 50% shorter than wild-type, showing a hypertrophic epithelial component while the stromal component was reduced in cellularity. To better understand the role of *Wnt5a*, we performed single-cell RNA-Sequencing (scRNA-Seq) of developing Müllerian ducts at 18.5 days post coitum (dpc). All identified cell clusters showed a similar cell composition between *Wnt5a*-/- and wild type Müllerian ducts, except for one cluster that was largely over-represented in *Wnt5a*-/- samples. To further understand the phenotype, we performed qPCR analysis and immunofluorescence staining, and found that the cranial portion of the uterine horns of *Wnt5a*-/- mice expressed markers of the fallopian tubes, suggesting a posterior to anterior homeotic transformation. Our results indicate additional roles for *Wnt5a* during mammalian reproductive development. These findings help to explain further the pathophysiology of Robinow syndrome and may prompt further investigations into the uterine anatomy of complex clinical cases.