During development, elements of the vertebrate limb are progressively determined in a proximal distal manner, with the autopod last to emerge. The autopod includes the wrist/ankle [carpals/tarsals] and digits [metacarpals/metatarsals and phalanges]. At autopod stages, digit precursors emerge as a series of alternating digital rays and interdigits (IDs). The IDs are secondary signaling centers that act on a discrete region of the distal digital ray called the Phalanx Forming Region (PFR) to determine digit identity (phalanx number and morphology). The PFR is molecularly defined by enrichment of phospho-SMAD 1, 5, 8 (p-SMAD) and each digital ray has a distinct p-SMAD 1, 5, 8 activity that correlates with a unique digit morphology. Bmp Receptor 1b is the major Bmp receptor necessary for PFR activity as phalanx development is inhibited following its genetic removal. Using these molecular markers of digit development, we explored how key molecular events and signaling pathways necessary for limb development related to establishment and maintenance of the PFR. Interestingly, we find that changes in gene expression previously proposed to signify termination of limb bud outgrowth are required for establishment of the PFR. Further, we uncovered a role for the Sonic hedgehog (Shh) transcriptional effector Gli3 in digit morphogenesis that is independent of Shh signaling. These results support an alternative molecular model of limb outgrowth that provides new insights into vertebrate autopod evolution and regeneration.

Males and females generally have different finger proportions. In males, digit 2 is shorter than digit 4 and in females digit 2 is the same length or longer than digit 4. The digit ratio (2D:4D) correlates with numerous sexually dimorphic behavioral and physiological conditions, and although it has been suggested that this reflects embryonic exposure to androgen, a role for sex hormones in digit development has never been demonstrated. Here we report that mice have sexually dimorphic digit proportions similar to humans, and that the 2D:4D ratio is controlled by the balance of androgen to estrogen signaling. Androgen and estrogen receptor activities are highest in digit 4, and the growth response of this digit alone determines the 2D:4D ratio. Modulation of sex steroid signaling during limb development shows that androgen and estrogen act on different phalanges within digit 4 and have opposite effects on the skeletogenic gene network and on chondrocyte proliferation. We find that altering the balance of androgen to estrogen activity in the developing digits can masculinize or feminize the 2D:4D ratio. These studies identify the first molecular dimorphisms between male and female limb buds and show that the digit ratio is a lifelong signature of prenatal hormonal exposure.

Program/Abstract # 220
Using forward genetics to advance our understanding of mouse limb development
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Despite our knowledge of signaling centers and genes controlling patterning along the primary axes in the developing limb, there are gaps in our understanding of how a limb is built. In an effort to broaden our insight into the genetic mechanisms underlying limb formation and patterning, our lab is currently focused on identifying new genes required during embryonic limb development. Work by multiple labs have verified that forward genetics screens are feasible in the mouse and are an excellent, unbiased method to uncover factors critical for a wide variety of developmental processes. We are currently working with several novel, recessive mouse mutants derived from chemical-based mutagenesis screens. One difficulty of forward genetics in mice has been the time required to identify the affected locus through standard meiotic mapping. Here we report that mice have sexually dimorphic digit proportions similar to humans, and that the 2D:4D ratio is controlled by the balance of androgen to estrogen signaling. Androgen and estrogen receptor activities are highest in digit 4, and the growth response of this digit alone determines the 2D:4D ratio. Modulation of sex steroid signaling during limb development shows that androgen and estrogen act on different phalanges within digit 4 and have opposite effects on the skeletogenic gene network and on chondrocyte proliferation. We find that altering the balance of androgen to estrogen activity in the developing digits can masculinize or feminize the 2D:4D ratio. These studies identify the first molecular dimorphisms between male and female limb buds and show that the digit ratio is a lifelong signature of prenatal hormonal exposure.

Program/Abstract # 222
Characterization of the mechanisms involved in the early specification and migration of Prox1-expressing lymphatic endothelial cells
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Prox1-expressing lymphatic endothelial cells (LEC) bud from the cardinal vein (CV) and migrate to form the lymph sacs, which is the first step of the formation of the entire lymphatic vasculature. Here we used immunohistochemistry to characterize the expression profile of the Prox1-expressing cells as they begin to leave the CV. We found that Podoplanin expression is initiated immediately after the LECs start to exit the CV, during which time Vwf expression is lost. At the same time, these moving LECs exhibit augmented expression of both Vegfr3 and Nrp2, likely mediators of the VEGF-C-promoted budding of differentiating LECs. Finally, we also show that the proliferation of Prox1-expressing LECs on the cardinal vein maintains a pool of LEC progenitors necessary for the formation of the lymphatic vasculature.

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Program/Abstract # 221
Developmental basis of sexually dimorphic digit proportions (2D:4D ratio)
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The embryonic vertebrate heart is built through two distinct phases of cardiomyocyte differentiation: an initial phase that establishes the primitive heart tube and a later phase that adds cardiomyocytes to both poles of the heart. Notably, the outflow tract (OFT) myocardium forms at the arterial pole of the heart tube through recruitment of late-differentiating cells derived from a