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Morphological Changes in Calcanei of Mice with *Hoxa11* and *Hoxd11* loss-of-function mutations

Samuel Goldman, Avani Bhavsar, Kelsey Kjosness, Sabrina Kistler, Emily Schuetz, Nicholas Stephens, Timothy Ryan, Philip Reno

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Abstract

Hox11 genes are expressed around the developing wrist and ankle and are known to substantially impact pisiform shape and length in mice. The calcaneus is a tarsal bone that is paralogous to the pisiform in the wrist, but previous descriptions of mice with *Hox11* mutations have suggested that little morphological change takes place unless *Hoxa11* and *Hoxd11* are both



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knocked out, at which point the calcaneus fails to form. However, these studies primarily relied on cleared and stained whole-mount specimens which limit resolution of morphological features. This study seeks to determine if calcaneus morphology is altered by three or fewer loss-of-function *Hoxa11* and *Hoxd11* alleles. We obtained microCT scans of 8 week old mice and compared calcaneus morphology in wild type mice and mice with one, two, and three *Hoxa11* and *Hoxd11* loss-of-function alleles. We used auto3dgm to conduct a 3D geometric morphometric analysis of shape variation using surface semi-landmarks. Principle components (PC) analysis indicates that calcaneus morphology is altered in mice with *Hoxa11* and *Hoxd11* loss-of-function mutations. PC1 accounts for 35.4% of shape variation and results from changes to the width and height of the calcaneal neck and shape of peroneal tubercle/process. PC2 accounts for 11.9% of shape variation and results from changes to the width of the calcaneal tuberosity and height of the posterior talar facet. Most specimens with either combination of three out of four *Hoxa11* and *Hoxd11* loss-of-function alleles cluster together. The other genotypes form a gradient of morphological change with WT, *Hoxd11* heterozygotes, and *Hoxd11* knockouts being most similar to each other and *Hoxa11* heterozygotes, *Hoxa11* knockouts, and heterozygotes for both genes being most similar to each other. These results suggest that Hox11 loss-of-function mutations result in altered calcaneus morphology and *Hoxa11* and *Hoxd11* loss-of-function mutations alter the shape of the calcaneus in different ways when fewer than three alleles are knocked out.

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