# *Hox* Expression in Murine Fatigue Loading Stress Fracture Model

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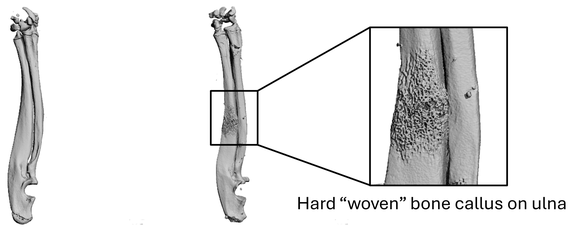
## Background

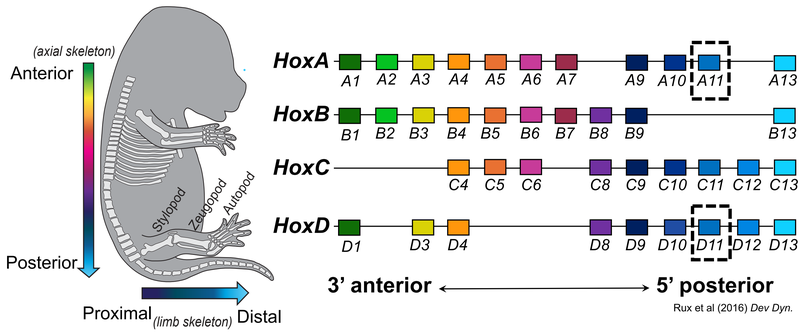
Bone is a highly dynamic tissue that responds to mechanical force. Following a stress fracture, the periosteum expands and a hard callus of “woven” (disorganized) bone quickly forms. Eventually, “lamellar” (organized) bone replaces and remodels it.

**Unloaded Forearm** **Stress Fracture Forearm**




New data suggests that *Hox* genes are expressed in the postnatal skeleton in the periosteum. *Hox* genes are a set of evolutionarily conserved transcription factors that are important in embryonic patterning.

*Hox* genes are separated into 13 paralogous groups, *Hox1-Hox13*, depending on the region of the body that they are expressed. *HoxA11* and *HoxD11* pattern the zeugopod region (radius/ulna, tibia/fibula) of the appendicular skeleton.




Rux et al (2016) *Dev Dyn.*

## Purpose

To determine the expression of *HoxA11* and *HoxD11* genes in the murine ulnar fatigue/stress fracture model to better understand bone healing mechanisms.

## Methods


Zweifler, Sinder, et. al. Bone 2024

**Stress Fracture Model**

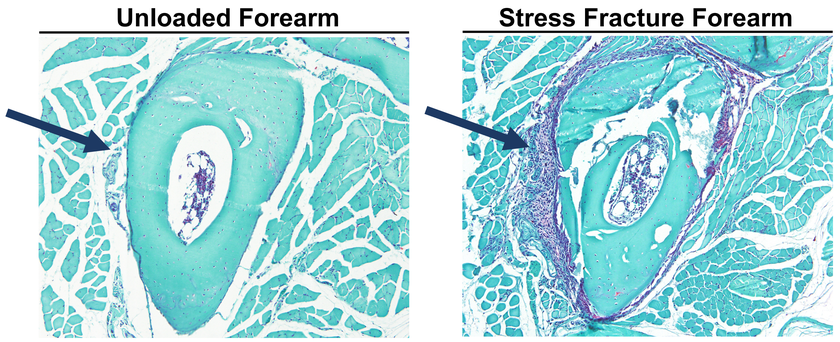
- Cyclically loaded right forearm of skeletally mature (~4month) mice at 2 Hertz to induce fatigue/stress fracture (n=9) Uthgenannt et. al. JBMR 2007
- Bones were collected 3 days after loading

**Histology**

- SafraninO FastGreen: Assessed periosteal expansion in stress fracture model
- RNAscope (In Situ Hybridization): Determined expression of both *HoxA11* and *HoxD11 (n=1)*

## Results: Stress Fracture Model

**Unloaded Forearm** **Stress Fracture Forearm**



SafraninO | Hematoxylin | FastGreen

**Figure 1.** SafraninO FastGreen Staining shows increased cell nuclei (Hematoxylin), without cartilage (SafraninO), around the stress fracture forearm. The ulna, which is in direct contact with the load cell, was analyzed.

SafraninO FastGreen Staining confirms the expanding periosteal stress fracture callus 3 days after loading.

## Results: *Hox* Gene Expression

**Unloaded Forearm** **Stress Fracture Forearm**

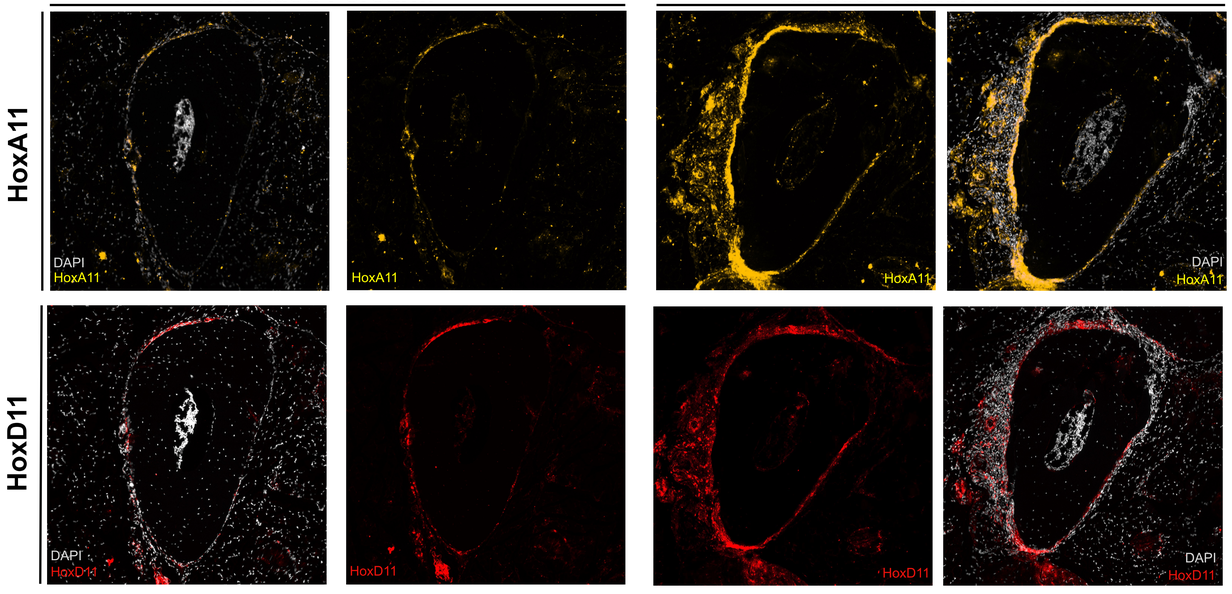



**Figure 2.** RNAscope for *HoxA11* (yellow) and *HoxD11* (red) expression. DAPI (white) stains for DNA, and shows dense cellular area in expanding callus.

*Hox* expression is upregulated on the periosteal surface in the stress fracture ulna.

## Conclusions

Preliminary data demonstrates that *HoxA11 and HoxD11* are expressed on the periosteal surface of early (3 day) stress fractures. This suggests that *Hox* genes become active during the stress fracture healing process.

## Future Directions

- Refining the stress fracture model (additional timepoints, increase group sizes, etc)
- Testing *Hox* expression in related models of bone loading (lamellar)
- Test function by deletion of *Hox* genes in the stress fracture model

## Acknowledgements

UConn Health Research Program (HRP), REP Award, and Dept of Orthopedic Surgery.