Functional Perturbation of Mustn1 in Cartilage

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NIH National Institutes of Health

TAP TO RETURN TO KIOSK MENU

The Gene: Mustn1

Mustn1, Mustang (Musculoskeletal Temporally Activated Novel Gene) is a novel pan-musculoskeletal marker discovered during an expression screen for upregulated genes during bone regeneration [1].

What we know about the gene so far:

- **It does not belong to any known gene family.**
- **Expression is localized to bone, cartilage, skeletal muscle, and tendon**
- **Mustn1 present in a wide range of pathologies such as arthritis, clubfoot, and duchenne muscular dystrophy.**

We hypothesize that Mustn1 is essential for development; deletion in chondrocytes is anticipated to impair cartilage formation and bone quality. ***Identifying the function of the Mustn1 gene in chondrogenesis would expand knowledge about the underlying pathophysiology for skeletal diseases.***

Prior Studies Have Shown:

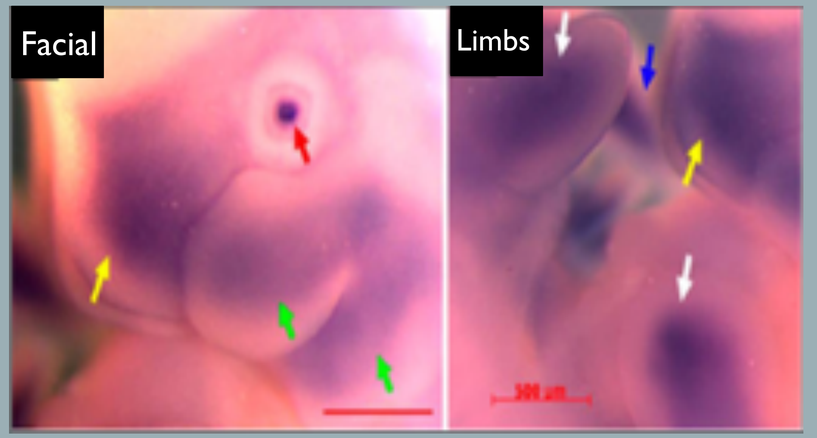

Fig. 1 – Mustn1 Expression.

Spatial expression of Mustn1 in branchial arches (green), frontonasal processes (yellow) limbs (white), lens (red) & tail bud (blue), in situ hybridization. [2]

Control

Unilateral Mustn1 Knockdown

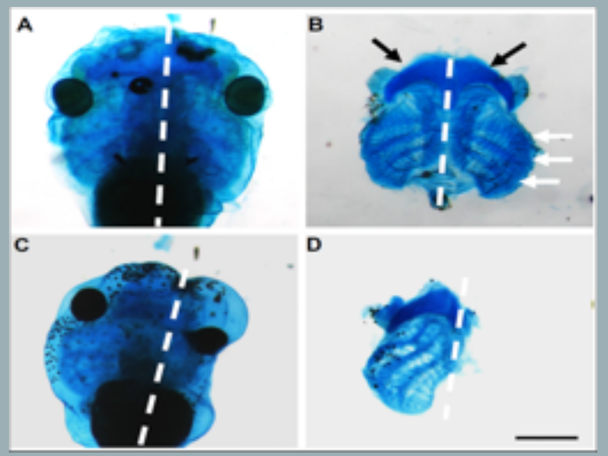

Fig. 2 – Knockdown effects.

Unilateral Mustn1 downregulation in Xenopus species (tadpoles) displayed craniofacial abnormalities, alteration of midline, and loss of cartilaginous structures. Morpholino injected embryo with Alcian blue staining. [3]

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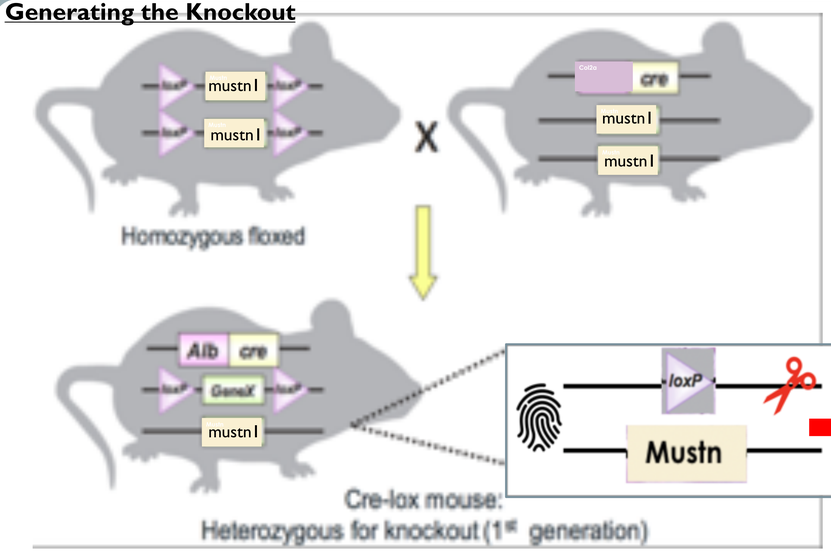
Developing the Knockout Model

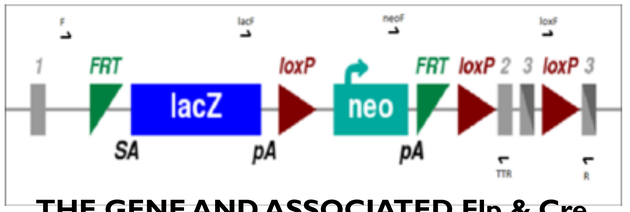
- The gold standard to study the *in vivo* function of an unknown gene is a knockout mouse model.
- **The Cre-lox & Flp-FRT system was used to generate a cartilage-specific Mustn1 conditional knockout.**
- Cartilage is predominant during embryonic and neonatal development, **newborn knockout (KO) mice age-matched to Wild Type (WT) mice.**

Knockout Viability:

- Mustn1 knockout mice were viable in the first generation (F1), and were able to successfully reproduce. However, in **the second generation (F2), all newborn knockout pups died shortly after birth.**
- Suspecting this may be due to parenting, **pups were moved to a foster wild-type mother directly after birth but they died days after.**
- Euthanized WT and dead F2 generation KO mice were processed for tissue and skeletal comparison.

Generating the Knockout

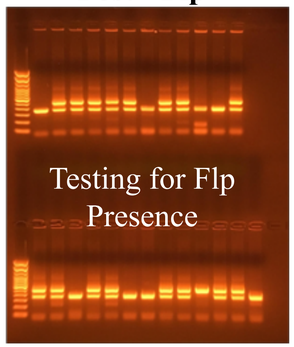




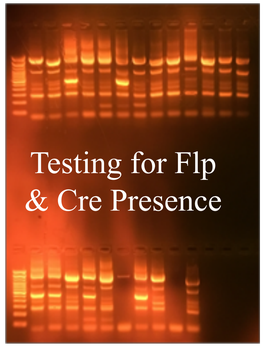
THE GENE AND ASSOCIATED Flp & Cre Binding Sites

Genotyping to Isolate the Knockout

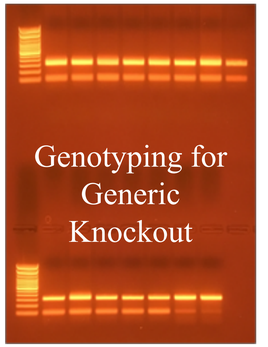
Post - Flp



Post Flp & Post-Cre



Post - CRE



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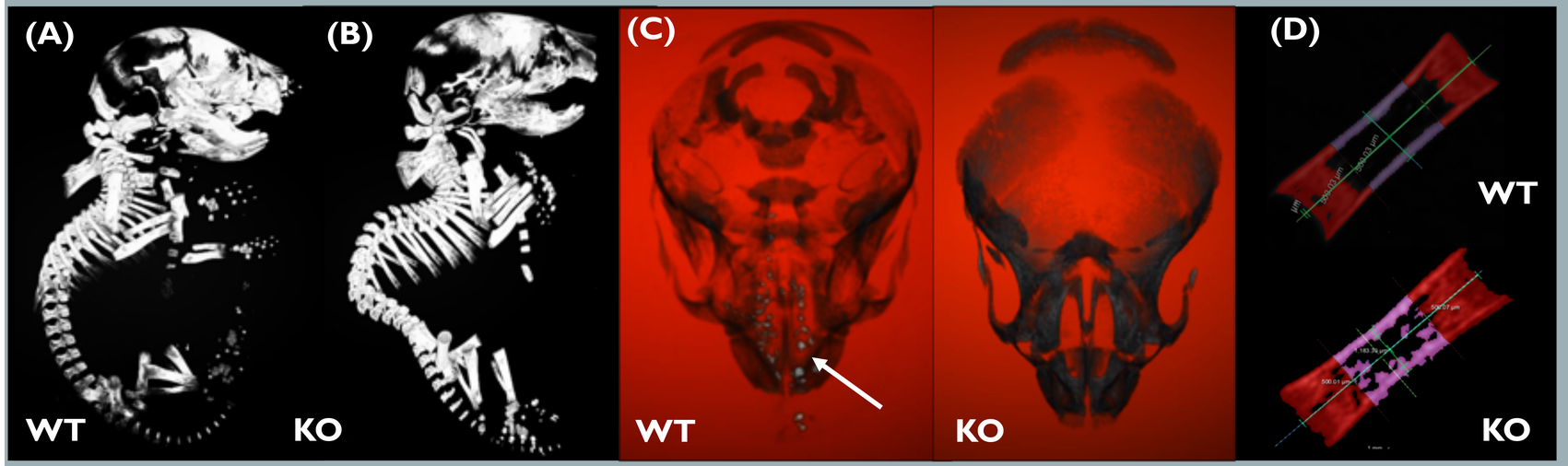


MicroCT Analysis

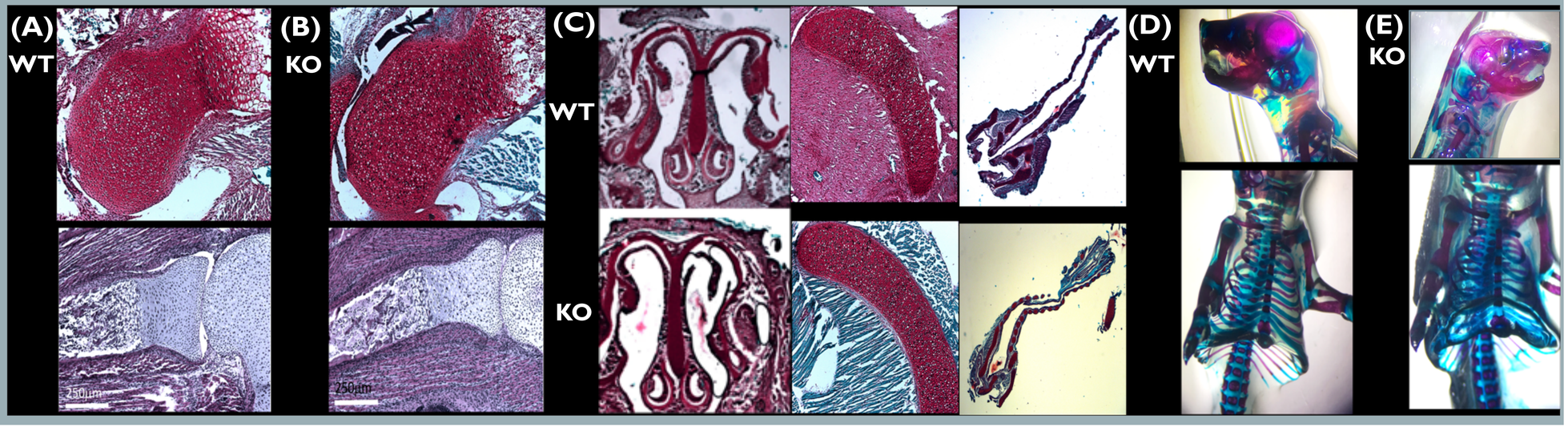
The SkyScan microCT scanner imaged samples while the software Dragonfly was used for reconstructive analysis. Skeletal microCT revealed qualitative structural differences.

- Compared to WT (A), KO mice exhibited a slight exaggeration of curvature in the spine (B).
- Nasal particles were noted in WT (C, left) not present in KO (C, right).
- There was little difference in WT (D, top) vs KO (D, bottom) femoral bone mineral density and bone volume but analyses are still being continued on all bones.

Histological Analysis:

Paraffin embedded samples were sectioned and stained using Safranin-O & Fast Green and Hematoxylin & Eosin.

- Staining revealed a qualitative increase, compared to WT mice (A), of empty space in the long bones of KO (B).
- Glenohumeral joint (A & B top), radial head (A & B bottom), nasal turbinates, rib, and trachea (C; WT top, KO bottom) showed little morphological differences.
- Alcian Blue & Alizarin Red staining of WT (D) and KO (E) are still being analyzed for differences.



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Discussion & Further Studies

- While no apparent physical differences are noted as of yet, the question as to why the newborn mice are failing to survive still needs to be answered.
- Further work analyzing the bone mineral density and bone volume via microCT, performing histology of the mice throughout their growth and development, and increasing the sample size of the knockout population would provide a greater foundation for deeper comparison of the effects of the genetic deletion.

- ***Clinical Significance:*** ***Through this genetic investigation, we hope to prove the functional significance of Mustn1 to genetic conditions of the skeletal system, providing the potential for expanding the understanding of skeletal pathophysiology.***

Osteopathic Significance:

Due to the presence of this gene in the development of the skeleton, if there were any mutations resulting in loss of gene function, there may be deficiencies in bone and even healing. It is essential to understand the function of this novel gene and its role in the mechanism of cartilage and bone development for the medical potential this knowledge can provide.

References

1. Lombardo F. et al. (2004). Molecular cloning and characterization of Mustang, a novel nuclear protein expressed during skeletal development and regeneration. *The FASEB Journal.*
2. Hadjiargyrou M. (2018). Mustn1:A Developmentally Regulated Pan-Musculoskeletal Cell Marker and Regulatory Gene. *International journal of molecular sciences*.
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Acknowledgements

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- Institutional Animal Care and Use Committee (IACUC) approved.