A novel zebrafish mutant to elucidate the consequences of Wnt16 cysteine deletion

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INTRODUCTION

- Osteoporosis is a common disease characterized by bone fragility that impacts over 200 million people worldwide[5]
- The WNT family of proteins has been implicated in numerous developmental and disease pathways[4]
- Mutations involving one of the 24 cysteines conserved across the WNT family often have functional consequences[4]
- WNT16 has been linked to genetic risk for osteoporosis[4]
- The impact of cysteine 10 (c10) alteration in any WNT remains unknown[4]
- Zebrafish are an ideal model for skeletal research[3]

METHODS

STEP 1: Creating somatic mutants using CRISPR-Cas9

- Cas9:gRNA injected into embryos at the 1- or 2-cell stage
- Mutations created at the *wnt16* locus at the c10 position

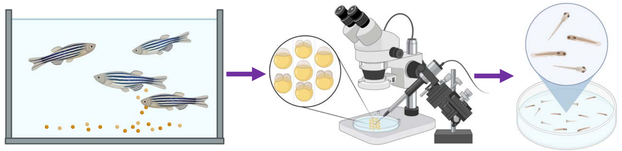


*Figure 1: Workflow for creating somatic mutants (breeding, injecting, and rearing).

STEP 2: Creating germline mutants

- Somatic mutants outcrossed with WT fish (F0)
- Heterozygous offspring of F0 selected and inbred (F1)
- Homozygous offspring of F1 used for experiments (F2)

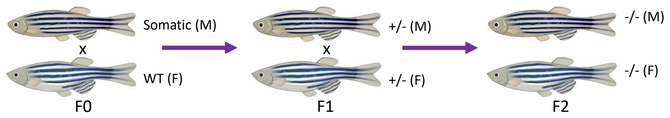


*Figure 2: Creating germline mutants via outcrossing, selecting, and inbreeding.

STEP 3: Confirming c10 deletion

- Sanger sequencing used to determine mutant genomic sequence
- Genomic sequence translated to protein sequence
- Consequences on protein sequence determined through sequence alignment

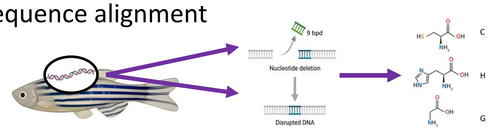


*Figure 3: 9 base pair deletion that resulted in the deletion of cysteine (C), histidine (H), and glycine (G).

*Figures 1, 2, and 3 created with BioRender.com

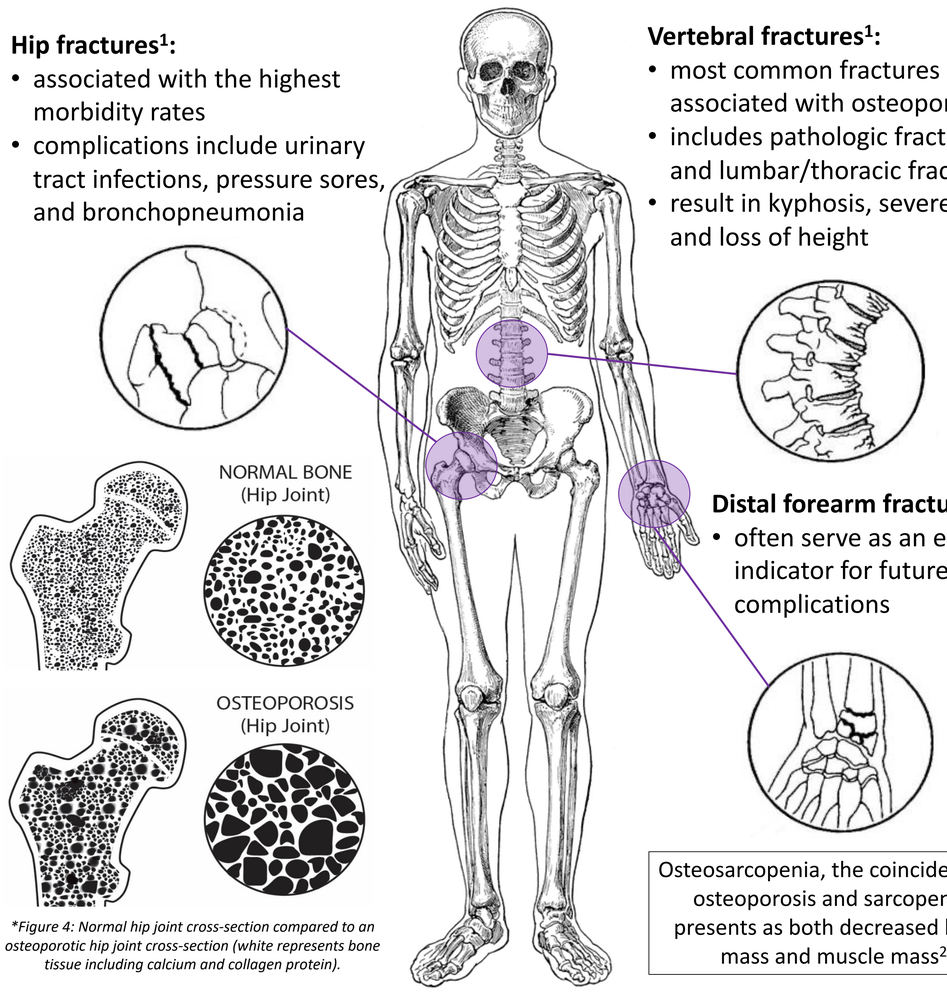
HYPOTHESIS & AIM

- **Hypothesis**: loss of c10 in Wnt16 will result in altered bone mass indicative of elevated osteoporosis risk.
- **Aim**: generate zebrafish mutants where c10 is deleted in Wnt16.

OSTEOPOROSIS: "POROUS BONE"

Hip fractures[1]:
- associated with the highest morbidity rates
- complications include urinary tract infections, pressure sores, and bronchopneumonia

Vertebral fractures[1]:
- most common fractures associated with osteoporosis
- includes pathologic fractures and lumbar/thoracic fractures
- result in kyphosis, severe pain, and loss of height



Distal forearm fractures[1]:
- often serve as an early indicator for future complications

Figure 4: Normal hip joint cross-section compared to an osteoporotic hip joint cross-section (white represents bone tissue including calcium and collagen protein).

Osteosarcopenia, the coincidence of osteoporosis and sarcopenia, presents as both decreased bone mass and muscle mass[2]

Figure 5: Prevalent fracture locations in osteoporotic individuals.
Adapted from Hurley, D. L., & Khosla, S. (1997). Update on Primary Osteoporosis. *Mayo Clinic Proceedings*, *72*(10), 943–949. https://doi.org/10.4065/72.10.943 and United States., United States., & Milwaukee Academy of Medicine. (1918). *Manual of surgical anatomy: Authorized by the Secretary of War and under the supervision of the Surgeon General and Council of National Defense*. Washington.

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REFERENCES

[1]Clynes, M. A., Harvey, N. C., Curtis, E. M., Fuggle, N. R., Dennison, E. M., & Cooper, C. (2020). The epidemiology of osteoporosis. *British Medical Bulletin*, ldaa005. https://doi.org/10.1093/bmb/ldaa005.

[2]Kirk, B., Zanker, J., & Duque, G. (2020). Osteosarcopenia: Epidemiology, diagnosis, and treatment—facts and numbers. *Journal of Cachexia, Sarcopenia and Muscle*, *11*(3), 609–618. https://doi.org/10.1002/jcsm.12567.

[3]Kwon, R. Y., Watson, C. J., & Karasik, D. (2019). Using zebrafish to study skeletal genomics. *Bone*, *126*, 37–50. https://doi.org/10.1016/j.bone.2019.02.009.

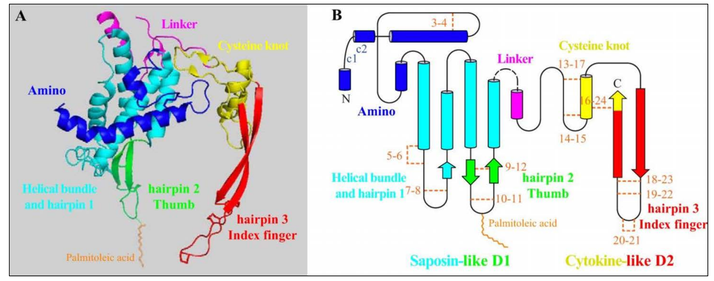
[4]MacDonald, B. T., Hien, A., Zhang, X., Iranloye, O., Virshup, D. M., Waterman, M. L., & He, X. (2014). Disulfide Bond Requirements for Active Wnt Ligands. *Journal of Biological Chemistry*, *289*(26), 18122–18136. https://doi.org/10.1074/jbc.M114.575027.

[5]Tański, W., Kosiorowska, J., & Szymańska-Chabowska, A. (2021). Osteoporosis – risk factors, pharmaceutical and non-pharmaceutical treatment. *European Review for Medical and Pharmacological Sciences*, *25*(9), 3557–3566. https://doi.org/10.26355/eurrev_202105_25838.

RESULTS + DISCUSSION

RESULTS

- 3 amino acid deletion discovered at Cys214, corresponding to c10 (p.Cys214_Gly216del)
- Previously characterized *Xenopus* Wnt8 crystal structure indicates c10 contributes to the integrity of WNT tertiary structures via a disulfide bond formed between c10 and c11[4]
- c10 and c11 exist in the hairpin 2 "Thumb" region, which directly interacts with Frizzled receptors[4]



[4]Figure 6: (A) Xwnt8 tertiary structure with subdomains shown. (B) Schematic of the 24 conserved cysteines of Wnt3a with known disulfide bonds modeled from the crystal structure of Xwnt8.

DISCUSSION

- We hypothesize that Wnt16 will have altered secretion/ activity in our novel mutant as a result of the c10 deletion
- Our novel animal model will help us understand the function of c10 in Wnt16
- Drug treatments for osteoporosis are only effective for half of all patients[5] (no known therapies for osteosarcopenia[2])
- This research will allow us to assist in developing new therapies that may alleviate a global health burden

NEXT STEPS

- Quantitatively evaluate bone phenotypic characteristics in *wnt16*^{w1012} mutants (using calcein staining and micro-computed tomography imaging)
- Compare *wnt16*^{w1012} mutants to *wnt16* knockouts previously characterized by our lab:

We predict that *wnt16*^{w1012} mutants will phenocopy *wnt16* knockouts, suggesting that c10 plays an essential role in WNT16 secretion and/or activity.

Musculoskeletal Systems Biology Lab: https://sites.uw.edu/msblgroup/