Investigation of ITGAM on Fracture Development and Vertebral Morphology in Zebrafish

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BACKGROUND

- *ITGAM* was recently identified as a commonly mutated gene in GWAS of humans with high fracture rates
- *ITGAM* codes for an integrin alpha M subunit, but its role in bone has not been clearly defined
- Zebrafish are a great model for studying skeletal abnormalities as they are rapidly developing, transparent organisms that share mammalian bone physiology[1]

The Goal of This Study: Investigate the relationship of the gene, *ITGAM*, on the vertebral column of zebrafish as it relates to fracture development and bone morphology.

METHODS

Subjects

- *itgam* germline knockout zebrafish were created using Crispr/Cas9
- 9 adult wildtype controls (*itgam*$^{+/+}$) and 13 adult homozygous mutants (*itgam*$^{-/-}$) were genotyped with DNA extracted from a fin clipping

Data Collection

- Zebrafish were then scanned using microCT at 136 days
- microCT scans were segmented by individual vertebra and assessed for bone morphology using FishCuT software[2]

Statistical Analysis

- Scans that were evaluated for quantitative differences using FishCuT were analyzed using a custom R script
- Measures analyzed were tissue volume, thickness, and mineral density of each vertebra centrum (Cent), haemal arch/ribs (Haem), and neural arch (Neur)
- All scans were also analyzed for qualitative differences using Fiji ImageJ
- Both types of analyses were performed blinded to genotype

RESULTS

- 2-Fold increase in average fractures per fish in mutants (Table 1)
- Centrum TMD was the only statistically significant variable (p=0.022) that demonstrated a pattern of difference between groups (not shown)
- *itgam*$^{+/+}$ were allometrically scaled to match *itgam*$^{-/-}$, which yielded no statistically significant patterns of difference for the 10 morphologic variables measured (Figure 2)

Genotype	n=	Subjects with Fx Calluses	% of subjects effected	Total Fx Calluses	Calluses/Subject
itgam$^{+/+}$	9	2	22.2%	4	0.444
itgam$^{-/-}$	13	4	30.8%	11	0.846

Table 1. Fracture rates observed in *itgam*$^{+/+}$ and *itgam*$^{-/-}$ zebrafish

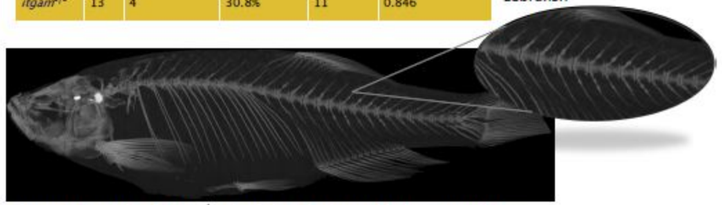

Figure 1. Example of itgam$^{-/-}$ zebrafish with observable fracture calluses

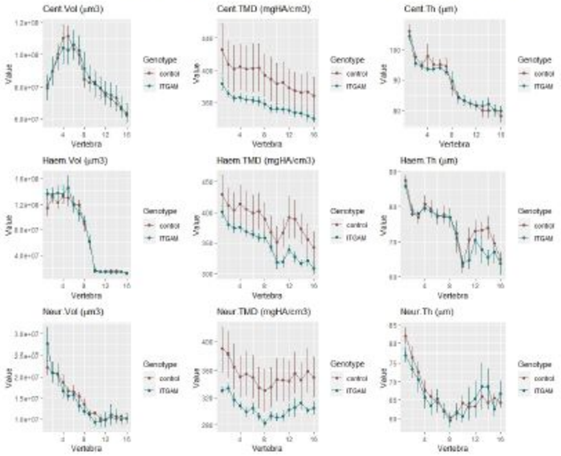

Figure 2. Analysis of itgam$^{-/-}$ fish. Phenotypic features plotted as a function of vertebra (mean ±SE, n = 8/group).

DISCUSSION

- We demonstrate increased fracture prevalence in *itgam* mutant zebrafish, which corroborates recent observations of increased fracture risk associated with human *ITGAM* genetic variants
- Despite demonstrating the increased fracture prevalence, our study exhibits no statistically significant difference in vertebral bone morphology (tissue mineral density, thickness, or volume) in *itgam*$^{-/-}$ zebrafish as compared to allometrically scaled control zebrafish
- Future studies of *itgam* will continue to study the association with the fracture phenotype to better understand the role of this gene within the vertebral column

CONCLUSIONS

Our preliminary research of *ITGAM*, a novel gene associated with increased fracture rates in humans, reveals an increased prevalence of fractures without demonstrating specific morphologic changes within the vertebral column.

Consequently, this lays the groundwork for continued study into the role of *ITGAM* as it relates to the development of fractures within the axial skeleton.

ACKNOWLEDGEMENTS

Special thanks to the Musculoskeletal System Biology Lab for their support and guidance.

REFERENCES

1. Watson C. J., Kwon R. Y. Osteogenic programs during zebrafish fin regeneration. BoneKEy Reports. 2015;4:p. 745. doi: 10.1038/bonekey.2015.114
2. Hur M, Gistelinck CA, Huber P, et al. MicroCT-Based Phenomics in the Zebrafish Skeleton Reveals Virtues of Deep Phenotyping in a Distributed Organ System. Zebrafish. 2018;15(1):77-78. doi:10.1089/zeb.2017.1540