Characterization of the Structural Effects of Global Deletion of LRP5 to Vertebral Bone and Intervertebral Disc in Rats

Tori Kroon[1], Devavrat Tarwala[2], Nilsson Holguin[1]
[1]Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY
[2]Bronxville High School, Bronx, NY
Tori.kroon@icahn.mssm.edu

Disclosures: Tori Kroon (N), Devavrat Tarwala (N), Nilsson Holguin (N).

SIGNIFICANCE/CLINICAL RELEVANCE: Large, genetically-manipulated animal models of the spine would help to test the pathophysiology of discogenic pain and potential therapies.

INTRODUCTION: 17 million people aged 65 and older suffer from at least one episode of lower back pain annually, and 35% of them experience chronic low back pain.[1] Aging promotes the replacement of healthy immature nucleus pulposus cells with mature nucleus pulposus cells, which are associated with intervertebral disc (IVD) degeneration. The Wnt signaling pathway regulates IVD cell proliferation and differentiation, and we have previously shown that a reduction of Wnt signaling in the IVD, as occurs with aging[2], by deletion of cell membrane receptor LRP5 in mice induces IVD degeneration.[2] The small size of the spine in mice is a limitation for surgically-induced discogenic pain and a larger animal model would be beneficial. Therefore, we hypothesize that global LRP5 deletion in rats will decrease vertebral bone structure and induce IVD degeneration, as occurs in mice.

METHODS: All experiments were IACUC-approved. The development of the animal model is previously published.[3] Briefly, CRISPR/Cas9 was used to make an 18 bp deletion in exon 2 of the LRP5 gene causing early termination. Wild-type (WT, 4 male, 4 female) and LRP5 KO (KO, 3 male, 4 female) rats on a Sprague-Dawley background were harvested at 6 months of age. L6 vertebrae were analyzed by microCT (Scanco) at a resolution of 9.6 µm. For cortical bone, the midpoint of the vertebra was identified and 15 slices up and 15 slices down were used to analyze for cortical thickness (Ct.Th). For trabecular bone, the growth plate was identified and 30 slices above were analyzed for bone volume fraction (Tb.BV/TV) and volumetric bone mineral density (vBMD). Subsequently, safranin-O-stained histological sections were analyzed by two-independent observers for IVD degeneration scores.[4] A 2-way ANOVA was conducted for statistical comparisons and statistical significance was set at a p-value less than 0.05.

RESULTS: Global deletion of LRP5 decreased cortical thickness (Ct.Th) by 37%~~, 33% in males and 42% females~~ ($p<0.001$) but there was no sex effect or interaction between deletion and sex (**Fig. 1A**). Similarly, LRP5 deletion decreased trabecular BV/TV by ~~45% in males and 16% in females~~31% ($p<0.05$) and there was no sex effect nor interactive effect (**Fig. 1B**). Lastly in bone, deletion of LRP5 reduced volumetric bone mineral density (vBMD) by ~~30% in the males and 17% in the females~~24% with a KO effect ($p<0.01$), with no sex effect or interaction (**Fig. 1C**). LRP5 deletion did not change IVD degeneration score, however there was a sex effect between groups with the total average degeneration score with a 67% decrease from males to females (**Fig. 1D**).

DISCUSSION: The decreases in cortical and trabecular bone parameters caused by the LRP5 deletion in rats mirrors the effects observed in mouse models and other rat bones.[2,5] Global LRP5 deletion did not statistically increase the IVD degeneration score in male or female rats but, while preliminary data suggest that the rat IVD expresses LRP5, further analysis will be needed to corroborate LRP5 deletion in the IVD and if any mechanical properties were affected by the LRP5 deletion. Overall, these data suggest we are able to study the effects of LRP5 deletion in a rat model and use this to further study IVD degeneration.

REFERENCES: [1] Wong, Arnold Y.L, et al. *Syst Rev*. 2018. [2] Holguin, N & Silva, M. *Faseb J*. 2019 [3] Ubels, John L, et al. *CRISPR J*. 2020. [4] Le Maitre, Christine, et al. *JOR Spine*. 2021. [5] Ubels, John L, et al. *ARVO*. 2019.

ACKNOWLEDGEMENTS: I would like to thank Dr. Bart O Williams for his contribution. This project was funded by NIH – (NH) R01 AR078764.

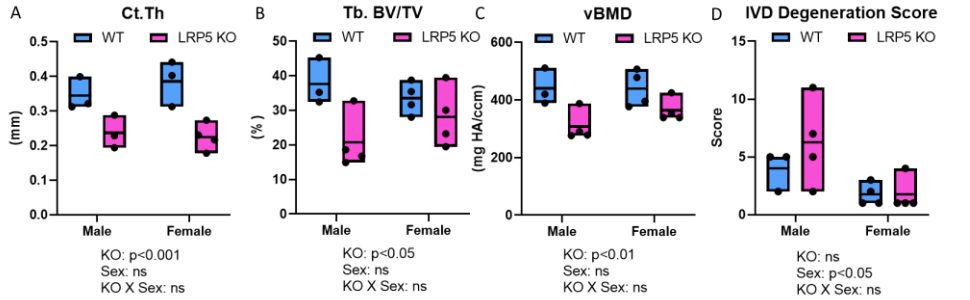

Figure 1. *(A) Cortical thickness (B) Trabecular bone volume fraction, (C) Volumetric bone mineral density and (D) IVD degeneration score of male and female WT and LRP5 KO rats.*

Commented [HN1]: One percentage by genotype if this is a sig main effect

Formatted: Highlight

Formatted: Highlight