Characterizing Cardiac Collagen Content in a Pre-Translational Mouse Model of Osteogenesis Imperfecta (*oim*)

Veterinary Research Scholars Program
University of Missouri

Ali McAllister-Day, Brittany Lafaver, Li Lee, Lixin Ma, Charlotte Phillips

College of Veterinary Medicine (McAllister-Day), Departments of Biochemistry (Lafaver, Phillips), Child Health (Phillips), and Radiology (Lee, Ma), University of Missouri, Columbia, MO; VA-Biomolecular Imaging Center (Lee, Ma), VA Hospital, Columbia, MO

Introduction

- Osteogenesis imperfecta (OI) is a heritable connective tissue disorder that results in reduced bone mineral density, bone deformity, increased bone fragility, and intrinsic muscle weakness. The incidence of OI is 1:10,000-20,000 births, with roughly 85% of OI cases due to defects in the type I collagen genes, *COL1A1* and *COL1A2*.
- The extracellular matrix of the heart is crucial to its function and is affected in many disease states. Approximately 80% of cardiac collagen is type I collagen. Cardiopulmonary complications are the second leading cause of death in OI patients.
- The osteogenesis imperfecta murine (*oim*) mouse models severe human OI due to a *Col1α2* gene defect, which prevents the formation of normal heterotrimeric type I procollagen comprised of two pro-α1(I) chains and one pro-α2(I) chain. This mutation results in the production of homotrimeric type I procollagen composed of three pro-α1(I) chains.
- Preliminary studies in *oim* mice demonstrate increased wet heart weights relative to body weights, as well as increased left ventricular blood volumes by 7T-MRI as compared to age- and sex-matched wildtype (Wt) littermates. Currently, male mice appear to be more greatly affected.
- The objective of the present study is to quantify the amount of collagen in *oim* and Wt age- and sex-matched mouse hearts.
- Total protein and hydroxyproline (an indirect measure of fibrillar collagen) content will be determined using hydrolyzed heart tissue. Collagen will also be assessed semi-quantitatively by evaluating cross-sections of 4-month-old male and female Wt and *oim* hearts fixed and stained with picrosirius red and analyzed using Image-J Fiji software.
- This study begins to characterize the impact of type I collagen alterations in the extracellular matrix to cardiac function in the *oim* hearts relative to Wt mouse hearts and their potential role to cardiac disease resulting in the early death of OI patients.
- **We hypothesize that *oim* will have reduced total collagen content in their hearts as compared to Wt littermates, with male mice more severely affected.**

Methods

Animals: Analyses were performed on adult 4-month-old (peak bone mass) male and female *oim/oim* and Wt C57 mice. *Oim* (*Col1a2*^{oim}) mice were bred and maintained on the congenic C57BL/6J background (Carleton et al., 2008). Mice were given ad libitum access to food and water while housed in an AAALAC facility at the University of Missouri. Four-month-old wildtype (Wt) and *oim/oim* mice underwent 7T MRI, followed by sacrifice. Heart tissue was collected after sacrifice.

7T MRI: Cardiac MRI was performed using a 7T/20 MRI (Bruker BioSpin, Billerica, MA, USA). Mice were anesthetized with isoflurane and respiratory rate was recorded to ensure adequate breathing. Data was analyzed using Segment (MedViso, Lund, Sweden).

Imaging: Sections of 4-month-old male and female age- and sex-matched hearts were stained with Picrosirius red, and images of the left ventricular wall and interventricular septum were acquired under brightfield and polarized light conditions using a Leica DM5500 microscope and attached Leica DFC290 camera (Leica, Wetzlar, Germany). Measurements of tunica media thickness, lumen area, and perivascular collagen were taken using Image-J Fiji software (version 2.5.0; WS Rasband, National Institute of Health, Bethesda, MD, USA) using area selection and threshold tools. Vessels were selected based on type, size, surrounding tissue, distance to neighboring vessels and other structures, and presence of artifact.

Total protein (Bicinchoninic Acid) assay: Homogenized heart tissue was diluted and working reagent added in a microplate. The plate was shaken for 2 minutes then incubated at 37°C for 30 minutes. Colorimetric analysis performed at 540 nm.

Statistics: A 2 way ANOVA was performed using GraphPad Prism 8 program (GraphPad Software, Inc., La Jolla, CA, USA)

7T MRI

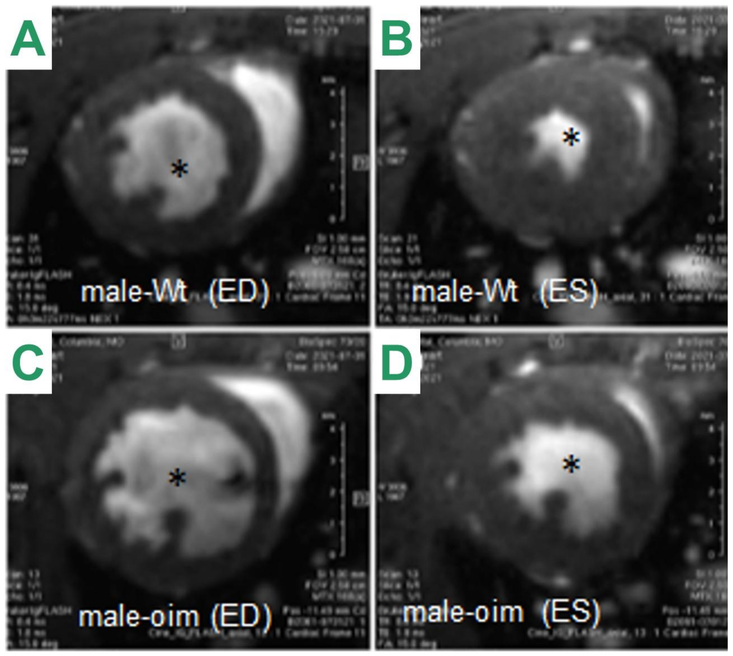

Fig. 1: Representative short axis slices of T7-MRI images from 4-month-old male Wt (A, B) and *oim* (C, D) hearts at the end of diastole (A, C) and end of systole (B, D). [* indicates left ventricle]

Histology

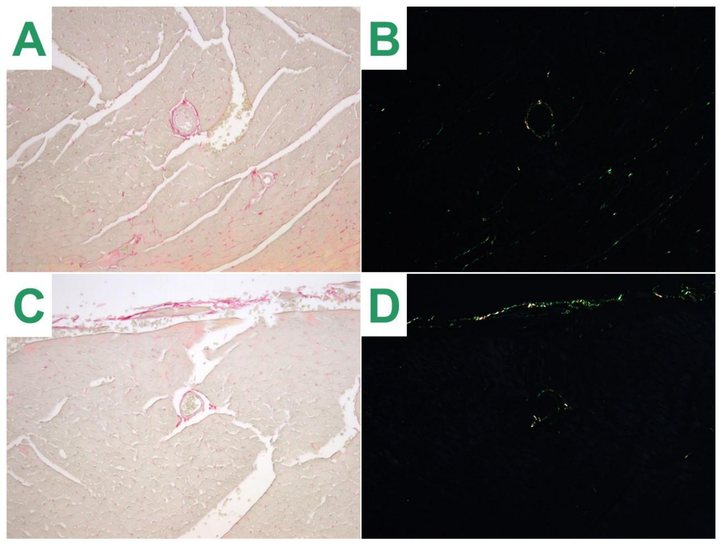

Fig. 2: Histological images of left ventricular myocardium of 4-month-old male Wt (A, B) and *oim* (C, D) mice stained with picrosirius red. Images shown as brightfield (A, C) and with polarized light filter demonstrating birefringence (B,D)

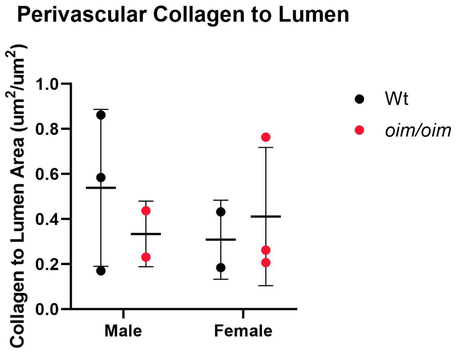

Fig. 3: Ratio of perivascular collagen to lumen area in male and female Wt and *oim/oim* mice. No statistically significant differences were found.

Discussion

- The amount of collagen in the heart is typically very small, approximately 1% by area in the ventricles. A large portion of cardiac collagen is in the cardiac skeleton, with a smaller amount in the perivascular space, and very little is in the interstitium. The low percentage of collagen can make quantification difficult, especially in a disease state with reduced collagen amounts.
- There are two forms of cardiac fibrosis: reactive and reparative
  - Reactive fibrosis is an adaptive response to hemodynamic stress that occurs in the interstitial and perivascular spaces. Excess fibrosis in the interstitium can result in reduced compliance and function of the myocardial wall as well as abnormal electrical conductance. Perivascular fibrosis can result in ischemia of the myocardium.
  - Reparative fibrosis refers to the formation of a scar due to loss of myocardium.
- Perivascular fibrosis has been implicated in its potential to impact oxygen delivery to the myocardium. Perivascular fibrosis is seen in multiple myocardial pathologic processes, including hypertrophic cardiomyopathy and possibly dilated cardiomyopathy.
- Previous research has noted a decrease of 45% in total collagen content in *oim* hearts compared to Wt. Patient history has shown an increased prevalence of aortic and mitral valve regurgitation associated with osteogenesis imperfecta.
- The ratio of type I collagen to type III collagen is critical for the structural integrity and function of the heart. These experiments quantified total collagen content in the heart, so it is possible that type III collagen is upregulated in the *oim* mouse as a potential compensatory mechanism, and this difference has not been elucidated.
- It is possible that the dilated phenotype is transient and changes to the collagen content occur later in life.
- Current data has relatively low sample size, therefore more experimentation is needed to determine differences.

Future Directions

- Analyze hydroxyproline content of hearts and compare to total protein content
- Differentiate between different types of collagen, e.g. qPCR

References

Carleton, Stephanie M., et al. “Role of Genetic Background in Determining Phenotypic Severity throughout Postnatal Development and at Peak Bone Mass in COL1A2 Deficient Mice (OIM).” *Bone*, vol. 42, no. 4, 2008, pp. 681–694., https://doi.org/10.1016/j.bone.2007.12.215.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. doi:10.1038/nmeth.2019

Acknowledgements

Light microscopy images were acquired at the University of Missouri Advanced Light Microscopy Core facility
National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases (R21 AR778132)
7T MRI facility at the VA-BIC was supported by Truman VA and University of Missouri.
Kansas City Consortium on Musculoskeletal Diseases (Collaborative Research for Neuromuscular/Musculoskeletal Disorders)
Stipend for Ali McAllister-Day provided by a grant from Boehringer Ingelheim