Abnormal Vascularity and Extracellular Matrix Remodeling are Associated with Impaired Secondary Ossification in Mucopolysaccharidosis VII

Zhirui Jiang PhD[1,2]
Casey P. Johnson, PhD[3]
Olli Nykänen, PhD[4]
Mikko Nissi, PhD[4,5]
Yian Khai Lau[1,2]
Meilun Wu[1,2]
Kai D. Ludwig, PhD[3]
Jutta Ellerman, MD[3]
Margret L. Casal, DVM, MS, PhD[6]
Lachlan J. Smith, PhD[1,2]

[1]Mckay Orthopedic Research Laboratory
University of Pennsylvania

[2]Department of Neurosurgery
University of Pennsylvania

[3]Department of Radiology
University of Minnesota

[4]Department of Applied Physics
University of Eastern Finland

[5]Department of Diagnostic Radiology
University of Oulu

[6]School of Veterinary Medicine
University of Pennsylvania

Introduction

Mucopolysaccharidosis VII is a genetic, lysosomal storage disease characterized by deficient beta-glucuronidase activity, which results in accumulation of poorly degraded glycosaminoglycans (GAGs) in cells and tissues.[1] MPS VII patients exhibit severe skeletal abnormalities, including dysplasia of the vertebrae and long bones. Resulting impaired mobility, pain and paralysis negatively impact quality of life.[2] Previous studies in our lab using the naturally occurring canine MPS VII model showed that formation of secondary ossification centers (SOCs) is markedly delayed in both vertebrae and long bones.[3] Conversion of cartilage to bone in SOCs is a multi-stage process that requires step-wise differentiation of chondrocytes, vascularization, cartilage matrix resorption and formation of mineralized bone matrix.[3] We showed previously that MPS VII chondrocytes in SOC epiphyseal cartilage exhibit impaired hypertrophic differentiation capacity.[4] Our objectives in the current study were to establish whether abnormal cartilage vascularity and impaired matrix resorption and mineralization also contribute to delayed SOC formation in MPS VII dogs, using novel, contrast-free MRI-based susceptibility-weight imaging (SWI)[5,6] and histological assays.

Methods

With IACUC approval, thoracic vertebrae were obtained postmortem from control (heterozygous) and MPS VII-affected dogs at 9 days-of-age.This is the age immediately preceding commencement of secondary ossification in controls.[4]

Cartilage Vascularity

Vertebrae (n = 3) were imaged on a 9.4T MRI scanner using a high-spatial-resolution (91 μm isotropic) 3D gradient echo sequence with magnetic susceptibility weighted to provide detailed visualization of epiphyseal cartilage vascularity.[5,6] Images were post-processed using a quantitative susceptibility mapping (QSM) pipeline to better visualize and quantify cartilage vessels,[5,6] Vessel density, thickness, branching and connectivity were then quantified using uCT Ray v4.0 software.

Cartilage Matrix Remodeling

For assessment of enzyme activity in epiphyseal cartilage, coronal, calcified cryosections of vertebrae (n = 5) were stained for either alkaline phosphatase (ALP, a marker of matrix mineralization) or tartrate-resistant acid phosphatase (TRAP, a marker of matrix resorption). The number of TRAP-positive chondroclasts per cartilage canal was quantified. Additional vertebrae (n = 5) were processed for paraffin immunohistochemistry, with sections stained for matrix metalloproteinase-9 (MMP-9, required for neovascularization).The number of MMP-9-positive epiphyseal chondrocytes was quantified

Statistical Analysis

Differences in quantitative metrics between control and MPS VII were established using Mann-Whitney tests ($p < 0.05$).

Results

Cartilage Vascularity

SWI and QSM were successfully applied to reveal detailed 3D renderings of vertebral epiphyseal cartilage vascularity (Figs 1A and B). While vessel thickness was similar in MPS VII compared to controls (Fig 1B), quantitative assessments revealed lower vessel density and connectivity (branching) in MPS VII vertebrae (~70% and 22% of control, respectively; Figs 1C and D), although the differences did not reach statistical significance.

Cartilage Matrix Remodeling

The number of MMP-9-positive chondrocytes was significantly lower for MPS VII (~33% of control, Fig 2).There was punctate ALP staining surrounding chondrocytes in control epiphyses; however, staining was completely absent in MPS VII (Fig 3A). Finally, the number of TRAP-positive chondroclasts per cartilage canal was a significantly lower in MPS VII (~13% of control, Figs 3B and C).

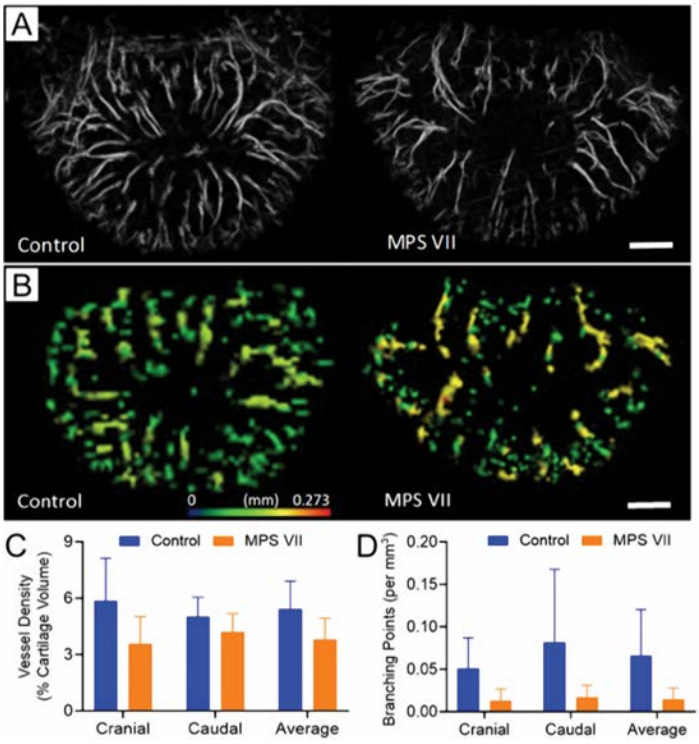

Figure 1. Analysis of vascularity in control and MPS VII dog vertebral epiphyseal cartilage using susceptibility-weighted MRI. **A.** 3D visualization of cartilage vessels (axial view). **B.** Heat map of vessel thickness. **C.** quantification of vessel density and **D.** number of branching points. Bar = 1mm

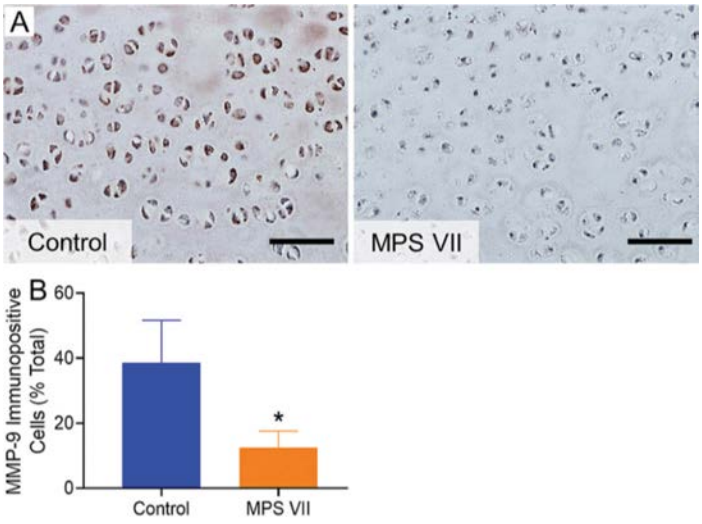

Figure 2. A. Representative MMP-9 immunostaining of control an d MPS VII epiphyseal chondrocytes. **B.** quantification of percent MMP-9 immuno-positive cells. *p<0.05 vs control. Bar = 100μ

Discussion

Vascularization and matrix remodeling are critical for effective cartilage-bone conversion during the process of endochondral ossification. We successfully applied novel, contrast-free SWI and QSM MRI-based techniques for detailed visualization of vascularity in canine vertebral epiphyseal cartilage. Preliminary findings suggest that vessel density and architecture may be abnormal in MPS VII, but this should be confirmed through analysis of additional samples and at additional skeletal sites. The lower number of MMP-9-positive chondrocytes in MPS VII supports our previous mRNA results,[8] and suggests impaired cartilage neovascularization, while altered TRAP and ALP expression likely reflect the diminished matrix resorption and mineralization capacity, respectively, of MPS VII cartilage cells. Ongoing studies seek to establish the molecular mechanisms linking lysosomal storage and GAG accumulation to altered cartilage vascularization and matrix remodeling, with the long term goal of developing improved therapies to normalize bone formation in MPS VII patients.

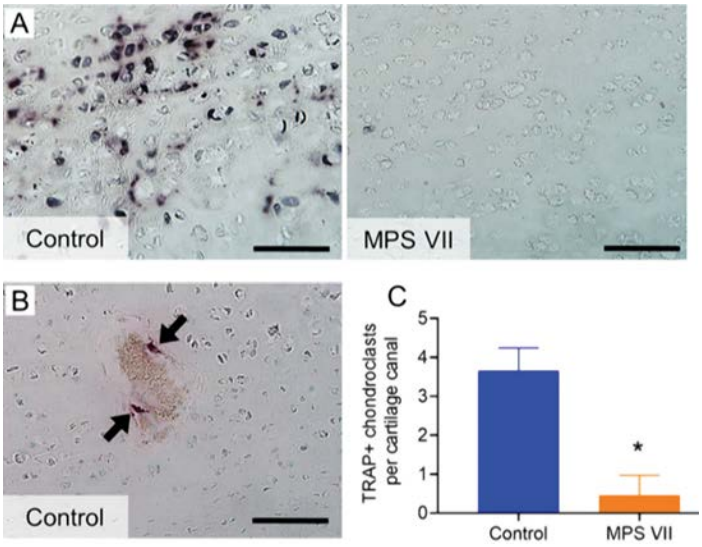

Figure 3. Impaired matrix turnover and mineralization in MPS VII epiphyseal cartilage. **A.** ALP staining is present in controls but absent in MPS VII cartilage. **B.** TRAP-positive chondroclasts (arrows) in a cartilage canal of a control. **C.** Number of TRAP-positive chondroclasts per cartilage canal. (*p<0.05 vs control). Bar = 100 μ

Significance

MPS VII patients exhibit crippling skeletal deformities for which there are no effective treatments. In this study we provide novel insights into the mechanisms underlying impaired bone formation in MPS VII using a clinically-relevant large animal model.

References

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