Aberrant Glycosaminoglycan Accumulation and Sulfation in Epiphyseal Cartilage in Mucopolysaccharidosis VII

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Introduction

The mucopolysaccharidoses (MPS) are a family of genetic, lysosomal storage diseases that are characterized by deficient activity of one of the 11 acid hydrolases responsible for degradation of glycosaminoglycans (GAGs). MPS VII is characterized by impaired β-glucuronidase activity, leading to the incomplete digestion and progressive accumulation of heparan, chondroitin, and dermatan sulfate GAG byproducts.[1] MPS VII presents with severe skeletal manifestations, which are particularly prevalent in the spine and include scoliosis, kyphosis, and spinal cord compression.[2-4] Previously, our lab established the presence of cartilaginous lesions in the vertebral bodies of MPS VII patients and dogs, which represent failed epiphyseal bone formation during postnatal development.[5] Using the naturally-occurring canine model, we identified the developmental window (between 9 and 14 days-of-age) when failed bone formation first manifests (Figure 1A), and showed that resident chondrocytes fail to undergo hypertrophic maturation. However, the links between chondrocyte dysfunction and aberrant GAG accumulation in MPS VII remain to be established. GAGs perform crucial roles in controlling the distribution and availability of many growth factors that regulate cell differentiation during endochondral ossification. The biological function of these GAGs, including binding to specific growth factors, is a function not only of their concentration, but also fine structure, including critical dependence on sulfation.[6-8] The objectives of this study were to 1) identify defects in GAG sulfation pathways in MPS VII epiphyseal cartilage using whole-transcriptome sequencing (RNA-Seq), 2) define the nature of GAG accumulation in MPS VII epiphyseal cartilage, and 3) validate an in vitro explant culture model for measuring GAG accumulation for future mechanistic studies of cellular dysfunction.

Methods

For this study, we used the naturally-occurring MPS VII canine model that mimics both the progression and pathological phenotype of the skeletal abnormalities found in human patients.[9] With IACUC approval, unaffected control and MPS VII dogs were euthanized at 9 and 14 days-of-age, and T12, L1, and L2 vertebrae were excised for analyses. Whole-Transcriptome Sequencing: Vertebral epiphyseal cartilage from T12 vertebrae of control and MPS VII dogs ($n = 5$, all groups) was collected, total RNA extracted, and RNA-Seq libraries prepared (Illumina TruSeq mRNA stranded kit). Paired-end, 100-base pair sequencing was performed (Illumina HiSeq 2500) and results mapped to the canine genome. Differential gene expression for GAG metabolic pathways was determined with DESeq2[10] (significance, $p < 0.05$). GAG Content and Disaccharide Composition: Cranial and caudal vertebral epiphyseal cartilage from L1 vertebrae of control and MPS VII animals at 9 days-of-age ($n = 3$, both groups) was excised, combined, and digested with collagenase until cells were released from the extracellular matrix. Digests were centrifuged to separate the supernatant (extracellular fraction) from the cell pellet (intracellular fraction). Total GAG content in each fraction was measured using the dimethylmethylene blue (DMMB) assay and normalized to total cell count. Disaccharide composition of chondroitin and dermatan sulfate (CS/DS) extracellular GAGs isolated as above from 9-day control and MPS VII epiphyseal cartilage ($n = 3$, both groups) was determined using UPLC. Significant differences in GAG composition ($p < 0.05$) were determined using unpaired t-tests. Explant Culture: Epiphyseal cartilage from L2 control ($n = 4$) and MPS VII ($n = 2$) vertebrae was cultured as explants for 5 days in serum-free medium (α-mem, 0.1% BSA, 1% PSF) and total GAG content in intracellular and extracellular fractions, and in media, was measured using the DMMB assay and normalized to total cell count, with significant differences between groups ($p < 0.05$) established using unpaired t-tests.

Results

Whole-Transcriptome Sequencing: RNA-Seq principal component analysis (PCA) of global gene expression showed distinct clustering (Figure 1B), indicating clear effects of both age and disease state between all groups. At 9 days-

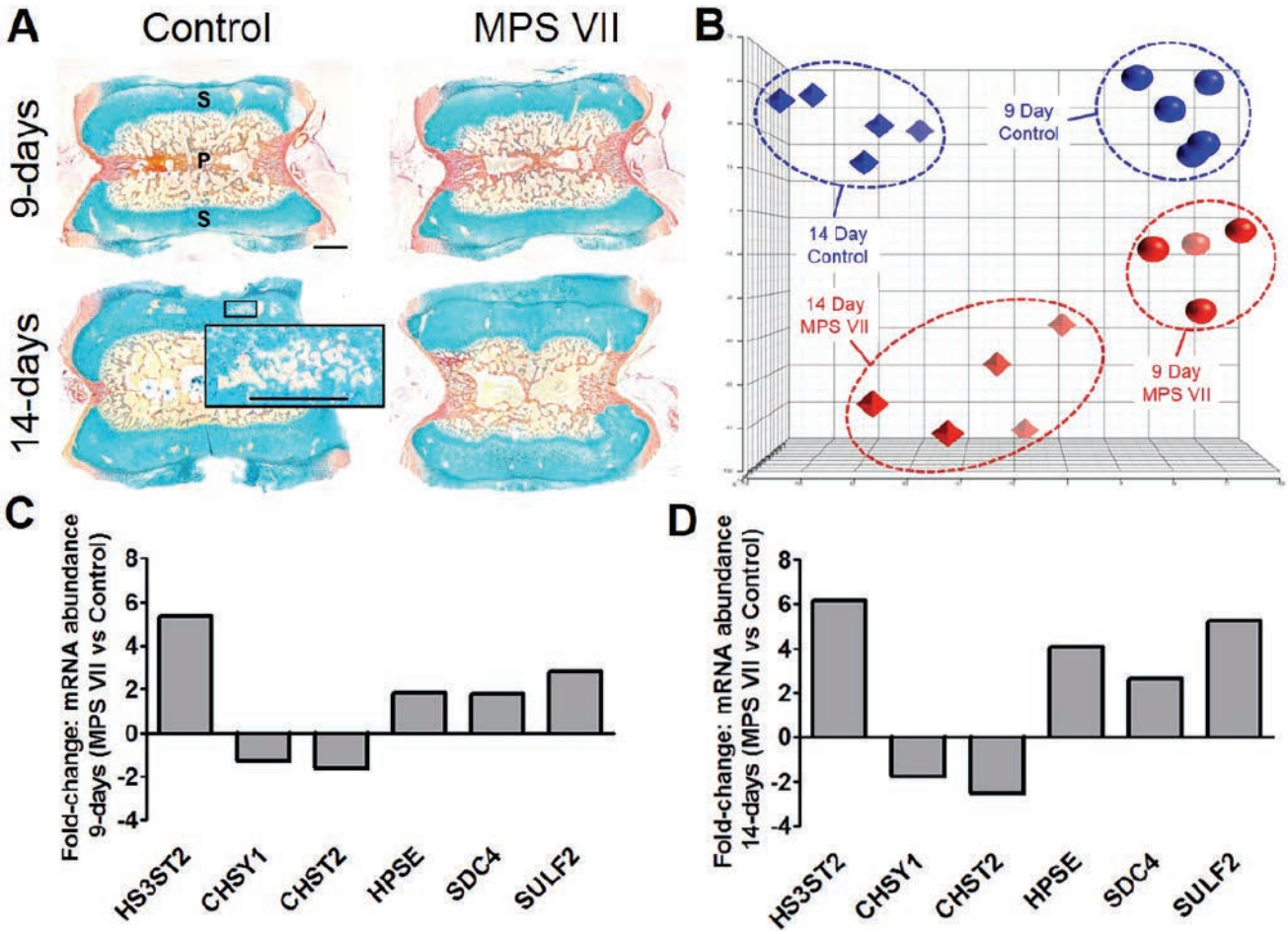

Figure 1. A. Representative mid-coronal ABPR-stained images of T11 vertebrae at 9 and 14 days-of-age. Inset: bone formation in secondary ossification centers in 14-day control animals. S: Secondary and P: Primary ossification center. B. RNA-Seq PCA plot. C. Fold-change of mRNA expression in MPS VII vertebral epiphyseal cartilage vs control at 9 days-of-age. D. Fold-change of mRNA expression in MPS VII vertebral epiphyseal cartilage vs control at 14 days-of-age. Scale = 1mm; n = 5; all $p < 0.05$.

of-age, there was significant differential mRNA abundance of key genes involved in GAG sulfation, and differences were even greater at 14 days-of-age (Figure 1C, D). GAG Content and Disaccharide Composition: Both intracellular and extracellular GAG content were significantly higher in MPS VII cartilage at 9 days (Figure 2A, B). There were also significant differences

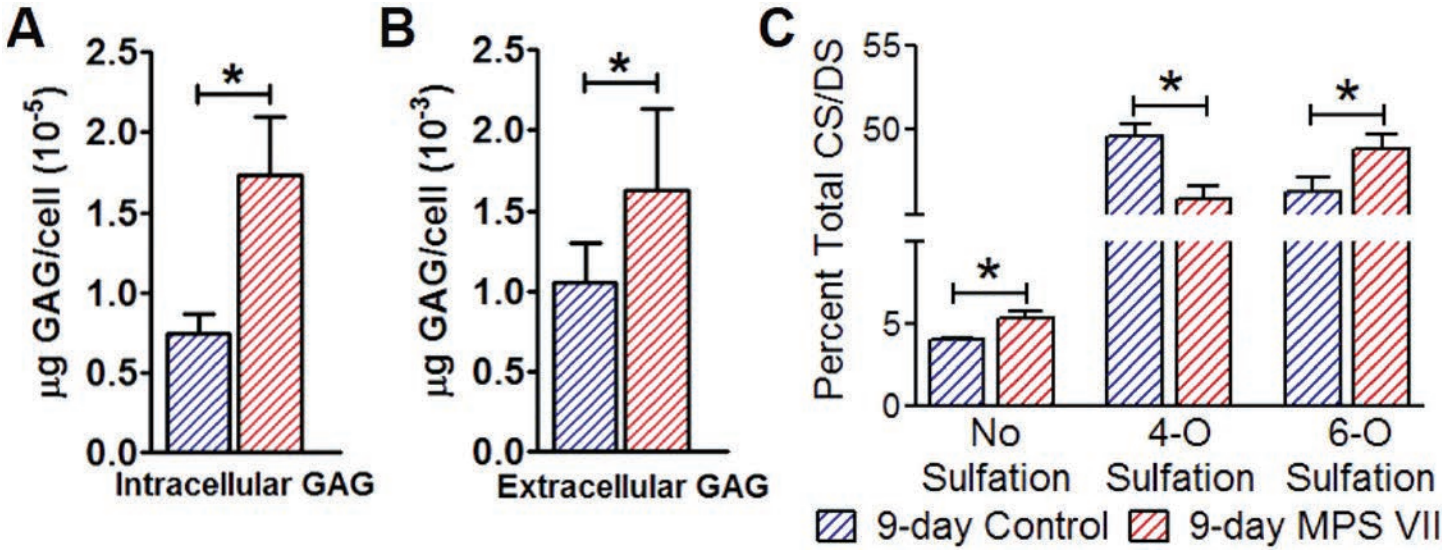

Figure 2. GAG analysis of 9-day vertebral epiphyseal cartilage. A. Intracellular GAG content. B. Extracellular GAG content. C. Extracellular GAG chondroitin and dermatan sulfate disaccharide composition. N = 3; *$p < 0.05$.

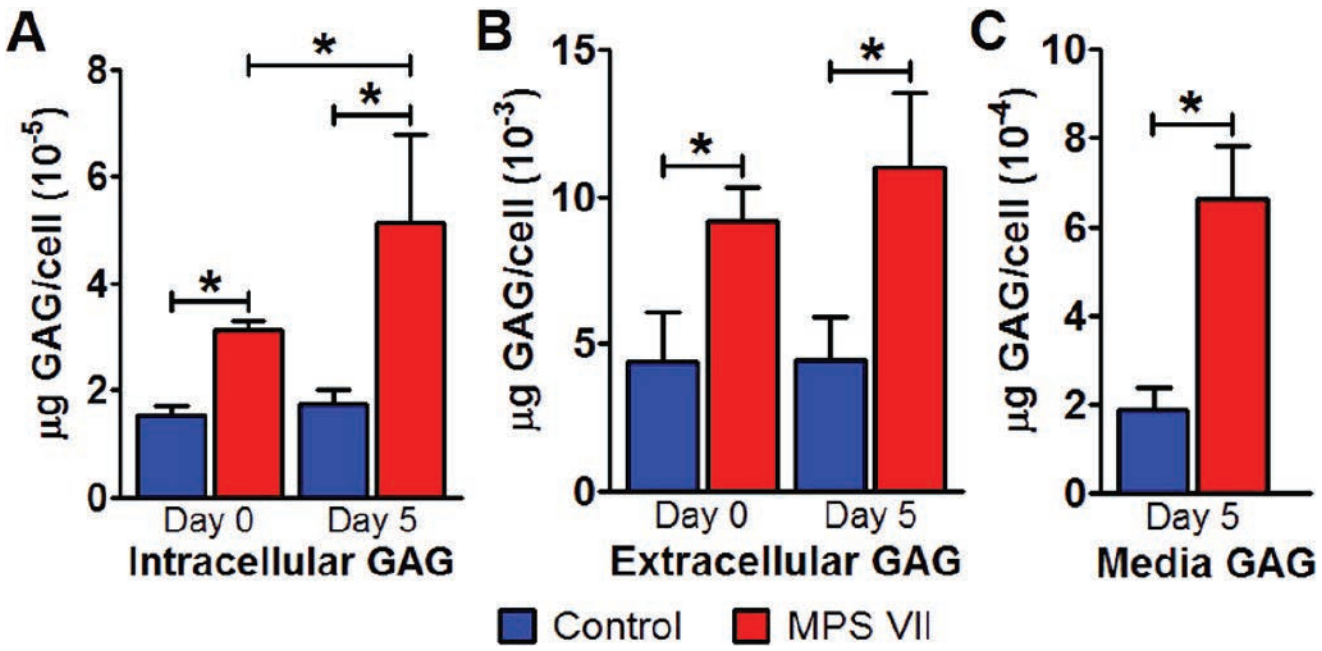

Figure 3. GAG content in epiphyseal cartilage explant model. A. Intracellular fraction. B. Extracellular fraction. C. Culture media, normalized to total cell count. Control (n=4), MPS VII (n = 2); *p < 0.05.

in CS/DS disaccharide composition between control and MPS VII vertebral epiphyseal extracellular GAGs, in both extent and position of sulfation (Figure 2C). Explant Culture: After 5 days in culture, MPS VII cartilage explants exhibited increased GAG content in both intracellular and extracellular fractions compared to controls, with intracellular GAG content significantly higher in MPS VII after 5 days of culture, while GAG content in controls remained stable over time (Figure 3A, B). Media GAG content after 5 days was also significantly higher for MPS VII compared to controls (Figure 3C).

Discussion

Results demonstrate that while tissue-level differences are not yet evident between control and MPS VII epiphyses at 9-days-age, molecular level abnormalities are present that may impact cell function and initiation of ossification. Differential expression of genes involved GAG sulfation indicates that there is broad dysregulation of GAG metabolic pathways in MPS VII that occurs secondary to the primary GUSB mutation. Elevated extracellular GAG content and abnormal GAG sulfation patterns in MPS VII cartilage may disrupt the signaling pathways that are necessary to initiate and sustain chondrocyte hypertrophic differentiation through altered growth factor binding and distribution. Elevated intracellular GAG content also likely contributes to cellular dysfunction by increasing cellular stress. Continued accumulation of GAGs in MPS VII explants over 5 days of in vitro culture suggest that resident cells remain metabolically active, validating this model for future mechanistic studies of abnormal GAG metabolism.

Significance

MPS VII is associated with severe skeletal disease for which there are no treatments. This study establishes the nature of aberrant GAG accumulation in MPS VII epiphyseal cartilage and identifies defects in GAG sulfation pathways, which likely contribute to cellular dysfunction and failed bone formation.

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