Progression of Vertebral Bone Disease in Mucopolysaccharidosis VII Dogs from Birth to Skeletal Maturity

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Introduction:

The mucopolysaccharidoses (MPS) are inherited lysosomal storage disorders characterized by mutations in hydrolases that degrade glycosaminoglycans (GAGs). GAGs accumulate in cells leading to multi-systemic clinical manifestations.[1] Many subtypes, including MPS VII (Sly Syndrome, beta-glucuronidase deficiency), exhibit severe skeletal abnormalities that are prevalent in the spine.[2-4] Previously, we showed the presence of cartilaginous lesions in MPS VII vertebrae that represent failed secondary ossification during postnatal development[4,5] and contribute to progressive spinal deformity.[6] To effectively target and optimize timing for therapeutic intervention for vertebral bone disease in MPS VII, it is critical to first elucidate temporal patterns of disease manifestation during postnatal development. Therefore, the objective of this study was to establish the nature, timing, and progression of vertebral bone disease in MPS VII from birth to skeletal maturity, using the naturally-occurring canine disease model.

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.[7] With IACUC approval, control and MPS VII dogs (n = 1-5) were euthanized at 9, 14, 30, 42, 90, 180, and 365 days, and lumbar and thoracic vertebrae were excised. Progression of vertebral bone formation of primary and secondary ossification centers were analyzed using μCT. Bone formation in vertebral secondary ossification centers was visualized through reconstructed images of the regions cranial and caudal to the growth plates. To quantify trabecular bone content in the primary ossification centers, standard 3D morphometric analyses were performed and bone volume fraction (BV/TV) and bone mineral density (BMD) were determined.[8] For non-invasive assessment of bone formation, serum was collected at 90, 180, and 365 days-of-age and bone-specific alkaline phosphatase (BAP) activity was measured using an ELISA kit. Significance for 9 and 14-day BV/TV and BMD measurements (n = 5 for each group) was established using 2-way analyses of variance and post-hoc Tukey's test ($p < 0.05$). Significance for 30 and 42-day BV/TV and BMD measurements (n = 2 for each group) were determined with unpaired t-test ($p < 0.05$).

Results:

Initiation of vertebral secondary ossification was markedly delayed in MPS VII animals compared to controls (Figure 1). While secondary ossification commenced by 14 days-of-age in controls, in MPS VII vertebrae, this did not occur until 30 days-of-age. Further, when it did commence, secondary ossification in MPS VII animals was highly irregular compared to the smooth, symmetrical phenotype in controls as seen from the axial images in Figure 1. Examining midsagittal cross-sectional images (Figure 2), bone formation in secondary ossification centers of the MPS VII animals was observed to cease progressing between 42 and 90 days-of-age. At 180 and 365 days-of-age, bone content in secondary ossification centers was greatly diminished compared to controls. Additionally, at 365 days, in control animals, growth plates were closed but remained open in MPS VII animals. Examining primary ossification centers, trabecular bone content was normal at early ages, but

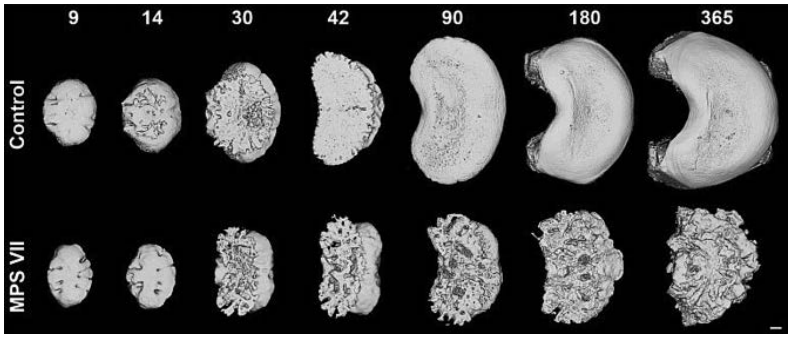

Figure 1. Representative axial μCT images showing delayed, incomplete, and non-uniform progression of secondary ossification in MPS VII vertebrae compared to controls. Numbers indicate postnatal days-of-age. Scale = 1mm.

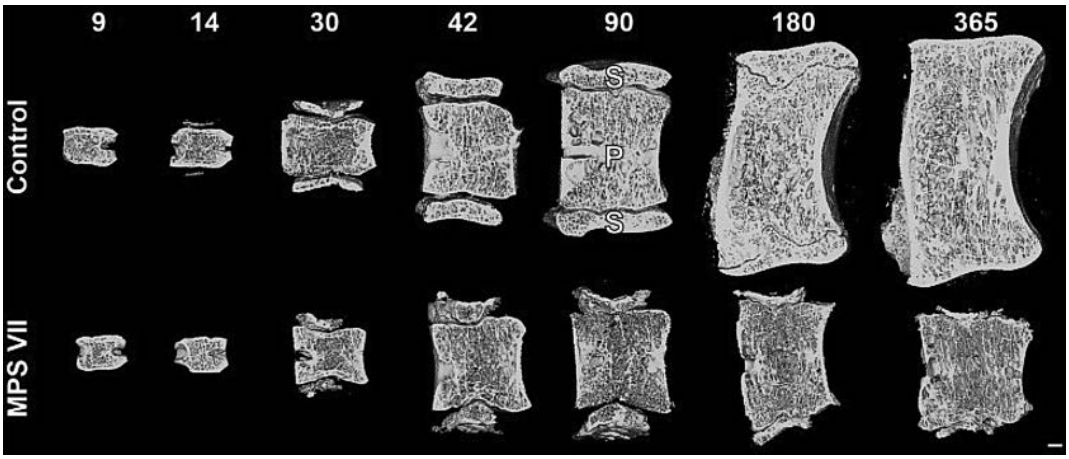

Figure 2. Representative midsagittal μCT images showing lower trabecular bone content in MPS VII vertebral primary ossification centers at older ages compared to controls. Numbers indicate postnatal days-of-age. Scale = 1mm. S: Secondary ossification center; P: Primary ossification center.

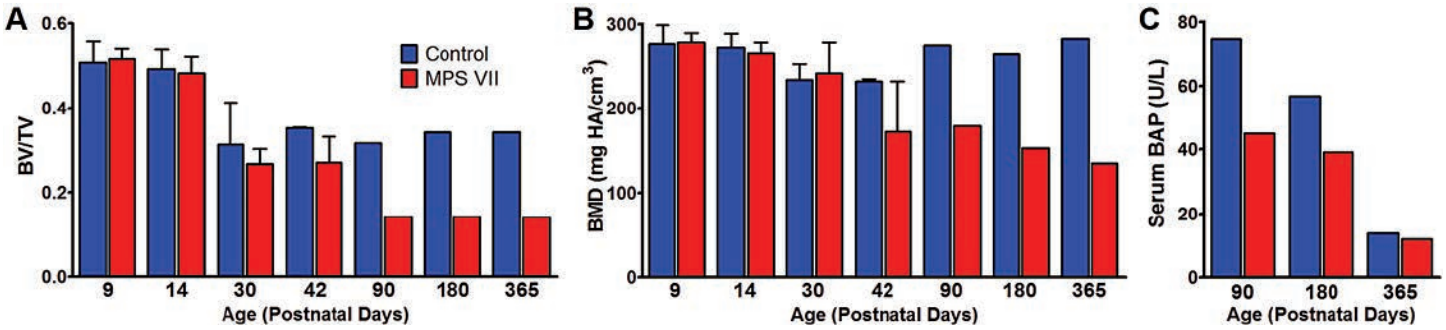

Figure 3. (A) Bone volume fraction (BV/TV) and (B) Bone mineral density (BMD) for control and MPS VII vertebral primary ossification center trabecular bone from birth to skeletal maturity. (C) Serum bone alkaline phosphatase (BAP) activity for control and MPS VII animals at older ages. 9 days (n = 5), 14 days (n = 5), 30 days (n = 2), 42 days (n = 2), 90 days (n = 1), 180 days (n = 1), and 365 days (n = 1).

beyond 30 days, MPS VII vertebrae exhibited lower BV/TV and BMD (Figure 2, Figures 3A and B). Finally, serum BAP levels were lower in MPS VII animals at 90 and 180 days (during skeletal growth), but not at 365 days (skeletal maturity) (Fig 3C).

Discussion:

This work establishes that vertebral bone disease in MPS VII manifests differently in primary and secondary ossification centers in a temporally-dependent manner, informing optimal targeting and timing for potential therapeutic interventions. Vertebral secondary ossification in MPS VII was found to be not only markedly delayed, but also highly non-uniform, suggesting that early developmental signals for bone formation are both impaired and spatially dysregulated. While primary ossification centers were not significantly affected at early ages, pathological changes (lower trabecular BV/TV and BMD) in MPS VII animals were evident at older ages. Lower serum BAP activity levels in MPS VII animals at 90 and 180 days may indicate reduced osteoblast activity during skeletal growth. Results also suggest that serum BAP may be a robust, non-invasive diagnostic tool for assessing bone disease progression in MPS patients.

Significance:

MPS VII is associated with severe skeletal disease for which there are currently no treatments. This study contributes to identification of optimal therapeutic windows for targeting bone disease in MPS VII and suggests a new diagnostic tool for assessing bone disease in MPS patients.

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