Impaired Chondrocyte Hypertrophic Differentiation is Associated with Failed Vertebral Bone Formation in Mucopolysaccharidosis VII Dogs

[1]Sun H. Peck
[1]Philip J.M. O'Donnell
[1]Eileen M. Shore
[2]Maurizio Pacifici
[1]Mark E. Haskins
[1]Neil R. Malhotra
[1]Lachlan J. Smith

[1]University of Pennsylvania, Philadelphia, PA, USA

[2]Children's Hospital of Philadelphia, Philadelphia, PA, USA

Introduction

Mucopolysaccharidosis (MPS) VII is associated with severe musculoskeletal manifestations, which are particularly prevalent in the spine. Vertebral dysplasia and accelerated intervertebral disc degeneration lead to kyphoscoliosis and spinal cord compression, directly impacting patient mortality and quality of life. A defining feature of spine disease in MPS VII is the presence of cartilaginous lesions in the vertebral bodies, indicative of failed cartilage-to-bone conversion during postnatal development; however, the underlying molecular mechanisms are poorly understood.[1,2] During normal vertebral bone formation, cartilaginous rudiments form a template where resident chondrocytes undergo distinct stages of differentiation (resting, proliferative, hypertrophic, terminal) regulated by a highly orchestrated pattern of growth factor signaling, culminating in ossification by osteoblasts. Glycosaminoglycans (GAGs) perform critical roles in regulating the activity of these growth factors. We hypothesize that abnormal GAG accumulation in MPS VII disrupts chondrocyte differentiation by interfering with growth factor signaling, thus preventing normal cartilage-to-bone conversion. Our objectives were to: 1) identify the earliest developmental age where altered bone formation is evident in MPS VII, and 2) establish the stage at which epiphyseal chondrocyte differentiation is impaired, using the naturally-occurring canine model.

Methods

We collected thoracic vertebrae from 9 day (n = 2) and 14 day old (n = 5) litter-matched MPS VII heterozygote and affected dogs for quantitative PCR and micro-computed tomography (microCT) analyses. This age range was selected based on previous radiographic, longitudinal studies of vertebral bone formation in MPS VII dogs.[2]

Results

Comparison of mRNA expression of chondrocyte differentiation (SOX9, RUNX2, COL10A1) and osteoblast (ALPL, BGLAP) markers, and microCT visualization of vertebral bodies of heterozygote and MPS VII affected dogs showed striking differences in bone formation (Figure 1) at 14 days whereas at 9 days, no significant differences besides a trend towards lower RUNX2 expression were detected (Figure 2). Interestingly, SOX9 expression was downregulated at 14 days for both heterozygote and affected dogs suggesting that both chondrocyte populations receive regulatory signals for proliferation but that MPS VII chondrocytes fail to progress into hypertrophy. Furthermore, bone volume fraction and bone mineral density quantification of the vertebral bone primary ossification centers showed no differences between heterozygote and affected dogs suggesting that at this time point, the most affected developmental pathways involve activation of secondary ossification centers. Since the epiphyses at these early ages are composed of cartilage and therefore high in GAG content, aberrant GAG accumulation in MPS VII is likely playing a role in failed ossification. Indian Hedgehog signaling plays a crucial role in regulating chondrocyte differentiation and is regulated in a highly GAG-dependent manner.[3,4,5]

Discussion

Our preliminary data showed differences in Hedgehog pathway mRNA expression levels between heterozygote and affected animals at both 9 and 14 days implicating this pathway in disease etiology. The results of this study lay the foundation for future mechanistic investigations into bone disease in all MPS subtypes.

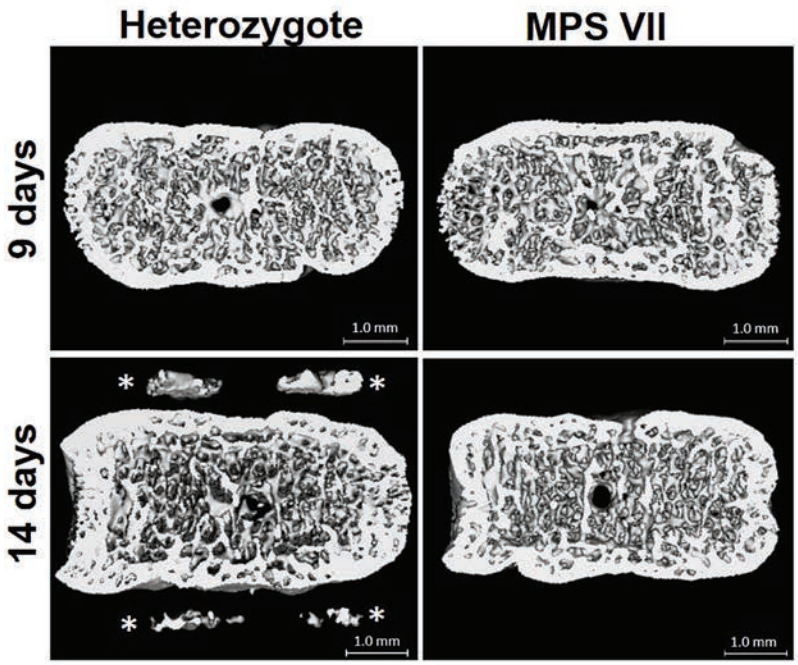

Figure 1.

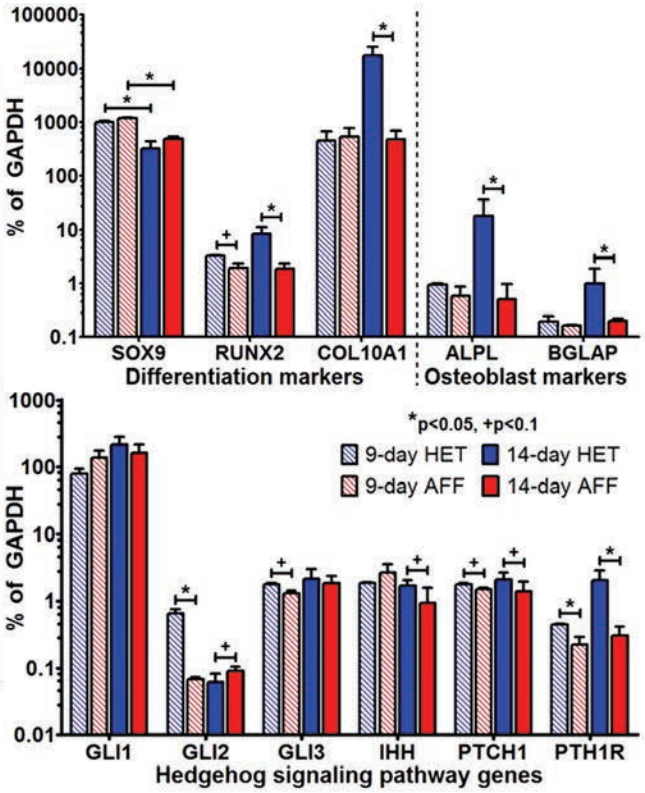

Figure 2.

Acknowledgements

Funding: NIH, National MPS Society, Penn Center for Musculoskeletal Disorders

References

1. Smith LJ, et al. Altered lumbar spine structure, biochemistry, and biomechanical properties in a canine model of mucopolysaccharidosis type VII. *J. Orthop. Res.* 28: 616-622 (2010).

2. Smith LJ, et al. Pathogenesis of lumbar spine disease in mucopolysaccharidosis VII. *Mol. Genet. Metab.* 107: 153-160 (2012).

3. Kronenberg HM. Developmental regulation of the growth plate. *Nature,* 423: 332-336 (2003).

4. Lin X. Functions of heparan sulfate proteoglycans in cell signaling during development. *Dev. Camb. Engl.* 131: 6009-6021 (2004).

5. Cortes M et al. Sulfation of chondroitin sulfate proteoglycans is necessary for proper Indian hedgehog signaling in the developing growth plate. *Dev. Camb. Engl.* 136: 1697-1706 (2009).