Lumbar Spine Segment Mechanical Properties, Composition and Gene Expression in Mucopolysaccharidosis VII Dogs Following Neonatal Gene Therapy

Methods
All experiments were performed with institutional IACUC approval. Three MPS VII-affected dogs were injected intravenously at 3 days of age with the hAAT-cGUSB-WPRE RV vector. Animals were euthanized at 7 months of age. For RNA analysis, tissue samples were obtained from the AF of the T12-L1 spine level with 1 hour of sacrifice and frozen on dry ice. Additional samples for RNA analysis were obtained from 4 untreated MPS VII and 4 normal (unaffected) dogs of similar age. Samples were homogenized in Trizol, and RNA isolated for gene expression analysis using real time rt-PCR. Target genes included cathepsins D and K, and MMPs 2 and 12. Gene expression in MPS VII dogs (treated and untreated) were plotted as percent normal values after comparison to β-actin levels, with inter-group comparisons made using unpaired t-tests (p<0.05, trend<0.10). Remaining lumbar spines of the 3 treated animals were divided into bone-disc-bone segments. Motion segment mechanical properties (compression and neutral zone stiffness, range of motion and creep displacement) were determined for the L1-2 spine level [2]. Samples for GAG and water content determination were obtained from the AF of the L5-6 inner and outer AF, and for GAG, water and calcium content determination from the anterior L6 vertebral epiphysis [2]. Motion segment mechanical properties and biochemical composition for the treated animals were compared to those of normal (n=4), and untreated MPS VII (n=4) dogs of a similar age, reported previously [2], using unpaired t-tests (p<0.05). L2-3 segments were processed for paraffin histology: mid-saggital sections were stained with alcin blue and picrosirius red for structural evaluation [2].

Results
At sacrifice, RV treated animals had 271±202% (mean±SD) of normal GUSB activity in serum. With respect to motion segment mechanical properties (Fig 1), compressive stiffness was not significantly different from either untreated MPS VII or normal. Neutral zone stiffness for treated animals showed a significant improvement (41% of normal compared with 19% of normal for untreated MPS VII). Both range of motion and creep displacement for treated animals exhibited small, though not significant improvements relative to untreated MPS VII animals, but remained significantly greater than normal.

With respect to biochemical composition (Fig 2), GAG content in the outer AF and the epiphysis was not significantly improved in treated samples relative to untreated samples, remaining elevated relative to normal. Water content for treated animals showed a significant decrease in the inner AF (89% of normal, compared with 108% in untreated MPS VII), and in the outer AF (104% of normal compared 124% of normal for untreated MPS VII). In the epiphysis there was no significant improvement in water content for treated animals. Calcium in the epiphysis remained significantly low (44% of normal), and unchanged compared with untreated MPS VII.

Discussion
Our results demonstrate that while neonatal gene therapy does not prevent the progression of spine pathology in MPS VII dogs, it does result in an improvement in mechanical function. This may be attributable to the measured decrease in intervertebral disc hydration relative to untreated samples, as water content plays a crucial role in determining viscoelastic mechanical properties. Interestingly, this decrease was not accompanied by a concomitant decrease in GAG, suggesting that the accumulated GAG may have reduced functional capacity. The inflammatory proteases shown to be upregulated in the AF of untreated MPS VII dogs have broad specificity for breaking down cartilage matrix proteins, including collagen, elastin and proteoglycans. Our results suggest that gene therapy may effectively lower the activity of cathepsin D. Inhibitors of this and other enzymes might represent an alternative approach to treating spine disease in MPS VII. In terms of composition, results suggest that gene therapy may not effectively improve matrix development in the vertebral epiphysis, although this will be examined more rigorously as part of ongoing investigations.

Acknowledgements Funding was received from the NH (DK54481, RR02512) and the Penn Center for Musculoskeletal Disorders.

References

Poster No. 1478 • 56th Annual Meeting of the Orthopaedic Research Society