INTRODUCTION:
Mucopolysaccharidosis VI (MPS VI, Maroteaux-Lamy syndrome) is an autosomal recessive lysosomal storage disorder with cellular accumulation of incomplete dermatan sulfate (DS) glycosaminoglycans (GAGs), originated from the deficiency of galactosamine-4-sulfate sulfatase (aryl sulfatase B) [1]. DS is important for the production of small leucine-rich repeat proteoglycans (e.g. decorin and biglycan), which are useful for the interaction with collagen fibrils to maintain the normal function of the intervertebral disc and articular cartilage. Spinal deformities (including scoliosis and kyphosis) are usually observed in patients with MPS VI disorder. Although the exact cause of spinal deformities has not been clearly determined, adverse changes were demonstrated resulting from the excessive accumulation of DS in MPS cartilage, include up-regulation of apoptotic chondrocytes, pro-inflammatory cytokines (tumor necrosis factor-a), nitric oxide, transforming growth factor-β as well as matrix metalloproteinase-2 and -9 [1-3]. The objective of this study was to investigate the structural, compositional and biomechanical alterations of lumbar spine in a naturally occurring MPS VI rat model, to determine the association with the development of spinal disorders. It is hypothesized that the MPS VI disorder significantly affects the structure, composition and function of the lumbar spine with alternations that are similar to those observed with disc degeneration.

METHODS:
Healthy (n=6) and MPS VI affected (n=6) rats were raised at the Mount Sinai School of Medicine under National Institute of Health and USDA guidelines for the care and use of animals in research. The animals were euthanized at 6-months of age using carbon dioxide inhalation. The lumbar spines of the animals were harvested for biomechanical, histological and biochemical analyses. For biomechanical analysis, the L3-6 motion segments were isolated and potted in stainless tubes using a custom-made tool to insure motion segments were accurately aligned and centered in the pots. Potted motion segments were tested in PBS using an Enduratec ELF 3200 (Bose Corporation) and custom grips with a 3 force-controlled loading stages: A) equilibration (-1.875N for 30 minutes), B) cyclic tension-compression (20 sinusoidal cycles from -6.25N to +6.25N), and C) creep (-6.25N for 60 minutes). The cyclic and creep displacement were fitted to a double sigmoid function [4] and stretched exponential function [5], respectively, for data analysis. The L3-4 motion segments were used for histological analysis. After fixation and processing, the specimens were embedded in methyl methacrylate, sectioned at 5µm, and a mid-sagittal section was used for Safranin O/fast-green/ hematoxylin staining. For biochemical analysis, nucleus pulposus (NP) and annulus fibrosus (AF) were isolated from L2-3 disc, weighed, lyophilized and digested in proteinase-k. GAG content was determined using dimethylmethylen blue assay and the results were normalized using dry weights. All results from the normal and MPS spine were compared using Student’s t-test with level of statistical significance set at p<0.05.

RESULTS:
Compared to normal spinal segment, the cyclic test showed that the MPS motion segment had significantly decreased compressive stiffness (41.6%), neutral zone stiffness (447.9%) and tensile stiffness (110.0%) as well as increased neutral zone length (54.3%) and total range of motion (58.7%). Creep time constant was significantly smaller for MPS VI samples (82.8%). Besides, the total displacement loss was significantly greater for the MPS VI samples (26.0%) (Figure 1). Histologically, MPS discs had abundant enlarged vacuolated nuclear and annular cells as well as thickened annulus layers, focal defects in the NP and increased disc height as compared to control discs (Figure 2). AF water content was significantly higher in MPS discs than controls (63.8±4 vs 56±4%, respectively). However, there were no significant differences in NP water content (69±11% vs 66±12%) and GAG content (NP: 338±199 vs 340±177 ug GAG/mg dry weight; AF: 90±15 vs 98±34 ug GAG/mg dry weight) for MPS vs normal discs (P>0.05).

DISCUSSION:
MPS VI animals demonstrated substantial alterations in disc structure, motion segment biomechanics, and composition, which may be associated with the development and progression of spinal pathology observed in this patient population. The histological results revealed important structural alterations in the annular layers that are likely associated with the increased annular water content, range of motion and the substantial loss of tensile stiffness. The sizeable defects in the NP may be associated with reduced compressive stiffness as well as neutral zone modifications. The reduced creep time also supports alterations in water retention capacity of MPS VI animals which may likely be associated with diminished functional GAGs, but could also be associated with loss of collagen integrity or altered endplate permeability [6]. Endplate and bone defects may contribute to the observed alterations in motion segment biomechanics, so that future investigations will target vertebral biomechanics to help distinguish bone and disc contributions. Size and geometric effects might contribute to the observed biomechanical alterations and will also be further studied. The MPS VI disorder affects metabolism, inflammation, and structural integrity of intervertebral discs and other organ systems [1-3]. Further investigations of MPS VI animals will focus on alterations in disc metabolism as well as accumulation of pro-inflammatory cytokines and matrix degrading enzymes to provide a mechanism for the alterations in collagen structure and biomechanical behaviors.

SIGNIFICANCE:
The investigation of this animal model system is of direct relevance to spinal dysfunction observed in MPS patients. This model may also be more generally applied to provide insight into how diminished GAG leads to dysfunction in the spinal system.

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