Relevance to clinical disorders: The most common reason for back pain is disc degeneration. Because the pathophysiology of disc degeneration is largely unknown the possibilities to heal spinal disorders are very limited. Genetic factors are believed to be one of the main etiologic factors of spinal disorders. One example of a hereditary syndrome that affects the locomotor system is the Stickler syndrome, which is an autosomally inherited disorder that affects the eyes, ears and the skeleton. The COL2A1 gene has been linked with the syndrome probably in more than half of the families analyzed (1, 2).

The aim of this study was to find out the significance of the lack of one allele of collagen type II gene on the structure and development of the spine.

Introduction: The major macromolecules of the intervertebral discs and articular cartilage are collagen and proteoglycans (PGs). Collagen network provides the tissue tensile strength, PGs have shock absorbing properties. The structure and concentration of these molecules change by age and degeneration. Specific defects in collagen genes lead to early osteoarthritis of joints. However the properties of spine and its reaction to physical activity in animal models with a collagen gene defect are seldom reported. The objective of this study was to determine if a targeted inactivation of one allele of the Col2a1 gene for collagen II (3) has an effect on the composition and morphology of the extracellular matrix of intervertebral discs and vertebral bone and if a long-term voluntary exercise prevents or predisposes to these changes.

Materials: The study material consisted of C57Bl male mice with inactivation of one allele of Col2a1 gene (n=115) and normal control mice (n=94). The ages of the animals were one month (n=61), 10 months (n=84) and 14 months (n=64). Half of the mice had a running wheel in their cages. At the age of two months the mean daily running distance of both mice groups was about 4 km.

Methods: The lengths and curvatures of the spines were measured from X-ray images. The PG concentration of the annulus fibrosus and nucleus pulposus of the intervertebral disc and the vertebral end-plates as well as the vertebral bodies was determined from optical density of the Safranin-O stained histological sections. Collagen orientation and morphology of the intervertebral discs were determined with quantitative polarization microscopy and light microscopy, respectively.

Results: At the age of seven months mean daily running distance of the control mice was 3.2 km/day and that of the mice with gene defect 1.8 km/day (p<0.001). The spines of the one-month-old mice with the targeted inactivation of the Col2a1 gene for collagen II were 4.1 % (p<0.05) shorter than in the controls. There was no difference in the curvatures of the spine between the groups. The irregularity of the end-plate was greater in the animals with the gene defect as compared with the controls at the age of one and ten months (p<0.01). The extent of ossification in the end-plate was significantly greater in the mice with the gene defect in the group of one-month-old (p<0.001) and ten-month-old mice (p<0.01) but not in the group of 14 months old mice. The one-month-old mice with gene defect had a significantly lower concentration of PGs than the control animals in the annulus fibrosus of the intervertebral discs (p<0.01), growth plates (p<0.01) and in the vertebral bodies (p<0.001) (Figure 1). However, this difference was not seen at the age of 10 or 14 months. In the nucleus pulposus concentration of PGs was lower in the mice with the gene defect only in ten-month-old mice. Exercise had no effect on the concentration of PGs in the spinal tissues. Furthermore there was no difference between the groups in the collagen orientation in the discs or trabecular bone of vertebral bodies.

Discussion: The inactivation of one allele of the Col2a1 gene had a significant effect on the matrix composition and morphology of the osteocartilaginous tissues of the spine in one-month-old mice. PG concentration was lower in the spinal tissues, a feature that compromises the shock absorbing properties of the tissues. The development of the spine was initially delayed resulting in abnormal morphology and histology of the spines of the affected mice up to the age of 10 months. Our hypothesis is that the production rate of the type II collagen in the spinal tissues of the gene defected mice was reduced and gradually with time the morphologic and histologic disturbances were compensated. This study indicates that normal production rate of type II collagen is essential for normal development and maturation of the spine.

References:

**Center for Gene Therapy, School of Medicine, MCP Hahnemann University, Philadelphia, USA.