

The effect of forced running exercise on the biomechanical and biochemical properties of the knee joint articular cartilage in Col9a1 deficient mice

M. Weyers¹, D. Mählich¹, C. Lorenz¹, M. Dreiner¹, T. Li², L. Han², B. Brachvogel^{3,4}, F. Zaucke⁵, A. Niehoff^{1,6}

¹Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Cologne, Germany; ²School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, United States; ³Department of Pediatrics and Adolescent Medicine, Experimental Neonatology, Medical Faculty, University of Cologne, Cologne, Germany; ⁴Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany; ⁵Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, Department of Orthopedics (Friedrichsheim), University Hospital Frankfurt, Goethe University, Frankfurt am Main, Germany; ⁶Cologne Center for Musculoskeletal Biomechanics, Medical Faculty, University of Cologne, Cologne, Germany; m.weyers@dshs-koeln.de

Disclosures: Miriam Weyers (N), Daniela Mählich (N), Charlotte Lorenz (N), Maren Dreiner (N), Thomas Li (N), Lin Han (N), Frank Zaucke (N), Anja Niehoff (N)

INTRODUCTION: Mechanical loading is a key factor for proper assembly and maintenance of the cartilage extracellular matrix (ECM). Collagen type IX (Col9) is a FACIT (fibril-associated with interrupted triple helices) collagen consisting of the three gene products alpha 1 (IX), alpha 2 (IX) and alpha 3 (IX). It is arranged on the surface of cartilage fibrils made of collagen type II and type XI [1]. By cross-linking with collagen fibrils, Col9 influences not only the fibril diameter but also the stability of the fibrils, whereby type IX molecules are also involved in the interaction of the fibrils with each other or with other ECM proteins [2-4]. Furthermore, it could be shown that the expression of Col9 is mechanosensitive [5]. Mutations in Col9 result in multiple epiphyseal dysplasia (MED), while Col9a1 deficient mice (Col9a1^{-/-}) have an abnormal growth plate structure, significantly wider bones in newborns and an increased incidence of osteoarthritis (OA) in older animals [6, 7]. The aim of this study was to analyze the effects of forced running exercise on the articular knee joint cartilage in Col9a1^{-/-} mice.

METHODS: Twelve weeks old female wildtype C57BL/6N (WT) and Col9a1^{-/-} mice [6] were randomly assigned into a control (CON) and forced running exercise (EXE) group (n = 10 per group and genotype). The exercise groups were trained for 6 weeks on a motorized treadmill (20% incline, 20m/min) for 40 min/day five times per week. The body mass of the mice was monitored weekly. The mice were sacrificed by cervical dislocation with an age of 18 weeks and the left hindlimbs were dissected. Bone structural parameters were measured using μ CT and mechanical properties of articular cartilage were analyzed by AFM-nanoindentation using a microspherical tip [8]. A modified OARSI score was used in a double-blinded manner to assess cartilage degeneration [9, 10]. Immunohistochemical staining and Western Blot using specific antibodies against cartilage oligomeric matrix protein (COMP), collagen II, matrix metalloproteinase 13 (MMP-13) and matrilin-1 to -4 was performed. All experimental protocols were carried out in accordance with the guidelines of the German animal protection law and were approved by a licensing committee (Institutional review Board: "Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein Westfalen", #81-02.04.2018.A272). For statistical analysis, the unpaired non-parametric Mann-Whitney U test was performed using GraphPad Prism 10 (GraphPad Software, Inc.). Data are presented as mean \pm SD and the level of significance was set at a value of $p < 0.05$.

RESULTS SECTION: Over the whole intervention, both Col9a1^{-/-} groups had a significantly lower body mass compared to the WT groups. Bone mineral density (BMD) was significantly ($p < 0.01$) reduced in both Col9a1^{-/-} groups but none of the studied groups showed cartilage degeneration in the lateral tibia compartment. Preliminary AFM results indicated an increase of the elastic indentation modulus in the Col9a1^{-/-} EXE group while the staining intensity of MMP-13 was significantly ($p < 0.05$) increased in the lateral tibial cartilage from the Col9a1^{-/-} CON mice compared to the WT CON mice. In addition, a significantly ($p < 0.05$) reduced COMP staining intensity and protein amount was detected in both Col9a1^{-/-} groups. Interestingly, the running exercise resulted in a reduction of the COMP staining intensity in the upper ($p < 0.001$) and deep ($p < 0.05$) zone of the lateral tibia cartilage of Col9a1^{-/-} EXE mice compared to the Col9a1^{-/-} CON mice. The staining intensity of matrilin 1, 3 and 4 showed a Col9a1-dependent reduction in the lateral tibia cartilage and the tibial growth plate, while only the protein expression of matrilin 1 seems to be significantly ($p < 0.001$) reduced in the Col9a1^{-/-} groups. The collagen II staining intensity and the protein amount was neither affected by the running exercise nor by the deficiency of Col9.

DISCUSSION: In 18-week-old female mice, running exercise seemed to have effects on the biomechanical and biochemical properties of the tibial cartilage of WT and Col9a1^{-/-} mice. The increased cartilage stiffness of Col9a1^{-/-} EXE mice suggests that the running exercise had a positive effect on the lateral tibial cartilage. On the other hand, the reduced presence of COMP in the lateral tibial cartilage of Col9a1^{-/-} EXE mice suggests an adaptation of the cartilage to running exercise by redistribution certain ECM components, such as COMP. These results show that further research is needed to determine the final effect of moderate loading on articular cartilage. Furthermore, the reduced levels of COMP and matrilins in Col9a1 deficient mice confirm Col9 as an important interaction partner in vivo that is needed to properly anchor these proteins in the cartilage ECM. In contrast, the running exercise and the deficiency of Col9 had no effect on the localization and intensity of the collagen II staining. Altered local catabolic activity was indicated by increased levels of MMP-13 in the Col9a1^{-/-} CON mice, which might impair cartilage integrity at later time points. These results suggest that the deficiency of Col9 led to structural changes in the ECM. Moreover, the reduced BMD in Col9a1^{-/-} mice indicates an effect of Col9 on bone homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE: Col9 plays an important role in skeletal development and its absence leads to premature OA. At the molecular level, Col9 is important for ECM homeostasis and its mechanosensitive expression makes it an interesting target for exercise-based therapies.

REFERENCES: [1] Wu JJ et al. J. Biol. Chem. 267: 23007-14, 1992. [2] Blaschke UK et al. J. Biol. Chem. 275: 10370-78, 2000. [3] Hagg R et al. J. Cell. Biol. 142: 285-294, 1998. [4] Shaw LM and Olsen BR Trends Biochem. Sci. 16: 191-194, 1991. [5] Ng KW et al. Cell. Mol. Bioeng. 2: 386-94, 2009. [6] Fässler R et al. Proc. Natl. Acad. Sci. U. S. A. 91: 5070-4, 1994. [7] Blumbach K et al. Matrix Biol. 27: 306-318, 2008. [8] Doyran, B et al. Osteoarthritis Cartilage 25: 108-117, 2017. [9] Glasson SS et al. Osteoarthritis Cartilage 3: S17-23, 2010. [10] Wang Q et al. Nat. Med. 17: 1674-1679, 2011

ACKNOWLEDGEMENTS: This project was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, project ID NI 1083/3-1 to AN).