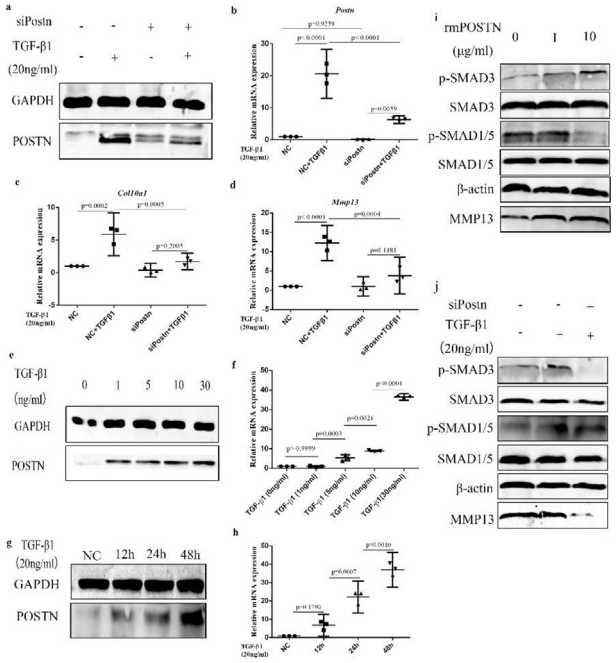

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3D OSTEOCYTE MORPHOLOGY AND VOLUME OF CHONDROCYTE CLUSTERS ARE MODULATED WITH OSTEOARTHRITIS SEVERITY

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Purpose: Osteoarthritis (OA) modulates bone remodeling by accelerating it in early phases followed by slower remodeling leading to subchondral bone sclerosis. During bone formation, osteoblasts are trapped in unmineralized osteoid and further differentiate to mature osteocytes. Osteocytes are known to adapt their shape and volume according to loading, age and matrix quality. The current knowledge about osteocyte morphology in OA is controversial, and the published studies are mainly based on 2D techniques or small sample volumes. Synchrotron micro-computed tomography (SRμCT) can be regarded as the gold standard for osteocyte imaging due to its capability to spatially resolve individual osteocytes in 3D. In this study, we utilized SRμCT to quantify osteocyte morphology and chondrocyte cluster volume in subchondral bone and calcified cartilage, respectively, at different histopathological OA grades. We hypothesized that volume and density of osteocytes and chondrocyte clusters increase along with OA severity.
Methods: 24 osteochondral cores with diameter of 4mm were collected from seven total knee replacement patients (approval no 78/2013, Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland) and two cadavers (approval no 134/2015 The Research Ethics Committee of the Northern Savo Hospital District, Kuopio, Finland). Osteochondral samples were imaged without any external contrast agent with SRμCT at the Canadian Light Source (Saskatoon, SK, Canada) on the BMIT-ID (05ID-2) beamline (parameters: 31 keV, 0.9 μm pixel size, and 720 projections from 180°). A distortion correction was applied to the projection images and cross-sectional images were reconstructed in NRecon software (v. 1.6.9, Bruker microCT, Kontich Belgium). Tissue mineral density and morphology of cell lacunae were analyzed inside the bone mask, from which bone marrow and open subchondral plate cavities were excluded. Lacunae were divided into osteocytes (500-5000 voxels) and chondrocyte clusters (over 5000 voxels) according to size. Individual object analysis was performed with the CTAn software (v.1.17.7.2, Brüker microCT, Kontich, Belgium). After SRμCT imaging, samples were subjected to conventional histology and OARSI histopathological grades were assessed from safranin O stained sections. Relationships between OARSI grade and both tissue mineral density and parameters of cellular morphology were studied by calculating Spearman's rho correlations.
Results: Tissue mineral density, osteocyte lacuna, volume ($p < 0.05$), thickness, structure model index and sphericity decreased significantly ($p < 0.01$) with increasing OARSI grade. Cellularity, major diameter of osteocyte lacuna, and osteocyte lacuna surface increased significantly ($p < 0.01$) with OA severity (Figure 1). Chondrocyte clusters in calcified cartilage increased already at OARSI grade 1.5 and their volume was highest at OARSI grade 3, after which their amount decreased.

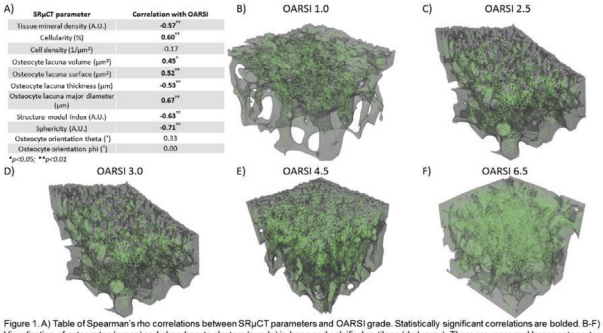

Figure 1. A) Table of Spearman's rho correlations between SRμCT parameters and OARSI grade. Statistically significant correlations are bolded. B-F) Visualization of osteocytes (green) and chondrocyte clusters (purple) in bone and calcified cartilage (dark grey). There are more and larger osteocytes with increasing OARSI grade. Chondrocyte clusters are detectable in moderate and advanced OA but not in healthy and late stage OA.

Conclusions: OA leads to larger, thinner and more elongated osteocytes in lower density bone tissue. This is most likely due to the presence of less mature bone tissue, associated with increased bone remodeling. A total volume of chondrocyte clusters appears to increase in early OA and decrease towards higher OARSI grades. This suggests that there are more hypertrophic chondrocytes in calcified cartilage in early and moderate OA and they disappear when cartilage becomes eroded.

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PROGRAMMED CELL DEATH AND TOLL-LIKE RECEPTOR ACTIVATION IN ARTICULAR CARTILAGE

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Purpose: Necroptosis and apoptosis have been suggested as possible modes of cell death in articular cartilage contributing to pathological changes in osteoarthritis. The release of cytosolic and nuclear factors during necroptotic cell death includes damage associated molecular patterns such as nucleic acids. Acting as ligand to Toll-like recpeptor-3 and -9 we hypothesize that programmed cell death causes TLR activation in articular cartilage.
Methods: dsDNA release from fractured murine hip caps was investigated using Pico green assay. C28/I2 chondrocytes were incubated with staurosporine to chemically induce programmed cell death and dsDNA release was also quantified using PicoGreen assay. FITC labelled CpG ssDNA and rhodamine labelled Poly-IC dsRNA was used to visualize nucleic acid uptake in primary murine chondrocytes and cartilage. Furthermore, human and murine cartilage explants were analysed for nucleotide binding TLRs using RT-PCR. Cell death was detected using the TUNEL assay on sections of human and murine trauma. Immunohistochemistry of human trauma and OA cartilage on the paraffin sections were performed for TLR-3 and -9. Only samples of recent human cartilage trauma (max. 2 weeks) were included. Furthermore, we performed qPCR, Western Blot and NFkB-Reporter-Luciferase-Assay using C28/I2 chondrocytes incubated with isolated ds DNA to detect TLR3 and -9 expression or NFkB activation. MMP3 expression upon dsDNA stimulation was analysed using an ELISA assay. Purity of isolated dsDNA for stimulation of cells was analysed using agarose gel and BCA assay.
Results: Trauma induced dsDNA release from murine hip caps. Staurosporine induced nucleic acid release dose dependently in C28/I2 chondrocytes. Semiquantitative RT-PCR revealed expression of nucleic acid binding TLR-3 and -9. Intracellular signal of FITC labelled CpG ssDNA and rhodamine labelled Poly-IC dsRNA showed in chondrocytes and tissue nucleic acid uptake. Trauma cartilage showed significantly more TLR-3 and-9 positive cells compared to healthy controls. This increase in TLR3 and -9 expression correlated with increasing cell death. There was a dose dependent up-regulation of TLR-3 and -9 after dsDNS

stimulation on mRNA level and of TLR-3 on protein level. Furthermore, dsDNA stimulation causes an increase in NFkB activity, ERK phosphorylation and MMP3 expression.
Conclusions: Programmed cell death after joint trauma can cause the release of damage associated molecular patterns such as nucleic acids. Toll-like receptor activation might be a possible pathway for inflammatory changes and development of posttraumatic osteoarthritis.

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STRAIN AND MODEL SPECIFIC DIFFERENCES IN MOUSE MODELS OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) has been proposed as a set of heterogeneous pathologies with a common endpoint; multi-factorial in it's etiology, and composed of multiple phenotypes. Many rodent studies of OA employ surgical models to induce a post-traumatic disease phenotype. However, post-traumatic OA only affects a subset of patients, and these results may not be generalized to other types of OA. Data from our laboratory and others have indicated genetic manipulation in the same tissue of interest using different models of OA (such as post-traumatic vs age associated) can have dramatically different effects. Additionally, groups may use different strains of mice to model OA yet the natural disease progression in some strains has not been characterized. CD1 mice are commonly used to test interventions, and for ex-vivo studies but little is known about their susceptibility to OA. Our first objective was to compare spontaneous age-associated OA in CD1 and C57/B6 mice in order to examine whether there are strain specific differences in OA pathology.
Our laboratory has previously shown that cartilage-specific inactivation of the nuclear receptor PPARdelta results in significantly decreased severity of OA in a mouse model of post-traumatic OA. Our second objective was to assess whether the protective effect of cartilage-specific PPARdelta inactivation was replicated in an aging model of OA (C57/B6 background).
Methods: CD1, C57/B6, and cartilage-specific PPARdelta knockout mice were aged to 6, 12 months, 20 months, or 24 months and then subjected to gait analysis. Knees, elbows, and ankles were harvested for histology. Mice were compared through classical measures of OA progression including Toluidine-blue staining with OARSI scoring of the cartilage, bone, meniscus, and joint capsule. Cartilage matrix breakdown products were assessed using immunohistochemistry. Quantitative measures of cartilage and synovial thickness, and subchondral bone structure were evaluated using histomorphometry. Picrosirius red staining was used to evaluate collagen structure and organization.
Results: 6 month old mice of either strain presented with minimal damage to joint structures. CD1 male mice at 12 months of age exhibited significant severe changes to the knee joint, including end-stage cartilage erosion to the Medial Femoral Condyle (MFC) in all animals, whereas C57/B6 male mice had little to no cartilage damage. Subchondral bone damage, and osteophyte formation pathological scores were also significantly increased in CD1 compared to C57/B6 male mice. Meniscal fissuring was evident in all CD1 male mice, with one half of these having completely degenerated menisci. CD1 male mice also had striking periarticular cartilage formation in the joint capsule. These significant changes in CD1 mice, were not present in the knee joints of C57/B6 male mice. Female mice of either strain presented with no significant differences at this age.
At 20 and 24 months of age, both male and female CD1 mice had severe OA in the medial compartment of the knee with persistent significantly elevated scores in MFC cartilage, meniscus, and periarticular cartilage formation. Conversely, male C57/B6 mice exhibited minor changes in articular cartilage at 20 and 24 months, consisting of focal proteoglycan loss and small fibrillations. At this age, there were also no observed differences in subchondral bone damage, and osteophyte formation between strains in male mice. All joint tissue histopathological parameters are significantly different between female CD1 and female C57/B6 mice. Cartilage-specific PPARdelta KO mice of both sexes demonstrate little to no incidence or progression of OA at 24 months of age, but display increased cartilage thickness in peripheral joints such as the ankle, when compared to wild type C57/B6 mice.
Conclusions: Our results suggest differing underlying physiological processes leading to OA in C57/B6 and CD1 strains. CD1 mice appear to have more severe earlier-onset spontaneous OA, initiated by capsular cartilage formation and meniscal changes, whereas C57/B6 mice have more moderate changes largely in the cartilage and subchondral bone. These profiles are distinct, demonstrating the importance of genetic background in both time course and mechanisms of OA pathogenesis. These data also illustrate the necessity to carefully choose which model is most appropriate for investigating disease development and therapeutic targets, as well as the need to use multiple models. In addition, our studies suggest a mild protective effect of PPARdelta loss in some joints during aging.

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FIBRILLIN-1 MUTANT MICE (TIGHT SKIN) SHOW INCREASED SPONTANEOUS AND TRAUMA-INDUCED OSTEOARTHRITIS DEVELOPMENT

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Purpose: Osteoarthritis development is a major chronic disorder affected >8million British people. Despite the high prevalence of OA, there are currently no therapies to slow disease progression. Fibrillin-1 is an extracellular matrix protein found in elastic fibres. One of its main roles is to control growth factor bioavailability, which play vital functions in joint homeostasis. Fibrillin-1 mutations, as those found in Tight Skin mice (TSK), can increase TGFß signalling, and leads to tight skin, myocardial hypertrophy, marfan-like skeletal phenotype and lung emphysema. The aim of this study is to assess OA development in TSK mice in spontaneous and in trauma-induced models.
Methods: Immunohistochemistry for Fibrillin-1 was performed in CBA and Str/ort mice (spontaneous OA), and in trauma-induced OA. For the ageing model, knees from 35wk-old TSK and littermate control male mice were collected, microCT scanned and used for histology. The non-invasive loading model was used and the right knee of male TSK and WT mice were loaded i) a single time at 7 or 9N loads and joints analysed 1 weeks later; or ii) repetitively for 2 weeks to induce OA progression, and joints assessed 6 weeks after the last loading episode. Histology was performed on these joints after decalcification (10% formic acid), serial sections cut across the whole joint at 6μm, and AC degradation severity assessed (OARSI grading system).
Results: Fibrillin-1 was found in the pericellular matrix of chondrocytes in both the growth plate (resting and hypertrophic cells) and in the uncalcified articular cartilage in healthy CBA mice. During the development of OA in Str/ort mice and in response to mechanical trauma, Fibrillin-1 immunolabelling was decreased. TSK mice showed spontaneous increased joint space mineralised tissue volume (including meniscus and ligament calcification) and increased AC degradation compared to WT at 35wks of age. In contrast, no significant differences were seen in tibial epiphyseal bone (medial and lateral; BV/TV, Trabecular Thickness, Trabecular Separation, Trabecular number). A single loading episode at both 9 and 7N produced AC lesions in the lateral femur in both WT and TSK mice, however severity of lesions created were more severe in TSK mice (preliminary data). Repetitive loading episodes lead to slight increase in mineralised joint space tissue between TSK and litter mate WT controls (preliminary data), which from our previous studies has shown to be correlated with OA severity.
Conclusions: This study shows that Fibrillin-1 is decreased from the pericellular matrix during OA development in spontaneous and trauma-induced OA, suggesting increased Fibrillin-1 degradation. In addition, mutations in Fibrillin-1 in the TSK mouse lead to increased spontaneous and trauma-induced OA development. These data suggest that normal Fibrillin-1 expression and function may play an important role in joint homeostasis and that abnormal expression or mutations promote OA development initiation and progression.

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CD14 DEFICIENCY DAMPENS OSTEOCLASTOGENESIS AND ALTERS BONE REMODELING IN A MURINE MODEL OF OSTEOARTHRITIS

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Purpose: Pathogenesis of osteoarthritis (OA) is accompanied by chronic inflammation evidenced by macrophage infiltration into the joint. We previously reported high levels in synovial fluid from OA patients of the soluble CD14, a macrophage co-receptor that facilitates Toll-like receptor signaling. Upon binding, the CD14/TLR complex can activate