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The dual pro-inflammatory and bone-protective role of calcitonin gene-related peptide alpha in age-related osteoarthritis

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Abstract

Background The vasoactive neuropeptide calcitonin gene-related peptide alpha (αCGRP) enhances nociception in primary knee osteoarthritis (OA) and has been shown to disrupt cartilage and joint integrity in experimental rheumatoid arthritis (RA). Little is known about how αCGRP may alter articular structures in primary OA. We investigated whether αCGRP modulates local inflammation and concomitant cartilage and bone changes in a murine model of age-dependent OA.

Methods Sixteen- to 18-month-old αCGRP-deficient mice (αCGRP^{-/-}_{aged}) were compared to, first, age-matched wild type (WT_{aged}) and, second, young 4- to 5-month-old non-OA αCGRP-deficient (αCGRP^{-/-}_{CTRL}) and non-OA WT animals (WT_{CTRL}). αCGRP levels were measured in serum. Knee and hip joint inflammation, cartilage degradation, and bone alterations were assessed by histology (OARSI histopathological grading score), gene expression analysis, and μ-computed tomography.

Results WT_{aged} mice exhibited elevated αCGRP serum levels compared to young WT_{CTRL} animals. Marked signs of OA-induced cartilage destruction were seen in WT_{aged} animals, while αCGRP^{-/-}_{aged} mice were mostly protected from this effect. Age-dependent OA was accompanied by an increased gene expression of pro-inflammatory *Tnfa*, *Il1b*, and *Il6* and catabolic *Mmp13*, *Adamts5*, *Ctsk*, *Tnfs11* (*Rankl*), and *Cxcl12/Cxcr4* in WT_{aged} but not in αCGRP^{-/-}_{aged} mice. αCGRP-deficiency however further aggravated subchondral bone sclerosis of the medial tibial plateau and accelerated bone loss in the epi- and metaphyseal trabecular tibial bone in age-dependent OA.

Conclusions Similar to its function in experimental RA, αCGRP exerts a dual pro-inflammatory and bone-protective function in murine primary OA. Although anti-CGRP treatment was previously not successful in reducing pain in OA clinically, these data underline a crucial pathophysiological role of αCGRP in age-related OA.

Keywords Calca, CGRP, Pain, Joint inflammation, Cartilage, Bone, Nociception

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Introduction

With a global prevalence of more than 15% in the adult population [1], osteoarthritis (OA) is the third most rapidly growing disease associated with disability [2], affecting more than 500 million people globally [3]. Intraarticular micro- and macro-injuries trigger repair processes that initiate pro-inflammatory immune cascades which contribute to progressive and irreversible joint destruction. The vicious cycle of inflammation and tissue damage results in cartilage degradation, pathological subchondral bone remodeling, and synovitis [4], causing debilitating pain, loss of mobility, and decreased quality of life.

Calcitonin gene-related peptide (CGRP) is a nociceptive neuropeptide that contributes to pain perception and sensitization in OA [5]. The 37 amino acid peptide is a member of the calcitonin (CT) peptide family and exists in two isoforms, α CGRP and β CGRP. Whereas α CGRP is encoded by the gene *Calca* and, among other tissues, expressed in the central and peripheral nervous system, β CGRP is encoded by the gene *Calcb* and primarily expressed in the intestine [6]. CGRP can be found in intraarticular perivascular sensory nerve fibers [5] and synovial fluid [7] of osteoarthritic joints. It has both, nociceptive/sensory and efferent/effector functions and arthritic pain develops partially through CGRP-mediated neurogenic vasodilation and inflammation [8]. The density of intraarticular CGRP-positive perivascular nerve fibers is positively correlated with OA severity [5].

We previously showed that α CGRP exhibits an independent pro-inflammatory role in antibody-mediated experimental rheumatoid arthritis (RA) [9]. While RA is however a systemic inflammatory auto-immune disease, OA is characterized by local low-grade inflammation with moderately elevated pro-inflammatory proteins in the plasma and synovial fluid [10]. Interestingly, the density of CGRP-positive nerve fibers is reportedly higher in the synovial tissue of knee joints from OA compared to RA patients [11]. Further, intraarticular CGRP can be secreted by fibroblast-like synoviocytes and its expression correlates with pain in OA [12]. Interestingly, CGRP seems to affect cartilage differently depending on the pre-existing phenotype of chondrocytes. In this regard, a chondro-protective and anti-apoptotic response was observed when CGRP was added to healthy chondrocytes, yet when added to OA-derived chondrocytes, collagen formation markers, and glycosaminoglycans were markedly reduced [13]. However, data from a placebo- and celecoxib-controlled clinical trial failed to show relevant pain relief following monoclonal CGRP-antibody therapy in knee OA patients [14]. A pathophysiological role of α CGRP in OA is therefore possible, yet in vivo evidence is scarce [15].

To explore the effects of α CGRP on intraarticular knee and hip joints during primary OA, α CGRP-deficient (α CGRP^{-/-}) and wild-type (WT) mice were exposed to age-dependent OA and compared to young control (CTRL) animals. Our results suggest an independent dual role of α CGRP contributing to pro-inflammatory and catabolic changes intraarticularly, while protecting bone structures from sclerosis and erosion.

Materials and methods

Animals and naturally occurring (primary) OA

Female α CGRP^{-/-} and WT mice were used for all experiments [16, 17] and backcrossed at least seven times to ensure a pure C57BL/6 J genetic background. Based on age, WT and α CGRP^{-/-} mice were separated in four groups: Young, 4- to 5-month-old WT_{CTRL} ($n=10$) and α CGRP^{-/-}_{CTRL} animals ($n=10$) and aged, 16- to 18-month-old WT_{aged} ($n=10$) and α CGRP^{-/-}_{aged} mice ($n=10$). The employed primary OA model is age-dependent and was previously described in animals with a C57BL/6 J genetic background [18, 19]. Aged WT animals develop spontaneous OA-like joint lesions with a prevalence of up to 90% [20–23]. α CGRP^{-/-} mice exhibit a normal skeletal phenotype, but develop mild spontaneous osteopenia, starting at the age of 4–6 months [24]. All animals were kept at a 12 h light/12 h dark cycle, fed a standard diet, and had access to water ad libitum. Ethical approval was obtained by the competent authority.

The body weight was recorded for all animals before euthanasia using a scale (EMB Scale Ø 150 mm, KERN&SOHN GmbH, Germany).

α CGRP serum protein analysis

Thirty microliters of snap frozen serum were analyzed with an ELISA kit (CSB-EQ027706MO, CUSABIO, Houston, TX, USA), according to the manufacturer's instructions.

Sample preparation

Both knee and hip joints were isolated when WT_{CTRL} and α CGRP^{-/-}_{CTRL} had reached 4 to 5 months and WT_{aged} and α CGRP^{-/-}_{aged} 16 to 18 months. Right knee and hip joints were fixed in paraformaldehyde (PFA) 4% for 48 h, washed and stored in phosphate-buffered saline (PBS) for μ -computed tomography (μ CT) analysis. Following μ CT scanning, samples were decalcified in 25% EDTA for 20 days, dehydrated (TP 1020 Tissue Processor, Leica Biosystems, Germany, protocol: ethanol 70% 1 h, 80% 3 h, 96% 4 h, 100% 7 h, xylol 2,5 h, paraffin 4 h), and embedded in paraffin (Surgipath Paraplast Plus, Leica Biosystems, Germany). Left knee joints were stripped of all muscle and soft tissue and snap-frozen in liquid nitrogen for RNA isolation and gene expression analysis.

Histology

Two-micrometer coronal sections of knee and hip joints were cut using a microtome (Rotary 3000 Compact, pfm medical, Germany) and stained with methylene blue (MB) and safranin O (SO). The previously established OARSI histopathological scoring system [25] from 0 to 6 was applied (Supplementary Data S1) by two blinded investigators (AH and TM). For the knee joint, each of the four quadrants of the knee joint: medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC), and lateral tibial plateau (LTP) were assessed separately and scored. For the hip joint, the femoral head (FH) and the acetabulum (AC) were assessed separately and scored. A total joint score was calculated as a mean of all individual scores obtained from MB- and SO-stained slides [25].

qRT-PCR

Snap-frozen knee joints were trimmed to thin corresponding articular surfaces consisting of synovium, cartilage, and the subchondral bone layer. RNA isolation and reverse transcription to complementary DNA (cDNA) were conducted as previously reported [26]. Snap-frozen joint samples were treated as previously described [9] and quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 384 well-plate reader in a 7900HT Fast Real-Time PCR System (Thermo Fisher). Raw data were analyzed with SDS v2.4 software (Applied Biosystems). Primers were designed as previously described [9] and provided by Eurofins Genomics GmbH. Primer sequences of assessed genes can be found in Supplementary Data S2. Data points for WT_{aged} and α CGRP^{-/-}_{aged} mice are displayed as mean fold changes of two pipetted runs for each sample relative to respective CTRL samples which were set to 1 according to the ddct method [27].

μ CT

Knee and hip joints were analyzed and reconstructed *post mortem* by μ CT (Skyscan 1172, Bruker, MA, USA). Parameters were set as follows: 70 kV, 142 μ A, slice thickness 5.1 μ m, filter 0.5 Al, rotation step 0.2, averaging frames 3, random movement 10. Raw data were reconstructed as previously described [9]. Systemic bone changes were evaluated in proximal tibiae where a volume of interest (VOI) of 1 mm in length was placed around the outer cortical bone layer, starting 0.5 mm below the most distal point of the growth plate. Assessed global bone parameters included bone volume/total volume (BV/TV) in % and bone density in mg hydroxyapatite (HA)/ccm, as well as trabecular bone parameters, including bone surface in μm^2 , trabecular number (Tb.N)

in $1/\mu\text{m}$, trabecular separation (Tb.Sp) in μm , and trabecular thickness (Tb.Th) in μm . For evaluation of subchondral bone sclerosis, parameters included cortical volume/total volume (Ct.V/TV) in %, subchondral bone, and pore (Bo&Po) density in mg HA/ccm, and average pore diameter (AvgPo.Dm) in μm [28]. For subchondral bone analysis of the hip joint, a VOI was placed around the femoral head, framing a plate of subchondral bone with a thickness of 80 μm . For radiological evaluation of the subchondral knee joint, the MTP and the epiphyseal trabecular bone were analyzed separately. A VOI was placed around the subchondral MTP, using the same technique as for the femoral head (Supplementary Data S3). The tibial epiphysis was evaluated using a separate VOI, excluding the subchondral bone plate, and avoiding the medial and lateral cortical bone and growth plates (Supplementary Data S3). A threshold of 70 mg HA/ccm for trabecular parameters and 80 mg HA/ccm for subchondral bone parameters was set. Bone density is displayed as 3D images using color maps with a maximum value of 130 mg HA/ccm.

Statistical analysis

We estimated mean OARSI histopathological grading scores of $2 \text{ SD} \pm$ of 1.0 for WT_{aged}, and mean scores of 0.75 for WT_{CTRL} animals. To obtain a power of 0.8 with an α of 0.05 we calculated that 10 animals per group would be necessary to show an effect size of 1.25. End-point comparisons between groups were performed in Prism 9 using the Wilcoxon-Mann-Whitney test. For group comparisons of the ordinal OARSI histopathological grading score and body weight, a non-parametric Kruskal-Wallis test with Dunn's test for multiple comparisons was performed. Outliers were included in the analysis. Unless stated otherwise, data are presented as median \pm minimum and maximum. Significance was accepted where $p < 0.05$. For data reporting and storage, we followed the internationally established ARRIVE guidelines [29].

Results

Serum α CGRP is elevated in OA and α CGRP-deficiency protects knee joints from histological signs of cartilage degradation in age-related OA

α CGRP was significantly elevated in serum samples of WT_{aged} compared to WT_{CTRL} animals ($p = 0.0410$) (Fig. 1A).

OA development was accompanied by a significant body weight gain in WT_{aged} ($p = 0.0012$) and α CGRP^{-/-}_{aged} animals ($p = 0.0025$) compared to their respective CTRL groups while no significant difference was seen between WT_{aged} and α CGRP^{-/-}_{aged} mice ($p > 0.9999$) (Fig. 1B).

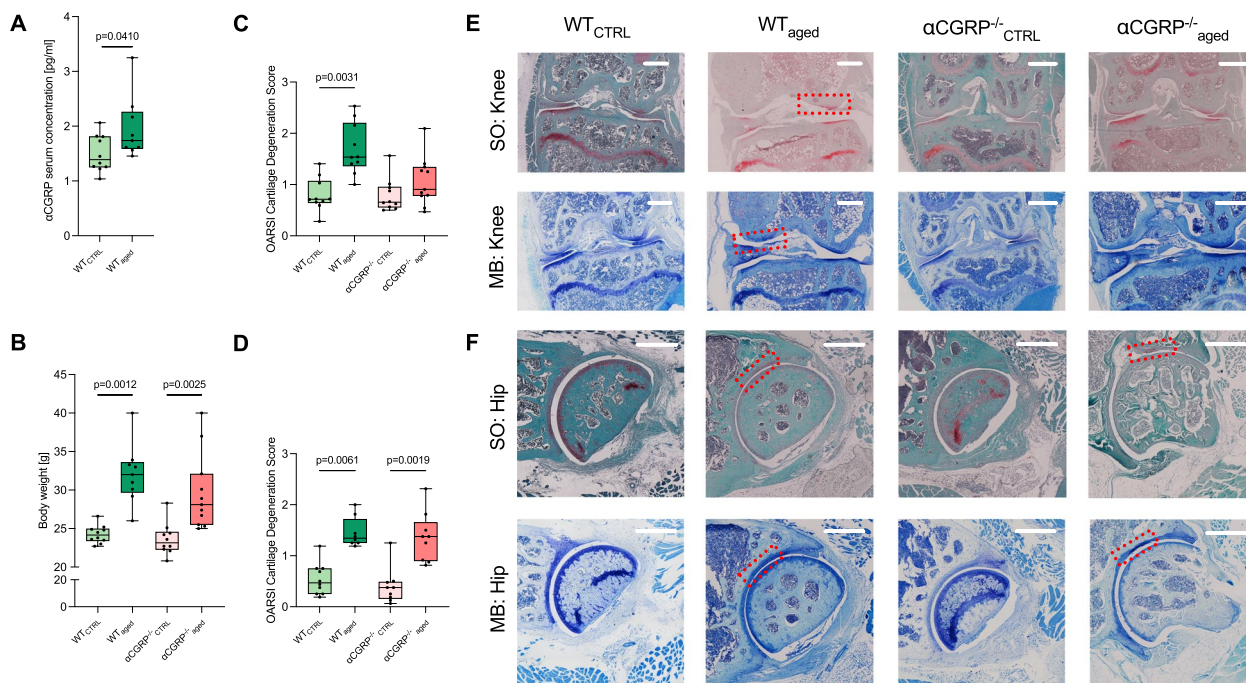


Fig. 1 Serum α CGRP is elevated in primary OA and knee but not hip joints of aged α CGRP-deficient mice are protected from cartilage degradation. **A** α CGRP serum concentrations and **B** body weight of indicated groups. **C** OARSI histopathological grading scores of knee and **D** hip joints of indicated groups. **E** Representative histological images of MB- and SO-stained sections of knee and **F** hip joints of indicated groups. Red dotted boxes indicate cartilage damage. Scale bars = 500 μ m. Given values are median \pm minimum and maximum. MB, methylene blue; SO, safranin O

In WT_{aged} animals, OA was evidenced by a marked loss of cartilage integrity in knee joints (WT_{aged} vs. WT_{CTRL} $p=0.0031$), which was also more pronounced when compared to α CGRP $^{-/-}$ $_{aged}$ animals, although not statistically significant ($p=0.867$). Further, no significant difference was seen between α CGRP $^{-/-}$ $_{aged}$ and CTRL mice ($p>0.9999$) (Fig. 1C, E). Overall, knee cartilage loss was most pronounced in the LTP and the MTP (data not shown).

OA was further observed in hip joints of WT_{aged} ($p=0.0061$) and α CGRP $^{-/-}$ $_{aged}$ ($p=0.0019$) when compared to CTRL animals (Fig. 1D, F). Cartilage loss was evident in both, the FH and the AC (data not shown).

α CGRP-deficiency prevents overexpression of inflammation markers in OA-affected knee joints

To assess molecular gene expression patterns in OA-affected knee joints, qRT-PCR analyses of osseocartilaginous knee joint samples were performed. WT_{aged} mice showed an increased expression of *Tnfa* ($p=0.0115$), *Il1b* ($p=0.0433$), and *Il6* ($p=0.0115$), when compared to WT_{CTRL} mice, while α CGRP-deficient animals were protected from this effect. Pro-inflammatory *Cxcl12* and *Cxcr4* were also increased in WT_{aged} compared to WT_{CTRL} animals, while only reaching statistical significance for the latter ($p=0.0355$) (Fig. 2A).

Expression of catabolic cartilage turnover markers is reduced in mice deficient for α CGRP in primary OA

Expression of catabolic cartilage turnover markers *Mmp13* ($p=0.0028$) and *Adams5* ($p=0.0062$), and bone resorption markers *Ctsk* ($p=0.0021$), *Tnfs11* (*Rankl*) ($p<0.0001$), and *Acp5* (*Trap*) ($p=0.0001$) were exclusively reduced in α CGRP $^{-/-}$ $_{aged}$ mice compared to CTRLs while *Casp3* was exclusively elevated in WT_{aged} compared to CTRLs ($p=0.0355$) (Fig. 2B). Further anabolic cartilage turnover markers also decreased with OA development including *Col2a1* (WT_{aged} vs. WT_{CTRL} $p=0.0021$; α CGRP $^{-/-}$ $_{aged}$ vs. α CGRP $^{-/-}$ $_{CTRL}$ $p<0.0001$) and *Acan* (WT_{aged} vs. WT_{CTRL} $p<0.0001$; α CGRP $^{-/-}$ $_{aged}$ vs. α CGRP $^{-/-}$ $_{CTRL}$ $p=0.0002$), however without differences between genotypes. The osteoblast transcription marker *Runx2* was exclusively reduced in α CGRP $^{-/-}$ $_{aged}$ mice compared to CTRLs ($p=0.0242$) (Fig. 2C).

α CGRP protects from medial tibial subchondral bone sclerosis in age-related OA

To evaluate OA-induced subchondral bone sclerosis and changes in cortical bone architecture, knee and hip joints were analyzed by μ CT. Increased cortical volume ($p<0.0001$) and subchondral bone density ($p=0.035$), both indicative of subchondral bone sclerosis of the MTP, were exclusively increased in α CGRP $^{-/-}$ $_{aged}$ mice but not

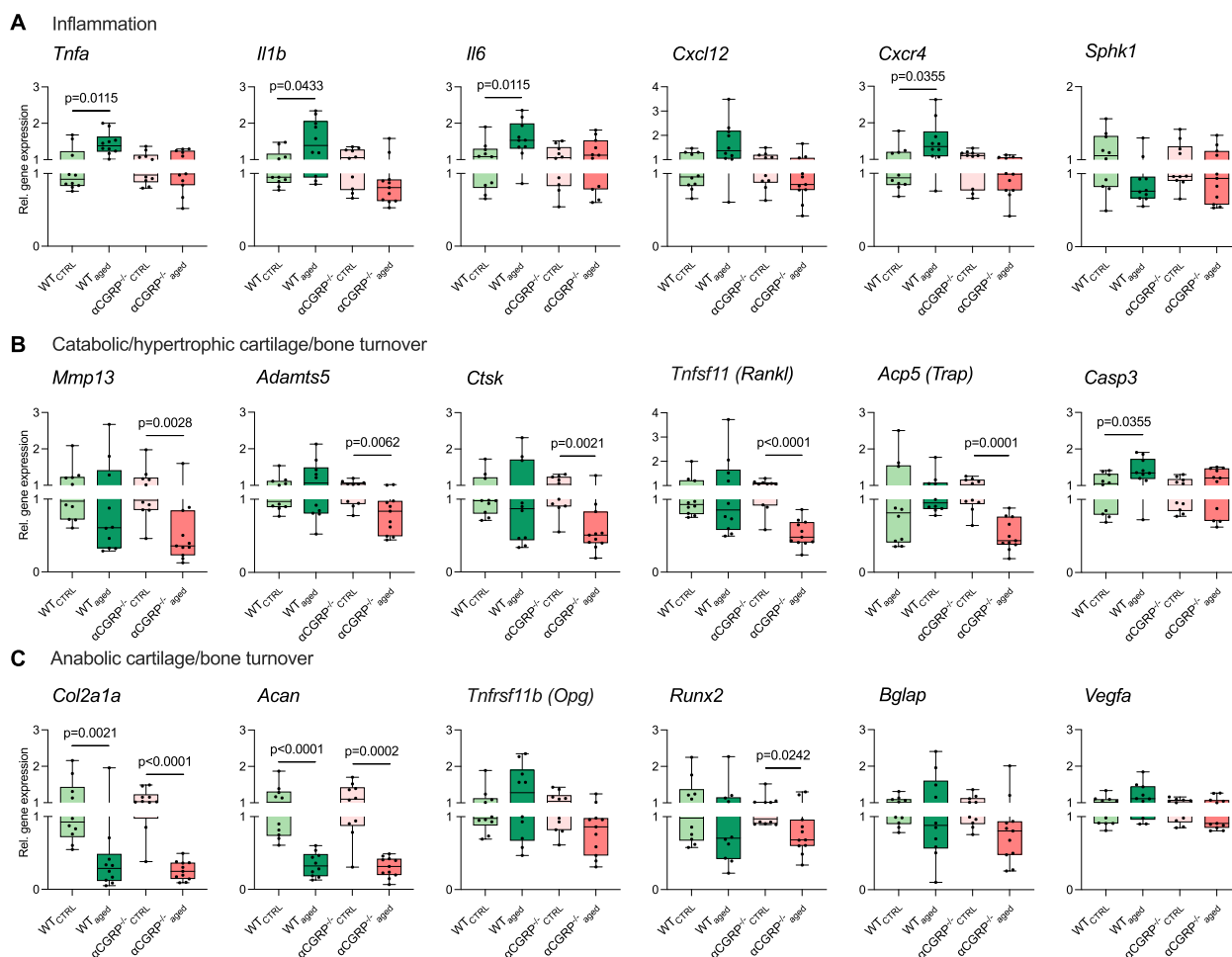


Fig. 2 αCGRP promotes intraarticular expression of pro-inflammatory and catabolic cartilage/bone turnover markers in OA-affected knee joints. **A** qRT-PCR gene expression analysis of inflammation markers, **B** catabolic/hypertrophic cartilage/bone turnover markers, and **C** anabolic cartilage/bone turnover markers in knee joint samples of indicated groups. Given values are median ± minimum and maximum. Values for WT_{aged} and αCGRP^{-/-}_{aged} mice are shown as relative fold changes with respect to CTRL groups that were set to 1

in WT_{aged} animals when compared to respective CTRLs (Fig. 3A + B). While cortical volume (WT_{aged} vs. WT_{CTRL} $p=0.0021$; αCGRP^{-/-}_{aged} vs. αCGRP^{-/-}_{CTRL} $p<0.0001$) and subchondral bone density (WT_{aged} vs. WT_{CTRL} $p=0.0355$; αCGRP^{-/-}_{aged} vs. αCGRP^{-/-}_{CTRL} $p=0.0089$) of the femoral head were elevated in both genotypes during OA, subchondral porosity was exclusively decreased in WT_{aged} animals compared to CTRLs ($p=0.0185$) (Fig. 3C + D).

αCGRP-deficiency promotes osteopenia and bone sclerosis in the tibial epi- and metaphysis during primary OA

To evaluate if age-induced OA further affects osseous structures distal to the subchondral bone, we investigated cortical and trabecular bone structures of the tibial epi- and metaphysis. αCGRP^{-/-}_{aged} mice showed marked signs of osteopenia accompanied by sclerotic changes of the tibial epiphysis, while bone integrity was

preserved in WT_{aged} mice. In particular, decreased bone volume ($p<0.0001$), bone surface ($p=0.0279$), and trabecular number ($p<0.0001$) and increased bone density ($p=0.0004$) and trabecular separation ($p<0.0001$) were observed in αCGRP^{-/-}_{aged} animals compared to CTRLs (Fig. 4A + B).

In the tibial metaphysis, bone density was also exclusively increased in αCGRP^{-/-}_{aged} mice ($p=0.0011$), while bone volume was reduced in both genotypes during OA (WT_{aged} vs. WT_{CTRL} $p=0.0029$; αCGRP^{-/-}_{aged} vs. αCGRP^{-/-}_{CTRL} $p<0.0001$) (Fig. 4C + D). Bone surface was lower in αCGRP^{-/-}_{aged} than in WT_{aged} mice ($p=0.0089$), while a loss of bone surface was seen in both genotypes compared to CTRLs ($p<0.0001$ for both). Trabecular deterioration was present in all aged animals when compared to CTRL mice, indicated by increased trabecular separation and decreased trabecular numbers ($p<0.0001$ for both genotypes).

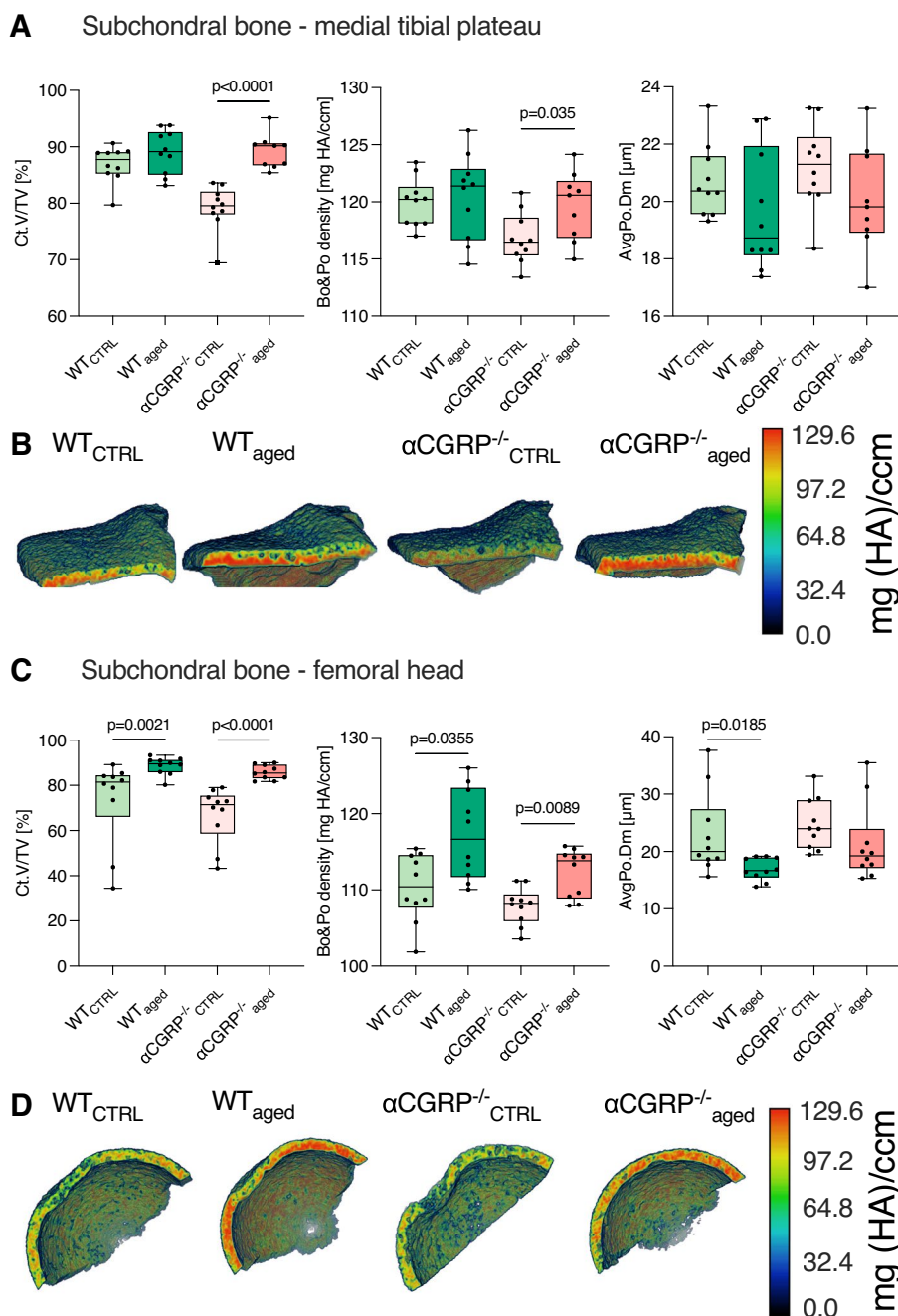


Fig. 3 αCGRP prevents the development of primary OA-related subchondral bone sclerosis in the medial knee joint. **A** μCT parameters of the subchondral bone plate of the MTP, and **B** representative 3D images of bone density of the MTP using color maps. **C** μCT parameters of the subchondral bone plate of the femoral head, and **D** representative 3D images of bone density of the femoral head using color maps. Given values are median ± minimum and maximum. Maximum values for color scales were set to 130 mg HA/ccm. MTP, medial tibial plateau

Discussion

In this study, the lifelong absence of αCGRP prevented cartilage degradation of the knee joint and decreased the expression of pro-inflammatory and catabolic cartilage markers in mice suffering from age-induced OA. Yet, aged αCGRP-deficient mice showed distinct signs

of tibial subchondral bone sclerosis, impaired bone quality of the epi- and metaphysis, and marked trabecular bone loss. Similar to the observed effects in experimental RA [9], we found αCGRP to display a dual pro-inflammatory and bone protective role in primary, age-dependent OA.

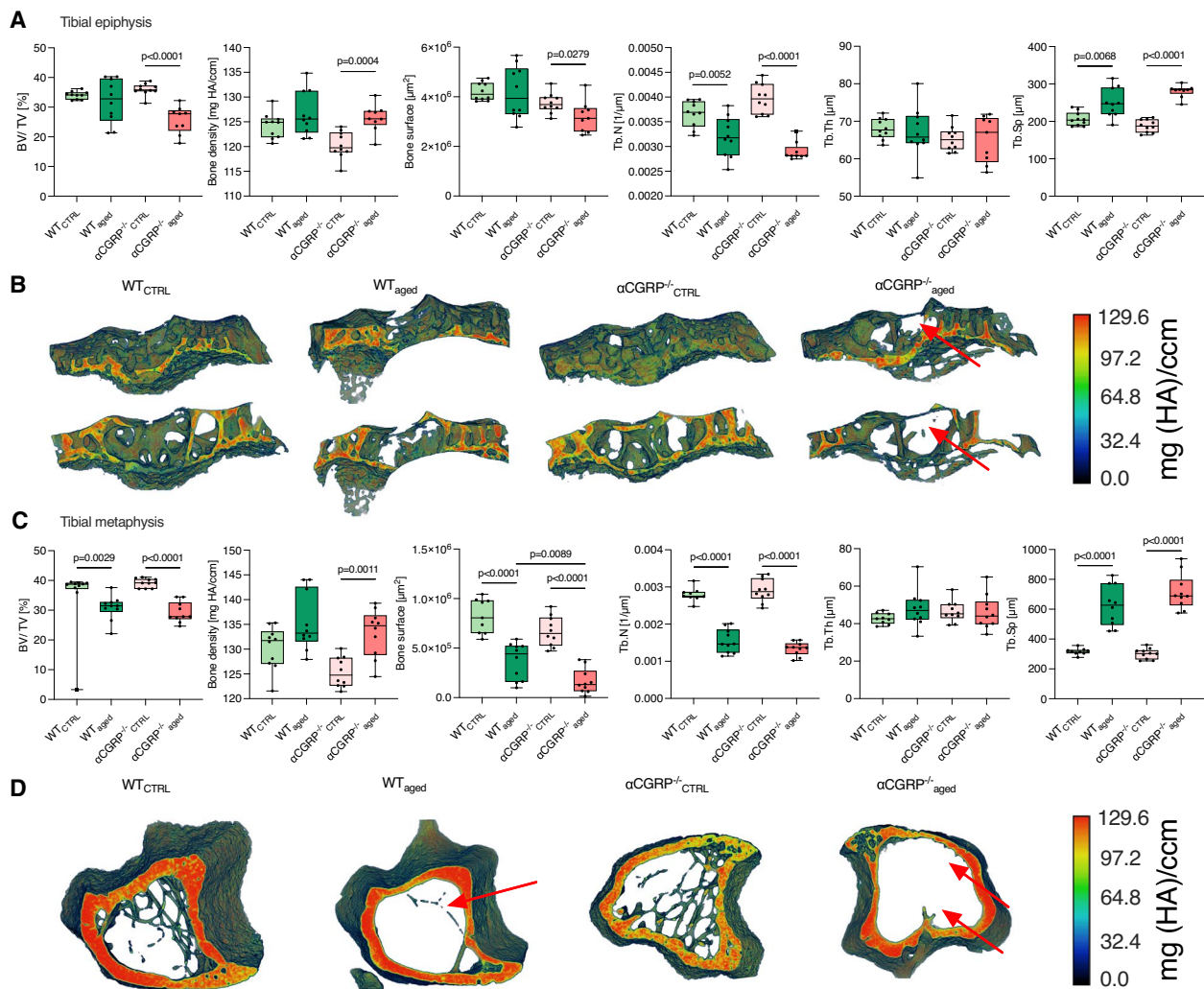


Fig. 4 αCGRP prevents radiological OA-related alterations in the tibial epi- and metaphysis. **A** μCT parameters of the tibial epiphysis, and **B** representative 3D images of bone density of the trabecular architecture of the tibial epiphysis using color maps (anterior and posterior sections). **C** μCT parameters of the tibial metaphysis, and **D** representative 3D images of bone density of the trabecular architecture of the tibial metaphysis using color maps. Red arrows indicate loss of trabecular bone mass. Given values are median ± minimum and maximum. Maximum values for color scales were set to 130 mg HA/ccm

Anti-CGRP therapy has recently been clinically introduced for the prevention and treatment of migraine [30]. Although the pharmacological blockade of CGRP was unable to provide clinically meaningful pain reduction in knee OA patients in a double-blind and placebo-controlled clinical trial [14], an independent pathological role of CGRP in OA—beyond its nociceptive function—is currently being debated [13, 15, 31].

While most in vivo studies employ surgically induced OA models [15, 31] to examine CGRP in OA, this study investigated the role of αCGRP in a murine model of naturally occurring primary OA, which resembles the most common form of OA to date [32].

We were previously able to show that αCGRP-deficiency is associated with decreased intraarticular expression of *Tnfa*, *Il1b*, and *Mmp13* in animals suffering from experimental RA [9]. Here, we again found elevated expression levels of *Tnfa*, *Il1b*, and *Il6* in knee joints of WT_{aged} mice while αCGRP^{-/-}_{aged} animals were protected from increased gene expressions of pro-inflammatory cytokines. TNFα, IL1β, and IL6 all contribute to low-grade inflammation and progressive cartilage loss in primary OA [4]. Chondrocytes show reduced proliferation when exposed to IL1β, which further increases the expression of catabolic markers (*Adams5* and *Mmp13*) and decreases the expression of anabolic markers (*Col2a1*

and *Acan*) in cartilage matrix [33]. Elevated serum concentrations of TNF α and IL6 further correlate with radiographic loss of cartilage volume in human knee OA [34].

Binding of the chemokine CXCL12/SDF-1 to its receptor CXCR4 upregulates MMP13, prevents apoptosis of intraarticular leukocytes, and has pro-sclerotic properties in OA-affected joints [35]. SDF-1 is elevated in the synovium of OA patients [36] and pharmacological disruption of SDF-1/CXCR4 signaling leads to a partial attenuation of cartilage damage in preclinical primary OA [37]. In line with these data, we detected an increased expression of SDF-1/CXCR4 signaling in WT_{aged} mice which was not the case in aged α CGRP-deficient mice.

Naturally occurring deterioration of cartilage is observed in mice between 3 and 9 months [20]. Previous in vitro data showed a chondroprotective effect of α CGRP in healthy chondrocytes but a contribution to cartilage deterioration in OA-altered chondrocytes [13]. Accordingly, we saw a protection from histological signs of cartilage destruction and a reduction of catabolic cartilage markers in α CGRP^{-/-}_{aged} mice exclusively, while anabolic *Col2a1* and *Acan* were reduced in α CGRP^{-/-}_{aged} and WT_{aged} mice alike. In line with our findings, Nakasa et al. showed that blocking CGRP pharmacologically reduced *Mmp13* expression levels and OA progression in vivo [31].

Cathepsin K was previously shown to promote cartilage degradation [38], and mice deficient in cathepsin K were partially protected from surgically induced OA [39]. In addition, receptor activator of NF- κ B ligand (RANKL) and Runx2 are overexpressed in OA cartilage [40, 41], and the cell-type specific deletion of Runx2 in chondrocytes protects from experimental OA [42]. Our study showed that α CGRP deficiency led to a marked decrease of *Mmp13*, *Adamts5*, *Ctsk*, *Tnfsf11* (*Rankl*), and *Runx2*, which further underlines the catabolic role of α CGRP for cartilage in primary OA.

While TRAP is traditionally understood an osteoclast marker, an intraarticular role for TRAP in cartilaginous tissues is being discussed. TRAP-positive chondroclasts were previously identified as cells capable of resorbing mineralized cartilage [43] and serum-TRAP was proposed as a clinically relevant and pain-associated biomarker for OA monitoring [44]. We observed a marked reduction of *Acp5* (*Trap*) in aged OA mice deficient for α CGRP, suggesting a protection from catabolic and pro-inflammatory cartilage changes through an inactivation of α CGRP.

As cartilage damage worsens during the course of OA, subchondral bone remodeling is initiated, causing increased sclerotic bone formation and ossification [45]. There is compelling evidence that subchondral bone stiffening further increases mechanical stress to the

overlying remaining articular cartilage [46]. In this study, α CGRP^{-/-}_{aged} mice showed pronounced signs of subchondral bone sclerosis of the MTP while WT_{aged} animals were partially protected from this effect. The limited extent of sclerotic changes of the MTP in WT mice was previously reported for surgically [15] and age-dependent OA [18, 47]. Together, these findings go in accordance with a reported subtle pro-sclerotic tendency in both, α CGRP-deficient mice receiving destabilizing meniscus surgery and animals receiving sham knee surgery [15]. As immunohistological data showed that the subchondral bone plate of the proximal tibia of healthy rat knee joints contains CGRP-positive nerve fibers [48] this may explain the observed bone-protective effect of α CGRP.

While some authors argue that bone sclerosis in knee OA is limited to the subchondral bone plate [45, 49], recent findings suggest that the epiphysis is also affected by OA-induced defects of the subchondral bone plate, causing a subsequent deterioration of the trabecular architecture beneath [50]. We saw an impaired epiphyseal and metaphyseal trabecular bone structure in all OA animals; however, α CGRP-deficient OA mice exclusively exhibited reduced bone volume and surface with an increased bone density of the epi- and metaphysis. As α CGRP^{-/-} mice develop mild spontaneous osteopenia with age [24], bone deterioration of the subchondral metaphyseal-, and potentially epiphyseal bone may be attributed to the genetically altered skeletal phenotype, while the increased sclerosis of the subchondral bone plate is likely to be caused by an interaction of α CGRP and OA.

Treatment with galcanezumab, an antibody against CGRP, was previously not successful in human OA [14]. The results of this well-conducted clinical trial were surprising, as a previously published preclinical study using the same antibody had shown a significant reduction in pain-related behavior in monoiodoacetate (MIA)-induced and meniscal tear (MT)-induced OA [51]. One potential reason for the observed differences is the choice of preclinical OA model. While the clinical trial was conducted in primary OA patients, all pre-clinical data were based on two secondary OA models [51]. The difference between primary and secondary OA is well researched as recently laid out by Poulsen et al., 2023 [52]. Further, current research in the OA field moves away from understanding OA as one disease and instead strives to identify different endo- and phenotypes which are likely to respond differently to available and newly developed treatments. This may explain in part why numerous “successful” preclinical studies can rarely confirm their findings clinically [53]. Interestingly, galcanezumab has not been tested in a primary OA model before.

We showed that a lifelong blockade of α CGRP signaling alleviates naturally occurring OA in female mice.

Our results imply that the inhibition of α CGRP signaling could be a promising therapeutic approach with translational potential in OA therapy when applied to the right disease and at the right disease stage.

The current study has several limitations. First, the radiological data sets do not allow to distinguish between OA-induced subchondral bone changes and calcified cartilage. The diameter and density of both, calcified cartilage and subchondral bone, increase similarly during progression of OA [15, 45]. This additional information has thus little relevance for the data presented in our study. Second, while joint samples were carefully and precisely dissected, the employed gene expression analyses lack tissue specificity, as samples were comprised of a mixture of cartilage, synovium, and small amounts of subchondral bone. When processing murine joint samples, a selective tissue examination is technically difficult, as joint samples are commonly crushed, minced, or digested [9, 12]. Third, we were only able to report changes that occurred until or during end-stage OA. OA is however a multi-stage disease with various origins and courses. This needs to be taken into consideration when interpreting the results. Fourth, our data did not include human samples. A lifelong blockade of α CGRP signaling, as utilized in our experiment, is an artificial model lacking direct translational potential. Further studies must include human samples, longitudinal disease evaluation, and pharmacological inhibition of α CGRP must be tested in primary and secondary OA. And finally, due to the higher prevalence of OA in women [1], we exclusively investigated female mice. The deterioration of estrogen activity during aging may however have impacted bone and cartilage quality additionally [54], warranting further studies on the role of α CGRP in the male organism.

Conclusions

In this study, we demonstrated that lifelong disruption of endogenous α CGRP-signaling protected animals from histological and molecular signs of cartilage degradation in primary, age-dependent OA. α CGRP serum levels were increased in primary OA and the inactivation of α CGRP impeded OA-associated overexpression of intraarticular inflammation and catabolic cartilage markers including IL1 β , IL6, TNF α , MMP13, ADAMTS5, Cathepsin K, RANKL, RUNX2, and SDF-1/CXCR4 in joint tissues. In contrast, lack of α CGRP was associated with subchondral bone sclerosis and tibial osteopenia in primary OA. Although the clinical application of anti-CGRP treatment did previously not meet the primary endpoint of pain reduction in knee OA patients, our study provides first-hand evidence for a dual pro-inflammatory and bone-protective role of α CGRP in naturally occurring OA.

Abbreviations

AC	Acetabulum
α CGRP	Calcitonin gene-related peptide alpha
AvgPo.Dm	Average pore diameter
BV/TV	Bone volume/total volume
CT	Calcitonin
cDNA	Complementary DNA
CTRL	Control
Ct.V/TV	Cortical volume/total volume
FH	Femoral head
HA	Hydroxyapatite
LFC	Lateral femoral condyle
LTP	Lateral tibial plateau
MFC	Medial femoral condyle
MTP	Medial tibial plateau
MIA	Monoiodoacetate
MT	Meniscal tear
MB	Methylene blue
OA	Osteoarthritis
PBS	Phosphate-buffered saline
qRT-PCR	Quantitative real-time polymerase chain reaction
RANKL	Receptor activator of NF- κ B ligand
RA	Rheumatoid arthritis
SO	Safranin O
Bo&Po	Subchondral bone and pore
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness
VOI	Volume of interest
WT	Wild-type
μ CT	μ -Computed tomography

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13075-023-03215-3>.

Additional file 1: S1. OARSI histopathological grading score. **S2.** Primer sequences. **S3.** Assessment of μ CT parameters.

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Authors' contributions

AH and TM take full responsibility for the integrity of the presented work, from the inception of the study to the finished article. Conception and design: TM and AH. Analysis and interpretation of the data: AH, JA, DJ, TD, JW, MMG, SJ, and TM. Drafting of the article: AH and TM. Critical revision of the article for important intellectual content: All authors. Final approval of the article: All authors. Provision of study materials: ST, JK, and TM. Statistical expertise: DJ, AH, and TM. Funding: TW, ST, JK, and TM. Administrative, technical, or logistic support: DJ, JA, and GND. Collection and assembly of data: AH, SZ, TD, JW, MMG, SJ, and TM.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations**Ethics approval and consent to participate**

This study was approved by the internal animal welfare committees and the number of animals duly notified to the competent authority (Landesamt für Gesundheit und Soziales, Berlin, Germany) (T-CH0009/20).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EClinicalMedicine*. 2020;29–30: 100587.
- Hawker GA. Osteoarthritis is a serious disease. *Clin Exp Rheumatol*. 2019;37(Suppl 120):3–6.
- Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. *Lancet*. 2020;396(10264):1711–2.
- Scanzello CR. Role of low-grade inflammation in osteoarthritis. *Curr Opin Rheumatol*. 2017;29(1):79–85.
- Ashraf S, Wibberley H, Mapp PI, Hill R, Wilson D, Walsh DA. Increased vascular penetration and nerve growth in the meniscus: a potential source of pain in osteoarthritis. *Ann Rheum Dis*. 2011;70(3):523–9.
- Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev*. 2014;94(4):1099–142.
- McNearney T, Chai X, Xu J, Chang CY, Collins E, Cox K, Anderson W, Mitchell P, Talbot J, Dage J, Miller B, Johnson K. calcitonin gene-related peptide (CGRP) levels are elevated in the plasma and knee synovial fluid of patients with knee osteoarthritis (OA). *Osteoarthritis Cartilage*. 2016;24:S80.
- Benemei S, Nicoletti P, Capone JG, Geppetti P. CGRP receptors in the control of pain and inflammation. *Curr Opin Pharmacol*. 2009;9(1):9–14.
- Maleitzke T, Hildebrandt A, Weber J, Dietrich T, Appelt J, Jahn D, Zocholl D, Baranowsky A, Duda GN, Tsitsilonis S, Keller J. Proinflammatory and bone protective role of calcitonin gene-related peptide alpha in collagen antibody-induced arthritis. *Rheumatology (Oxford)*. 2021;60(4):1996–2009.
- Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, Sokolove J. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(10):580–92.
- Dirmeier M, Capellino S, Schubert T, Angele P, Anders S, Straub RH. Lower density of synovial nerve fibres positive for calcitonin gene-related peptide relative to substance P in rheumatoid arthritis but not in osteoarthritis. *Rheumatology*. 2008;47(1):36–40.
- Takano S, Uchida K, Inoue G, Minatani A, Miyagi M, Aikawa J, Iwase D, Onuma K, Mukai M, Takaso M. Increase and regulation of synovial calcitonin gene-related peptide expression in patients with painful knee osteoarthritis. *J Pain Res*. 2017;10:1099–104.
- Stockl S, Eitner A, Bauer RJ, König M, Johnstone B, Grassel S. Substance P and Alpha-Calcitonin Gene-Related Peptide Differentially Affect Human Osteoarthritic and Healthy Chondrocytes. *Front Immunol*. 2021;12:722884.
- Jin Y, Smith C, Monteith D, Brown R, Camporeale A, McNearney TA, Deeg MA, Raddad E, Xiao N, de la Pena A, Kivitz AJ, Schnitzer TJ. CGRP blockade by galcanezumab was not associated with reductions in signs and symptoms of knee osteoarthritis in a randomized clinical trial. *Osteoarthritis Cartilage*. 2018;26(12):1609–18.
- Muschter D, Fleischhauer L, Taheri S, Schilling AF, Clausen-Schaumann H, Grassel S. Sensory neuropeptides are required for bone and cartilage homeostasis in a murine destabilization-induced osteoarthritis model. *Bone*. 2020;133: 115181.
- Lu JT, Son YJ, Lee J, Jetton TL, Shiota M, Moscoco L, Niswender KD, Loewy AD, Magnuson MA, Sanes JR, Emeson RB. Mice lacking alpha-calcitonin gene-related peptide exhibit normal cardiovascular regulation and neuromuscular development. *Mol Cell Neurosci*. 1999;14(2):99–120.
- Huebner AK, Keller J, Catala-Lehnen P, Perkovic S, Streichert T, Emeson RB, Amling M, Schinke T. The role of calcitonin and alpha-calcitonin gene-related peptide in bone formation. *Arch Biochem Biophys*. 2008;473(2):210–7.
- de Hooge AS, van de Loo FA, Bennink MB, Arntz OJ, de Hooge P, van den Berg WB. Male IL-6 gene knock out mice developed more advanced osteoarthritis upon aging. *Osteoarthritis Cartilage*. 2005;13(1):66–73.
- McNulty MA, Loeser RF, Davey C, Callahan MF, Ferguson CM, Carlson CS. Histopathology of naturally occurring and surgically induced osteoarthritis in mice. *Osteoarthritis Cartilage*. 2012;20(8):949–56.
- Akoun J, Tahiri K, Corvol MT, Borderie D, Étienne F, Rannou F, Nguyen C. Aging Cartilage in Wild-Type Mice: An Observational Study. *Cartilage*. 2021;13(2_suppl):1407s–11s.
- Lapveteläinen T, Hyttinen M, Lindblom J, Långsjö TK, Sironen R, Li SW, Arita M, Prockop DJ, Puustjärvi K, Helminen HJ. More knee joint osteoarthritis (OA) in mice after inactivation of one allele of type II procollagen gene but less OA after lifelong voluntary wheel running exercise. *Osteoarthritis Cartilage*. 2001;9(2):152–60.
- Takahashi K, Kubo T, Goomer RS, Amiel D, Kobayashi K, Imanishi J, Teshima R, Hirasawa Y. Analysis of heat shock proteins and cytokines expressed during early stages of osteoarthritis in a mouse model. *Osteoarthritis Cartilage*. 1997;5(5):321–9.
- van Osch GJ, van der Kraan PM, Vitters EL, Blankevoort L, van den Berg WB. Induction of osteoarthritis by intra-articular injection of collagenase in mice. Strain and sex related differences. *Osteoarthritis Cartilage*. 1993;1(3):171–7.
- Schinke T, Liese S, Priemel M, Haberland M, Schilling AF, Catala-Lehnen P, Blicharski D, Rueger JM, Gagel RF, Emeson RB, Amling M. Decreased bone formation and osteopenia in mice lacking alpha-calcitonin gene-related peptide. *J Bone Miner Res*. 2004;19(12):2049–56.
- Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010;18(Suppl 3):S17–23.
- Keller J, Catala-Lehnen P, Huebner AK, Jeschke A, Heckt T, Lueth A, Krause M, Koehne T, Albers J, Schulze J, Schilling S, Haberland M, Denninger H, Neven M, Hermans-Borgmeyer I, Streichert T, Breer S, Barvencik F, Levkau B, Rathkolb B, Wolf E, Calzada-Wack J, Neff F, Gailus-Durner V, Fuchs H, de Angelis MH, Klutmann S, Tsourdi E, Hofbauer LC, Kleuser B, Chun J, Schinke T, Amling M. Calcitonin controls bone formation by inhibiting the release of sphingosine 1-phosphate from osteoclasts. *Nat Commun*. 2014;5:5215.
- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. *Nat Protoc*. 2008;3(6):1101–8.

28. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res*. 2010;25(7):1468–86.
29. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;8(6): e1000412.
30. Chiang CC, Schwedt TJ. Calcitonin gene-related peptide (CGRP)-targeted therapies as preventive and acute treatments for migraine-The monoclonal antibodies and gepants. *Prog Brain Res*. 2020;255:143–70.
31. Nakasa T, Ishikawa M, Takada T, Miyaki S, Ochi M. Attenuation of cartilage degeneration by calcitonin gene-related peptide receptor antagonist via inhibition of subchondral bone sclerosis in osteoarthritis mice. *J Orthop Res*. 2016;34(7):1177–84.
32. Aboulenain S, Saber AY. Primary Osteoarthritis. Treasure Island: StatPearls; 2022.
33. He L, He T, Xing J, Zhou Q, Fan L, Liu C, Chen Y, Wu D, Tian Z, Liu B, Rong L. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. *Stem Cell Res Ther*. 2020;11(1):276.
34. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. Circulating levels of IL-6 and TNF- α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cartilage*. 2010;18(11):1441–7.
35. Villalvilla A, Gomez R, Roman-Blas JA, Largo R, Herrero-Beaumont G. SDF-1 signaling: a promising target in rheumatic diseases. *Expert Opin Ther Targets*. 2014;18(9):1077–87.
36. Kanbe K, Takagishi K, Chen Q. Stimulation of matrix metalloproteinase 3 release from human chondrocytes by the interaction of stromal cell-derived factor 1 and CXC chemokine receptor 4. *Arthritis Rheum*. 2002;46(1):130–7.
37. Wei F, Moore DC, Wei L, Li Y, Zhang G, Wei X, Lee JK, Chen Q. Attenuation of osteoarthritis via blockade of the SDF-1/CXCR4 signaling pathway. *Arthritis Res Ther*. 2012;14(4):R177.
38. Morko JP, Söderström M, Säämänen AM, Salminen HJ, Vuorio EI. Up regulation of cathepsin K expression in articular chondrocytes in a transgenic mouse model for osteoarthritis. *Ann Rheum Dis*. 2004;63(6):649–55.
39. Soki FN, Yoshida R, Paglia DN, Duong LT, Hansen MF, Drissi H. Articular cartilage protection in *Ctsk*^{-/-} mice is associated with cellular and molecular changes in subchondral bone and cartilage matrix. *J Cell Physiol*. 2018;233(11):8666–76.
40. Martinez-Calatrava MJ, Prieto-Potin I, Roman-Blas JA, Tardio L, Largo R, Herrero-Beaumont G. RANKL synthesized by articular chondrocytes contributes to juxta-articular bone loss in chronic arthritis. *Arthritis Res Ther*. 2012;14(3):R149.
41. Zhong L, Huang X, Karperien M, Post JN. Correlation between Gene Expression and Osteoarthritis Progression in Human. *Int J Mol Sci*. 2016;17(7):1126.
42. Liao L, Zhang S, Gu J, Takarada T, Yoneda Y, Huang J, Zhao L, Oh CD, Li J, Wang B, Wang M, Chen D. Deletion of *Runx2* in Articular Chondrocytes Decelerates the Progression of DMM-Induced Osteoarthritis in Adult Mice. *Sci Rep*. 2017;7(1):2371.
43. Khan NM, Clifton KB, Lorenzo J, Hansen MF, Drissi H. Comparative transcriptomic analysis identifies distinct molecular signatures and regulatory networks of chondroclasts and osteoclasts. *Arthritis Res Ther*. 2020;22(1):168.
44. Nwosu LN, Allen M, Wyatt L, Huebner JL, Chapman V, Walsh DA, Kraus VB. Pain prediction by serum biomarkers of bone turnover in people with knee osteoarthritis: an observational study of TRAcP5b and cathepsin K in OA. *Osteoarthritis Cartilage*. 2017;25(6):858–65.
45. Burr DB, Gallant MA. Bone remodelling in osteoarthritis. *Nat Rev Rheumatol*. 2012;8(11):665–73.
46. Brown TD, Radin EL, Martin RB, Burr DB. Finite element studies of some juxta-articular stress changes due to localized subchondral stiffening. *J Biomech*. 1984;17(1):11–24.
47. Ouhaddi Y, Najjar M, Paré F, Lussier B, Urade Y, Bendoricour M, Pelletier JP, Martel-Pelletier J, Fahmi H. L-PGDS deficiency accelerated the development of naturally occurring age-related osteoarthritis. *Aging (Albany NY)*. 2020;12(24):24778–97.
48. Imai S, Tokunaga Y, Maeda T, Kikkawa M, Hukuda S. Calcitonin gene-related peptide, substance P, and tyrosine hydroxylase-immunoreactive innervation of rat bone marrows: an immunohistochemical and ultrastructural investigation on possible efferent and afferent mechanisms. *J Orthop Res*. 1997;15(1):133–40.
49. Botter SM, Glasson SS, Hopkins B, Clockaerts S, Weinans H, van Leeuwen JP, van Osch GJ. ADAMTS5^{-/-} mice have less subchondral bone changes after induction of osteoarthritis through surgical instability: implications for a link between cartilage and subchondral bone changes. *Osteoarthritis Cartilage*. 2009;17(5):636–45.
50. Oláh T, Reinhard J, Gao L, Haberkamp S, Goebel LKH, Cucchiariini M, Madry H. Topographic modeling of early human osteoarthritis in sheep. *Sci Transl Med*. 2019;11(508):eaax6775. <https://doi.org/10.1126/scitranslmed.aax6775>.
51. Benschop RJ, Collins EC, Darling RJ, Allan BW, Leung D, Conner EM, Nelson J, Gaynor B, Xu J, Wang XF, Lynch RA, Li B, McCarty D, Nisenbaum ES, Oskins JL, Lin C, Johnson KW, Chambers MG. Development of a novel antibody to calcitonin gene-related peptide for the treatment of osteoarthritis-related pain. *Osteoarthritis Cartilage*. 2014;22(4):578–85.
52. Poulsen RC, Jain L, Dalbeth N. Re-thinking osteoarthritis pathogenesis: what can we learn (and what do we need to unlearn) from mouse models about the mechanisms involved in disease development. *Arthritis Res Ther*. 2023;25(1):59.
53. Mobasher A, Thudium CS, Bay-Jensen AC, Maleitke T, Geissler S, Duda GN, Winkler T. Biomarkers for osteoarthritis: Current status and future prospects. *Best Pract Res Clin Rheumatol*. 2023;101852.
54. van der Kraan PM. Factors that influence outcome in experimental osteoarthritis. *Osteoarthritis Cartilage*. 2017;25(3):369–75.

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