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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 ($\alpha CGRP^{-/-}{}_{aged}$) were compared to, first, age-matched wild type (WT_{aged}) and, second, young 4- to 5-month-old non-OA αCGRP-deficient ($\alpha CGRP^{-/-}{}_{CTRL}$) 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 $\alpha CGRP^{-/-}{}_{aged}$ 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 $\alpha CGRP^{-/-}{}_{aged}$ 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 reparation 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, αCGRP and βCGRP. Whereas αCGRP is encoded by the gene *Calca* and, among other tissues, expressed in the central and peripheral nervous system, βCGRP 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 αCGRP 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 αCGRP in OA is therefore possible, yet in vivo evidence is scarce [15].

To explore the effects of αCGRP on intraarticular knee and hip joints during primary OA, αCGRP-deficient ($\alpha CGRP^{-/-}$) 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 αCGRP 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 $\alpha CGRP^{-/-}$ 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 $\alpha CGRP^{-/-}$ mice were separated in four groups: Young, 4- to 5-month-old WT_{CTRL} ($n=10$) and $\alpha CGRP^{-/-}{}_{CTRL}$ animals ($n=10$) and aged, 16- to 18-month-old WT_{aged} ($n=10$) and $\alpha CGRP^{-/-}{}_{aged}$ 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]. $\alpha CGRP^{-/-}$ 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 $\alpha CGRP^{-/-}{}_{CTRL}$ had reached 4 to 5 months and WT_{aged} and $\alpha CGRP^{-/-}{}_{aged}$ 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 (μCT) analysis. Following μCT 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 $\alpha CGRP^{-/-}{}_{aged}$ 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].

µCT

Knee and hip joints were analyzed and reconstructed *post mortem* by µCT (Skyscan 1172, Bruker, MA, USA,). Parameters were set as follows: 70 kV, 142 µA, slice thickness 5.1 µm, 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/µm, 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 SD ± 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. Endpoint 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 ± 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 αCGRP is elevated in OA and αCGRP-deficiency protects knee joints from histological signs of cartilage degradation in age-related OA

αCGRP 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 $\alpha CGRP^{-/-}{}_{aged}$ animals ($p = 0.0025$) compared to their respective CTRL groups while no significant difference was seen between WT_{aged} and $\alpha CGRP^{-/-}{}_{aged}$ mice ($p > 0.9999$) (Fig. 1B).

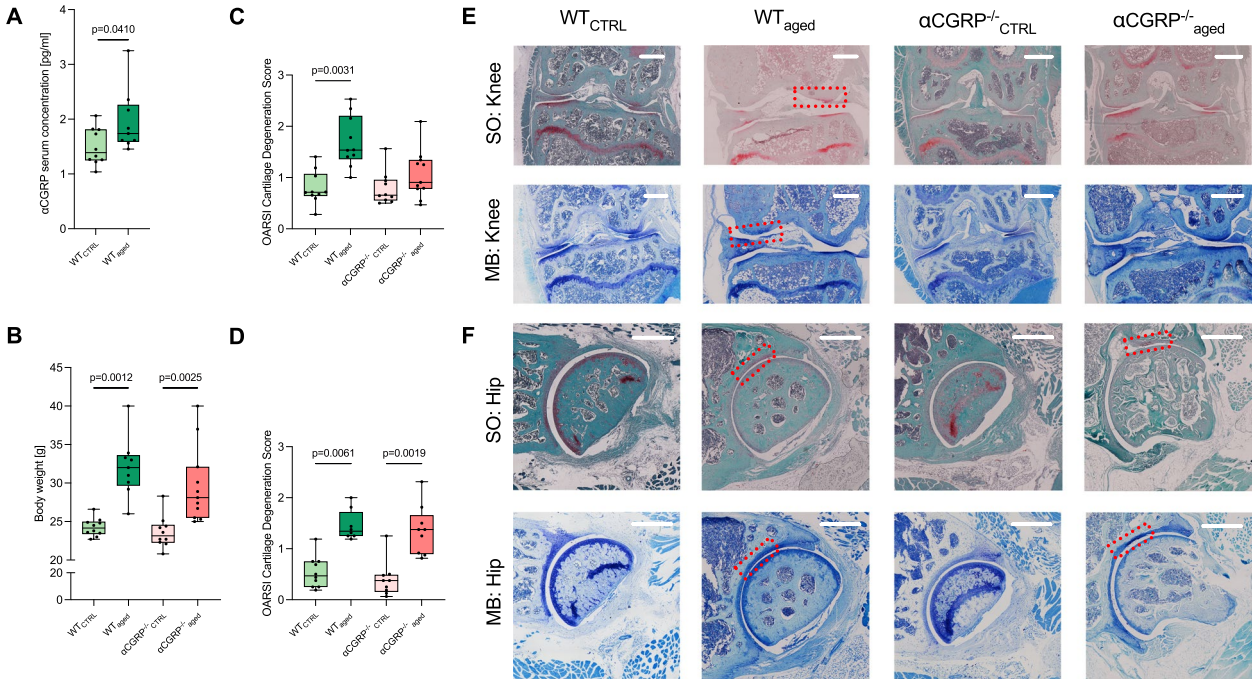

Fig. 1 Serum αCGRP is elevated in primary OA and knee but not hip joints of aged αCGRP-deficient mice are protected from cartilage degradation. **A** αCGRP serum concentrations and **B** body weight of indicated groups. **C** OARSI histopathological grading scores of knee and **D** hip joints of indicated 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 µm. Given values are median ± 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 $\alpha CGRP^{-/-}{}_{aged}$ animals, although not statistically significant ($p=0.867$). Further, no significant difference was seen between $\alpha CGRP^{-/-}{}_{aged}$ 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 $\alpha CGRP^{-/-}{}_{aged}$ ($p=0.0019$) when compared to CTRL animals (Fig. 1D, F). Cartilage loss was evident in both, the FH and the AC (data not shown).

αCGRP-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 αCGRP-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 αCGRP in primary OA

Expression of catabolic cartilage turnover markers *Mmp13* ($p=0.0028$) and *Adamts5* ($p=0.0062$), and bone resorption markers *Ctsk* ($p=0.0021$), *Tnfs11* (*Rankl*) ($p<0.0001$), and *Acp5* (*Trap*) ($p=0.0001$) were exclusively reduced in $\alpha CGRP^{-/-}{}_{aged}$ 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} $p=0.0021$; $\alpha CGRP^{-/-}{}_{aged}$ vs. $\alpha CGRP^{-/-}{}_{CTRL}$ $p<0.0001$) and *Acan* (WT_{aged} vs. WT_{CTRL} $p<0.0001$; $\alpha CGRP^{-/-}{}_{aged}$ vs. $\alpha CGRP^{-/-}{}_{CTRL}$ $p=0.0002$), however without differences between genotypes. The osteoblast transcription marker *Runx2* was exclusively reduced in $\alpha CGRP^{-/-}{}_{aged}$ mice compared to CTRLs ($p=0.0242$) (Fig. 2C).

αCGRP 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 µCT. 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 $\alpha CGRP^{-/-}{}_{aged}$ mice but not

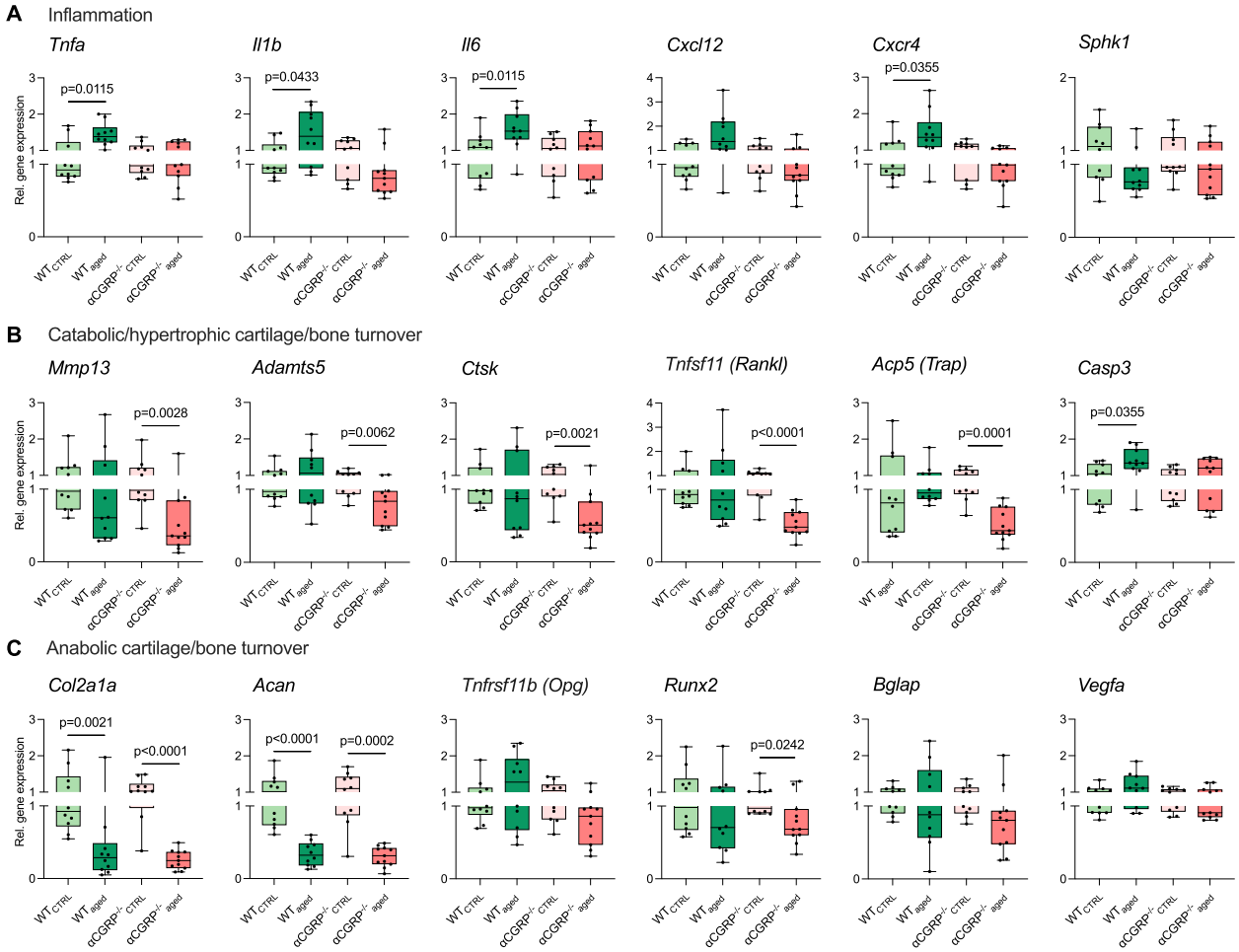

Fig. 2 αCGRP promotes intraarticular expression of pro-inflammatory and catabolic cartilage markers in OA-affected knee joints. **A** qRT-PCR gene expression analysis of inflammation markers, **B** catabolic/hypertrophic cartilage/bone turnover markers, and **C** anabolic cartilage/bone turnover markers 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).

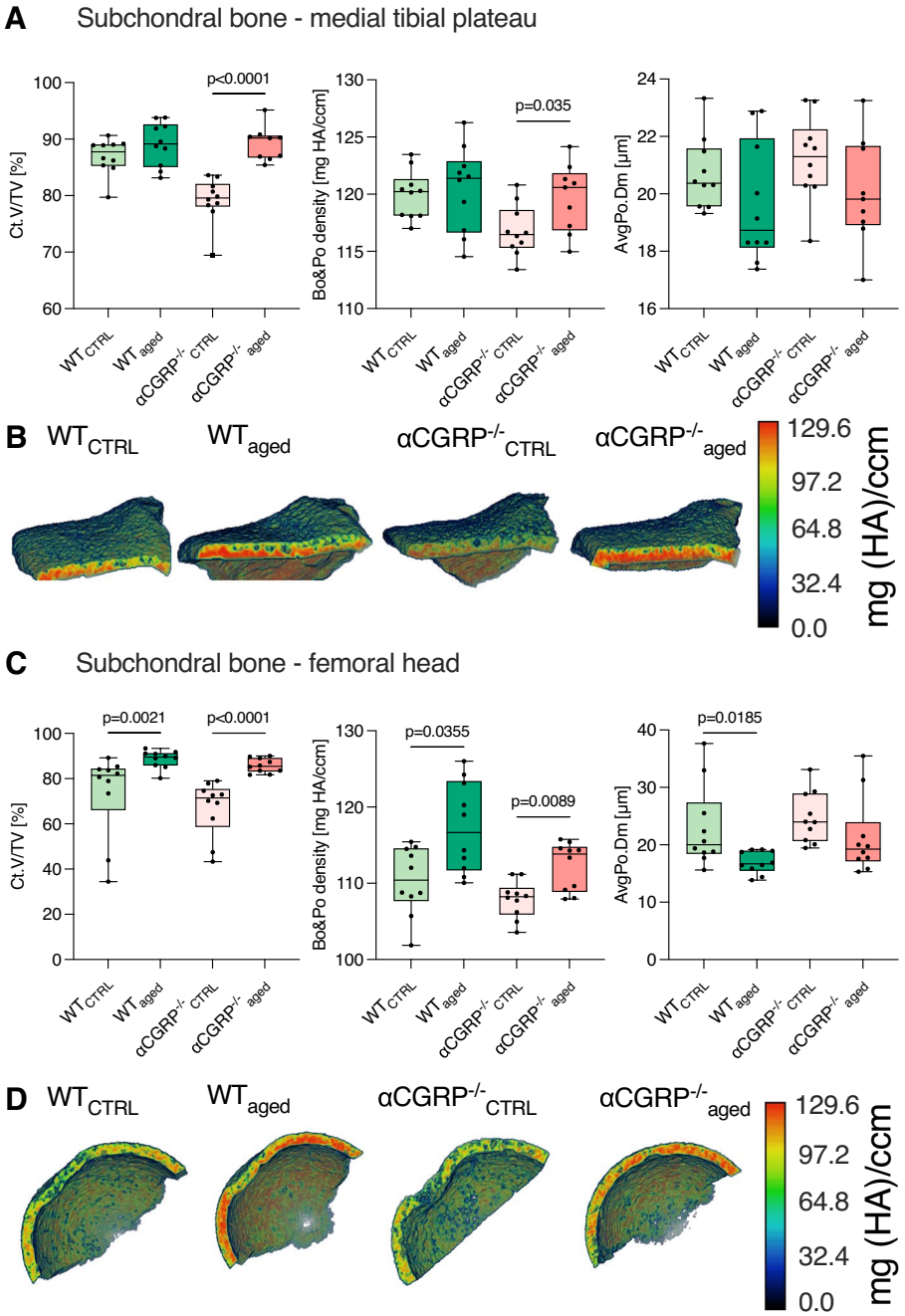

Fig. 3 αCGRP prevents the development of primary OA-related subchondral bone sclerosis in the medial knee joint. **A** µCT parameters of the subchondral bone plate of the MTP, and **B** representative 3D images of bone density of the MTP using color maps. **C** µCT parameters of the subchondral bone 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.

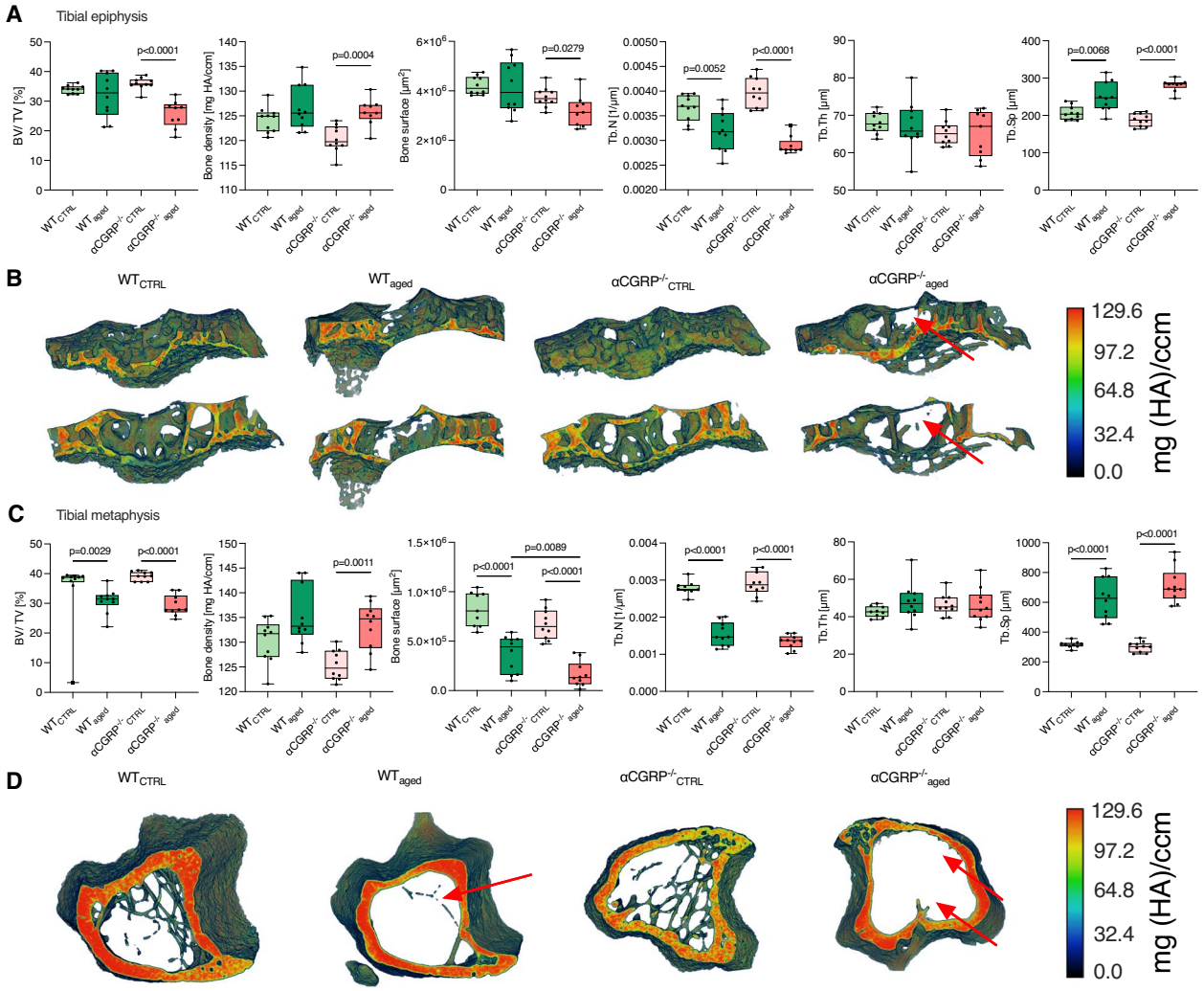

Fig. 4 αCGRP prevents radiological OA-related alterations in the tibial epi- and metaphysis. **A** µCT parameters of the tibial epiphysis, and **B** representative 3D images of bone density of the trabecular architecture of the tibial epiphysis using color maps (anterior and posterior sections). **C** 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 $\alpha CGRP^{-/-}{}_{aged}$ 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 (*Adamts5* and *Mmp13*) and decreases the expression of anabolic markers (*Col2a1*

and *Acan*) in cartilage matrix [33]. Elevated serum concentrations of TNFα 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 αCGRP-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 αCGRP 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 $\alpha CGRP^{-/-}{}_{aged}$ mice exclusively, while anabolic *Col2a1* and *Acan* were reduced in $\alpha CGRP^{-/-}{}_{aged}$ 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-κB 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 αCGRP deficiency led to a marked decrease of *Mmp13*, *Adamts5*, *Ctsk*, *Tnfsf11* (*Rankl*), and *Runx2*, which further underlines the catabolic role of αCGRP 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 αCGRP, suggesting a protection from catabolic and pro-inflammatory cartilage changes through an inactivation of αCGRP.

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, $\alpha CGRP^{-/-}{}_{aged}$ 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, αCGRP-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 αCGRP.

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, αCGRP-deficient OA mice exclusively exhibited reduced bone volume and surface with an increased bone density of the epi- and metaphysis. As $\alpha CGRP^{-/-}$ 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 αCGRP 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 αCGRP signaling alleviates naturally occurring OA in female mice.

Our results imply that the inhibition of αCGRP 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 αCGRP 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 αCGRP 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 αCGRP in the male organism.

Conclusions

In this study, we demonstrated that lifelong disruption of endogenous αCGRP-signaling protected animals from histological and molecular signs of cartilage degradation in primary, age-dependent OA. αCGRP serum levels were increased in primary OA and the inactivation of αCGRP impeded OA-associated overexpression of intraarticular inflammation and catabolic cartilage markers including IL1β, IL6, TNFα, MMP13, ADAMTS5, Cathepsin K, RANKL, RUNX2, and SDF-1/CXCR4 in joint tissues. In contrast, lack of αCGRP 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 αCGRP in naturally occurring OA.

Abbreviations

AC	Acetabulum
αCGRP	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-κB 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
μCT	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 μCT 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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