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Accelerated osteoarthritis in the temporomandibular joint of biglycan/fibromodulin double-deficient mice

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Summary

Objective: To investigate whether the absence of biglycan and fibromodulin, two proteoglycans expressed in cartilage, bone and tendon, resulted in accelerated osteoarthritis in the temporomandibular joint (TMJ).

Methods: Histological sections of TMJ from 3-, 6-, 9- and 18-month-old wild-type (WT) and biglycan/fibromodulin double-deficient (DKO) mice were compared. Immunostainings for biglycan, fibromodulin and proliferating cell nuclear antigen (PCNA) were performed.

Results: Biglycan and fibromodulin were highly expressed in the disc and articular cartilage of the TMJ. At 3 months of age, both WT and DKO presented early signs of cartilage degeneration visible as small acellular areas under the articular surfaces and superficial waving. From 6 months of age, DKOs developed accelerated osteoarthritis compared to WT. At 6 months, small vertical clefts in the condylar cartilage and partial disruption of the disk were visible in the DKO. In addition, chondrocytes had lost their regular columnar organization to form clusters. At 9 months, these differences were even more pronounced. At 18 months, extended cartilage erosion was visible in DKOs when by comparison the thickness of the articular cartilage in WT controls was basically intact. PCNA staining was stronger in 3-month-old WT TMJ fibrocartilage than in 3-month-old DKO TMJ fibrocartilage suggesting that chondrocyte proliferation might be impaired in DKOs.

Conclusion: The biglycan/fibromodulin double knock-out mouse constitutes a useful animal model to decipher the pathobiology of osteoarthritis in the TMJ.

Key words: Temporomandibular joint (TMJ), Biglycan, Fibromodulin, Proliferation, Osteoarthritis, Mice.

Introduction

Temporomandibular joint disorders (TMD) are comprised of a number (over 20) of pathologic conditions. Whether some of these conditions are part of the natural history of the same pathological process or each condition exists as a separate entity is unknown. One of the most prevalent conditions of TMD is osteoarthritis of the temporomandibular joint (TMJ OA)[1,2]. Currently, there are no biomarkers or treatments for TMJ OA. Hence, diagnosis of TMJ OA is made by imaging techniques only after there is irreversible damage to the joint. Due to the limited capacity of joints to regenerate, it would be beneficial if one could detect and treat TMJ OA at an early stage. Since it is practically impossible to obtain human pre-osteoarthritic TMJ samples, the generation of animal models, covering the whole disease process is necessary.

The TMJ is composed of two bones, the mandibular condyle and the glenoid fossa of the temporal bone, separated by a fibrocartilaginous disc, the TMJ disc. Unlike most diarthrodial joints in which the articular surfaces are covered by pure hyaline cartilage, the articulating surfaces of the bones are made of fibrocartilage[3]. Interspersed among these tissues are members of the small leucine repeat proteoglycan (SLRP) family. Members of this family are characterized by a small protein core that consists predominantly of the repetition of leucine-rich regions[4]. Four of them, biglycan (Bgn), fibromodulin (Fmod), lumican and decorin are found in bone, cartilage and tendons, have been shown to be able to bind transforming growth factor (TGF)-beta and play a role in regulating collagen fibrillogenesis (see Ameye and Young[5] for a review).

We have previously shown that mice double deficient in Bgn and Fmod have severe and premature knee OA, while mice single deficient in Bgn or Fmod have a milder form of it[6]. Mice single deficient in lumican and mice double deficient in lumican and fibromodulin have also been shown to develop premature knee OA but again more slowly than the mice double deficient in Bgn and Fmod[7]. Mice single deficient in decorin, in contrast to mice single deficient in Bgn, Fmod or lumican, do not develop OA. We have previously shown that knees from mice double deficient in Bgn and Fmod displayed a progressive degeneration of the articular cartilage from early fibrillation to complete erosion, subchondral sclerosis, osteophytes and bone cysts, the hallmarks of human OA[6]. In this study, we investigated

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whether the absence of Bgn and Fmod resulted in accelerated TMJ OA. We chose to use the Bgn/Fmod double-deficient mouse instead of the other SLRP deficient mice not only because they were available to us, but also because the more rapid progression of the disease in this mouse makes it a more convenient animal model to study. We found by histology, that mice that were deficient in both Bgn and Fmod have accelerated OA in their TMJ compared to control mice. We also characterized mandibular condylar cartilage proliferation and found that the double-deficient mice had a significant decrease in condylar chondrocyte proliferation compared to WT before TMJ OA was overt.

Methods and materials

GENERATION OF Bgn AND Fmod SINGLE AND DOUBLE-DEFICIENT MICE

All experiments were performed under an institutionally approved protocol for the use of animals in research (#NIDCR-IRP-98-058 and 01-151). Mice deficient in Bgn or Fmod were generated by gene targeting in embryonic stem cells as described previously[6]. Heterozygous Bgn/Fmod-deficient mice were produced by breeding a homozygous Bgn-deficient female (*Bgn−/−/Fmod+/+*) with an Fmod-deficient male (*Bgn+/0/Fmod−/−*); Bgn males are designed as Bgn−/0 since the Bgn gene is located on the X chromosome and absent from the Y chromosome. F2 Bgn/Fmod double-deficient (male *Bgn−/0/Fmod−/−* and female *Bgn−/−/Fmod−/−*) mice were obtained by interbreeding F1 heterozygous Bgn/Fmod mice and will be referred to hereafter as Bgn/Fmod DKO mice.

GENOTYPING

All mice were genotyped for Bgn and Fmod alleles by polymerase chain reaction (PCR) analysis as described previously[6]. PCR products were resolved by electrophoresis through 1.8% agarose gels, yielding bands of 212 bp for the WT Bgn allele, 310 bp for the disrupted Bgn allele, 280 bp for the WT Fmod allele, and 603 bp for the disrupted Fmod allele.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

For each genotype and age, heads from at least three animals were dissected in two halves. After removal of the brain, the specimens were fixed for 2 weeks at room temperature in 10% formalin. After being washed in tap water for 5 min, they were decalcified in formic acid bone decalcifier solution (Immunocal from Decal Corporation, Tallman, NY, USA) for 4 weeks. Specimens were then washed in tap water for 5 min and fixed for 3 days in buffered zinc formalin (Z-fix from Anatech Ltd, Battle Creek, MI, USA) before being classically processed for histology and sectioned sagittally. Sections were stained by hematoxylin and eosin (H&E) or by Safranin O[8].

Tissue sections were deparaffinized with xylene. Following rehydration, with graded ethanol, endogenous peroxidase activity was blocked by treating the sections in 3% peroxide in methanol for 20 min. In order to expose the antigen, sections were predigested in chondroitinase ABC (cat# KE01502, Seikagaku Corp, Tokyo, Japan) at a concentration of 0.015 units/ml for 1 h at 37°C. Non-specific binding was reduced by incubating the sections in 10% goat serum for 30 min. Sections were then incubated for 1 h at 37°C with primary antibody at a 1: 200 dilution. Fibromodulin and biglycan antibodies (LF 150 and LF 106, respectively) were kind gifts from Larry Fisher[9], whereas proliferating cell nuclear antigen (PCNA) antibody clone PC 10 was purchased from DAKO Cytomation (Carpinteria, CA, USA). Biotinylated goat anti-rabbit secondary antibody was used and visualized by a streptavidin–peroxidase solution in presence of AEC chromagen.

The data presented in this paper were reproduced in at least three different animals of the same genotype at each age. Serial sections through the whole joints were obtained for each animal and observations were confirmed in different interspaced serial sections chosen to cover the whole joint. In this way, it was ensured that the reported observations were genuine and not local random abnormalities. In order to minimize experimental variability, WT and DKO were processed in parallel at the same time for histology and immunohistostainings.

EVALUATION OF PCNA STAINING

Images from sections immunostained with PCNA were captured using an AxioCam MR camera (Zeiss). A rectangular box was constructed to evaluate the percentage of PCNA positive cells in the mandibular condylar cartilage. The number of PCNA positive cells were counted and divided by the total number of cells within the box. For each section, three areas were analyzed, corresponding to anterior, center and posterior portions of the mandibular condylar cartilage. Eleven sections from three different 3-month-old DKO mice and 10 sections from three different 3-month-old WT mice were evaluated.

Results

Immunohistochemistry revealed that Bgn and Fmod were abundantly expressed within the TMJ of 3-month-old WT mice (Fig. 1). Both were found in the disc, and in the articular cartilage of the mandibular condyle. In the disc, fibromodulin was found in the anterior and posterior attachment parts, while biglycan was found predominantly in the anterior attachment portion of the disk. Regarding their expression in the articular cartilages of the glenoid fossa and the mandibular condyle, both proteoglycans were more highly expressed in the anterior and posterior parts of these cartilages than in the central parts [Fig. 1(a and b)]. At the same time, they were also more highly expressed in the superficial zone than in the deeper zones. This pattern of differential expression was more marked for Bgn than for Fmod: Bgn was not expressed in the central parts and in the deepest zones of these articular cartilages, while Fmod was expressed although at a relatively low level [Fig. 1(c and d)]. As the mice aged, the localization of Bgn and Fmod remained the same; however, there was an overall decrease in the intensity of their immunohistochemical staining (data not shown).

In order to examine the function of Bgn and Fmod in the TMJ, the morphology of TMJ from WT and double-deficient mice was compared at 3, 6, 9 and 18 months of age. At 3 months, early and similar signs of degeneration were present in the articular cartilage of the mandibular condyle from deficient and WT TMJs. These defects consisted of the presence of small acellular areas under the articular surfaces and superficial waving (Fig. 2). While little differences were apparent at 3 months of age between the WT and deficient TMJs, they became obvious at 6 months of age due to the accelerated degeneration of the articular

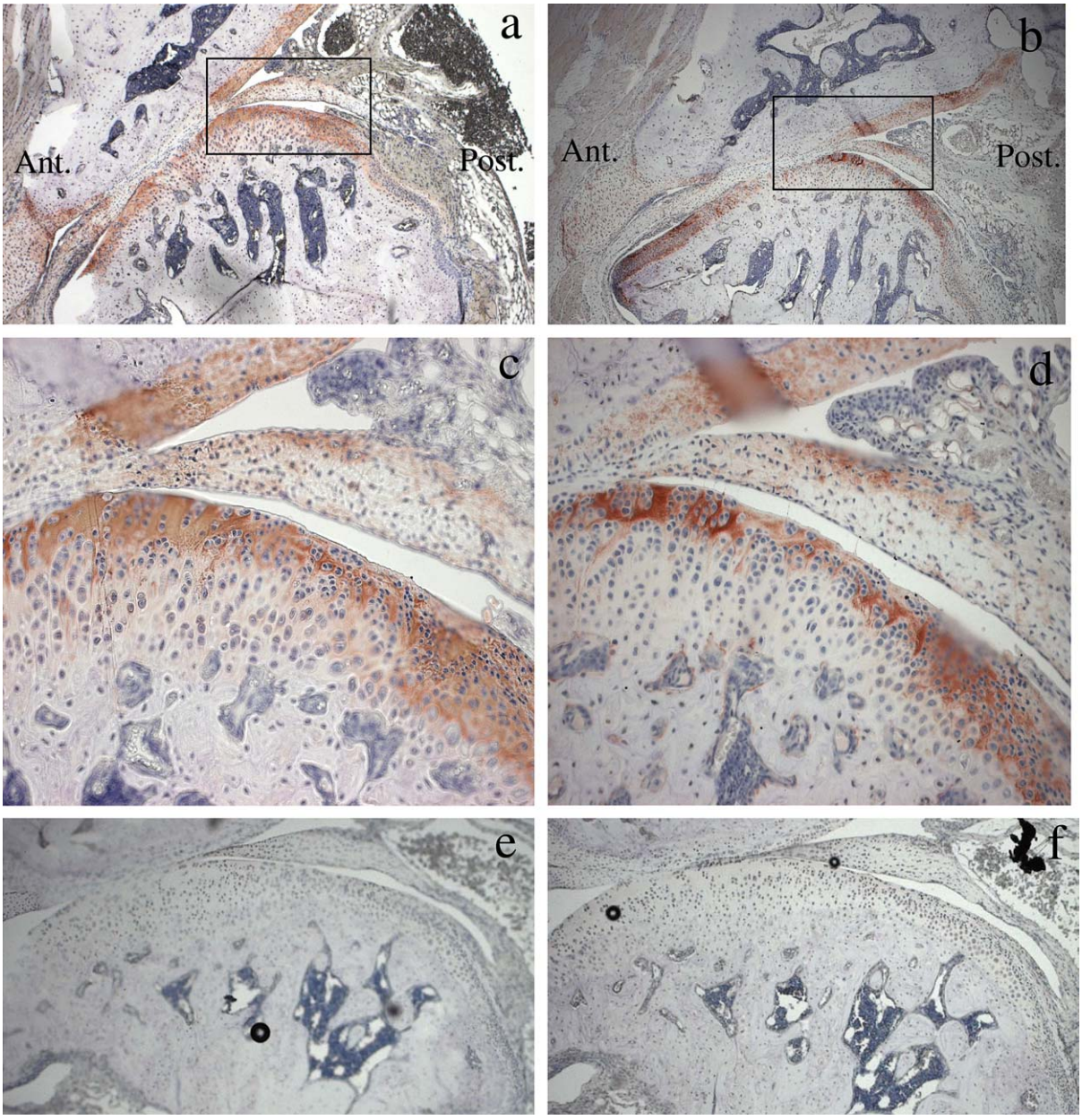

Fig. 1. (a) Immunohistochemistry of fibromodulin (Fmod) performed on sagittal sections of the TMJ area of WT 3-month-old mice; 5× magnification. (b) Immunohistochemistry of biglycan (Bgn) performed on the sagittal sections of the TMJ area from 3-month-old WT mice; 5× magnification. (c) 20× magnification of boxed area shown in panel a. (d) 20× magnification of boxed area shown in panel b. (e and f). Negative controls picturing immunohistostaining of Fmod (e) and Bgn (f) on sagittal sections of the TMJ area from 3-month-old Bgn/Fmod DKO mice.

cartilages in the deficient TMJ. At this age in the Bgn/Fmod DKO, partial disruption of the disk was sometimes observed while in the articular cartilage of the condyle, small vertical clefts were visible and chondrocytes started to lose their regular columnar organization and form clusters [Fig. 3(d)]. At 9 months, the vertical clefts and chondrocyte clusters became more severe in the condylar cartilage of the Bgn/Fmod DKO mice, while in the WT there was no evidence of either of these two characteristics (Fig. 4). Moreover, while quite extensive acellular areas were present in the deficient articular cartilage, such areas remained small in the WT articular cartilage [see Fig. 4(c and d), for comparison]. By 18 months, there was almost complete destruction of the articular cartilage of the condyle in the DKO mice [Fig. 5(b, d and f)]. The erosion on the articular surface of the condyle was so extended that it resulted in a significant decrease in cartilage thickness [see Fig. 5(a–b and e–f) for comparison]. In addition, the chondrocytes had completely lost their vertical columnar organization. Finally, partial disruption of the disc was often observed [Fig. 5(b and d)]. In contrast, in the WT, the articular cartilage displayed some small clefts, but retained more or less the normal columnar chondrocyte organization [Fig. 5(a, c and e)].

In parallel to the articular cartilage degeneration, osteophyte formation was observed in the double-deficient mice [Fig. 6(b and d)] but not in the WT [Fig. 6(a and c)].

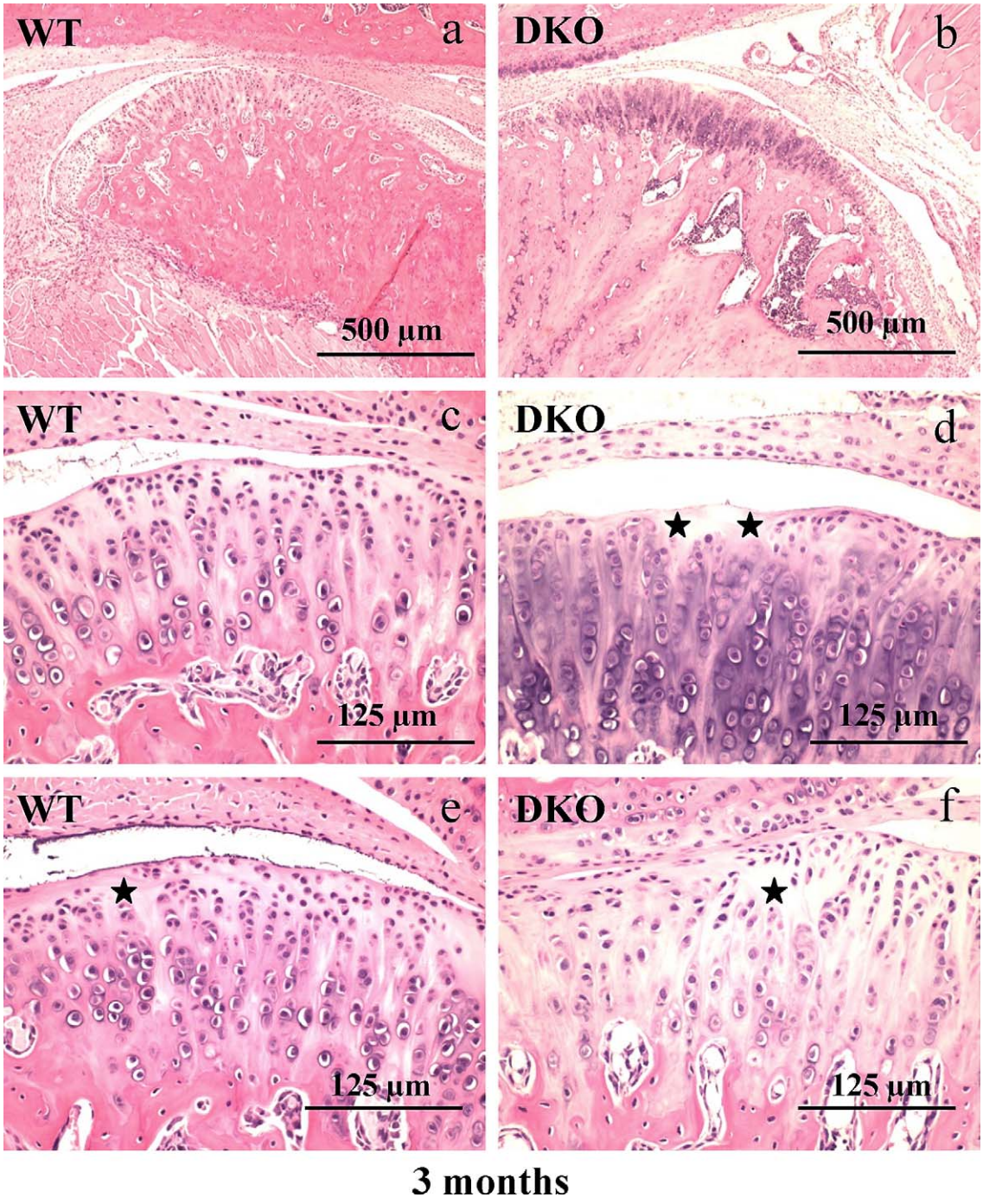

Fig. 2. Sections of the TMJ from 3-month-old WT (a, c and e) or Bgn/Fmod DKO mice (b, d and f) stained with hematoxylin and eosin (H&E). The asterisk shows the small acellular areas under the articular surfaces. Note the waving of the articular cartilage surface in (d–f).

Osteophytes started to form on the mandibular condyle and the glenoid fossa of the temporal bone from 6 months of age. They were well developed by 18 months of age, particularly on the posterior side of the condyle [Fig. 6(b or d)]. High inter-individual variability in the thickness of the subchondral bone did not allow us to determine whether or not subchondral sclerosis was more pronounced or even present in the double-deficient mice compared to the WT.

To further characterize the development of TMJ OA in the double-deficient mice compared to WT, TMJ sections were stained with Safranin O. Safranin O is a cationic dye that binds to the negatively charged glycosaminoglycans. At 18 months in the DKO mice but not in the WT, some of the fibrocartilaginous cells of the temporomandibular disc changed phenotype and started to synthesize glycosaminoglycans as suggested by their pericellular staining with Safranin O [Fig. 6(f)].

We next examined if we could identify any defects in cellular proliferation in the condylar articular cartilage of the double-deficient mice. Immunohistochemistry against PCNA (a measure of proliferation) revealed that there was

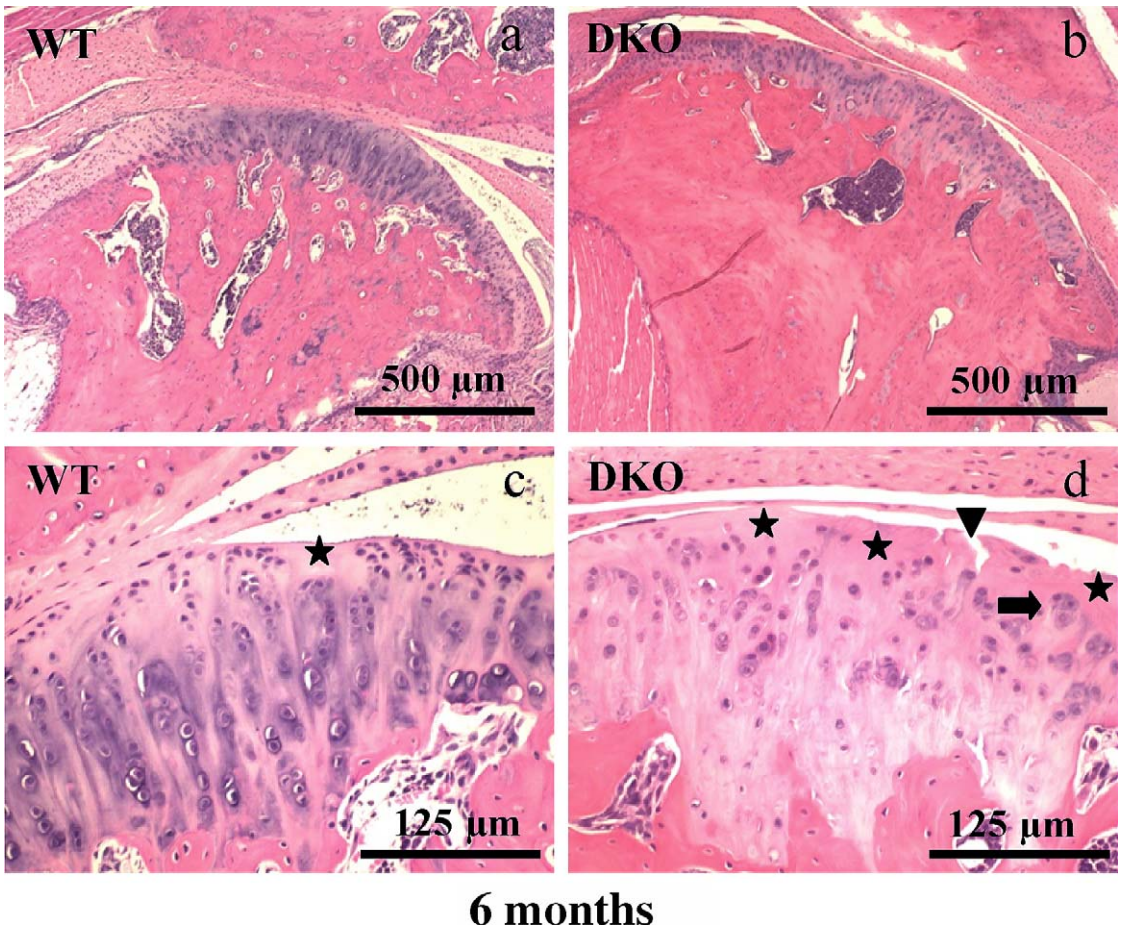

Fig. 3. Sections of the TMJ from 6-month-old WT (a and c) or Bgn/Fmod DKO mice (b and d) stained with hematoxylin and eosin (H&E). The arrowhead points to small vertical clefts, the asterisks show the small acellular areas under the articular surfaces and the arrow shows regions where chondrocytes lose their regular columnar organization and form clusters.

a significant decrease in the number of positive cells in the condylar cartilage of 3-month-old DKO compared to WT [Fig. 7(d)]. This decrease in proliferation occurred before the double-deficient mice showed any histological evidence of accelerated TMJ OA. However, once the double-deficient mice had developed signs of accelerated TMJ OA (6-month-old), PCNA staining revealed a high number of positive cells in the chondrocyte clusters of their condylar cartilage [Fig. 7(f)].

Discussion

The majority of animal models for TMJ OA involve chemical or physical trauma to the joint. They include the local application of Freud's adjuvant[10], the application of carrageenan[11], forced bite opening[12] and surgical manipulation of the TMJ structures[13–16]. Unlike traumatic models of TMJ OA, genetically modified animal models provide information on why certain populations are more likely to develop the disease. Because both genetic and environmental factors may be precisely defined and controlled, genetic mouse models hold the potential to reveal the molecular pathways that influence the degeneration of the TMJ. Besides our model, there are four other reported genetic mouse models of TMJ OA[17–20]. Two of the models have collagen mutations: the transgenic Del1 mouse, which has mutations in the Col2a1 gene, and the transgenic cho mouse, which has a mutation in the alpha1 chain of type collagen XI gene[19,20]. The fourth genetic model is the Institute for Cancer Research mouse strain whose genetic etiology for the development of OA is unknown. Thus, animal models with alterations in Bgn, Fmod, TNF-alpha, collagen type II and collagen type XI expression develop TMJ OA. This suggests that TMJ OA could be a genetically inherited disease although twin studies argue against this hypothesis by showing no difference in the prevalence of TMJ disorders between monozygotic twins and dizygotic twins[21,22].

In the articular hyaline cartilage, OA increases the expression levels of Bgn and Fmod[23–25] whereas in the meniscal fibrocartilage, OA increases Bgn expression, but decreases Fmod expression[26]. Therefore, OA seems to affect Fmod expression differently depending on the type of cartilage. In the TMJ specifically, Bgn had been shown to be expressed in the disk and in the fibrocartilage of the mandibular condyle[27–29] but this is the first report, that we are aware of, describing the expression of Fmod in the TMJ.

We have previously shown that mice double deficient in Bgn and Fmod had severe and premature knee OA[6]. In this study, we found that these mice also develop premature TMJ OA although the osteoarthritic changes in the knee

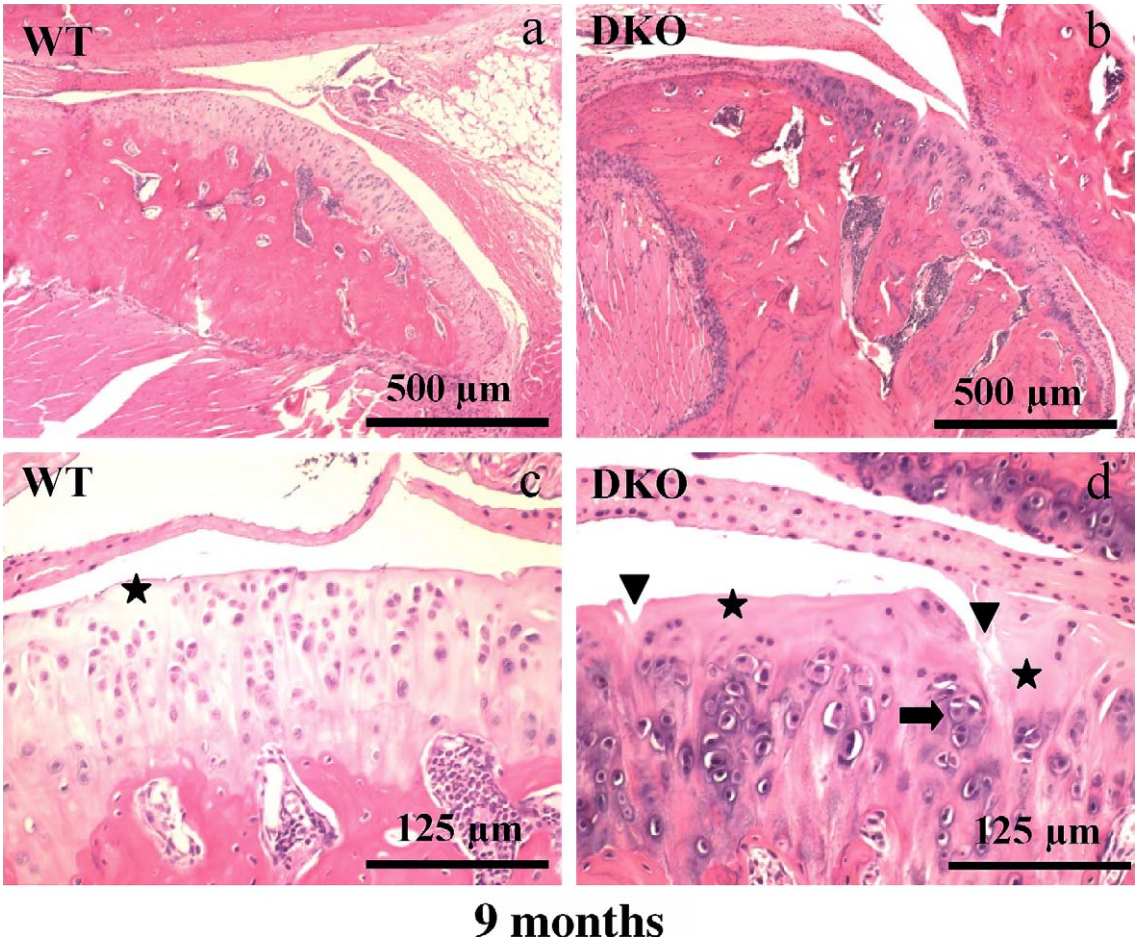

Fig. 4. Sections of the TMJ from 9-month-old WT (a and c) or Bgn/Fmod DKO mice (b and d) stained with hematoxylin and eosin (H&E). The arrowheads show vertical clefts, the asterisks show the small acellular areas under the articular surfaces and the arrow shows regions where chondrocytes lose their regular columnar organization and form clusters.

occurred more rapidly than in the TMJ. In the knee, there were no osteoarthritic lesions detected in 1-month-old double-mutant mice. However, by 6 months, there was almost complete erosion of the articular cartilage. In contrast, in the TMJ at 6 months, only early histological evidence of osteoarthritic lesions started to become visible. In the knee, ectopic ossification of tendons and ligaments was detected before significant cartilage lesions develop[6]. In contrast, we did not detect any ectopic ossification in the TMJ although the chondrocytic-like phenotype displayed by some fibrocartilageneous cells of the disc at 18 months, as evidenced by Safranin O staining, is reminiscent of the early stages of formation of ectopic bones as observed in the tendons and ligaments of knees from Bgn and Fmod double-deficient mice.

The primary purpose of this study was to investigate if the absence of Bgn and Fmod resulted in accelerated TMJ OA. Originally, after establishing the timing of progression of TMJ OA in the double-deficient mice, our plan was to continue with the study of TMJ OA in the two single deficient mice to determine to which extent each single deficiency contributes to the phenotype. However, we later changed our mind in view of the slow progression of the disease in the TMJ and of its relative mildness compared to the knee. Based on our former knee observations in these single and double-deficient mice, it is probable that the Bgn and Fmod single deficient mice develop a very mild form of TMJ OA, similar to the one displayed by the WT. Indeed, we have shown that the single deficient Bgn and the single deficient Fmod mice develop a milder form of knee OA than the double-deficient mice[6]. We were able to detect significant differences in a semi-quantitative grading score for knee OA at 3 months for the Bgn-deficient mice and at 9 months for the Fmod-deficient mice compared to the knee of WT mice. However, unlike the TMJ, the knee of 9-month-old WT mice shows no signs of OA[6], a characteristic, which helped to clearly demonstrate the presence of OA in the single deficient mice. In the presence of TMJ OA in the WT and in view of the slow progression of TMJ OA in the double-deficient mouse, it is probable that even by 2 years of age, the difference in severity of TMJ OA between the WT and the two single deficient mice will be too small to clearly determine to which extent each deficiency contributes to the TMJ phenotype of the double-deficient mouse. Hence, we can only extrapolate that similarly to what happens for knee OA, each deficiency partially contributes to the accelerated TMJ OA detected in the Bgn/Fmod double-deficient mice.

The mechanisms by which the absence of Bgn and Fmod accelerates the development of OA are currently unknown. One possible mechanism is that the absence of Bgn and Fmod would make the joint structures less suited to withstanding mechanical loading and therefore more prone

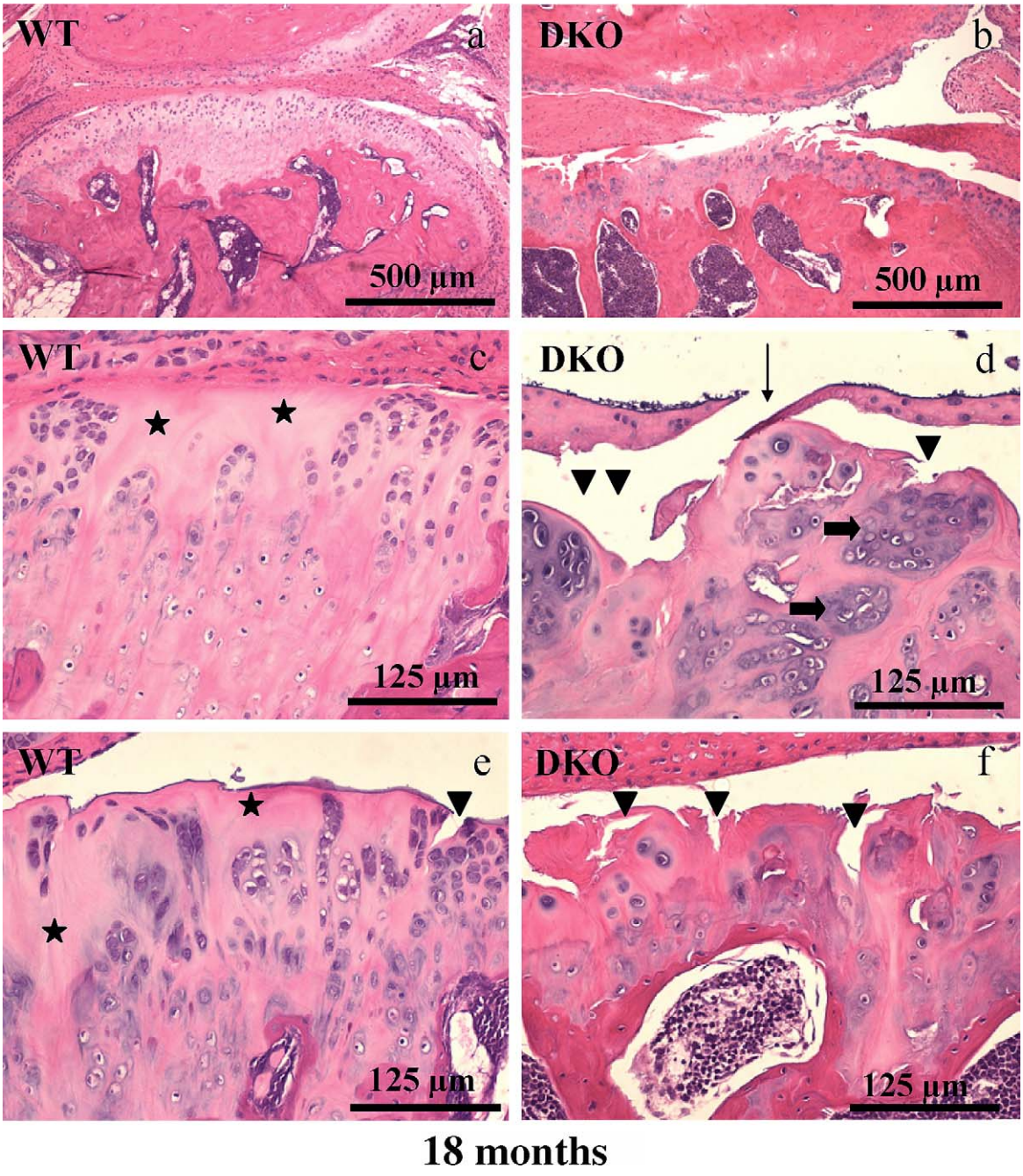

Fig. 5. Sections of the TMJ from 18-month-old WT (a, c and e) Bgn/Fmod DKO (b, d and e) mice stained with hematoxylin and eosin (H&E). The arrowheads point to the extensive destruction of the articular cartilage in the condyle of the Bgn/Fmod DKO mice. Asterisks show acellular areas. Large arrows point to large chondrocyte clusters. Thin arrow shows disc disruption.

to osteoarthritic lesions. In support of this, a recent study has shown that the viscoelastic properties of the temporomandibular disc decrease with the reduction of proteoglycan content[30]. Structurally weak tendons were also suggested to cause knee OA in the Bgn/Fmod double-deficient mice because gait problems, as well as structurally and mechanically compromised tendons, were present before OA started to develop[6]. A similar mechanism could also take place in the TMJ. Finally, it is also possible that OA arises indirectly from changes in the underlying bone. Mice deficient in Bgn have been shown to have age-dependent osteoporosis[31].

Changes in the biomechanical properties of cartilage, tendons and bone could arise from the ability of Bgn and Fmod to regulate collagen fibrillogenesis (see Ameye and Young[5] for review). It has been shown that the distribution in size of the collagen fibrils in the quadriceps tendon of the Bgn deficient, Fmod-deficient and Bgn/Fmod double deficient are different compared to the tendons from WT mice[6]. Alternatively or additionally, changes in these tissues could also

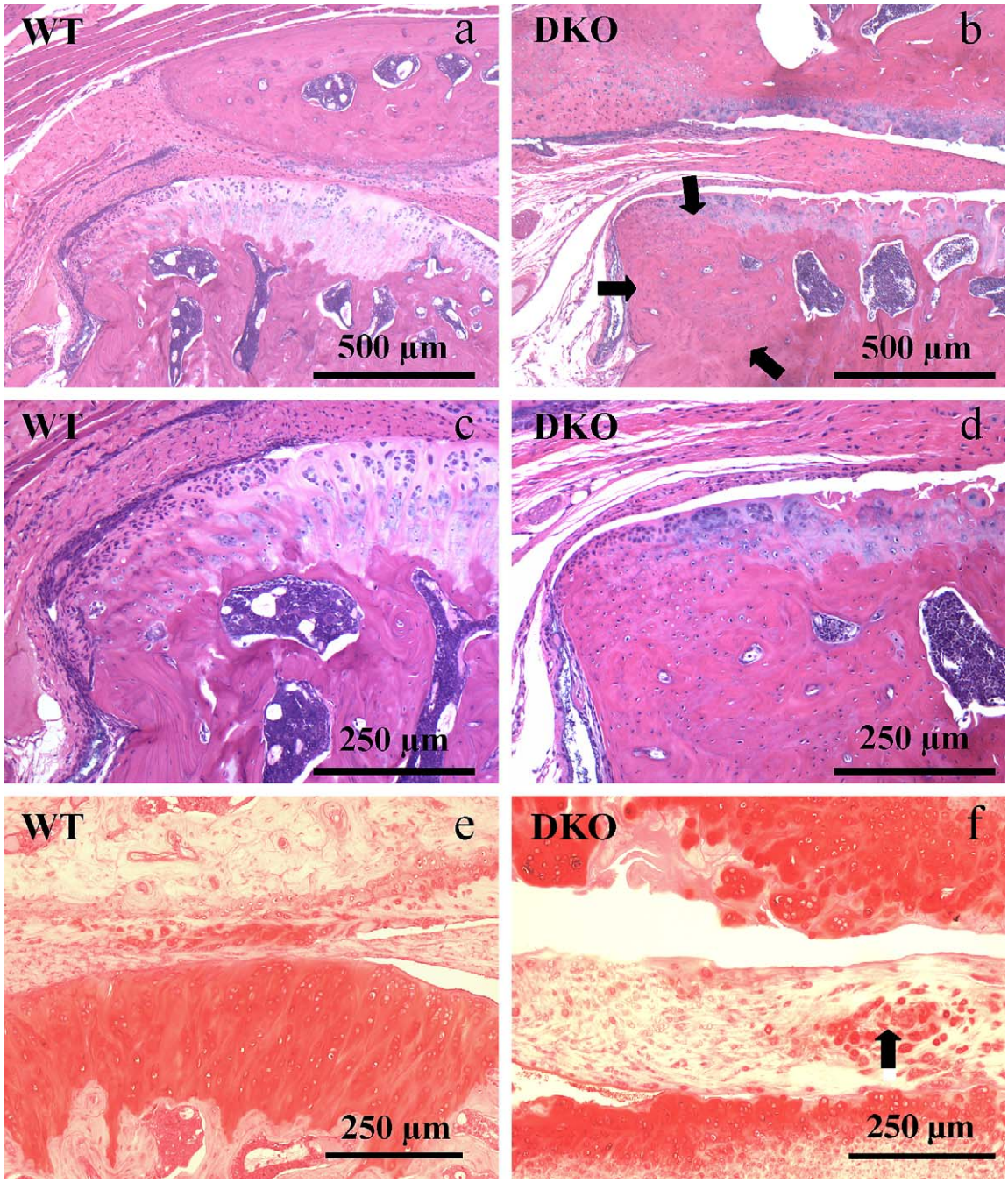

Fig. 6. (a–b) Eighteen-month-old Bgn/Fmod DKO mice (b) displays osteophytes (arrows) contrary to 18-month-old WT mice (a). (c–d) Higher magnification of (a) and (b), picturing the posterior part of the mandibular condyle. (e–f) Safranin O staining of TMJ sections from 18-month-old WT (e) and Bgn/Fmod DKO (f) mice. Arrows show the strong pericellular staining present around some of the cells of the disc.

result from the abilities of Bgn and Fmod to modulate the actions of members of the TGF-beta superfamily with their receptors. On one hand, Bgn and Fmod can both bind to members of the TGF-beta family[32] and may regulate their activity by sequestering TGF-beta family members into the extracellular matrix[32], thereby inhibiting their interactions with their cellular receptors. On the other hand, Bgn has been shown to improve the presentation of bone morphogenetic protein 4 (BMP-4) to its receptor in bone marrow stromal cells[33]. Through such interactions with members of the TGF-beta family or with cell receptors, small leucine-rich proteoglycans are known to participate in the regulation of cell proliferation (see Kresse and Schonherr[34], for a review). For example, overexpression of biglycan causes an increase in the proliferation of vascular smooth muscle cells[35] while its deficiency decreases the proliferation of bone marrow stroma cells[36]. Biglycan has a nuclear localization sequence and has been found in the nuclei of neuronal cells where it has been suggested to be involved in cell proliferation[37]. In this context, we report here a decrease in chondrocyte

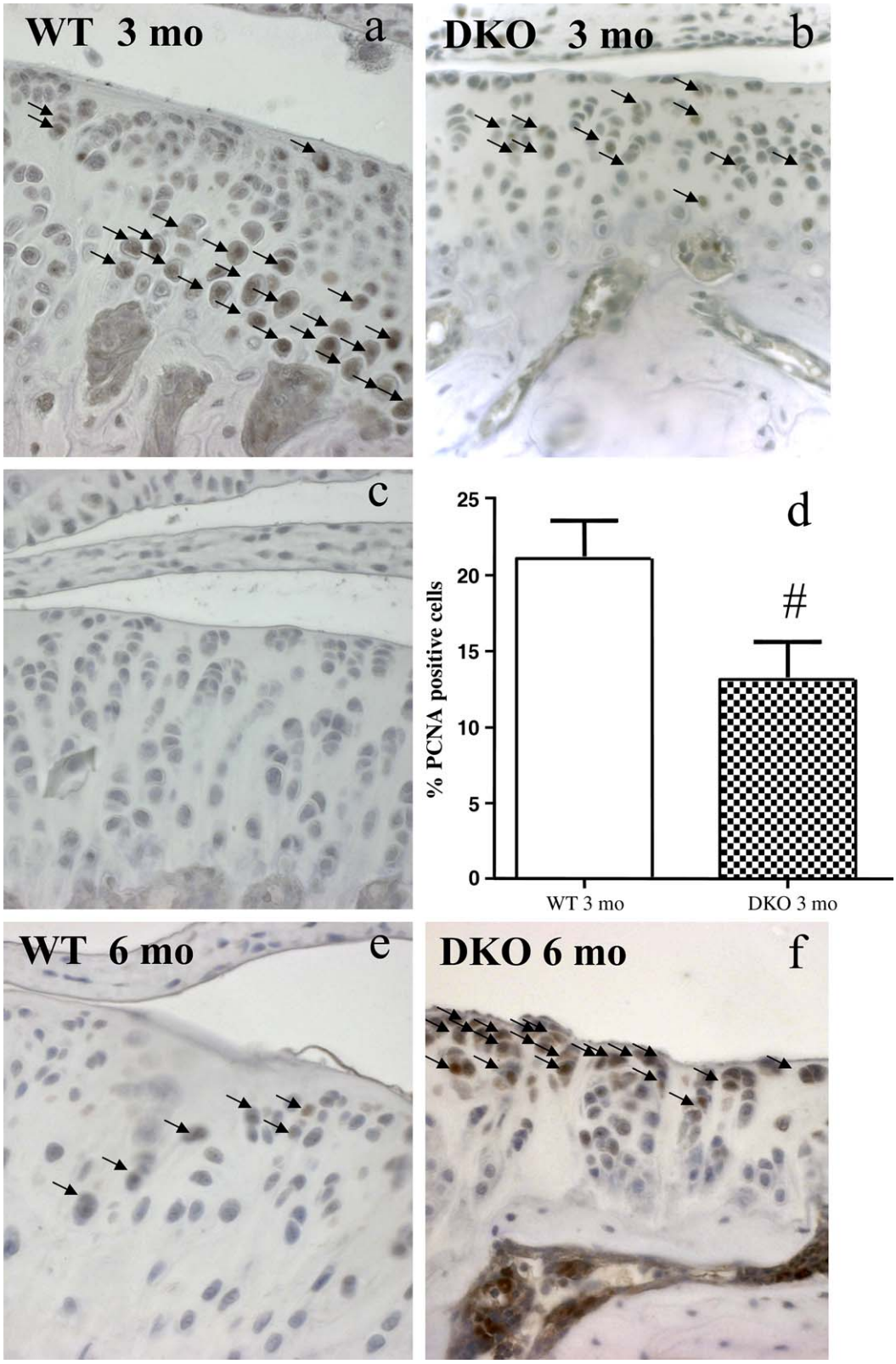

Fig. 7. Immunohistochemistry of proliferating cellular nuclear antigen (PCNA) performed on sagittal sections of the TMJ area of WT (a, c and e) and Bgn/Fmod DKO (b and f) mice at the age of 3 months (a, b, c) or 6 months (e, f). (c) Negative control where primary PCNA antibody was omitted on the TMJ area of 3-month-old WT mice. Arrows indicate positive PCNA staining. Panel d shows histogram representing the percentage of PCNA positive cells in mandibular condylar cartilage from three WT (white bar) and three DKO (checkered bar) 3-month-old mice. Three to four slides were taken from each animal. #$P < 0.05$.

proliferation in the double-deficient cartilage before overt osteoarthritic lesions developed suggesting that the impairment in chondrocyte proliferation induced by the Bgn and Fmod deficiency could be a key event in the early development of TMJ OA in this animal model.

A similar decrease in chondrocyte proliferation has been shown in a diminished mechanical loading model of the TMJ. In this model, the experimental group was fed a soft diet and the incisors were shortened regularly to keep them out of occlusion, while the controls were fed a hard diet. The experimental group had reduced chondrocyte proliferation after 12–24 h and it occurred before there was any evidence of cartilage breakdown (as measured by matrix metalloproteinase staining), which appeared only after 9 days[38]. Similar to what has been described in other models of TMJ OA, there was an increase in cell proliferation observed in the chondrocyte clusters of double-deficient condylar cartilage after there was overt OA, which may be part of an attempted repair response[13,39].

In conclusion, this report describes a new animal model for TMJ OA and confirms the important role that is played by small leucine-rich proteoglycans in the biological homeostasis of joints in general and in the pathobiology of OA in particular. The observation that cell proliferation in the articular cartilage is decreased at the onset of OA in the absence of Bgn and Fmod creates exciting new opportunities of investigation to further decipher the functions of small leucine-rich proteoglycans in the biology of articular cartilage.

References

1. Israel HA, Diamond B, Saed-Nejad F, Ratcliffe A. Osteoarthritis and synovitis as major pathoses of the temporomandibular joint: comparison of clinical diagnosis with arthroscopic morphology. J Oral Maxillofac Surg 1998;56(9):1023–7; discussion 28.
2. Zarb GA, Carlsson GE. Temporomandibular disorders: osteoarthritis. J Orofac Pain 1999;13(4):295–306.
3. Benjamin M, Ralphs JR. Biology of fibrocartilage cells. Int Rev Cytol 2004;233:1–45.
4. Hocking AM, Shinomura T, McQuillan DJ. Leucine-rich repeat glycoproteins of the extracellular matrix. Matrix Biol 1998;17(1):1–19.
5. Ameye L, Young MF. Mice deficient in small leucine-rich proteoglycans: novel *in vivo* models for osteoporosis, osteoarthritis, Ehlers–Danlos syndrome, muscular dystrophy, and corneal diseases. Glycobiology 2002; 12(9):107R–16R.
6. Ameye L, Aria D, Jepsen K, Oldberg A, Xu T, Young MF. Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. FASEB J 2002; 16(7):673–80.
7. Jepsen KJ, Wu F, Peragallo JH, Paul J, Roberts L, Ezura Y, *et al*. A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. J Biol Chem 2002;277(38):35532–40.
8. Kiraly K, Lammi M, Arokoski J, Lapvetelainen T, Tammi M, Helminen H, *et al*. Safranin O reduces loss of glycosaminoglycans from bovine articular cartilage during histological specimen preparation. Histochem J 1996;28(2):99–107.
9. Fisher LW, Stubbs JT, Young MF. Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. Acta Orthop Scand Suppl 1995;266(66):61–5.
10. Harper RP, Kerins CA, McIntosh JE, Spears R, Bellinger LL. Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant. Osteoarthritis Cartilage 2001;9(7): 619–24.
11. Lundeberg T, Alstergren P, Appelgren A, Appelgren B, Carleson J, Kopp S, *et al*. A model for experimentally induced temperomandibular joint arthritis in rats: effects of carrageenan on neuropeptide-like immunoreactivity. Neuropeptides 1996;30(1):37–41.
12. Fujisawa T, Kuboki T, Kasai T, Sonoyama W, Kojima S, Uehara J, *et al*. A repetitive, steady mouth opening induced an osteoarthritis-like lesion in the rabbit temporomandibular joint. J Dent Res 2003;82(9): 731–5.
13. Axelsson S, Holmlund A, Hjerpe A. An experimental model of osteoarthrosis in the temporomandibular joint of the rabbit. Acta Odontol Scand 1992;50(5):273–80.
14. Imai H, Sakamoto I, Yoda T, Yamashita Y. A model for internal derangement and osteoarthritis of the temporomandibular joint with experimental traction of the mandibular ramus in rabbit. Oral Dis 2001;7(3): 185–91.
15. Kubota Y, Takatsuka S, Nakagawa K, Yamamoto E. A model for temporomandibular joint disc repositioning surgery. J Oral Maxillofac Surg 2001;59(12):1443–51.
16. Tominaga K, Hirashima S, Fukuda J. An experimental model of osteoarthrosis of the temporomandibular joint in monkeys. Br J Oral Maxillofac Surg 2002;40(3): 232–7.
17. Puzas JE, Landeau JM, Tallents R, Albright J, Schwarz EM, Landesberg R. Degradative pathways in tissues of the temporomandibular joint. Use of *in vitro* and *in vivo* models to characterize matrix metalloproteinase and cytokine activity. Cells Tissues Organs 2001; 169(3):248–56.
18. Silbermann M, Livne E. Age-related degenerative changes in the mouse mandibular joint. J Anat 1979; 129(3):507–20.
19. Rintala M, Metsaranta M, Saamanen AM, Vuorio E, Ronning O. Abnormal craniofacial growth and early mandibular osteoarthritis in mice harbouring a mutant type II collagen transgene. J Anat 1997;190(Pt 2): 201–8.
20. Xu L, Flahiff CM, Waldman BA, Wu D, Olsen BR, Setton LA, *et al*. Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho). Arthritis Rheum 2003;48(9):2509–18.
21. Michalowicz BS, Pihlstrom BL, Hodges JS, Bouchard TJ Jr. No heritability of temporomandibular joint signs and symptoms. J Dent Res 2000;79(8):1573–8.
22. Heiberg A, Heloe B, Heiberg AN, Heloe LA, Magnus P, Berg K, *et al*. Myofascial pain dysfunction (MPD) syndrome in twins. Community Dent Oral Epidemiol 1980;8(8):434–6.
23. Bock HC, Michaeli P, Bode C, Schultz W, Kresse H, Herken R, *et al*. The small proteoglycans decorin and biglycan in human articular cartilage of late-stage osteoarthritis. Osteoarthritis Cartilage 2001;9(7): 654–63.
24. Liu W, Burton-Wurster N, Glant TT, Tashman S, Sumner DR, Kamath RV, *et al*. Spontaneous and

experimental osteoarthritis in dog: similarities and differences in proteoglycan levels. J Orthop Res 2003;21(4):730–7.
25. Dourado GS, Adams ME, Matyas JR, Huang D. Expression of biglycan, decorin and fibromodulin in the hypertrophic phase of experimental osteoarthritis. Osteoarthritis Cartilage 1996;4(3):187–96.
26. Hellio Le Graverand MP, Vignon E, Otterness IG, Hart DA. Early changes in lapine menisci during osteoarthritis development: Part II: molecular alterations. Osteoarthritis Cartilage 2001;9(1):65–72.
27. Scott PG, Nakano T, Dodd CM. Small proteoglycans from different regions of the fibrocartilaginous temporomandibular joint disc. Biochim Biophys Acta 1995; 1244(1):121–8.
28. Mizoguchi I, Scott PG, Dodd CM, Rahemtulla F, Sasano Y, Kuwabara M, *et al*. An immunohistochemical study of the localization of biglycan, decorin and large chondroitin-sulphate proteoglycan in adult rat temporomandibular joint disc. Arch Oral Biol 1998; 43(11):889–98.
29. Kuwabara M, Takuma T, Scott PG, Dodd CM, Mizoguchi I. Biochemical and immunohistochemical studies of the protein expression and localization of decorin and biglycan in the temporomandibular joint disc of growing rats. Arch Oral Biol 2002;47(6): 473–80.
30. Tanaka E, Aoyama J, Tanaka M, Van Eijden T, Sugiyama M, Hanaoka K, *et al*. The proteoglycan contents of the temporomandibular joint disc influence its dynamic viscoelastic properties. J Biomed Mater Res 2003;65A(3):386–92.
31. Xu T, Bianco P, Fisher LW, Longenecker G, Smith E, Goldstein S, *et al*. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. Nat Genet 1998;20(1):78–82.
32. Hildebrand A, Romaris M, Rasmussen LM, Heinegard D, Twardzik DR, Border WA, *et al*. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. Biochem J 1994;302(Pt 2):527–34.
33. Chen XD, Fisher LW, Robey PG, Young MF. The small leucine-rich proteoglycan biglycan modulates BMP-4-induced osteoblast differentiation. FASEB J 2004; 18(9):948–58.
34. Kresse H, Schonherr E. Proteoglycans of the extracellular matrix and growth control. J Cell Physiol 2001; 189(3):266–74.
35. Shimizu-Hirota R, Sasamura H, Kuroda M, Kobayashi E, Hayashi M, Saruta T. Extracellular matrix glycoprotein biglycan enhances vascular smooth muscle cell proliferation and migration. Circ Res 2004;94(8): 1067–74.
36. Chen XD, Shi S, Xu T, Robey PG, Young MF. Age-related osteoporosis in biglycan-deficient mice is related to defects in bone marrow stromal cells. J Bone Miner Res 2002;17(2):331–40.
37. Liang Y, Haring M, Roughley PJ, Margolis RK, Margolis RU. Glypican and biglycan in the nuclei of neurons and glioma cells: presence of functional nuclear localization signals and dynamic changes in glypican during the cell cycle. J Cell Biol 1997; 139(4):851–64.
38. Pirttiniemi P, Kantomaa T, Sorsa T. Effect of decreased loading on the metabolic activity of the mandibular condylar cartilage in the rat. Eur J Orthod 2004;26(1): 1–5.
39. Sharawy MM, Ali AM, Choi WS. Immunohistochemical localization and distribution of proliferating cell nuclear antigen in the rabbit mandibular condyle following experimental induction of anterior disk displacement. CRANIO 2002;20(2):111–5.