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Structural Associations of Symptomatic Knee Osteoarthritis

Laura A. Stoppiello,[1] Paul I. Mapp,[1] Deborah Wilson,[2] Roger Hill,[2] Brigitte E. Scammell,[1] and David A. Walsh[3]

Objective. **Structural changes of osteoarthritis (OA) may occur in the absence of pain. In this study, we aimed to identify histopathologic features that are associated with symptomatic knee OA.**

Methods. **Medial tibial plateaus and synovium samples were obtained at the time of total knee replacement (TKR) surgery for OA (advanced OA group) or were obtained postmortem from subjects who had not sought medical attention for knee pain during the last year of life (non-OA control group). To identify features of OA, we compared the patients with advanced OA with the age-matched non-OA controls (n = 26 per group). To identify OA features associated with symptoms, we compared two additional groups of subjects who were matched for severity of chondropathy (n = 29 per group): patients undergoing TKR for symptomatic OA (symptomatic chondropathy group) and postmortem subjects with similar severity of chondropathy who were asymptomatic during the last year of life (asymptomatic chondropathy group). The histologic features of the samples were graded, and immunoreactivities for macrophages (CD68) and nerve growth factor (NGF) in the synovium were quantified. The cellular localization of synovial NGF was determined by double immunofluorescence analysis.**

Results. **Advanced OA cases displayed more severe changes in the synovium (synovitis, increased synovial NGF, and CD68-immunoreactive macrophages) and cartilage (loss of cartilage surface integrity, loss of proteoglycan, tidemark breaching, and alterations in chondrocyte morphology) than did the non-OA controls. Synovial NGF was localized predominantly to fibroblasts and to some macrophages. The symptomatic chondropathy group displayed greater levels of synovitis, synovial NGF, and loss of cartilage integrity, in addition to alterations in chondrocyte morphology, than did the asymptomatic chondropathy group ($P < 0.05$ for each comparison).**

Conclusion. **Synovitis, increased synovial NGF, alterations in chondrocyte morphology, and loss of cartilage integrity are features of knee OA that may be associated with symptoms.**

Osteoarthritis (OA) is the most common joint disease in the elderly population. Pathologic features include articular cartilage degradation, synovial inflammation, osteophytes, and bone marrow lesions. Pain is the main reason sufferers seek clinical assistance. Patients with end-stage OA may eventually benefit from total knee replacement (TKR) surgery.

Although joint pathology makes an important contribution to OA pain, the precise relationship between pain and structural pathology is not entirely clear (1,2). Radiographic features of OA are often weakly associated with pain, suggesting that structural, cellular, or biochemical changes that directly mediate pain may not be easily detected on radiographs. Imaging studies have indicated possible associations between pain and synovitis (3–6) or subchondral changes (6–9), but associations with histopathology have not been explored in detail. Such studies have previously been limited by age

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[1]Laura A. Stoppiello, BSc(Hons), Paul I. Mapp, PhD, Brigitte E. Scammell, DM, FRCS(Orth): Arthritis Research UK Pain Centre and University of Nottingham, Nottingham, UK; [2]Deborah Wilson, RGN, Roger Hill: Sherwood Forest Hospitals NHS Foundation Trust, Sutton in Ashfield, UK; [3]David A. Walsh, PhD, FRCP: Arthritis Research UK Pain Centre and University of Nottingham, Nottingham, UK, and Sherwood Forest Hospitals NHS Foundation Trust, Sutton in Ashfield, UK.

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Address correspondence to Laura A. Stoppiello, BSc(Hons), Arthritis Research UK Pain Centre, Clinical Sciences Building, City Hospital, Hucknall Road, Nottingham NG5 1PB, UK. E-mail: mbxls1@nottingham.ac.uk.

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differences between OA patients and normal controls and by limited access to joint tissue from people with OA whose pain is less than that which would lead them to undergo joint replacement surgery.

Synovitis may contribute to OA knee pain. The cause of synovitis in OA is incompletely understood, but has been observed in both early (10) and late disease (11). Synovitis may mediate pain through the production of factors that sensitize or activate sensory nerves.

Recent evidence has suggested an important role of nerve growth factor (NGF) in mediating inflammatory pain through increasing nociceptor sensitivity or facilitating sensory nerve growth (12). NGF blockade was shown to improve the degree of pain from knee OA (13–15), indicating a causal link between NGF and OA pain.

NGF is elevated in a variety of rheumatic diseases, particularly in the synovial fluid of patients with inflammatory arthritis (16,17). NGF is expressed by synovial macrophages from patients with rheumatoid arthritis, spondyloarthritis, and to a lesser extent, in OA (17), as well as by synovial fibroblasts from patients with OA (18). However, the expression and cellular profile of NGF in synovial biopsy samples from people with knee OA has not been explored in detail. Synovial fibroblasts can be visualized using immunohistochemistry with antibodies directed against Hsp47, a collagen-specific molecular chaperone (19). Macrophages can be identified by the transmembrane glycoprotein CD68 (20).

Our aim in the present study was to first validate the measurement of histopathologic characteristics of knee OA by comparing samples obtained at the time of TKR surgery for OA (advanced OA) with samples collected postmortem from age-matched non-OA controls. Second, we aimed to identify histopathologic features associated with symptomatic knee OA by comparing cases with similar macroscopic chondropathy, half of whom had sought medical care for knee pain and had undergone TKR (symptomatic chondropathy) and the other half had not sought medical care for knee pain but had died of an unrelated illness (asymptomatic chondropathy). We hypothesized that symptomatic OA is mediated by specific structural and biochemical changes within the knee joint, whereas other changes may be coincidental or even protective.

PATIENTS AND METHODS

Patient samples. The joint tissue repository of the Arthritis Research UK Pain Centre, which contains samples from >1,700 subjects, was screened first to select tissues obtained at the time of TKR for OA (advanced OA group) and tissues obtained postmortem from age-matched subjects who had not sought medical attention for knee pain during the last year of life (non-OA control group) (n = 26 per group) and second to select tissues from patients who had undergone TKR for symptomatic OA (symptomatic chondropathy group) and tissues with similar macroscopic chondropathy scores obtained postmortem from subjects who had not sought medical attention for knee pain during the last year of life (asymptomatic chondropathy group) (n = 29 per group). One TKR case was included in both the advanced OA group and the symptomatic chondropathy group and was therefore counted twice.

Patients undergoing TKR fulfilled the American College of Rheumatology classification criteria for OA (21) at the time of surgery. Subjects from whom samples were obtained postmortem were recently deceased, had no history of rheumatoid arthritis or pseudogout, and had not previously sought help for knee pain during the last year of life, as determined by interviews with the relatives and review of case notes. Exclusion criteria for non-OA controls consisted of a history of OA, Heberden's nodes identified on clinical examination, macroscopic chondropathy lesions of grade 3 or 4 in the medial tibiofemoral compartment, or osteophytes on direct visualization of the dissected knee.

Body mass index (BMI; kg/m^2) in the TKR cases was calculated from measurements of height and weight that were documented in the cases notes. BMI in the postmortem cases could not be calculated because height and weight measurements were not available.

Informed consent was obtained from the TKR patients and from the next of kin of the postmortem subjects. The study was approved by the UK National Research Ethics Service (Nottingham Research Ethics Committee 1 [05/Q2403/24] and Derby Research Ethics Committee 1 [11/H0405/2]).

Sample processing. Midcoronal sections of the middle one-third of the medial tibial plateau were fixed in neutral-buffered formalin and then decalcified in 10% EDTA in 10 m*M* Tris buffer (pH 6.95; at 4°C) prior to embedding in wax. Surgeons and technicians were instructed to collect synovium from the medial joint line. Synovial tissues were fixed in formalin and embedded in wax without decalcification or were frozen without fixation in melting isopentane and embedded in OCT mounting medium (Raymond A. Lamb Ltd.).

Macroscopic chondropathy score. Following tissue harvesting, the articular surfaces of the medial and lateral tibial plateaus and femoral condyles were evaluated for the extent and severity of loss of surface integrity by a single assessor (RH), as described by Walsh et al (22) based on the method of Ayral et al (23). Briefly, articular surface defects were graded from 0 (normal, smooth, unbroken surface) to 4 (subchondral bone exposure), and the proportion of each articular surface area corresponding to each grade was used to calculate a chondropathy score (range 0–100). Scores for each of the 4 compartments were summed to give a total tibiofemoral chondropathy score (range 0–400). Surgical damage precluded the calculation of macroscopic chondropathy scores for some TKR cases.

Radiographic OA severity score. Radiographic severity scores for OA were derived using preoperative posteroanterior knee radiographs as previously described (22). An atlas of line drawings of the knee joint was used to grade medial and lateral joint space narrowing and osteophytes (24). The scores for joint space narrowing (range 0–6) and osteophytes (range

Table 1. Clinical and pathologic characteristics of the study groups*

Characteristic	Features of OA		Features of symptomatic OA	
	Non-OA control (n = 26)	Advanced OA (n = 26)	Asymptomatic chondropathy (n = 29)	Symptomatic chondropathy (n = 29)
Age, years	61 (48–67)	61 (50–67)	71 (66–79)	68 (61–74)†
% male	62	31†	55	55
BMI, kg/m^2	NA	32 (29–40)	NA	31 (28–37)
Macroscopic chondropathy score (range 0–400)	51 (40–69)	NA	199 (177–215)‡	223 (185–234)‡
Total radiographic score (range 0–18)§	NA	12 (10–13)	NA	12 (10–13)
JSN score (range 0–6)	NA	5 (4–5)	NA	5 (4–5)
Osteophyte score (range 0–12)	NA	7.5 (5–8)	NA	8 (5.7–8)
Histologic features				
Total Mankin score (range 0–14)¶	5 (3–7)	8 (7–9)#	7 (7–8)	8 (7.5–9)
Cartilage surface integrity (range 0–6)	2 (2–3)	4 (4–4)‡	4 (3–4)	4 (4–4)†
% with breached tidemark	35	68†	66	67
Proteoglycan loss (range 0–4)	1 (1–1)	1 (1–2)†	1 (1–2)	1 (1–2)
Chondrocyte morphology (range 0–3)	1 (0–3)	2.5 (2–3)†	3 (1.5–3)	3 (2–3)†
% with subchondral bone marrow replacement	0	19	18	32
Synovitis (range 0–3)	0 (0–0)	0 (0–3)†	0 (0–0.25)	2 (0–3)#
Fractional areas**				
Macrophage	3.4 (1.8–4.6)	4.8 (3.1–8.7)†	7.4 (4.2–13.2)	13.1 (6.6–15.3)
Nerve growth factor	1.4 (0.4–1.8)	4.0 (1.5–8.3)†	8.7 (5.9–12.4)	12.9 (9.4–18.6)†

* Tissues were obtained at the time of total knee replacement for osteoarthritis (OA) or were obtained postmortem from cadaver donors. Data for the body mass index (BMI) were available for 23 patients with advanced OA and 20 patients with symptomatic chondropathy. (One patient with TKR was included in both groups and was therefore counted twice.) Statistical comparisons for the immunohistochemical and histomorphometric data were not made between the features of OA and the features of symptomatic OA subgroups because staining was carried out in separate experiments. Except where indicated otherwise, values are the median (interquartile range). NA = not available.

† $P < 0.05$ versus the corresponding subgroup (i.e., non-OA control group or asymptomatic chondropathy group).

‡ $P \leq 0.001$ versus the non-OA control group.

§ The total radiographic score is the sum of the scores for tibiofemoral joint space narrowing (JSN) and osteophytes.

¶ The total Mankin score is the sum of the scores for cartilage surface integrity, tidemark breaching, proteoglycan loss, and chondrocyte morphology. Tidemark breaching is expressed as the percentage of subjects in whom cartilage integrity permitted assessment (25 in the advanced OA group and 24 in the symptomatic chondropathy group).

# $P < 0.01$ versus the corresponding subgroup.

** Fractional areas are the percentage of the synovial area occupied by CD68-immunoreactive macrophages or nerve growth factor–immunoreactive cells. Fractional areas are reported only for samples that displayed synovial lining (n = 15–26 per group).

0–12) were summed to provide a total radiographic OA severity score (range 0–18). Radiographs were not available for postmortem cases.

Histologic assessment and grading. Formalin-fixed tissue sections (5 μm) were stained with hematoxylin and eosin or, to visualize proteoglycan content in the medial tibial plateaus, with Safranin O–fast green. Articular cartilage changes of OA were graded as 4 components according to the Mankin scoring system (25): cartilage surface integrity was graded on a scale of 0–6 (where 0 = normal and 6 = complete disorganization), tidemark integrity was graded 0 or 1 (where 0 = intact and 1 = crossed by vessels), chondrocyte morphology was graded on a scale of 0–3 (where 0 = normal and 3 = hypocellular), and proteoglycan loss was graded on a scale of 0–4 (where 0 = normal, no loss of Safranin O staining and 4 = complete loss of staining). The presence of subchondral bone marrow replacement by fibrovascular tissue was assessed in each case as either present (pathologic) or absent (normal). Synovial inflammation was graded on a scale of 0–3 (where 0 = normal and 3 = severe inflammation) by assessing the degree of synovial lining hyperplasia, inflammatory cell infiltrate, and cellularity (11).

Immunohistochemistry. Sections of formalin-fixed synovium underwent antigen retrieval (1 mg/ml of pepsin in 0.5*M* acetic acid at 37°C for 2 hours), incubated with monoclonal mouse anti-human CD68 (clone PG-M1; Dako), followed by biotinylated horse anti-mouse IgG secondary antibody (Vector). NGF immunoreactivity was visualized after citrate buffer antigen retrieval (90°C for 20 minutes). Sections were blocked with 5% bovine serum albumin (BSA) containing goat serum, followed by incubation with rabbit monoclonal antibody to NGF (clone EP1320Y; Abcam) and biotinylated goat anti-rabbit IgG secondary antibody (Vector). Visualization of both immunoreactivities used avidin–biotin complex peroxidase (Vector) with nickel-enhanced diaminobenzidine development (26).

Synovium samples from 3 TKR patients, 3 postmortem cases with high levels of chondropathy, and 2 non-OA controls were simultaneously double-stained for NGF and either macrophage or fibroblast markers. Frozen synovial tissue sections were fixed in 4% paraformaldehyde, followed by acetone and then were blocked with 5% BSA containing goat serum and horse serum. Sections were incubated overnight with a mixture of antibodies directed toward NGF (as above) and either CD68 (as above) or Hsp47 (M16.10A1; Enzo Life Sciences), followed by incubation with a mixture of secondary antibodies: Texas Red–labeled horse anti-mouse IgG and fluorescein-labeled goat anti-rabbit IgG (Vector). Sections were counter-

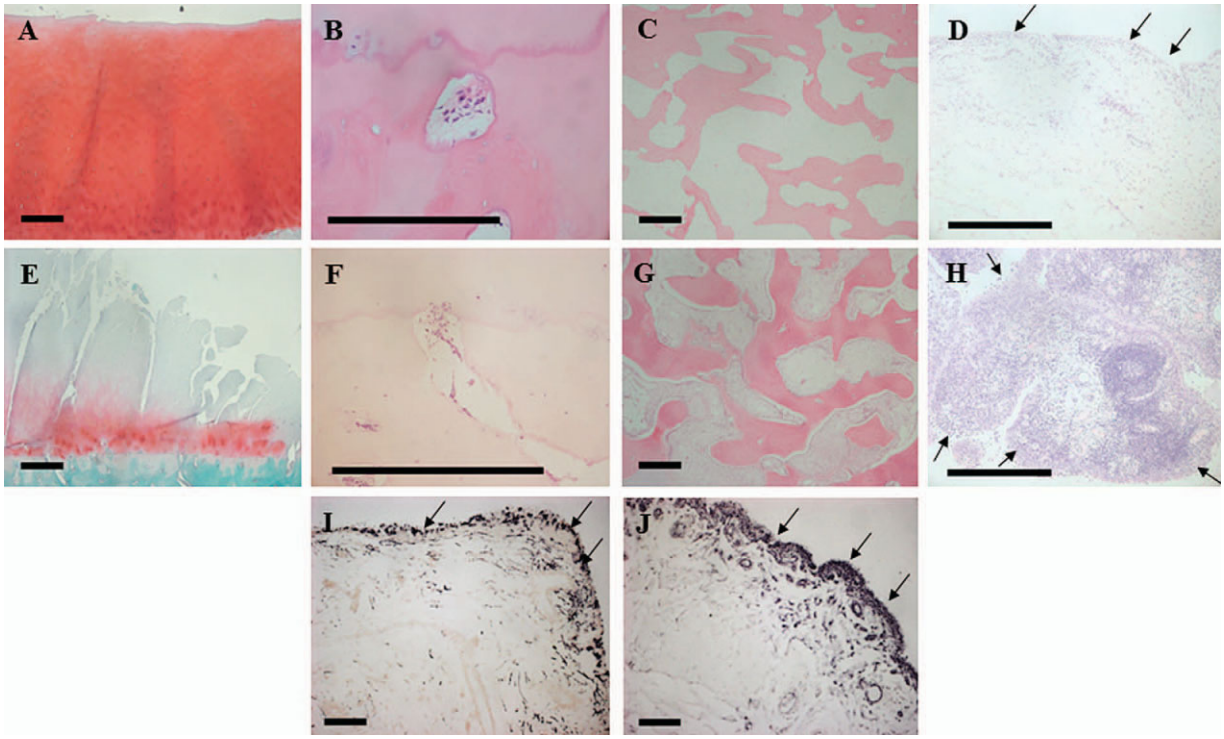

Figure 1. Histopathologic features of non-osteoarthritic (non-OA) (**A–D**) and OA (**E–J**) knees. Non-OA knees typically displayed mild cartilage surface irregularities of the superficial zone, with normal proteoglycan staining (**A**), whereas in some patients with advanced OA of the knee, there were deep clefts in the articular cartilage surface and severe proteoglycan loss (**E**) (Safranin O–fast green stained). Channels can be seen in the calcified cartilage beneath an intact tidemark in a non-OA knee (**B**), whereas an OA knee shows a channel breaching the tidemark and entering the noncalcified cartilage (**F**) (hematoxylin and eosin [H&E] stained). Normal subchondral bone marrow spaces are filled with fatty tissue in a non-OA knee (**C**), whereas subchondral bone marrow is replaced by fibrovascular tissue in an OA knee (**G**) (H&E stained). Mild synovial lining hyperplasia is shown in a sample from a non-OA knee (**D**), whereas severe synovitis, characterized by hyperplasia and hypertrophy of the synovial lining and sublining hypercellularity, can be seen in an OA knee (**H**) (H&E stained). Macrophage immunoreactivity for CD68 (**I**) and immunoreactivity for nerve growth factor (**J**) in OA knees was localized to the synovial lining and sublining regions (staining [blue-black] developed with nickel-enhanced diaminobenzidine). **Arrows** indicate the synovial surface. Bars = 500 μm.

stained with DAPI, and autofluorescence was blocked with 1% Sudan black (Sigma-Aldrich) in 70% ethanol. Sections were mounted in phosphate buffered saline/glycerol.

Image analysis. All histologic and immunohistologic scoring was carried out by a single observer (LAS) who was blinded with regard to the diagnostic group and clinical details. A Zeiss Axioscop-50 microscope was used. Macrophage and NGF fractional areas were quantified using a 3-CCD video camera and KS300 image analysis software. Areas of the synovium with the highest density of positive staining for CD68 or NGF were identified. Briefly, an image was captured, and area of interest, which included the synovial lining and sublining, was delineated. Positive staining was differentiated from background by thresholding the image according to hue in order to create a mask. The area of positive staining and the total area were measured. The fractional area was determined as the percentage of synovial area positive for CD68 or NGF immunoreactivity within the total area. Data were collected using a 20× objective lens in 4 different fields of view. Only samples with synovial lining were included in the analyses.

Statistical analysis. Data were analyzed using SPSS v.21 software. The Mann-Whitney U test was used to compare TKR and postmortem groups for each variable, except for age (compared by *t*-tests) and the presence of subchondral bone marrow replacement (compared by chi-square test) between groups. Associations between BMI and outcome measures were evaluated using linear regression, adjusting for age and experimental group. Statistical significance was accepted when the *P* value was less than 0.05. Logistic regression was used to adjust for sex differences in the non-OA control and age-matched advanced OA group comparisons and for age differences in the asymptomatic and symptomatic chondropathy group comparisons.

The intraobserver reliability for the histology and fractional area scores was assessed by rescoring 20–25 randomly selected cases on 2 separate occasions 6 months apart. For reproducibility analyses, the kappa statistic (StatsDirect software) (27) was used for cartilage, subchondral bone, and synovial scores. The intraclass correlation coefficient (SPSS software) was used for the fractional area values.

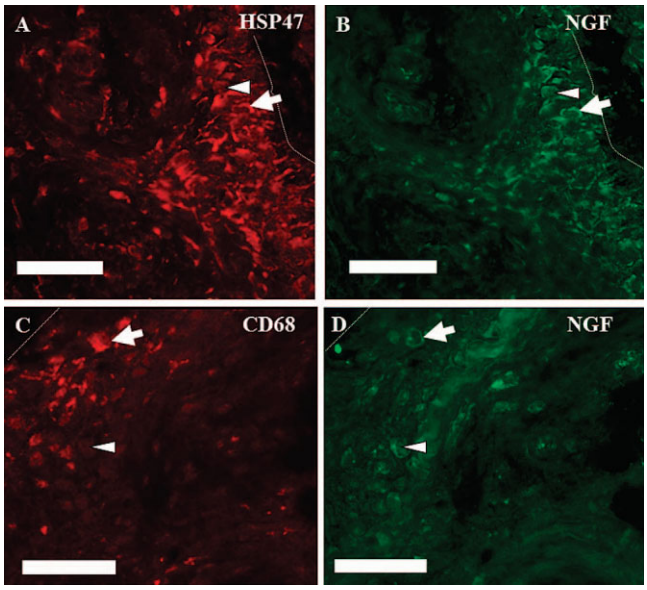

Figure 2. Cellular localization of nerve growth factor (NGF) immunoreactivity in human synovium. **A,** Hsp47 immunoreactivity localized to cells with fibroblast morphology in the lining and sublining regions of synovium from a patient with osteoarthritis (OA). **Arrow** indicates a fibroblast colocalized to NGF (same cell as indicated in **B**); **arrowhead** indicates a fibroblast not colocalized to NGF. **B,** NGF immunoreactivity colocalized with Hsp47 (**arrow**) and in a cell that is not immunolabeled for Hsp47 (**arrowhead**) in the section shown in **A**. **C,** CD68 immunoreactivity of macrophages in synovium from a patient with OA. **Arrow** indicates a macrophage colocalized to NGF (same cell as indicated in **D**); **arrowhead** indicates a macrophage not colocalized to NGF. **D,** NGF immunoreactivity colocalized with CD68 (**arrow**) and in a cell that is not immunolabeled for CD68 (**arrowhead**) in the section shown in **C**. Double immunohistochemistry was visualized by Texas Red (Hsp47 and CD68 [red]) and fluorescein (NGF [green]) staining. Dotted lines indicate the synovial surface. Bars = 50 μm.

RESULTS

Histopathologic features of knee OA. Patient demographics are reported in Table 1, and histopathologic features are illustrated in Figure 1. Samples displayed a range of cartilage changes, including mild clefts to the articular surface with normal proteoglycan content (Figure 1A), as compared with deep clefts and loss of proteoglycan in some advanced OA cases (Figure 1E). Channels from the subchondral bone were found in close proximity to the tidemark (Figure 1B) and also breached the tidemark to invade the noncalcified cartilage (Figure 1F). Subchondral bone spaces mostly contained normal fatty tissue (Figure 1C); however, in some cases, this was replaced by fibrovascular tissue (Figure 1G). Synovial inflammation was sometimes marked, with lining hyperplasia and lymphoid aggregates (Figure 1H). CD68+ (Figure 1I) and NGF+ (Figure 1J) cells were evident in the synovial lining and sublining regions and surrounded the blood vessels.

NGF immunoreactivity in the synovium was predominantly localized to Hsp47+ spindle-shaped and mononuclear cells, consistent with fibroblast morphology (Figures 2A and B). However, some NGF+Hsp47– and NGF–Hsp47+ cells were also observed. Some CD68+macrophages in the lining and sublining regions also displayed NGF immunoreactivity (Figures 2C and D). Similar distributions were found in advanced OA patients and non-OA controls.

Intraobserver reliability for histologic scoring was moderate or substantial, as determined using the kappa statistic (range 0.53–0.76). For fractional area measurements, the intraclass correlation coefficient was 0.85, indicating good reproducibility.

Patients with advanced OA displayed more severe cartilage surface changes, proteoglycan loss, tidemark breaching, changes in chondrocyte phenotype, and synovitis than did non-OA controls ($P < 0.05$ for each comparison) (Figure 3). Tidemark breaching was observed in 17 of 25 (68%) of the advanced OA patients and 9 of 26 (35%) of the non-OA controls ($P = 0.012$). Subchondral fibrovascular replacement was present in 5 of 26 (19%) of the advanced OA patients and was absent from the non-OA controls; however, significance was lost after adjusting for sex differences. The synovial fractional areas positive for CD68 and NGF immunoreactivities were greater in patients with advanced OA than in the non-OA controls ($P = 0.031$ and 0.035, respectively) (Figures 3E and F).

Histopathologic features of symptomatic knee OA. Both the asymptomatic and symptomatic chondropathy groups displayed evidence of structural OA, with higher macroscopic chondropathy scores than the non-OA controls (Table 1). Patients in the symptomatic chondropathy group displayed macroscopic chondropathy features similar to the subjects in the asymptomatic chondropathy group, indicating appropriate matching (Table 1). Severe macroscopic chondropathy lesions graded as 3 or 4 (deep cartilage fibrillation or subchondral bone exposure, respectively) were present in the medial tibiofemoral compartment of 28 of 29 (97%) subjects with asymptomatic chondropathy and 29 of 29 (100%) patients with symptomatic chondropathy.

Loss of cartilage surface integrity and alterations in chondrocyte morphology were greater in the symptomatic chondropathy group than in the asymptomatic chondropathy group (Table 1 and Figures 4A and B), whereas proteoglycan loss, tidemark integrity, and subchondral bone marrow replacement by fibrovascular tissue did not differ significantly between groups.

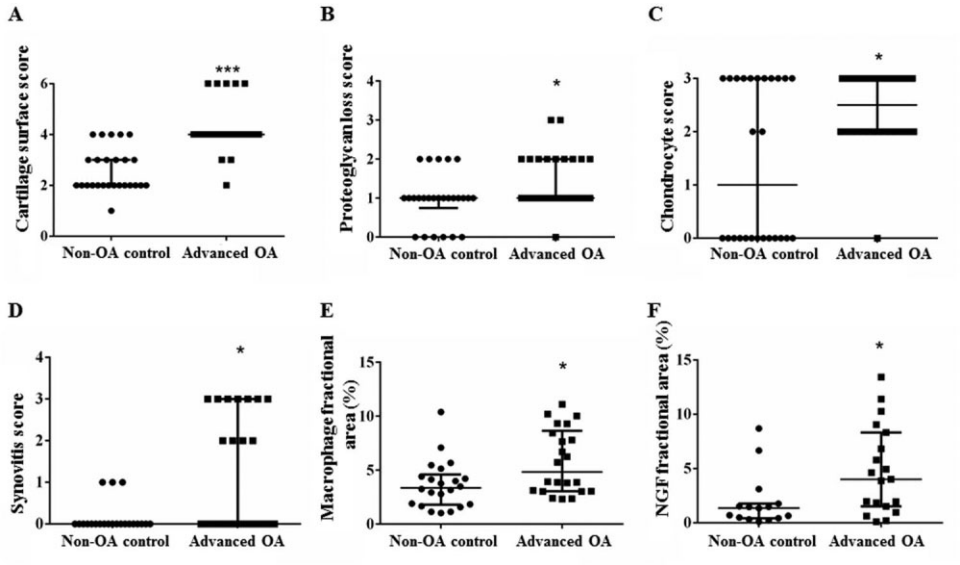

Figure 3. Histopathologic features of osteoarthritis (OA). Scatterplots illustrate the differences between the non-OA control group and the advanced OA group for the scores on cartilage surface integrity (**A**), proteoglycan loss (**B**), chondrocyte morphology (**C**), and synovitis (**D**), and for the synovial fractional area positive for CD68-immunoreactive macrophages (**E**) and nerve growth factor (NGF) (**F**). Each symbol represents an individual subject; horizontal lines and error bars show the median and interquartile range. $* = P < 0.05$; $*** = P < 0.001$ versus the non-OA control group.

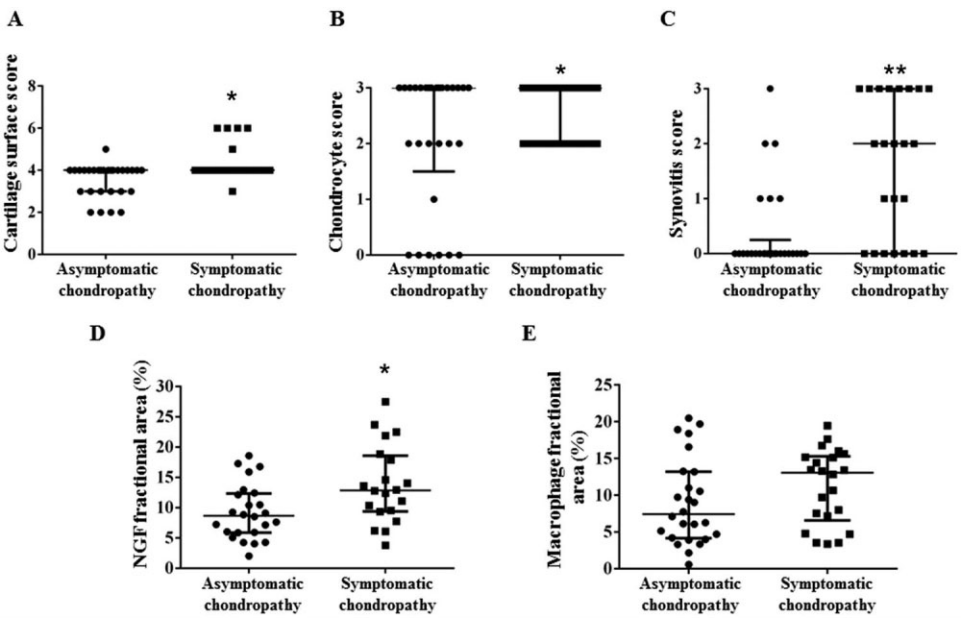

Figure 4. Histologic associations of symptomatic osteoarthritis (OA). Scatterplots illustrate the differences between subjects with similar macroscopic chondropathy scores who did not (asymptomatic chondropathy) or did (symptomatic chondropathy) undergo total knee replacement surgery. The cartilage surface integrity score (**A**), chondrocyte morphology score (**B**), synovitis score (**C**), and synovial fractional area positive for nerve growth factor (NGF) (**D**) were greater in the symptomatic chondropathy group, but differences in the synovial fractional area positive for CD68-immunoreactive macrophages (**E**) did not reach statistical significance. Each symbol represents an individual subject; horizontal lines and error bars show the median and interquartile range. $* = P < 0.05$; $** = P < 0.01$ versus the asymptomatic chondropathy group.

Synovitis scores were greater in patients in the symptomatic chondropathy group than in subjects in the asymptomatic chondropathy group (Table 1 and Figure 4C). Severe inflammation with perivascular lymphoid aggregates was observed in 8 of 29 symptomatic chondropathy patients (28%) compared with 1 of 29 asymptomatic chondropathy subjects (3%) ($P = 0.021$). The synovial area positive for NGF was greater in symptomatic chondropathy patients than in asymptomatic chondropathy patients (Table 1 and Figure 4D). Differences in macrophage infiltration between groups did not reach statistical significance (Table 1 and Figure 4E). We used logistic regression analysis to examine whether the association between symptomatic OA and synovitis was explained by increased synovial NGF immunoreactivity and/or macrophage fractional area. Associations between synovitis and symptomatic chondropathy remained significant in each regression model ($P \leq 0.008$).

BMI (kg/m^2) was calculated for both TKR groups (advanced OA and symptomatic chondropathy). Data were available for 42 of 54 patients (78%). Of these, 29 patients (69%) were classified as obese (BMI ≥30), 11 patients (26%) were classified as overweight (BMI 25–29.9), and 2 patients (5%) were a healthy weight (BMI 18.5–24.9). Linear regression was used to determine associations between BMI and outcome measures, combining data from both TKR groups. Age and experimental group were used as independent covariates. BMI was not significantly associated with any histopathologic or immunohistologic parameter.

DISCUSSION

We found that a range of histopathologic features in the synovium and tibial plateaus were associated with OA. Of these, synovitis, increased synovial NGF immunoreactivity, chondrocyte changes, and loss of cartilage surface integrity were associated with symptomatic OA, as compared with postmortem subjects with similar macroscopic joint surface appearances who had not sought TKR during life. Increased synovitis and synovial NGF expression by fibroblasts and some macrophages may be key features associated with pain in OA.

The prevalence and severity of OA are strongly associated with increasing age, raising doubts in earlier studies as to whether the histopathologic features are specifically associated with OA, rather than being characteristics of healthy aging. We addressed this by carefully age-matching the patients with advanced OA and the non-OA controls, a process that required a large source repository from which to select cases.

Synovitis, the densities of CD68-immunoreactive macrophages or NGF-immunoreactive cells within the synovium, proteoglycan loss, chondrocyte changes, loss of tidemark integrity, and tidemark breaching were all greater in patients with advanced OA than in age-matched non-OA controls. This finding indicates that in this study, these pathologic features of OA were not explained by aging alone. However, each of these features was also observed to a lesser extent in our age-matched control group, indicating the presence of either "preclinical" early OA in the control group or the presence of normal structural features in the advanced OA group that are exaggerated by the disease. A key question, therefore, is which pathologic features are associated with clinical OA?

The relevance of structural changes to OA symptoms has been difficult to determine. Evidence of synovitis on magnetic resonance imaging (MRI) has been associated with pain in people who have OA or those who are at high risk of developing OA (5). Furthermore, a recent study demonstrated that fluctuation in synovitis corresponds to a fluctuation in knee pain, providing further evidence of the contribution of synovitis to symptomatic OA (28). Patients with greater amounts of synovitis display more severe chondropathy, and synovitis may predict progressive structural damage in knee OA (22,29). It can therefore be difficult to be certain as to whether synovitis itself is a cause of pain or whether it acts as a surrogate for other aspects of OA pathology. Our work extends these previous findings by indicating that synovitis is associated with symptomatic OA independently of the severity of chondropathy.

In the present study, we explored the characteristics of synovitis that have been associated with symptomatic OA. We confirmed the previous findings of increased macrophage infiltration in OA (10), but the presence of macrophages may not be sufficient to explain OA pain. Levels of NGF are elevated in the synovial fluid of patients with rheumatoid arthritis (16,30), and NGF is present to a lesser extent in patients with OA (17), but a previous study did not localize NGF to the synovium in the single OA case reported (31). Nevertheless, monoclonal antibodies directed against NGF have been shown to reduce pain and improve function in people with OA (13–15).

We showed that in OA, NGF immunoreactivity is present in the synovium, predominantly in Hsp47+ fibroblasts, and in some macrophages. NGF immunoreactivity was greater in the synovial tissue of the symptomatic chondropathy group than in that of the asymptomatic chondropathy group. These findings suggest an important contribution of fibroblast-derived

NGF in the generation of OA pain. The proinflammatory cytokines interleukin-1β and tumor necrosis factor α (TNFα) can stimulate the release of NGF from synovial fibroblasts, and NGF can modulate the release of TNFα (18). NGF may act on sensory nerves to increase pain in OA. After we adjusted for NGF and/or CD68 immunoreactivity, the significant association of synovitis with symptomatic OA remained, suggesting that increased NGF and macrophage infiltration, either alone or together, do not completely explain the pain. The production of pain mediators, for example, by fibroblasts, may yet be driven by macrophages or other aspects of inflammation.

Previous histopathologic studies of OA have often relied on samples obtained at joint replacement surgery, which display a range of OA structural severity (22). The severity of chondropathy overlaps to a small extent between postmortem cases and surgical cases (22), and therefore, our study of pathologic associations in symptomatic OA required selection of cases with matched degrees of macroscopic chondropathy from a large tissue repository. Radiographic scores in our symptomatic chondropathy group were similar to those in our advanced OA group, and macroscopic chondropathy scores in the symptomatic and asymptomatic chondropathy groups were significantly higher than those in the non-OA control group, indicating that both of the chondropathy groups had pathologic features of knee OA.

Although the two chondropathy groups were matched for similarity of macroscopic chondropathy scores, microscopic loss of cartilage surface integrity was greater in the symptomatic chondropathy group. Chondropathy cases were selected on the basis of similar total macroscopic chondropathy scores of the tibiofemoral joint, where the appearance of the 4 articular surfaces was evaluated: the medial and lateral femoral condyle and tibial plateau. Histologic grading was examined in one midcoronal section of the middle one-third of the medial tibial plateau because this is an important weight-bearing area that is characteristically affected by OA. Macroscopic and microscopic analyses therefore reflect distinct but related aspects of OA pathology (22).

Not all histopathologic features associated with OA were associated with symptomatic OA. Tidemark breaching was found in twice as many asymptomatic chondropathy cases than non-OA controls, suggesting that it may be an early feature of OA, but it was no more common in the symptomatic chondropathy group than the asymptomatic chondropathy group. In contrast, osteochondral vascularity was previously associated with pain behavior in rats with OA following transection of the medial meniscus (32). Our current data suggest that tidemark breaching alone is insufficient to cause OA symptoms and that additional factors, such as sensory innervation or local expression of NGF, may be necessary mediators of pain from osteochondral channels (12). Our current study provides novel insights into the incidence of tidemark breaching in a subgroup of asymptomatic postmortem cases with macroscopic chondropathy similar to patients who underwent TKR. Further work would be required to determine the time course and mechanisms by which channels breaching the tidemark may predispose to pain.

Fibrovascular replacement of the subchondral bone marrow was twice as common in the symptomatic chondropathy group as it was in the asymptomatic chondropathy group, but this difference did not reach statistical significance after adjusting for age differences between groups. Bone marrow replacement has been associated with bone marrow lesions on MRI (33), which in turn, has been associated with pain in knee OA (7,34). Our study may have had insufficient power to detect differences between groups, and further work would be required to define the structural and biochemical features of bone marrow lesions that may explain their association with pain.

Obesity is a major risk factor for OA (35–37) and has been associated with pain (38) and cartilage changes (39,40). The majority of patients with advanced OA in our cohort were classified as overweight or obese, but histopathologic factors associated with symptoms were not significantly associated with BMI in our study.

Our study is necessarily subject to a number of limitations. We assumed that the subjects in the asymptomatic chondropathy group had experienced less pain than the patients in the symptomatic chondropathy group, since to the best of our knowledge, these subjects had not sought medical attention for knee pain during the last year of life. Clinical data acquisition in postmortem cases relies on case notes, where knee pain may not have been reported, and interviews with bereaved relatives, who may not have been aware of the deceased's symptoms. Histologic analysis was performed on only 1 section of osteochondral tissue per case, and the sample was obtained from the midcoronal section of the middle one-third of the medial tibial plateau, a key weight-bearing site. It is possible that there may be different structural associations with symptoms at other sites within the joint. Furthermore, our patients with symptomatic OA all had late-stage disease (undergoing TKR), and different structural features may be associated with pain in early OA.

Our study design avoided confounding by age in our characterization of features associated with OA, but

we were unable to simultaneously match for sex. Similarly, matching for the severity of chondropathy precluded age matching between our symptomatic and asymptomatic chondropathy groups, even when using a source repository containing samples from >1,700 cases. However, we adjusted for age or sex differences in our analyses.

We classified postmortem cases as having OA based on the presence of cartilage changes comparable to those seen in patients undergoing TKR for OA, and we selected small, homogeneous patient groups in order to undertake detailed histopathologic experiments. Our work extends imaging and arthroscopic studies indicating the relative importance of synovitis (3,5) and macroscopic cartilage changes (41,42) to symptoms in the broader OA population.

We have identified histopathologic features of knee joint tissues that are associated with OA; however, not all of these features may cause pain. Symptomatic OA was associated with synovitis, synovial NGF immunoreactivity, changes in chondrocyte morphology, and loss of cartilage surface integrity as compared with the asymptomatic chondropathy group showing a similar macroscopic joint surface appearance who had not sought TKR during life. Different pathologic features of OA typically coincide, and better understanding of the structural features that are associated with symptomatic OA would help in refining strategies for developing structure-modifying treatments (disease-modifying OA drugs) by focusing on those which are most likely to improve symptoms. Synovitis and synovial NGF expressed by fibroblasts and some macrophages may be key mediators associated with OA knee pain.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Ms Stoppiello had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Stoppiello, Mapp, Wilson, Hill, Scammell, Walsh.

Acquisition of data. Stoppiello.

Analysis and interpretation of data. Stoppiello, Mapp, Scammell, Walsh.

REFERENCES

1. Bedson J, Croft PR. The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. BMC Musculoskelet Disord 2008;9:116.
2. Dieppe PA, Cushnaghan J, Shepstone L. The Bristol 'OA500' study: progression of osteoarthritis (OA) over 3 years and the relationship between clinical and radiographic changes at the knee joint. Osteoarthritis Cartilage 1997;5:87–97.
3. Hill CL, Hunter DJ, Niu J, Clancy M, Guermazi A, Genant H, et al. Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. Ann Rheum Dis 2007;66:1599–603.
4. Hill CL, Gale DG, Chaisson CE, Skinner K, Kazis L, Gale ME, et al. Knee effusions, popliteal cysts, and synovial thickening: association with knee pain in osteoarthritis. J Rheumatol 2001;28:1330–7.
5. Baker K, Grainger A, Niu J, Clancy M, Guermazi A, Crema M, et al. Relation of synovitis to knee pain using contrast-enhanced MRIs. Ann Rheum Dis 2010;69:1779–83.
6. Torres L, Dunlop DD, Peterfy C, Guermazi A, Prasad P, Hayes KW, et al. The relationship between specific tissue lesions and pain severity in persons with knee osteoarthritis. Osteoarthritis Cartilage 2006;14:1033–40.
7. Felson DT, Chaisson CE, Hill CL, Totterman SM, Gale ME, Skinner KM, et al. The association of bone marrow lesions with pain in knee osteoarthritis. Ann Intern Med 2001;134:541–9.
8. Szebenyi B, Hollander AP, Dieppe P, Quilty B, Duddy J, Clarke S, et al. Associations between pain, function, and radiographic features in osteoarthritis of the knee. Arthritis Rheum 2006;54:230–5.
9. Hernandez-Molina G, Neogi T, Hunter DJ, Niu J, Guermazi A, Reichenbach S, et al. The association of bone attrition with knee pain and other MRI features of osteoarthritis. Ann Rheum Dis 2008;67:43–7.
10. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 2005;64:1263–7.
11. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, et al. Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 2003;48:2173–7.
12. Walsh DA, McWilliams DF, Turley MJ, Dixon MR, Franses RE, Mapp PI, et al. Angiogenesis and nerve growth factor at the osteochondral junction in rheumatoid arthritis and osteoarthritis. Rheumatology (Oxford) 2010;49:1852–61.
13. Brown MT, Murphy FT, Radin DM, Davignon I, Smith MD, West CR. Tanezumab reduces osteoarthritic knee pain: results of a randomized, double-blind, placebo-controlled phase III trial. J Pain 2012;13:790–8.
14. Lane NE, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL, Smith MD, et al. Tanezumab for the treatment of pain from osteoarthritis of the knee. N Engl J Med 2010;363:1521–31.
15. Sanga P, Katz N, Polverejan E, Wang S, Kelly KM, Haeussler J, et al. Efficacy, safety, and tolerability of fulranumab, an anti-nerve growth factor antibody, in the treatment of patients with moderate to severe osteoarthritis pain. Pain 2013;154:1910–9.
16. Halliday DA, Zettler C, Rush RA, Sciccitano R, McNeil JD. Elevated nerve growth factor levels in the synovial fluid of patients with inflammatory joint disease. Neurochem Res 1998;23:919–22.
17. Barthel C, Yeremenko N, Jacobs R, Schmidt RE, Bernateck M, Zeidler H, et al. Nerve growth factor and receptor expression in rheumatoid arthritis and spondyloarthritis. Arthritis Res Ther 2009;11:R82.
18. Manni L, Lundeberg T, Fiorito S, Bonini S, Vigneti E, Aloe L. Nerve growth factor release by human synovial fibroblasts prior to and following exposure to tumor necrosis factor-α, interleukin-1β and cholecystokinin-8: the possible role of NGF in the inflammatory response. Clin Exp Rheumatol 2003;21:617–24.
19. Izquierdo E, Canete JD, Celis R, Del Rey MJ, Usatequi A, Marsal

S, et al. Synovial fibroblast hyperplasia in rheumatoid arthritis: clinicopathologic correlations and partial reversal by anti–tumor necrosis factor therapy. Arthritis Rheum 2011;63:2575–83.
20. Holness CL, Simmons D. Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. Blood 1993;81:1607–13.
21. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
22. Walsh DA, Yousef A, McWilliams DF, Hill R, Hargin E, Wilson D. Evaluation of a Photographic Chondropathy Score (PCS) for pathological samples in a study of inflammation in tibiofemoral osteoarthritis. Osteoarthritis Cartilage 2009;17:304–12.
23. Ayral X, Gueguen A, Listrat V, Bahuaud J, Beaufils P, Beguin J, et al. Simplified arthroscopy scoring system for chondropathy of the knee (revised SFA score). Rev Rhum [Engl Ed] 1994;61:88–90.
24. Nagaosa Y, Mateus M, Hassan B, Lanyon P, Doherty M. Development of a logically devised line drawing atlas for grading of knee osteoarthritis. Ann Rheum Dis 2000;59:587–95.
25. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.
26. Shu S, Ju G, Fan L. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 1988;85:169–71.
27. Viera A, Garrett J. Understanding interobserver agreement: the kappa statistic. Fam Med 2005;37:360–3.
28. Zhang Y, Nevitt M, Niu J, Lewis C, Torner J, Guermazi A, et al. Fluctuation of knee pain and changes in bone marrow lesions, effusions, and synovitis on magnetic resonance imaging. Arthritis Rheum 2011;63:691–9.
29. Ayral X, Pickering EH, Woodworth TG, Mackillop N, Dougados M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis: results of a 1 year longitudinal arthroscopic study in 422 patients. Osteoarthritis Cartilage 2005;13:361–7.
30. Raychaudhuri SP, Raychaudhuri SK, Atkuri KR, Herzenberg LA, Herzenberg LA. Nerve growth factor: a key local regulator in the pathogenesis of inflammatory arthritis. Arthritis Rheum 2011;63:3243–52.
31. Aloe L, Tuveri MA, Carcassi U, Levi-Montalcini R. Nerve growth factor in the synovial fluid of patients with chronic arthritis. Arthritis Rheum 1992;35:351–5.
32. Mapp PI, Walsh DA, Bowyer J, Maciewicz RA. Effects of a metalloproteinase inhibitor on osteochondral angiogenesis, chondropathy and pain behavior in a rat model of osteoarthritis. Osteoarthritis Cartilage 2010;18:593–600.
33. Zanetti M, Bruder E, Romero J, Hodler J. Bone marrow edema pattern in osteoarthritic knees: correlation between MR imaging and histologic findings. Radiology 2000;215:835–40.
34. Felson DT, Niu J, Guermazi A, Roemer F, Aliabadi P, Clancy M, et al. Correlation of the development of knee pain with enlarging bone marrow lesions on magnetic resonance imaging. Arthritis Rheum 2007;56:2986–92.
35. Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis: the Framingham Study. Ann Intern Med 1988;109:18–24.
36. Ackerman IN, Osborne RH. Obesity and increased burden of hip and knee joint disease in Australia: results from a national survey. BMC Musculoskelet Disord 2012;13:254.
37. Jiang L, Tian W, Wang Y, Rong J, Bao C, Liu Y, et al. Body mass index and susceptibility to knee osteoarthritis: a systematic review and meta-analysis. Joint Bone Spine 2012;79:291–7.
38. Goulston LM, Kiran A, Javaid MK, Soni A, White KM, Hart DJ, et al. Does obesity predict knee pain over fourteen years in women, independently of radiographic changes? Arthritis Care Res (Hoboken) 2011;63:1398–406.
39. Roemer FW, Zhang Y, Niu J, Lynch JA, Crema MD, Marra MD, et al, for the Multicenter Osteoarthritis (MOST) Study Investigators. Tibiofemoral joint osteoarthritis: risk factors for MR-depicted fast cartilage loss over a 30-month period in the Multicenter Osteoarthritis Study. Radiology 2009;252:772–80.
40. Antony B, Ding C, Stannus O, Cicuttini F, Jones G. Association of baseline knee bone size, cartilage volume, and body mass index with knee cartilage loss over time: a longitudinal study in younger or middle-aged adults. J Rheumatol 2011;38:1973–80.
41. Hunter DJ, March L, Sambrook PN. The association of cartilage volume with knee pain. Osteoarthritis Cartilage 2003;11:725–9.
42. Sowers MF, Hayes C, Jamadar D, Capul D, Lachance L, Jannausch M, et al. Magnetic resonance-detected subchondral bone marrow and cartilage defect characteristics associated with pain and X-ray-defined knee osteoarthritis. Osteoarthritis Cartilage 2003;11:387–93.