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Osteoarthritic changes in vervet monkey knees correlate with meniscus degradation and increased matrix metalloproteinase and cytokine secretion

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SUMMARY

Objective: Meniscus injury increases osteoarthritis risk but its pathobiology in osteoarthritis is unclear. We hypothesized that older adult vervet monkeys would exhibit knee osteoarthritic changes and the degenerative menisci from these animals would secrete matrix metalloproteinases (MMPs) and pro-inflammatory cytokines that contribute to the development of osteoarthritis.
Design: In a cross sectional analysis of healthy young adult (9–12 years) and old (19–26 years) adult female vervet monkeys, knees were evaluated *in vivo* with computed tomography (CT) imaging, and joint tissues were morphologically graded at necropsy. Meniscus explants were subsequently cultured to evaluate meniscal MMP and cytokine secretion.
Results: CT images revealed significant bony osteoarthritic changes in 80% of older monkeys which included increases in osteophyte number and meniscal calcification. Meniscus and cartilage degradation scores were greater in the older monkeys and were positively correlated ($r > 0.7$). Menisci from older animals exhibiting osteoarthritic changes secreted significantly more MMP-1, MMP-3, and MMP-8 than healthy menisci from younger monkeys. Older menisci without significant osteoarthritic changes secreted more IL-7 than healthy young menisci while older osteoarthritic menisci secreted more IL-7 and granulocyte-macrophage colony-stimulating factor than healthy older menisci.
Conclusions: Aged vervets develop naturally occurring knee osteoarthritis that includes involvement of the meniscus. Degenerative menisci secreted markedly increased amounts of matrix-degrading enzymes and inflammatory cytokines. These factors would be expected to act on the meniscus tissue and local joint tissues and may ultimately promote osteoarthritis development. These finding also suggest vervet monkeys are a useful animal model for studying the progression of osteoarthritis.

Introduction

Osteoarthritis is the most common type of arthritis affecting older adult humans and is widely prevalent in several species of non-human primates, including baboons, rhesus and cynomolgus macaques, chimpanzees and gorillas[1–5]. Several studies identified an increased prevalence of knee osteoarthritis associated with increased age in nonhuman primates[6–10]. The gross and radiographic appearance of osteoarthritis in these nonhuman primates is

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similar to humans in that they progress through mild to severe osteoarthritis with symptomatic activity limitation and the development of osteophytes and joint space narrowing[3,11–14]. Nonhuman primates offer a unique but established opportunity to model human disease processes[15] and detailed examination of osteoarthritis in nonhuman primates may elucidate new insights into biomolecular processes underlying human disease.

Osteoarthritis is increasingly recognized as a disease of the whole joint with a shared environment comprised of cartilage, synovium, ligaments and the meniscus[2,16–19]. The impact of cytokine stimulation on articular cartilage and subsequent extracellular matrix degradation is well documented[18,20–22]; however, the role of the meniscus in this process is unclear. Meniscus injury is a known predisposing factor for osteoarthritis[11,17], and meniscus biology is likely impacted by exposure to inflammatory factors produced by knee tissues secondary to acute or chronic injury, such as age-associated repetitive microtrauma[12,21,23]. Certain aspects of meniscus biology are pathologically altered in meniscus injury, in aging and in the development of osteoarthritis, including increased release of matrix-degrading enzymes and pro-inflammatory cytokines and chemokines[24–27]. Thus, the meniscus likely also has a biologic role in osteoarthritis development. Release of matrix metalloproteinases (MMPs) and pro-inflammatory factors from the meniscus could negatively affect the nearby articular cartilage and synovium. In this cross-sectional analysis, we evaluated knee joints from young adult and older adult vervets for the presence of osteoarthritis pathology and tested the hypothesis that aged and damaged menisci would produce catabolic factors, including MMPs and pro-inflammatory cytokines, which may contribute to the development of osteoarthritis in vervet monkeys.

Methods

All animal procedures were approved and in accordance with federal and institutional animal care and use guidelines. Five young adult (9.4–11.8 years) and five old (19.8–26.4 years) otherwise healthy (lacking clinically significant disease conditions) adult female African green vervet (*Chlorocebus aethiops sabaeus*) monkeys were included in each of the first two analysis groups. These monkeys were part of a unique vervet colony established from animals captured on St. Kitts Island in the 1970s[28,29] and raised in social groups to study relationships between behavior, genetics, and metabolic parameters with aging. The vervet ages roughly correspond to young adult and older adults[3,13,22,30]. These animals underwent detailed assessments of physical functioning and determinants of immune system function and physiologic parameters of relevance to human health as a part of a multi-investigator pilot study. Prior to 2008 animals were housed in octagonal outdoor enclosures, ~15 m across, with access to shelter, and a grassy floor, at the Sepulveda Veterans Administration Medical Center Nonhuman Primate Laboratory in California[29]. Since 2008, animals were housed at Wake Forest School of Medicine in social groups of approximately 15–40 animals, allowed to roam freely in large inside/outside pens (30 m^2) which contain perches, platforms, elevated climbing structures and a base composed of smooth stones. Animals were fed a Chow based diet and water *ad libitum*.

Although females in this colony are fertile into their late teens, animals above 20 years of age are generally reproductively senescent and are likely peri- or post-menopausal. As part of colony management animals are given annual physicals with collection blood CBC and chemistries on animals >10 years old. Vervets are tested for tuberculosis three times per year. During tuberculosis testing, animals were weighed and tested for pregnancy via transabdominal ultrasound with additional blood collection. Two older animals were identified as having osteoarthritis and two had elevated fasting glucoses during regular health exams, though this was not part of the selection criteria for the study. It is not possible to determine if prior trauma may have led to knee osteoarthritis, as animals were active during their time in the colony and had almost unlimited opportunities for leaping, jumping, climbing with the potential for falls and trauma. Non-human primates are generally stoic and do not exhibit behaviors reflecting pain unless significantly injured. The assessment of animal mobility and physical function was previously described[30].

Whole body computed tomography (CT) scans were obtained with anesthetized animals using a Toshiba Aquilon 32 Slice CT (0.5 mm slice thickness) and analyzed with HU thresholding and 3D reconstructions using AquariusNet Viewer v.4.4.8.85 (TeraRecon, Inc.). Qualitative subjective evaluation of the CT scans demonstrated a high incidence of significant bony osteoarthritic changes in the older animals.

Vervets were euthanized as a part of the parent protocol in order to harvest an array of tissues for detailed histologic and molecular evaluations. Knees from these vervet monkeys were obtained opportunistically as the vervets were undergoing euthanasia for other studies. Vervets were euthanized with IV sodium pentobarbital (60–100 mg/kg) to attain deep surgical anesthesia and exsanguination in accordance with guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. Tissues were then harvested from the euthanized vervets in the necropsy suite by a skilled surgical team who rapidly deliver them on ice to the respective study groups. Knees from young adult and old vervets were analyzed for knee osteoarthritis on macroscopic examination and meniscus tissue was harvested for MMP and cytokine analysis. A third group of knees from old animals ($n = 6$, 17.4–25.0 years) was opportunistically acquired and these specimens were graded and tissue harvested in the same fashion. All knee ligaments were intact at the time of specimen acquisition and no gross evidence of fracture or malalignment was present.

Quantitative analysis of CT images was completed using the AquariusNet software. The tibial osteophyte and calcified meniscal volume were quantified from both knees of each vervet. Knee reconstructions were repositioned to acquire a coronal plane relative to the tibia aligned with the intercondylar eminence. The 10 mm-long region of interest (ROI) extended 5 mm anterior and posterior to this landmark. Tibial osteophyte and calcified meniscus volume were calculated from measures collected at 1 mm increments throughout the ROI with a threshold set for bone at Window Width = 2200 and Window Length = 200.

All vervet knees were disarticulated and macroscopically graded for cartilage degradation using the International Cartilage Repair Society scoring system (http://www.cartilage.org/index.php?pid=223) and with a meniscus adaption as previously published[23]: Grade 0, normal meniscus; Grade 1, near-normal with minor fibrillations; Grade 2, small, scattered cracks or fissures, some fibrillations; Grade 3, small tear, excessive fibrillations; Grade 4, large tear, extensive fibrillations. The first eight knees (totaling 48 measurements) were individually graded by two authors and demonstrated an interobserver reliability to be Kappa = 0.90 ± ($P < 0.001$, 95% CI 0.84–0.97). The remainder of specimens were graded by one of the two initial raters since the pilot assessment demonstrated significant agreement. Osteoarthritis scoring was calculated through the summation of cartilage and meniscus severity scores. Meniscus weights were obtained immediately *ex vivo* to avoid any confounding factors, such as swelling in culture (Supplementary Fig. 1). Meniscus specimens were individually cultured as whole explants in twelve-well culture plates in DMEM/F12 media (Gibco) with 10% fetal bovine serum (FBS; Gibco). After overnight acclimation, explants were changed to serum-free media to eliminate growth factors and cultured for 48 h.

Conditioned explant media was then collected and target proteins analyzed.

For protein analysis, equal volumes of conditioned media were separated by SDS-PAGE (BioRad), transferred to nitrocellulose (Odyssey, Invitrogen) and probed with the primary antibodies [anti-MMP1 (PAB12708, Abnova); anti-MMP3 (AB2963, Millipore); anti-MMP8 (MAB3316, Millipore); anti-MMP13 (AB84594, Abcam); anti-GM-CSF (AP10690c, Abgent); anti-IL7 (MAB207, R&D Systems)] and secondary antibody (CellSignal). Immunoblots were visualized with chemiluminesence (Amersham ECL, GE Life Sciences). Conditioned media was also analyzed with a cytokine array (#AAH-CYT-1; RayBiotech) according to the manufacturer's protocol. A total of 23 cytokines were tested in duplicate with the array. Processed films were imported into Photoshop v7.0 (Adobe). Densitometry measurements were completed with ImageJ 1.44p (NIH) and normalized to explant wet weight.

Statistical analysis was performed with SigmaPlot v10.0 (Systat Software) and Prism v5.02 (GraphPad Software). An inter-observer reliability analysis for vervet knee morphology scores was analyzed using linear weighted Cohen's Kappa statistic. Vervet knee morphology scores were analyzed using Pearson product moment correlation while meniscal MMP protein secretion data was analyzed using analysis of variance (ANOVA), and post-hoc analyses. Significance was set at $P < 0.05$ for main effects and where appropriate, Bonferroni corrections were applied for multiple comparisons (for 3 comparisons, $P < 0.017$). Each vervet monkey was counted as a unique individual ($n = 1$).

Results

Older adult vervets exhibited typical morphological features of osteoarthritis evident on gross pathology that included cartilage degradation with bony eburnation, osteophyte formation and meniscal degradation. Radiographic analysis demonstrated bony hypertrophy with osteophyte formation, subchondral sclerosis, and bone cyst formation (Figs. 1 and 2). These changes were seen in 4 of the 5 older adult animals studied but not in any of the young adult animals. The changes on CT correlated with changes noted in the knee joint tissues removed from these same animals. Tabulated vervet knee morphologic scores are presented in Fig. 1 (complete data set in Table I). Older vervets demonstrated gross evidence of osteophyte formation in 80% of knee specimens (Table I). In vervets with osteoarthritic changes, medial compartment morphologic scores were consistently high, but lateral compartment scores were more variable (see Table 1).

Cartilage and medial meniscus scores (Table I) correlated with age (right knee $r = 0.77$, $P = 0.007$; left knee $r = 0.73$ $P = 0.013$). Meniscus degradation correlated with femoral and tibial cartilage degradation in the respective medial compartments (right femoral $r = 0.93$, $P < 0.001$; right tibial $r = 0.85$, $P < 0.001$; left femoral $r = 0.89$, $P < 0.001$; left tibial $r = 0.79$, $P = 0.005$) and lateral compartments (right femoral $r = 0.73$, $P = 0.013$; right tibial $r = 0.63$ $P = 0.048$; left femoral $r = 0.72$, $P = 0.016$; left tibial $r = 0.072$, $P = 0.013$). Meniscus degradation scores were significantly greater in old vs young adult vervets in both compartments ($P = 0.002$). Medial and lateral compartment cartilage degradation was significantly greater in old vervets ($P < 0.001$). Medial meniscus degradation scores correlated with the lateral cartilage scores ($r = 0.858$, $P < 0.001$), but medial and lateral meniscus scores were not significantly different within an age group. Osteophyte analysis of the vervet tibial plateaus demonstrated significantly greater mean osteophyte volume in old vervets compared to young adult vervets (Fig. 2, $P = 0.009$) and the left medial tibial plateau demonstrated significantly greater osteophyte volume than the other compartments ($P = 0.01$). Menisci from older vervets contained more calcific changes than young adult vervets ($P < 0.001$) and medial menisci had greater calcific changes than lateral menisci ($P = 0.01$).

Vervet activity was assessed as previously described[30] and evaluated in relation to knee morphology. Increased total knee osteoarthritis degradation scores were significantly correlated with increased time spent moving [Fig. 3(A); $R^2 = 0.78$, $P = 0.008$]. Time spent moving was also individually correlated with medial meniscus degradation scores (right, $R^2 = 0.70$, $P = 0.023$; left $R^2 = 0.69$, $P = 0.027$) and lateral meniscus degradation scores (right, $R^2 = 0.73$, $P = 0.018$; left $R^2 = 0.89$, $P < 0.001$). Increased right medial tibial plateau degradation scores negatively and significantly correlated with average walking speed ($R^2 = -0.65$,

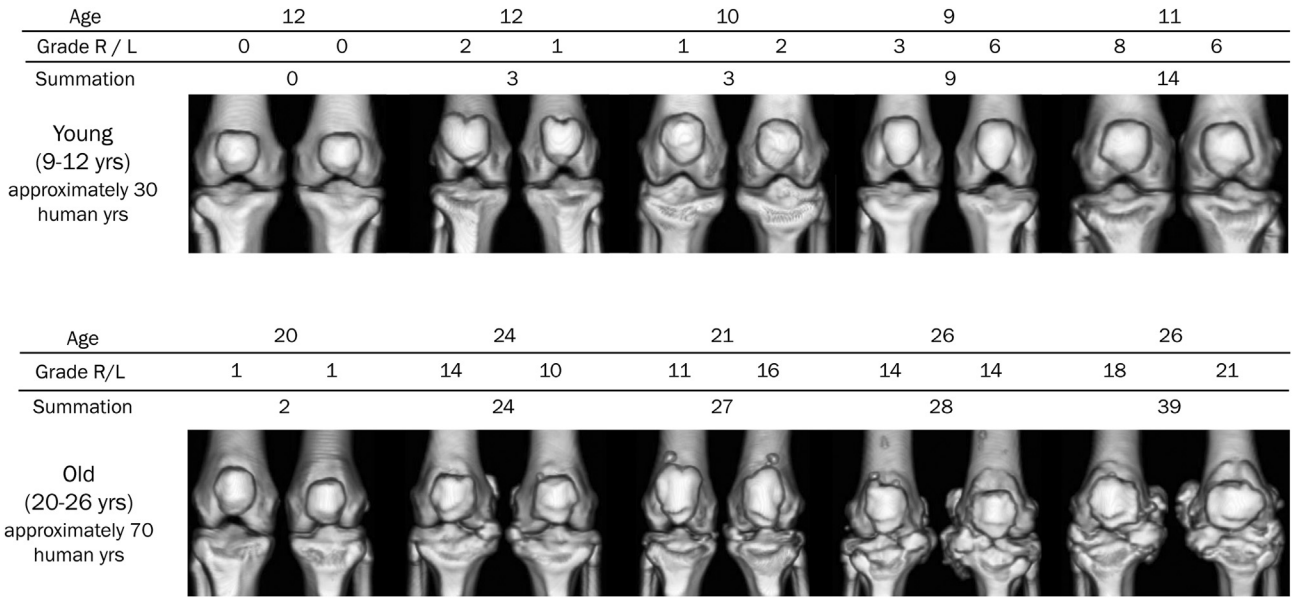

Fig. 1. CT scans and accompanying morphologic scores from young adult and older adult vervets. CT reconstructions from young adult (9.3–11.6 years) and older (19.7–26.2 years) vervet knees. Morphology scores were tabulated for each limb as a measure of disease severity. Tabulated scores are reported above the CT scans.

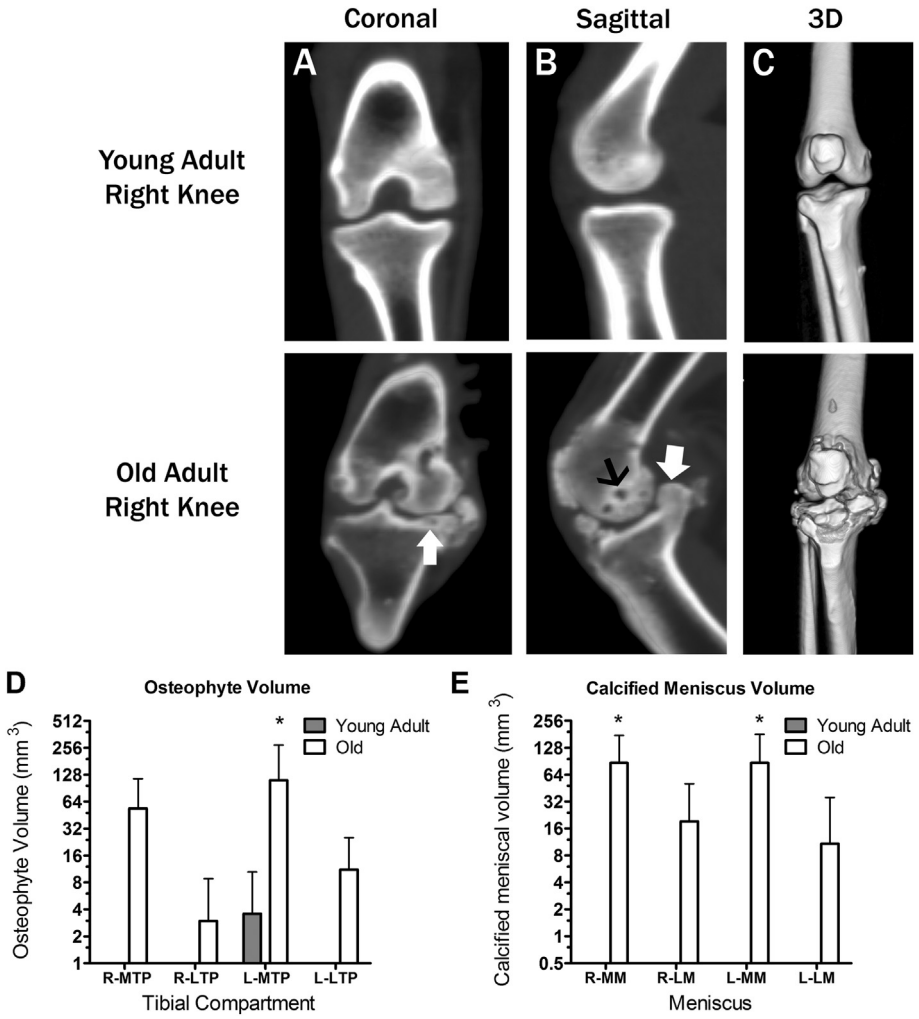

Fig. 2. CT analysis and with osteophyte and calcified meniscal volume in young adult and old vervets. Coronal (A), Sagittal (B) and three dimensional (C) CT reconstructions demonstrating radiographic differences between young adult and old vervet knees. Old vervet knees demonstrated subchondral sclerosis (A, white arrow), subchondral cysts (B, black arrow) and osteophyte formation (B, white arrow). Osteophyte (D) and calcific meniscus volume (E) measured using CT reconstructions from young adult ($n = 5$; 9.3–11.6 years) and older ($n = 5$; 19.7–26.2 years) vervet knees. Young adult vervets did not demonstrate calficied meniscal voulume in right MTP, right LTP or left LTP osteophytes. Older vervets demonstrated significantly greater mean osteophyte volume ($P = 0.009$) and the left medial tibial plateau demonstrated significantly greater osteophyte volume than the other compartments ($P = 0.01$). Menisci from older vervets contained more calcific changes than young adult vervets ($P < 0.001$) and medial menisci had greater calcific changes than lateral menisci ($P = 0.01$). R-Right; L-Left; MTP-medial tibial plateau; LTP-lateral tibial plateau; MM-medial meniscus; LM lateral meniscus.

Table I
Vervet knee cartilage and meniscus scores

			Right knee								Left knee							
			Morphologic scores						Osteophytes	Meniscal calcifications	Morphologic scores						Osteophytes	Meniscal calcifications
	Age (yr)	Wt (kg)	MTP	LTP	MFC	LFC	MM	LM			MTP	LTP	MFC	LFC	MM	LM		
	9.3	5.53	1	1	1	0	0	0	None	None	1	2	1	2	0	0	None	None
	10.4	7.12	1	0	0	0	0	0	None	None	0	0	1	0	1	0	None	None
	11.4	6.42	2	1	2	1	1	1	None	None	1	1	2	1	1	0	None	None
	11.5	5.63	0	0	0	0	0	0	None	None	0	0	0	0	0	0	None	None
	11.6	6.62	2	0	0	0	0	0	None	None	1	0	0	0	0	0	None	None
Mean	**10.8**	**6.26**	**1.2**	**0.4**	**0.6**	**0.2**	**0.2**	**0.2**			**0.6**	**0.6**	**0.8**	**0.6**	**0.4**	**0**		
	19.7	4.66	1	0	0	0	0	0	None	None	1	0	0	0	0	0	None	None
	21.3	5.85	3	1	3	1	2	1	Moderate	Moderate	3	3	3	4	2	1	Moderate	Moderate
	23.6	6.03	3	1	4	1	3	2	Moderate	Moderate	2	1	4	1	2	0	Moderate	Moderate
	25.6	4.13	3	4	3	4	4	4	Severe	Severe	3	4	3	4	3	4	Severe	Severe
	26.2	4.92	4	2	3	3	2	0	Severe	Severe	4	2	3	2	3	0	Severe	Severe
Mean	**23.3**	**5.12**	**2.8**	**1.6**	**2.6**	**1.8**	**2.2**	**1.4**			**2.6**	**2**	**2.6**	**2.2**	**2**	**1**		

MTP, medial tibial plateau; LTP, lateral tibial plateau; MFC, medial femoral condyle; LFC, lateral femoral condyle; MM, medial meniscus; LM, lateral meniscus.

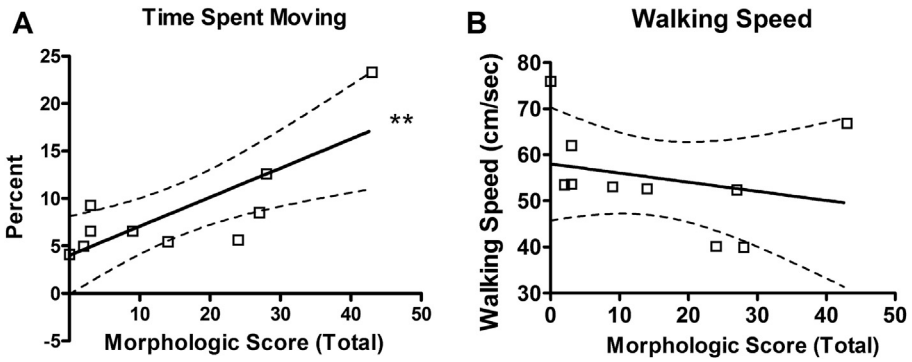

Fig. 3. Vervet activity versus total knee osteoarthritis morphologic scores. (A) Percent of time spent moving was significantly positively correlated with knee osteoarthritis scores (**$R^2 = 0.6095$, $P = 0.008$). (B) Walking speed was significantly negatively correlated with right medial tibial plateau degradation scores ($R^2 = -0.65$, $P = 0.04$) and negatively, but not significantly, correlated with left medial tibial plateau ($R^2 = -0.52$, $P = 0.13$) and total knee osteoarthritis degradation scores ($R^2 = -0.26$, $P = 0.47$). The dotted line represents the 95% confidence interval.

$P = 0.04$). Left medial tibial plateau scores demonstrated a trend to negatively correlate with walking speed ($R^2 = -0.52$, $P = 0.13$). Walking speed was negatively but not significantly associated with increased total knee osteoarthritis degradation scores [Fig. 3(B), $R^2 = -0.26$, $P = 0.47$].

Vervet menisci were cultured as explants to assess MMP and cytokine secretion. Compared to explants from young adult animals, osteoarthritic meniscus explants from older animals demonstrated increased secretion of MMP-1, -3, and -8 (respectively $P = 0.019$, $P = 0.048$, $P = 0.049$; Fig. 4). Medial menisci produced greater amounts of MMP-1 and -8 than the lateral meniscus, which paralleled the higher grade degenerative changes in the medial compartments (Table I).

Inflammatory cytokine production in meniscal explant media was also analyzed. Explants from both young adult and old animals produced IL-8, and GRO family chemokines (GRO antibody binds CXCL1(GROα), CXCL2(GROβ), CXCL3(GROγ); Fig. 5). Six of the 23 cytokines were above the limit of detection. Older menisci demonstrated a slight increase in IL-6 production but a substantial increase in IL-7. The higher morphologic grade medial meniscus also secreted granulocyte-macrophage colony-stimulating factor (GM-CSF).

A third set of knees from old vervets was analyzed to assess the differences between osteoarthritic and normal menisci and to confirm the secretion of IL-7 and GM-CSF identified on the cytokine array. The cartilage and meniscus scores are shown in Table II. These

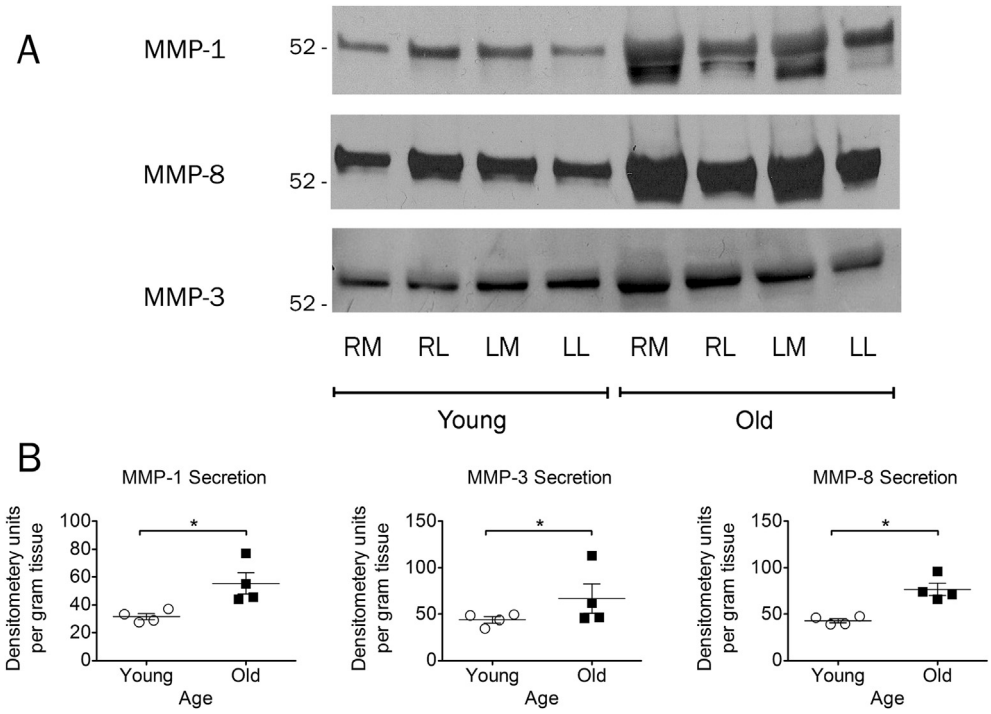

Fig. 4. MMP protein secretion in meniscal explant cultures from young adult and old vervets. *A) Representative immunoblots of conditioned media from meniscus explant culture*. MMP-1 and MMP-8 and MMP-3. [Right (R) or left (L) modified with medial (M) or lateral (L)]. *B) Densitometric analysis of MMP blots*. Each data point is the mean response of the menisci from each compartment of $n = 3$ animals (right medial, left medial or right lateral, left lateral compartments) and the line is the mean for all young or old knee compartments. Densitometry units were normalized to explant tissue wet weight. MMP-1: $P = 0.0192$; MMP-3:$P = 0.0483$; MMP-8: $P = 0.0495$. Error bars are standard error of the mean. Meniscus specimens obtained young adult (9.3–11.6 years) and older (19.7–26.2 years) vervet knees.

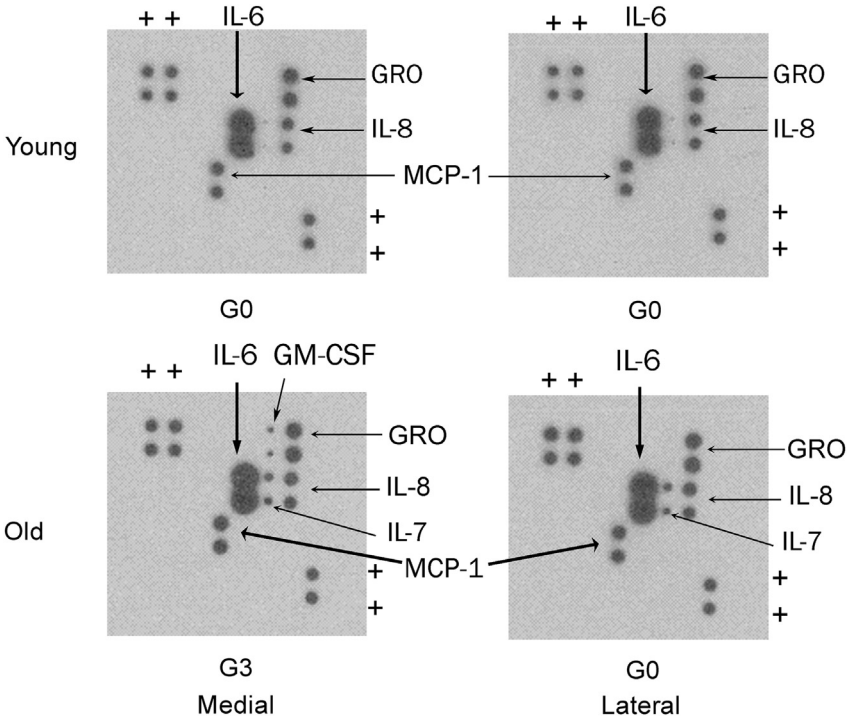

Fig. 5. Cytokine protein array from young adult and old monkey menisci. Conditioned media from meniscal explant cultures was incubated with cytokine protein array membranes. ($n = 1$ medial and lateral meniscus from a young and old vervet; + positive control; G0: grade 0; G3: grade 3).

monkeys were identified to have an approximately 50% prevalence of osteoarthritic changes based on gross analysis of knee specimen pathology. Osteoarthritic menisci secreted increased IL-7 ($P = 0.002$) and GM–CSF ($P = 0.004$) compared to healthy menisci (Fig. 6). Aged vervet menisci secreted IL-7 which was significantly increased with worsening disease of the meniscus. GM-CSF appeared to be secreted by only diseased meniscus. A higher meniscus osteoarthritis grade positively and significantly correlated with increased secretion of IL-7 ($R = 0.774$, $P < 0.001$) and GM-CSF ($R = 0.655$, $P < 0.001$). IL-7 and GM-CSF secretion were also positively and significantly correlated ($R = 0.698$, $P < 0.001$).

Discussion

Older adult vervet knees demonstrated pathologic changes consistent with the well-documented relationship between age and osteoarthritis development. CT scans demonstrated the typical pathology of osteoarthritis which was accompanied by cartilage and meniscus degeneration subsequently observed during examination of the knee joint tissues. These changes were similar to human osteoarthritis, and consistent with established reports of idiopathic and surgically induced osteoarthritis in non-human primates[2–5,31].

Age-related increases in knee osteoarthritis was identified in Rhesus macaques[10,32,33] and Cynomolgus macaques[6] and noted to be more severe in older baboons compared to younger baboons with osteoarthritis changes[31]. Knee arthritis and the development of osteophytes were found to increase with age in nonhuman primates[6,8,31,32,34]. Radiographic evidence of osteoarthritis was found in both baboons and rhesus macaques. In our vervet population, osteophytes were associated with greater meniscus and cartilage degradation, which differs from the lack of reliable association reported in baboons[31]. One additional reason why our analysis identified a correlation of osteophytes and disease progression more similar to those found in humans, may be from our gross analysis of both the femur and the tibia, rather than exclusive analysis of the distal femur as previously reported[31]. Osteoarthritic changes, including glenoid retroversion and joint space narrowing,

Table II
Vervet knee cartilage and meniscus scores from old monkeys (second cohort)

		Right knee								Left knee							
		Morphologic scores						Osteophytes	Meniscal calcifications	Morphologic scores						Osteophytes	Meniscal calcifications
	Age (yr)	MFC	LFC	MTP	LTP	MM	LM			MFC	LFC	MTP	LTP	MM	LM		
	18.1	2	1	2	1	2	1	None	None	2	1	2	1	2	1	None	None
	17.4	2	2	2	2	2	2	None	None	2	2	2	2	2	1	None	None
	21.5	1	1	1	1	1	1	None	None	2	1	1	1	2	1	None	None
Mean	**19.0**	**1.7**	**1.3**	**1.7**	**1.3**	**1.7**	**1.3**			**2.0**	**1.3**	**1.7**	**1.3**	**2.0**	**1.0**		
	21.4	4	2	4	3	4	2	Moderate	Moderate	4	3	4	3	4	2	Moderate	Moderate
	21.4	4	4	4	4	4	4	Severe	Severe	4	3	4	4	4	3	Severe	Severe
	25	4	3	4	4	4	3	Severe	Severe	4	2	4	2	4	2	Severe	Severe
Mean	**22.6**	**4.0**	**3.0**	**4.0**	**3.7**	**4.0**	**3.0**			**4.0**	**2.7**	**4.0**	**3.0**	**4.0**	**2.3**		

MTP, medial tibial plateau; LTP, lateral tibial plateau; MFC, medial femoral condyle; LFC, lateral femoral condyle; MM, medial meniscus; LM, lateral meniscus.

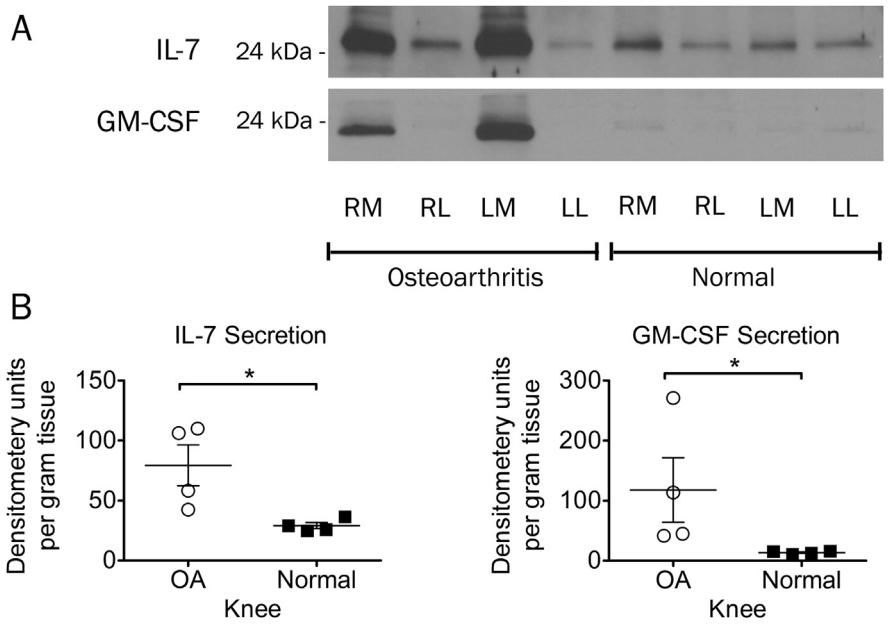

Fig. 6. Cytokine protein secretion in meniscal explant cultures from osteoarthritic and healthy knees. *A) Representative immunoblots of conditioned media from meniscus explant culture*. Interleukin-7 (IL-7) and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). [Right (R) or left (L) modified with medial (M) or lateral (L)]. *B) Densitometric analysis of IL-7 and GM-CSF blots*. Each data point is the mean response of the menisci from each compartment of $n = 3$ animals (right medial, left medial or right lateral, left lateral compartments) and the line is the mean for all osteoarthritic (OA) or healthy knee compartments. Densitometry units were normalized to explant tissue wet weight. Error bars are the 95% confidence interval. IL-7 ($P = 0.002$) and GM–CSF ($P = 0.004$). Vervet ages 17.4–25.0 years.

were previously identified in the vervet shoulder[3]. In our vervet population, weight did not correlate with increasing severity of osteoarthritis, which is consistent with reports of cartilage degradation in cynomolgus macaques[6], but differs from the association seen in female (but not male) baboons[31]. We did see a high prevalence of osteoarthritis in the older, post-menopausal vervets in our study (64%, 7/11), which is consistent with the high reported rate of post-menopausal knee osteoarthritis in baboons[31]. In our study, osteoarthritis changes were associated with increased vervet movement. Pain responses are difficult to quantify in non-human primates; however, since walking speed was negatively correlated with increased osteoarthritic scores, the increased rate of movement may be secondary to pain[35]. These factors in the development, pathologic presentation and radiographic markers a great deal of similarity to the progression of osteoarthritis in humans.

The clinical importance of the meniscus in osteoarthritis development is well documented[11,36]; however, meniscus pathology in osteoarthritis is largely attributed to mechanically mediated loss of structural integrity[37]. These biomechanical stress factors may lead to “osteoarthritis in the meniscus” which is proposed to be responsible for MRI changes seen in the meniscus during the early development of osteoarthritis[37]. Recent evidence suggests the meniscus may play an active role in the whole joint pathology of osteoarthritis[25,26]. Our data support previous gene expression reports and identifies biologic contributions from the meniscus implicated in osteoarthritis pathogenesis[17,23–25].

Our cross sectional analysis suggested that pathologic degradation of the meniscus corresponded to more severe bony changes and cartilage degradation. Meniscus degenerative changes have been previously correlated with osteoarthritic changes in cartilage and articular cartilage loss[7,12,36,38,39]. In a magnetic resonance imaging (MRI) evaluation of human knees, meniscus degeneration preceded or accompanied severe articular cartilage loss[39]. A larger MRI study identified an increased risk of cartilage loss that was associated with an increase in meniscus abnormalities[38]. We identified similar correlations in meniscus and cartilage pathology and found that higher degeneration scores were associated with increased secretion of matrix metalloproteinases (MMPs) and cytokines.

Increased production of MMPs, cytokines and chemokines are believed to be responsible for propagating the catabolic responses in joint tissues that ultimately lead to osteoarthritis[16,18–20,24,27,40,41]. In our study, we identified increased secretion of MMP-1, MMP-3 and MMP-8 by osteoarthritic vervet meniscus that is consistent with elevated catabolic activity in stimulated human meniscus cells and human osteoarthritic meniscus cells[23]. The observed increase in MMP-1 secretion by diseased menisci may be partially responsible for the structural compromise of osteoarthritic menisci since MMP-1 degrades collagen type I, the primary constituent of the meniscus[42]. MMP3 (stromelysin-1) was identified in early osteoarthritis pathology and was observed in diseased meniscus tissue acquired during partial meniscectomy[20,26,27]. MMP-8 activity in osteoarthritis pathogenesis is less well studied, but was identified as increased in expression and secretion in human osteoarthritis meniscus cells and can be stimulated to increase by pro-inflammatory stimulation[23]. MMP-8 degrades collagen, is associated with neutrophil infiltration, and co-localizes with IL-1β and type II collagen cleavage in osteoarthritic cartilage[20]. Similar inflammatory pathway activation has also been identified in pig meniscus explants with increased MMP1 activity, proteoglycan release and nitric oxide release in response with IL-1 stimulation[21]. MMP-1 and MMP-3 were also previously demonstrated to be upregulated in pig and human meniscus in response to pro-inflammatory stimulation, which supports an active biologic role for the meniscus disease process[19].

Diseased meniscus explants secreted matrix-degrading enzymes which may impact cartilage, but the tissue interaction is likely part of a more dynamic signaling network. We identified age- and disease-dependent cytokine production by meniscus explants that paralleled behaviors seen in human meniscus cell culture studies[23]. Chemokines CXCL1, CXCL2, CXCL3 (identified by the GRO antibody) and IL-8 were secreted by both young and old menisci

which may not independently initiate osteoarthritic changes but may contribute to the propagation of inappropriate inflammatory cycles after injury[16,20,43]. These genes were recently identified as part of the meniscus pathology following injury and were up-regulated in pro-inflammatory stimulated human meniscus[23–25]. In mouse articular chondrocytes, alterations in the CXCR1/2 signaling pathway (which binds CXCL1, -2 and -3 ligands) were associated with a disruptions of articular cartilage phenotype and the development of osteoarthritic changes, including decreased matrix production and the development of more severe osteoarthritis in the destabilized medial meniscus mouse model[43].

IL-6 secretion appeared to be elevated in older and higher grade vervet menisci but was also present in younger vervet menisci. Increased IL-6 production stimulates catabolic responses in cartilage tissue, but as we recently demonstrated, also increases catabolic activity in human meniscus cells[16,20]. The elevated vervet IL-6 secretion in young vervet menisci was unexpected, but could be evidence of early stage inflammatory processes.

GM-CSF was increased in more degenerated menisci. GM-CSF is linked to the inflammatory process in rheumatoid arthritis and has been observed in human osteoarthritic synovium[14]. Future investigations may link GM-CSF production to the more fibroblastic cell phenotype in the meniscus. IL-7 was also shown to be produced by aged and degenerative vervet menisci. IL-7 stimulates human chondrocyte MMP-13 production, extracellular matrix degradation, and proteoglycan release from human cartilage explants[18]. Chondrocytes not only respond to IL-7 with increased MMP-13 production, but they may also be stimulated to produce IL-7 by IL-1 and IL-6. Additionally, IL-7 is reportedly higher in humans during earlier stages of osteoarthritis and is also elevated in patients with synovitis and older patients with osteoarthritis[16,44]. Increased secretion of IL-7 may be more detrimental to joint tissues since IL-7 receptor expression is significantly increased in human menisci associated with chondrosis[17]. Increases in IL-8 secretion, such as those observed in aged vervet knees, are thought to contribute to chondrocyte hypertrophy, calcification and crystals in the joint, and the development of subchondral bone sclerosis[16].

Our results confirm many of the trends recently reported in an analysis of gene expression in meniscus tears in patients undergoing partial meniscectomy[25]. Patients younger than 40 years with a meniscus tear demonstrated increased expression of IL-1β and the matrix-degrading enzymes MMP-1, MMP-9, MMP-13, and ADAMTS-5 compared to older patients with meniscus tears. Gene expression of cytokines (IL-1β and TNF-α), chemokines (CCL3 and CCL3L1) and MMP-13 were increased in patients with a meniscus tear and concomitant anterior cruciate ligament tear compared to a meniscus tear alone. These findings support the concept that meniscus tears that are of a traumatic etiology may be more prone to inflammatory changes and a biologic rationale for the increased risk of development of osteoarthritis after meniscal injury. Our findings in the vervet are also congruent with the increased matrix degrading enzyme and cytokine secretion observed in human meniscus cells treated with pro-inflammatory stimulation[23]. Human meniscus cells treated with pro-inflammatory stimulants, including interleukin and fibronectin fragments, increased their secretion of cytokines, chemokines, and matrix degrading enzymes, while also increasing their cellular catabolic activity through the NF-κB pathway[45]. Older, more degenerative menisci in our vervet analysis produced greater basal amounts of matrix-degrading enzymes and pro-inflammatory factors than younger vervets. It is possible that degenerative menisci contribute to the pro-inflammatory environment of idiopathic osteoarthritis in a similar fashion to the acutely injured meniscus. We observed age related increases in meniscal inflammatory cytokine release and MMP production; and menisci from osteoarthritic older vervets produced greater amounts of cytokines than their age matched counterparts with less severe osteoarthritis. Meniscal secretion of enzymes and cytokines likely stimulate catabolic activity in both cartilage and synovium, potentially contributing to osteoarthritis development, especially after meniscal injury. Inhibition of MMPs stimulated by cytokines improved *in vitro* meniscal repairs, which suggests a critical role for MMPs in connective tissue degradation[46]. Increased catabolic secretion in aged individuals may better explain high rates of failure for meniscus repair in older patients[25,47–49].

Our findings are additionally supported by a recent study examining the transcriptome alterations in injured and degenerative human menisci[17]. Inflammatory and matrix-degrading responses were elevated in injured menisci and associated with chondrosis, and degenerative menisci demonstrated decreased expression of matrix proteins with an age associated increase in inflammatory pathway expression[17]. In a whole joint analysis in mice, age associated alterations in gene expression demonstrated a decrease in matrix-associated genes with a concomitant increase in inflammatory gene expression which would support a predominately catabolic environment in the aged and diseased joint[45]. Menisci in older patients with a previous meniscus injury likely produce increased matrix degrading enzymes as a function of both the initial injury and age.

Our study has advantages as well as common limitations of laboratory and animal models. While quadrupedal non-human primate skeletal anatomy and biomechanics are not identical to humans, the vervet biomechanics and disease progression are more similar to humans than those of rodent models[50]. Our study is limited by the ability only to analyze female vervet monkeys, since no male vervets were available to study; however, osteoarthritis is highly prevalent in women. Osteoarthritis is more prevalent in female macaques[10] and progresses more rapidly in female baboons[31]. While certain nonhuman primate models do not demonstrate gender-related differences in the prevalence in osteoarthritis[6], we believe that we have selected an appropriate model for evaluation of the meniscus in osteoarthritis pathobiology. Despite these limitations, the animals exhibit many similar morbidities associated with human aging and decline, including physiologic and pathologic alterations in skeletal muscle and physical function[13,22,30], despite a lifelong consumption of a healthy, low-fat 'chow' diet.

Knee joints were analyzed using macroscopic and CT evaluation. The explant culture and handling precluded histologic analysis of the specimens; however, previous examination of knee osteoarthritis in rhesus macaques demonstrated significant correlation between macroscopic and histologic examination of knee arthritis[8]. Whole meniscal explants were used to assess the contributions of both the inner and outer meniscus zones to protein secretion that would be expected in the intact joint. This study sought to identify catabolic patterns in menisci and place them in the context of the natural disease progression in a monkey model.

A final, but important consideration is that we cannot exclude a contribution from post-traumatic arthritis. While all ligamentous structures were intact at the time of specimen acquisition and no gross evidence of previous fracture or joint malalignment was present, it is not possible to confirm that all vervets remained uninjured during their lifetime. Post-traumatic arthritis would be expected to occur after significant ligamentous injury or fracture, since these mechanisms are associated with chondral damage. Our observational data and detailed physicals did not demonstrate evidence of severe injuries, but non-human primates are known to be stoic despite pain[35]. In consideration of these factors, we favor an idiopathic etiology for the development of osteoarthritis, but a portion may be attributable to post-traumatic arthritis; regardless of the precise etiology, all vervet arthritis was naturally occurring and not intentionally induced.

The role of the meniscus in osteoarthritis pathogenesis remains to be defined, and this cross sectional analysis sought to better understand the biologic activity of the meniscus in osteoarthritis. The production of matrix degrading enzymes was clearly associated with morphologic changes in naturally occurring vervet knee arthritis. While the detailed cellular and molecular mechanisms cannot be elucidated from these *in vitro* studies, the data suggest that increased meniscal secretion of MMPs and inflammatory cytokines likely play a significant role in osteoarthritis pathogenesis in the meniscus and articular cartilage of the knee joint. Further exploration of molecular events associated with the progression of osteoarthritis in the aging nonhuman primate model has great potential to identify key factors involved in early osteoarthritis pathology, and hopefully lead to new approaches to prevent, or at least attenuate, the development of osteoarthritis.

Author contributions

Stone: Conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding, collection and assembly of data.

Willey: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, collection and assembly of data.

Vanderman: Critical revision of the article for important intellectual content, final approval of the article, collection and assembly of data.

Long: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article.

Register: Conception and design, *in vivo* imaging, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

Shively: Conception and design, physical function assessments, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

Stehle: Conception and design, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

Loeser: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

Ferguson: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining of funding.

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Conflict of interest

The authors do not have competing interests directly or indirectly related to the publication of this work.

Supplementary data

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