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Original article

Expression of mitochondrial *TSPO* and *FAM173B* is associated with inflammation and symptoms in patients with painful knee osteoarthritis

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Abstract

Objectives. To characterize the expression profiles of two nuclear-encoded mitochondrial genes previously associated with chronic pain, the translocator protein (*TSPO*) and family with sequence similarity 173B (*FAM173B*), in different knee compartments from patients with painful knee OA. Also, to examine their association with the joint expression of inflammatory cytokines/chemokines and clinical symptoms.

Methods. The study was performed on 40 knee OA patients and 19 postmortem (PM) controls from which we collected the knee tissues: articular cartilage (AC), synovial membrane (SM) and subchondral bone (SB). Quantitative real-time polymerase chain reaction was used to determine the relative mRNA levels of *TSPO*, *FAM173B*, and inflammatory mediators *IL6*, *IL8*, *IL10*, *IL12*, *MCP1*, *CCL11* and *CCL17*. OA patients rated their pain intensity (visual analogue scale), severity of knee-related outcomes (KOOS) and pain sensitivity assessed by pressure algometry.

Results. The gene expression of *TSPO* in SM was elevated in OA patients compared with control subjects while there were no group differences in AC and SB. Expression of *FAM173B* was reduced in SM but elevated in SB in OA patients compared with controls. The expression of *TSPO* and *FAM173B* in SM and SB was associated with the expression of inflammatory substances, but not in AC. Synovial expression of *TSPO* correlated with lower pain intensity and *FAM173B* with increased pressure pain sensitivity in OA.

Conclusion. Our results suggest that altered expression of *TSPO* and *FAM173B* is associated with joint expression of inflammatory mediators and with clinical symptoms indicating the relevance for the pathophysiology of knee OA.

Key words: osteoarthritis (OA), mitochondrial dysfunction, synovial inflammation, translocator protein (TSPO), family with sequence similarity 173B (FAM173B), joint pain

CLINICAL SCIENCE

Rheumatology key messages

- Synovial expression of *TSPO* is elevated in OA patients and associated with reduced pain intensity (VAS-global).
- Synovial *FAM173B* expression in OA patients is associated with increased pressure pain sensitivity in knees.
- Synovial and subchondral bone expression of *FAM173B* and *TSPO* is associated with inflammation in OA.

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Introduction

OA is the most prevalent joint disease characterized by inflammation of synovial joints and cartilage destruction [1, 2] with pain being a dominant symptom. Joint inflammation was found to contribute to pain and cartilage degradation through increased release of inflammatory substances such as cytokines and chemokines from synoviocytes in the SM, chondrocytes in articular

cartilage (AC), osteoblasts and osteoclasts in the subchondral bone (SB), and infiltrating macrophages [3–6]. Growing evidence suggests that mitochondrial dysfunction in the joint cells can lead to changes in a local production of inflammatory mediators [7, 8]. In particular, in cultured human chondrocytes, inhibition of mitochondrial respiratory chain activity was shown to increase the production of cytokines IL1, IL6 and IL18, prostaglandin E2 (PGE2), the chemokines IL8 and monocyte chemotactic protein 1 (MCP1), as well as the metalloproteases MMP1, MMP3 and MMP13 [9, 10]. Mitochondrial dysfunction induced by inhibition of the respiratory chain was also shown to increase the release of inflammatory substances PGE2 and IL8 in human synoviocytes [11]. Furthermore, exposure to inflammatory cytokines such as IL1β and TNF-α induced increased mitochondrial DNA damage in OA chondrocytes by stimulating the production of reactive oxygen species and nitric oxide [12]. Additionally, chondrocytes from patients with knee OA had decreased protein levels of mitochondrial biogenesis mediators and reduced mitochondrial mass [13]. Hence, these studies suggest the presence of a feedback mechanism between mitochondrial dysfunction and synovial inflammation in patients with OA. However, the association between mitochondrial dysfunction and joint pain is currently unknown. We postulated that mitochondrial dysfunction due to the abnormal expression of mitochondrial genes could lead to altered expression profiles of inflammatory cytokines and chemokines in the joint tissues and thus ultimately to the joint pain. Therefore, we wanted to examine the expression profiles of two nuclear-encoded mitochondrial genes, both previously associated with chronic pain, i.e. the translocator protein (*TSPO*) and the family with sequence similarity 173B (*FAM173B*), in patients with painful knee OA.

TSPO is an outer mitochondrial membrane protein found to be up-regulated in activated brain microglia and astrocytes and involved in the regulation of inflammation, synthesis of neurosteroids, oxidative stress and cell survival [14, 15]. So far, TSPO has been associated with different painful conditions, including chronic low back pain [16–18], fibromyalgia [19, 20] and rheumatoid arthritis [21]. We have recently shown that *TSPO* gene expression in intervertebral discs was lower in patients suffering from pain due to lumbar disc herniation (LDH) compared with patients with painful degenerative disc disease. The lower *TSPO* expression in the discs of LDH patients was associated with high expression of proinflammatory cytokines as well as the higher intensity of low back pain [18]. Since elevated TSPO ligand binding was also reported in neuroforamina and lumbar spinal cord from LDH patients [16], TSPO mechanisms seem to be relevant both in peripheral tissues and within the nervous system.

The gene *FAM173B* encodes a mitochondrial lysine-specific methyltransferase, which is responsible for the methylation of ATP synthase, an essential protein in cellular ATP production [22]. In a genome-wide association study, a genetic polymorphism of the *FAM173B* gene was linked to joint-specific chronic widespread pain [23]. Furthermore, the expression of *FAM173B* was up-regulated in the spinal cord of mice following induction of peripheral inflammatory pain using two different models [23]. In an elegant series of experiments Willemen *et al.* demonstrated that FAM173B is involved in persistent inflammatory and neuropathic pain through its lysine-specific methyltransferase activity in mitochondria of sensory neurons promoting macrophage/microglia activation through a reactive oxygen species-dependent pathway [24].

Furthermore, a loss of *TSPO* and *FAM173B* was previously linked to mitochondrial dysfunction. Deficiency of *TSPO* in mouse glioma GL261 cells leads to decreased global ATP production and reduction in mitochondrial respiratory capacities [25]. Knock-out of *FAM173B* in human HAP1 cells was associated with impaired assembly of the mitochondrial ATP synthase complex [22] and increased mitochondrial respiration [26].

In the current study, we aimed to profile the expression of these two nuclear-encoded mitochondrial genes, *TSPO* and *FAM173B*, in different knee compartments in OA patients compared with controls. Furthermore, we examined the associations between expression of *TSPO* and *FAM173B* and inflammatory cytokines/chemokines in different joint tissues, as well as the associations between the expression of these mitochondrial genes and clinical symptoms. Finally, we also characterized potential sex differences as sex-specific innate immune mechanisms are clinically relevant in patients with chronic pain [27–31], including knee OA [32].

Methods

Study participants

Forty consecutive patients with painful knee OA (17 women and 23 men, average age 64.5 years, range 49–73 years) were recruited from the waiting list for total knee replacement (TKR) at Ortho Center, Upplands Väsby, Sweden. The inclusion criteria were 25–75 years of age, radiologically verified knee OA, OA pain as the dominant pain complaint and of sufficient severity to merit TKR. The exclusion criteria were the presence of chronic pain due to causes other than knee OA (fibromyalgia, degenerative disc disease, disc herniation, inflammatory rheumatic disease or neurological disease) or previous knee surgery at the knee planned for TKR. Information regarding medication was collected from all patients. Eight patients were taking analgesics (three codeine, two tramadol, two buprenorphine plaster, one strong opioid orally), 14 were taking acetaminophen and 18 had previously been taking NSAIDs at demand; however, these had stopped due to the surgical procedure. All patients received 2 g acetaminophen (paracetamol) and 10 mg oxycodone orally as premedication before surgery.

As a control group, we used 19 postmortem (PM) subjects (six women and 13 men, average age

43.6 years, range 25–68 years) that were subject to forensic autopsy. On average, the autopsy took place within 49 (15) h after the death. Exclusion criteria for PM controls were: known history of chronic pain such as fibromyalgia, OA, degenerative disc disease, disc herniation, inflammatory rheumatic disease or neurological disease and macroscopic signs of OA during cartilage examination at autopsy.

The study was approved by the ethical committee (2011/2036-31/1; 2012/2006-32) and informed consent was obtained from all contributing individuals according to the Declaration of Helsinki.

Questionnaires and sensory testing protocol

The OA patients completed the questionnaires within a week before the surgery. A 100 mm visual analogue scale (VAS) where 0 indicated 'no pain' and 100 indicated 'the worst imaginable pain' was used to rate the global intensity of the average weekly pain (VAS-global) and pain in the affected knee (VAS-knee). The severity of patient-reported symptoms was assessed by the Knee injury and Osteoarthritis Outcome Score (KOOS), which consists of five subscales: (i) pain, (ii) other symptoms, (iii) activity in daily living, (iv) function in sport and recreation, and (v) knee-related quality of life [33, 34]. Each KOOS subscale contains questions scored from 0–4 summarized into continuous scores ranging from 0 (worst) to 100 (best), and the average score of all five KOOS subscales was calculated and used for the analysis.

Furthermore, typically within a week before the surgery, pressure pain sensitivity was determined by a pressure algometer (Somedic Sales AB, Hörby, Sweden) with a flat circular tip area of 1 cm^2 and a constant pressure increase of ~50 kPa/s using visual feedback [35]. To assess pressure pain thresholds (PPTs) subjects were asked to press a button as soon as the pressure became painful. PPTs were assessed at the medial epicondyle of the femur, close to the joint space (PPT-knee). To obtain a measure of the general pain sensitivity, PPTs were also assessed once per site, bilaterally, at the trapezius muscle (mid-point of the upper border) and glutaeal muscle (upper outer quadrants of buttocks in anterior fold of muscle) and the average of these assessments was calculated for each participant (PPT-average).

Sample collection and storage

SM, subchondral bone (SB) and articular cartilage (AC) were collected from OA patients during the TKR and from PM controls at the medial side during the autopsy. All the tissues were immediately frozen at −80°C for future analysis. AC tissues from PM controls were macroscopically examined during the autopsy for any characteristic signs of OA.

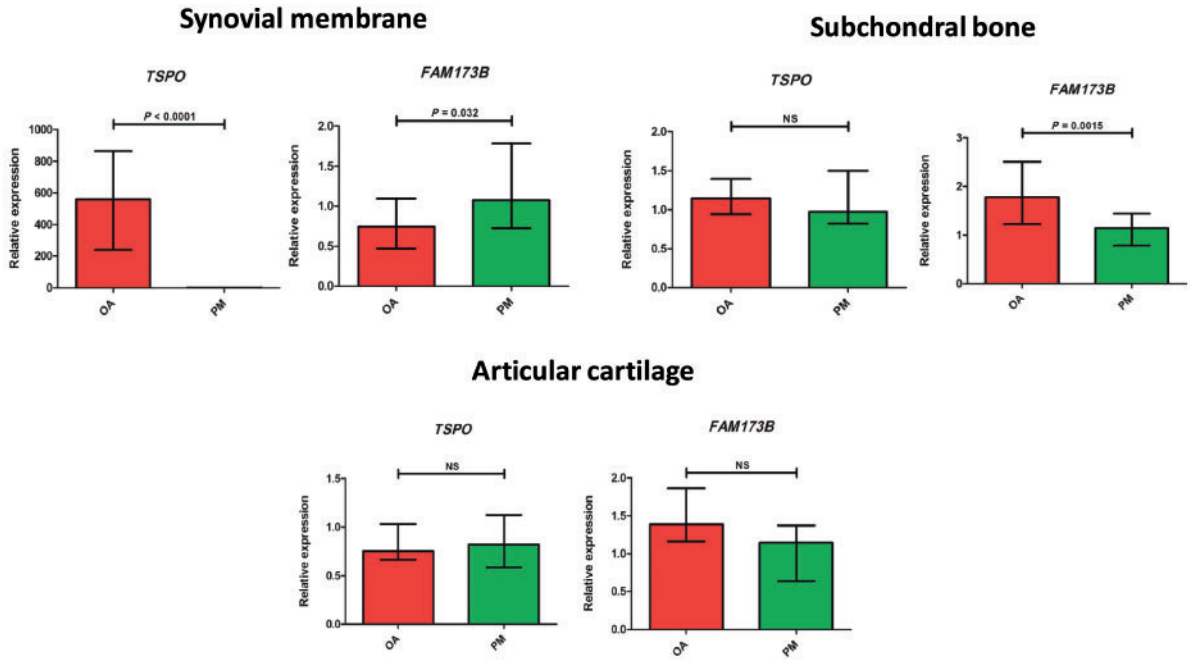
Quantitative real-time polymerase chain reaction

Frozen tissues collected from OA patients and PM controls were homogenized by Mikro-dismembrator (B. Braun Biotech International, Berlin, Germany) and dissolved in 2–3 volumes of Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was extracted using the RNeasy MiniKit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. Due to technical and handling issues, it was possible to extract total RNA from SM for 38/40 OA patients and from the AC for 30/40 OA patients and 7/19 PM controls. Quantity of RNA was determined using a Nanodrop ND-1000 spectrophotometer (Isogen Life Science, De Meern, Netherlands) and the quality of extracted RNA was assessed using the Experion automated electrophoresis system (Bio-Rad, Hercules, CA, USA). There were no differences in RNA quality index between patients and PM controls for SM and AC tissues, with mean values measured for SM as 8.04 (0.5) for OA and 7.83 (0.5) for PM controls, and for AC as 7.30 (1.54) for OA and 7.41 (1.42) for PM controls. However, the RNA integrity number for SB was reduced in PM controls [3.62 (1.74)] compared with OA patients [6.65 (2.33)]. The first-strand cDNA was synthesized from 1 μg of total RNA using a first-strand cDNA Synthesis Kit (Roche, Basel, Switzerland). Quantitative real-time PCR was performed with the StepOne Plus System (Thermo Fisher Scientific, Waltham, MA, USA) using TaqMan fast PCR master mix (Thermo Fisher Scientific, Waltham, MA, USA). Specific primers (Thermo Fisher Scentific, Waltham, MA, USA) for *IL6* (Hs00174131_m1), *IL8* (Hs00174103_m1), *MCP1* (Hs00234140_m1), *IL10* (Hs00961622_m1), *IL12* (Hs01011518_m1), *CCL11* (Hs00237013_m1), *CCL17* (Hs00171074_m1), *TSPO* (Hs00559362_m1) and *FAM173B* (Hs00291497_m1) were used to detect the targets. The C_t values were calculated by StepOne Software v2.3 (Thermo Fisher Scientific). Relative gene expression was analysed using the $2^{-\Delta Ct}$ method and the C_t values were normalized to HPRT1 (Hs02800695_m1) as the reference. The cDNA from primary human fibroblasts-like synoviocytes was used as positive control. Genes with relative expression below the limit of quantification (LOQ) were excluded from the further analysis.

Statistical analysis

The data distribution was tested by the D'Agostino–Pearson omnibus normality test. Since the data were not normally distributed, the Mann–Whitney *U*-test was used for between group comparisons and the Wilcoxon signed-rank test was used for within group comparisons. The significance of correlations was determined by Spearman's rank correlation test (two-tailed). A univariate analysis of covariance was applied to observe age- and gender-related group differences. The results with *P*-values <0.05 were considered statistically significant. The data analysis and visualization were performed by Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

FIG. 1 Comparison of *TSPO* and *FAM173B* gene expression in the knee tissues from OA patients and PM controls



Data are presented as bars with medians of relative gene expression with 25% and 75% percentile values. NS: not significant ($P > 0.05$); PM: postmortem controls.

Results

Gene expression of mitochondrial markers in OA knee tissues

The expression of *TSPO* in SM was significantly higher in OA patients compared with control subjects ($P < 0.0001$), while there were no group differences in AC and SB (Fig. 1, Table 1). OA patients had higher *TSPO* gene expression in SM compared with SB and AC ($P < 0.0001$) and in SB compared with AC ($P < 0.0001$) (Fig. 2, Table 1). There were no statistically significant correlations in *TSPO* expression between the knee compartments in OA patients as well as no gender differences (Table 1).

Compared with controls, *FAM173B* expression in OA patients was higher in SB ($P = 0.0015$), lower in SM ($P = 0.032$), while no group differences were seen in AC (Fig. 1). In OA patients, the expression of *FAM173B* was significantly lower in SM compared with the other two knee compartments ($P < 0.0001$) while no difference in expression was seen between SB and AC (Fig. 2, Table 1). No significant association in *FAM173B* gene expression between the three knee compartments was found in OA patients, and no gender differences were observed (Table 1).

A significant positive correlation was seen for the expression of *TSPO* and *FAM173B* in all OA knee tissues (AC: $r = 0.472$, $P = 0.0085$; SB: $r = 0.556$, $P = 0.00029$; SM: $r = 0.383$, $P = 0.015$).

Gene expression of inflammatory substances in knee OA tissues

Expression profiles of inflammatory substances in OA and PM tissues are provided in Table 1. In SM, expression of *IL6* ($P = 0.0247$) and *IL10* ($P = 0.0001$) was significantly increased and expression of *MCP1* ($P < 0.0001$) and *CCL17* ($P < 0.0001$) reduced in OA patients compared with PM controls. In SB, we detected increased expression of *IL6* ($P = 0.0003$), *IL8* ($P < 0.0001$), *MCP1* ($P < 0.0001$) and *CCL17* ($P = 0.00046$) in the patients *vs* PM controls. Finally, we found reduced expression of *MCP1* ($P < 0.02$) in AC of OA patients compared with controls.

No gender differences regarding the expression of inflammatory substances in the knees were seen (Table 1). Expression of *IL12* and *CCL11* was below LOQ in all the tissues as well as expression of *IL6* and *CCL17* in AC.

Correlation of *TSPO* and *FAM173B* expression in OA knees with inflammatory substances and clinical symptoms

The expression of *TSPO* in the patients was positively correlated with *IL6* ($r = 0.465$, $P = 0.002$) and *MCP1* ($r = 0.432$, $P = 0.005$) in SB. Additionally, we found a negative correlation of *TSPO* with the expression of *IL8* ($r = -0.313$, $P = 0.049$) and positive correlation with higher expression of *IL10* ($r = 0.397$, $P = 0.0135$) in SM

Table 1 Relative gene expression of mitochondrial markers and inflammatory substances in the knee tissues

Gene	SM					SB				AC				
	OA ($n = 38$)	PM ($n = 19$)	*P*-value	OA men ($n = 21$)	OA women ($n = 17$)	*P*-value	OA ($n = 40$)	PM ($n = 19$)	*P*-value	OA men ($n = 23$)	OA women ($n = 17$)	*P*-value	OA ($n = 30$)	PM ($n = 7$)	*P*-value	OA men ($n = 16$)	OA women ($n = 14$)	*P*-value
TSPO	559.4	1.03	<0.0001	601	429.5	NS	1.14	0.97	NS	1.07	1.25	NS	0.75	0.82	NS	0.72	0.87	NS
FAM173B	0.74	1.074	0.0322	0.58	0.85	NS	1.78	1.14	0.0015	1.71	2.27	NS	1.385	1.14	NS	1.25	1.68	NS
IL6	0.11	0.04	0.0247	0.098	0.1	NS	2.8	0.82	0.0003	2.7	4.3	NS				Below LOQ		
IL8	0.71	0.94	NS	0.62	0.81	NS	42.36	0.78	<0.0001	46.28	36.33	NS	0.003	0.002	NS	0.001	0.005	NS
IL10	4.92	1.51	0.0001	4.91	4.93	NS	0.71	1.19	NS	0.67	0.93	NS	1.3	0.515	NS	1.26	2.03	NS
IL12			Below LOQ						Below LOQ						Below LOQ			
MCP1	0.036	0.16	0.0001	0.039	0.031	NS	8.36	0.93	<0.0001	8.44	7.49	NS	0.005	0.011	P=0.0168	0.004	0.007	NS
CCL11			Below LOQ						Below LOQ						Below LOQ			
CCL17	0.12	0.95	0.0001	0.16	0.13	NS	2.34	0.98	= 0.0046	1.9	2.51	NS			Below LOQ			

Data are presented as the medians of relative gene expression. AC: articular cartilage; LOQ: limit of quantification; NS: not significant; PM: postmortem controls; SB: subchondral bone.

while there were no significant associations for *TSPO* expression in AC. The elevated expression of *TSPO* in SM was associated with lower average weekly pain intensity ($r = -0.369$, $P = 0.025$; VAS-global) (Table 2, Fig. 3).

There was a positive correlation between expression of *FAM173B* in SM with increased expression of *IL6* ($r = 0.444$, $P = 0.005$) and *IL10* ($r = 0.4$, $P = 0.014$) in patients with knee OA. Also, we observed that expression of *FAM173B* in SB is positively associated with higher expression of *IL6* ($r = 0.511$, $P = 0.001$), *MCP1* ($r = 0.537$, $P = 0.000356$) and *CCL17* ($r = 0.456$, $P = 0.003$) in OA. There were no significant associations between the expression of *FAM173B* and inflammatory substances in AC. The expression of *FAM173B* in SM was associated with higher sensitivity to pressure pain in the affected knees ($r = -0.365$, $P = 0.029$; PPT-knee) (Table 2, Fig. 3).

Correlation between the knee expression of inflammatory substances and clinical symptoms

Regarding SM, there was a correlation between *IL6* ($r = -0.371$, $P = 0.024$) and *IL8* expression ($r = -0.34$, $P = 0.04$) to more severe knee-related outcomes (KOOS) (Table 2). The *MCP1* expression in SM was associated with less intense knee pain ($r = -0.34$, $P = 0.046$; VAS-knee) whereas *CCL17* expression was associated with increased pressure pain sensitivity in the knee ($r = -0.368$, $P = 0.027$; PPT-knee). Expression of *IL6* in SM from male OA patients was associated with more severe knee-related outcomes ($r = -0.477$, $P = 0.029$; KOOS) while *CCL17* expression was associated with higher pain intensity ($P = 0.0086$, $r = 0.558$; VAS-global) and increased pressure pain sensitivity in the knee ($P = 0.003$, $r = -0.629$; PPT-knee) in OA males. Expression of *MCP1* in SM from OA females was associated with less intense knee pain ($r = -0.65$, $P = 0.006$; VAS-knee).

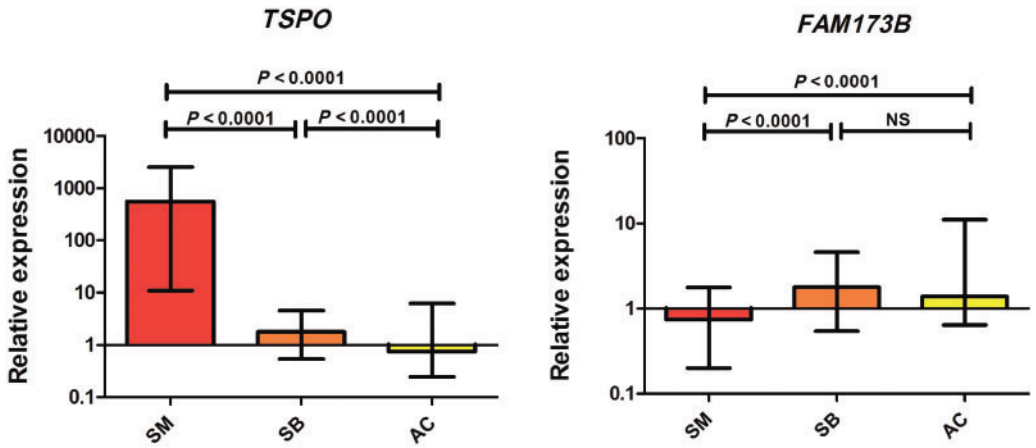
In SB, expression of *IL6* was associated with reduced sensitivity to pressure pain in the knees ($r = 0.433$, $P = 0.43$; PPT-knee) and *IL10* was associated with less intense knee pain ($r = -0.519$, $P = 0.039$; VAS-knee) in males (Table 2).

Finally, we didn't find any significant association between the expression of inflammatory cytokines and chemokines in AC and clinical symptoms.

Discussion

To our knowledge, this is the first study investigating the pain-related nuclear-encoded mitochondrial genes *FAM173B* and *TSPO* in patients with knee OA as well as the first clinical study on *FAM173B* expression in human subjects suffering from pain. The main finding was that OA patients had altered expression of both mitochondrial genes in SM and of *FAM173B* in SB. The expression of these genes was also associated with changes in the expression of inflammatory cytokines/chemokines

Fig. 2 Relative gene expression of *TSPO* and *FAM173B* in different knee tissues from OA patients



Data are presented as bars with medians of relative gene expression with 25% and 75% percentile values. AC: articular cartilage; NS: not significant ($P > 0.05$); SB: subchondral bone.

and with symptoms indicating their relevance for the pathophysiology in knee OA. However, the two genes had different effects. More specifically, the gene expression of *TSPO* was elevated in the SM of OA patients and was associated with elevated expression of anti-inflammatory cytokine *IL10*, reduced expression of pro-inflammatory cytokine *IL8* and lower pain intensity. On the other hand, the expression of *FAM173B* in SM was reduced in OA patients, and there was a positive correlation between *FAM173B* expression and the expression of *IL6* and *IL10* and a negative correlation with pressure pain thresholds. Contrary to SM, the expression of *FAM173B* was elevated in the SB of OA patients and both *FAM173B* and *TSPO* expression in SB were positively correlated with the expression of *IL6* and *MCP1* in SB while *FAM173B* was also associated with the expression of *CCL17*. No associations between the expression of the mitochondrial genes and inflammatory substances were found in AC. Our results identify the synoviocytes in SM and osteoblasts in SB as promising candidates for driving the mitochondria-related expression of inflammatory substances in knee OA since there were no significant associations between the expression of inflammatory mediators in AC to the clinical symptoms and no correlation between AC inflammatory substances and mitochondrial genes. Furthermore, these findings suggest that TSPO and FAM173B might play an important role in local knee homeostasis between pro-inflammatory and anti-inflammatory mechanisms in patients with painful knee OA.

Synovial TSPO is associated with expression of anti-inflammatory cytokine IL-10, reduced expression of pro-inflammatory IL-8 and lower pain intensity

Growing evidence suggests that OA should be considered a metabolic disorder caused by metabolic adaptation of chondrocytes and synoviocytes to the inflammatory microenvironment in the inflamed OA joints [7, 36]. The existence of a feedback mechanism between the cytokine-induced synovial inflammation and mitochondria-promoted cartilage degeneration was reported both for synoviocytes and chondrocytes in OA [9–11, 37, 38]. In line with these previous studies, we identified the association between the expression of *TSPO* and *FAM173B* to the expression profile of inflammatory cytokines and chemokines in SM and SB tissues from patients with knee OA.

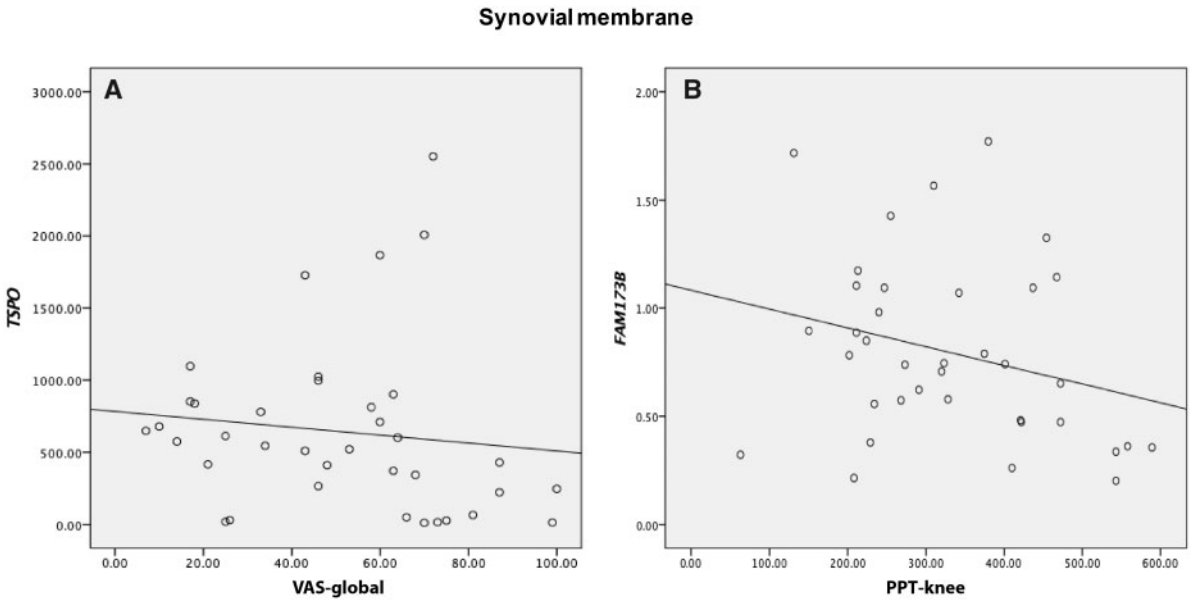
In the current study, we found that elevated expression of *TSPO* in SM from OA patients was associated with reduced average weekly pain intensity. There are several possible explanations for this finding. First, we documented a negative correlation between synovial *TSPO* expression and synovial expression of the pro-inflammatory cytokine IL-8 and the synovial *IL8* expression was associated with more severe knee-related outcomes (KOOS). These results tally with our previously reported finding from the same cohort of knee OA patients, namely that IL-8 concentrations in the synovial fluid were associated with increased pressure pain sensitivity, and in women also with increased knee pain [30]. Another possible explanation is the association between increased synovial TSPO expression and the elevated expression of the anti-inflammatory and chondroprotective cytokine IL-10 in our patients compared with controls [39]. IL-10 has been associated with reduced secretion of metalloproteinases from human macrophages [40], decreased synovial levels of cartilage degradation markers in OA patients after exercise [41] and increased survival of human chondrocytes due to reduced caspase activity [42]. Furthermore, our *TSPO* results are in line with the recent report of elevated *TSPO* gene expression and protein levels in anti-inflammatory M2 type of synovial macrophages derived from patients with rheumatoid arthritis [43]. Taken together, these findings indicate that

Table 2 Correlation of relative gene expression to clinical symptoms

	SM					SB					AC					
Gene	VAS-global	VAS-knee	PPT-average	PPT-knee	KOOS	VAS-global	VAS-knee	PPT-average	PPT-knee	KOOS	VAS-global	VAS-knee	PPT-average	PPT-knee	KOOS	
TSPO	$r = -0.369$, $P = 0.025$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
FAM173B	NS	NS	NS	$r = -0.365$, $P = 0.029$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
IL6	NS	NS	NS	NS	$r = -0.371$, $P = 0.024$	NS	NS	NS	$r = 0.433$, $P = 0.43$	NS	NS	NS	NS	NS	NS	
IL8	NS	NS	NS	NS	$r = -0.34$, $P = 0.04$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
IL10	NS	NS	NS	NS	NS	NS	NS[a]	NS	NS	NS	NS	NS	NS	NS	NS	
IL12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
MCP1	NS	$r = -0.34$, $P = 0.046$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
CCL11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
CCL17	NS[a]	NS	NS	$r = -0.368$, $P = 0.027$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Spearman correlation coefficients (r) and P-values are shown. [a]Significant only in male patients. AC: articular cartilage; KOOS: severity of knee related outcomes (0–100; 0 = extreme symptoms; 100 = no symptoms); NS: not significant ($P > 0.05$); PPT-average: general pressure pain sensitivity; PPT-knee: pressure pain sensitivity in the affected knees; SB: subchondral bone; VAS-global: global pain intensity of the average weekly pain; VAS-knee: pain intensity in the knee.

Fig. 3 Correlation of mRNA levels of nuclear-encoded mitochondrial genes *TSPO* and *FAM173B* to clinical symptoms



Two-tailed Spearman's rank correlation test. (**A**) Correlation between *TSPO* gene expression in SM and average weekly pain intensity ($r = -0.369$; $P = 0.025$; VAS-global). (**B**) Correlation between *FAM173B* gene expression in SM and pressure pain sensitivity in the affected knees ($r = -0.365$; $P = 0.029$; PPT-knee). PPT: pressure pain threshold; VAS visual analogue score.

TSPO might promote anti-inflammatory mechanisms in SM from patients with knee OA and could play an important role in the maintenance of homeostatic balance between anti-inflammatory and pro-inflammatory substances in the inflamed joints.

Synovial *FAM173B* gene expression is associated with expression of pro-inflammatory cytokine IL-6 and increased pain sensitivity

Our results suggest that TSPO and FAM173B have different effects on the profile of inflammatory substances

in SM from patients with knee OA. Unlike the elevated expression of *TSPO* in the SM, the expression of synovial *FAM173B* was decreased, and there was a positive correlation between expression of *FAM173B* and expression of the pro-inflammatory cytokine *IL6*, which was higher in our patients compared with controls. Furthermore, there was a negative correlation between the expression of *FAM173B* and pressure pain thresholds, meaning that *FAM173B* expression was associated with increased pain sensitivity. The latter might be mediated by IL-6 as *IL6* expression was associated with more severe knee-related outcomes in our cohort (KOOS), which is in accordance with our previously reported findings from the same cohort where IL-6 concentrations in the synovial fluid were associated with pain and more severe knee-related outcomes as assessed by KOOS [32]. In addition, in SB, our patients had a higher expression of *FAM173B* as well as *IL6* compared with controls, and there was an association between the expression of these substances. Therefore, our *FAM173B* data suggest the existence of a feedback loop between mitochondrial dysfunction and inflammation that can contribute to the joint pain in humans, which tallies the results from a genome-wide association study [23] and fits with previous data from pain animal models [24]. Our study also indicates that pharmacological targeting of inflammation-associated mitochondrial genes [44] should be considered in future studies as new strategies to not only prevent the destruction of cartilage but also provide the OA patients with pain relief.

Study limitations

For ethical reasons, we had to use a postmortem control group, which was not optimal. Although no significant differences in the quality of isolated RNA between PM controls and OA patients were found, we cannot exclude that the results were influenced by postmortem artefacts and differences in the age and BMI. Moreover, the expression of certain genes might have been affected by cellular infiltrates. The type-I error might be pronounced in the absence of a multiple comparison correction. Our findings should be considered as exploratory to identify promising therapeutic targets for future studies. The identified associations between gene expression and clinical symptoms should be replicated in additional cohorts of patients with knee OA.

Conclusions

Overall, our results suggest that altered synovial expression of the nuclear-encoded mitochondrial genes *TSPO* and *FAM173B* is associated with changes in the expression of cytokines/chemokines and pain mechanisms in the affected knees of patients with painful knee OA. Our results support the presence of feedback mechanism between mitochondrial dysfunction and synovial inflammation in patients with OA and suggest that targeting inflammation-associated mitochondrial proteins could become a new strategy in the treatment of OA.

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