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

Vinko Palada1, Aisha Siddiqah Ahmed2, Anders Hugo3, Maja R. Radojčić4, Camilla I. Svensson1,* and Eva Kosek2,*

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

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 chemoattractant protein 1 (MCP1), as well as the metalloproteinases 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]. Moreover, 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 [16]. 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/microglial 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 2g acetaminophen (paracetamol) and 10mg 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 cm² 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 gluteal 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 Scientific, 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 Cq values were calculated by StepOne Software v2.3 (Thermo Fisher Scientific). Relative gene expression was analysed using the 2-ΔΔCt method and the Cq 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).
**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.
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...
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 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 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.

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...
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.

### Table 2

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

Spearman correlation coefficients ($r$) and $P$-values are shown. *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

**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.

Two-tailed Spearman’s rank correlation test.
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.

Acknowledgements

The authors thank orthopaedic surgeons Ingemar Gladh and Per Gerdin for patient recruitment and taking tissue samples during surgery at Ortho Center, Stockholm. Furthermore, we thank Carola Skärvinge, research nurse at Ortho Center, for excellent logistic assistance and Azar Baharpoor, Department of Physiology and Pharmacology, Karolinska Institutet for support and laboratory assistance.

Funding: This work was supported by Stockholm County Council, Swedish research Council (K2013-52X-22199-01-3 for E.K. and 542–2013-8373 for C.I.S.), Knut and Alice Wallenberg Foundation (C.I.S.), Swedish Rheumatism Association (A.S.A.), King Gustav V Foundation Sweden (A.S.A.), IBSA Foundation (V.P.), Petrus and Augusta Hedulds Foundation (V.P.), Ulla and Gustaf af Ugglas Foundation (V.P.), KI Rheumatology Foundation (V.P.), Lars Hiertas Minne Foundation (V.P.) and Eli Lilly. The research was also funded from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 602919 and from a donation from the Lundblad family. The funding sources had no influence on study design or scientific content of this manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

References

1. Martel-Pelletier J, Barr AJ, Cicuttini FM et al. Osteoarthritis. Nat Rev Dis Primers 2016;2:16072.
2. Eitner A, Hofmann GO, Schaible HG. Mechanisms of osteoarthritic pain. Studies in humans and experimental models. Front Mol Neurosci 2017;10:349.
3. Rainbow R, Ren W, Zeng L. Inflammation and joint tissue interactions in OA: implications for potential therapeutic approaches. Arthritis 2012;2012:741582.
4. Miller RE, Miller RJ, Malfait AM. Osteoarthritis joint pain: the cytokine connection. Cytokine 2014;70:185–93.
5. Ahmed AS, Gedin P, Hugo A et al. Activation of NF-κB in synovium versus cartilage from patients with advanced knee osteoarthritis: a potential contributor to inflammatory aspects of disease progression. J Immunol 2018;201:1918–27.
6. Zhu S, Zhu J, Zhen G et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. J Clin Invest 2019;129:1076–93.
7. Blanco FJ, Rego I, Ruiz-Romero C. The role of mitochondria in osteoarthritis. Nat Rev Rheumatol 2011;7:161–9.
8. Mobasher A, Rayman MP, Guallullo O et al. The role of metabolism in the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2017;13:302–11.
9. Vaamonde-García C, Riveiro-Naveira RR, Valcárcel-Ares MN et al. Mitochondrial dysfunction increases inflammatory responsiveness to cytokines in normal human chondrocytes. Arthritis Rheum 2012;64:2927–36.
10 Ciller-Pastor B, Rego-Pérez I, Oreiro N, Fernandez-Lopez C, Blanco FJ. Mitochondrial respiratory chain dysfunction modulates metalloproteases-1, -3 and -13 in human normal chondrocytes in culture. BMC Musculoskelet Disord 2013;14:235.

11 Valcárcel-Ares MN, Riveiro-Naveira RR, Vaamonde-Garcia O et al. Mitochondrial dysfunction promotes and aggravates the inflammatory response in normal human synoviocytes. Rheumatology (Oxford) 2014;53:1332–43.

12 Kim J, Xu M, Xo R et al. Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes. Osteoarthritis Cartilage 2010;18:424–32.

13 Wang Y, Zhao X, Lotz M, Terkeltaub R, Liu-Bryan R. Mitochondrial biogenesis is impaired in osteoarthritic chondrocytes but reversible via peroxisome proliferator-activated receptor-γ coactivator 1α. Arthritis Rheumatol 2015;67:2141–53.

14 Pozzo E, Costa B, Martini C. Translocator protein (TSPO) and neurosteroids: implications in psychiatric disorders. Curr Mol Med 2012;12:426–42.

15 Gattiffi J, Campanella M. TSPO is a REDOX regulator of cell mitophagy. Biochem Soc Trans 2015;43:543–52.

16 Loggia ML, Chonde DB, Akeju O et al. Evidence for brain glial activation in chronic pain patients. Brain 2015;138:604–15.

17 Albrecht DS, Ahmed SU, Kettnier NW et al. Neuroinflammation of the spinal cord and nerve roots in chronic radicular pain patients. Pain 2018;159:968–77.

18 Palada V, Ahmed AS, Finn A et al. Characterization of neuroinflammation and periphery-to-CNS inflammatory cross-talk in patients with disc herniation and degenerative disc disease. Brain Behav Immun 2019;75:60–71.

19 Kosek E, Martinsen S, Gerdle B et al. The translocator protein gene is associated with symptom severity and cerebral pain processing in fibromyalgia. Brain Behav Immun 2016;58:527–38.

20 Albrecht DS, Forsberg A, Sandstrom A et al. Brain glial activation in fibromyalgia – a multi-site positron emission tomography investigation. Brain Behav Immun 2019;75:72–83.

21 Narayan N, Owen DR, Mandhair H et al. Translocator protein as an imaging marker of macrophage and stromal activation in RA pannus. J Nucl Med 2018;59:1125–32.

22 Malecki JM, Willemen HLDM, Pinto R et al. Lysine methylation by the mitochondrial methyltransferase FAM173B optimizes the function of mitochondrial ATP synthase. J Biol Chem 2019;294:1128–41.

23 Peters MJ, Broer L, Willemen HL et al. Genome-wide association study meta-analysis of chronic widespread pain: evidence for involvement of the 5p15.2 region. Ann Rheum Dis 2013;72:427–36.

24 Willemen HLDM, Kavelaars A, Prado J et al. Identification of FAM173B as a protein methyltransferase promoting chronic pain. PLoS Biol 2018;16:e2003452.

25 Fu Y, Wang D, Wang H et al. TSPO deficiency induces mitochondrial dysfunction, leading to hypoxia, angiogenesis, and a growth-promoting metabolic shift toward glycolysis in glioblastoma. Neuro Oncol 2020;22:240–52.

26 Malecki JM, Willemen HLDM, Pinto R et al. Human FAM173A is a mitochondrial lysine-specific methyltransferase that targets adenine nucleotide translocase and affects mitochondrial respiration. J Biol Chem 2019;294:11654–64.

27 Levine JD, Khasar SG, Green PG. Neurogenic inflammation and arthritis. Ann N Y Acad Sci 2006;1069:155–67.

28 Karshikoff B, Lekander M, Soop A et al. Modality and sex differences in pain sensitivity during human endotoxemia. Brain Behav Immun 2015;46:35–43.

29 Karshikoff B, Jensen K, Kosek E et al. Why sickness hurts: a central mechanism for pain induced by peripheral inflammation. Brain Behav Immun 2016;57:38–46.

30 Doyle HH, Murphy AZ. Sex differences in innate immunity and its impact on opioid pharmacology. J Neurosci Res 2017;95:487–99.

31 Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. J Neurosci Res 2017;95:500–8.

32 Kosek E, Finn A, Uttenius C et al. Differences in neuroimmune signalling between male and female patients suffering from knee osteoarthritis. J Neuroimmunol 2018;321:49–60.

33 Roos EM, Roos HP, Lodhman LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)—development of a self-administered outcome measure. J Orthop Sports Phys Ther 1998;28:88–96.

34 Roos EM, Toksvig-Larsen S. Knee injury and osteoarthritis outcome score (KOOS) – validation and comparison to the WOMAC in total knee replacement. Health Qual Life Outcomes 2003;1:17.

35 Kosek E, Ekholm J, Nordemar R. A comparison of pressure pain thresholds in different tissues and body regions. Long-term reliability of pressure algometry in healthy volunteers. Scand J Rehabil Med 1993;25:117–24.

36 Loeser RF, Collins JA, Dieckman BO. Ageing and the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016;12:412–20.

37 Jovanovic DV, Mineau F, Notoya K et al. Nitric oxide induced cell death in human osteoarthritic synoviocytes is mediated by tyrosine kinase activation and hydrogen peroxide and/or superoxide formation. J Rheumatol 2002;29:2165–75.

38 Reed KN, Wilson G, Pearsall A, Grishko VI. The role of mitochondrial reactive oxygen species in cartilage matrix destruction. Mol Cell Biochem 2014;397:195–201.

39 Wojdasiewicz P, Poniatowski LA, Szuikiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014;2014:561459.

40 Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP1 production in human mononuclearphagocytes. J Clin Invest 1995;96:2304–10.

41 Helmark IC, Mikkelsen UR, Berglum J et al. Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a
randomized controlled trial. Arthritis Res Ther 2010;12: R126.

42 John T, Müller RD, Oberholzer A et al. Interleukin-10 modulates pro-apoptotic effects of TNF-α in human articular chondrocytes in vitro. Cytokine 2007;40: 226–34.

43 Narayan N, Mandhair H, Smyth E et al. The macrophage marker translocator protein (TSPO) is down-regulated on pro-inflammatory ‘M1’ human macrophages. PLoS One 2017;12:e0185767.

44 Blanco FJ, Rego-Pérez I. Mitochondria and mitophagy: biosensors for cartilage degradation and osteoarthritis. Osteoarthritis Cartilage 2018;26:989–91.