The association between changes in synovial fluid levels of ARGs aggrecan fragments, progression of radiographic osteoarthritis and self-reported outcomes: a cohort study

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SUMMARY

Objective: To investigate whether change in concentrations over time of aggrecanase generated ARGs aggrecan in synovial fluid (SF ARGs) associates with progression of radiographic knee osteoarthritis (OA) and patient-reported outcome in subjects with previous meniscectomy.

Methods: We studied 141 subjects at two time points after meniscectomy. Time point A was on average 18 years after meniscectomy, time point B was on average 7.5 years later; 74 subjects had SF available from both examinations. We measured SF ARGs by an electrochemiluminescence immunoassay, graded radiographic features of tibiofemoral or patellofemoral OA according to the Osteoarthritis Research Society International (OARSI) atlas, and scored patient-reported outcomes using the Knee Injury and Osteoarthritis Outcome Score (KOOS). Using logistic regression (adjusted for age, gender, body mass index, time between examinations, and SF ARGS at first examination) we assessed associations between change in SF ARGs between first and second examinations and progression of radiographic OA and KOOS.

Results: In subjects with decreasing SF ARGs between examinations, the likelihood of loss of joint space and worsening of KOOS pain between examinations was increased 6- and 4-fold respectively compared to those increasing in SF ARGs (odds ratio (OR) 5.72; 95% confidence interval (CI) 1.53–21.4 and 3.66; 1.01–13.2, respectively). No significant associations were seen between decreasing SF ARGs and progression of osteophytes (OR 0.88; 0.28–2.78), or for patient-reported outcomes other than KOOS pain.

Conclusion: Having decreasing levels of SF ARGs over time was associated with an increased risk of loss of joint space and pain worsening, but showed no association with other patient-reported outcomes or osteophyte progression.

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Introduction

Osteoarthritis (OA) is distinguished by loss of cartilage, alterations of subchondral bone, formation of osteophytes, and is associated with pain and function limitations. Much effort has been put into the development of OA biomarkers in search of tools to enable earlier diagnosis and prognosis. Aggrecan is a major constituent in articular cartilage and is of crucial importance for load distribution and shock-absorption. Two aggreganolytic enzymes with increased expression and activity in pathology are the aggrecanases a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS-4) and ADAMTS-5 (also known as aggrecanase 1 and 2, respectively). They both cleave aggrecan at the Glu392-Ala393 bond (amino acid numbering based on the accession number [Swiss-Prot: P16112] for human aggrecan) in the interglobular domain resulting in the release of AGS neoepitope fragments carrying the majority of the fixed negative charges of aggrecan.

Joint injury predisposes individuals to develop OA. We have shown that the release from cartilage into synovial fluid (SF) of aggrecanase generated AGS aggrecan is highly elevated after knee trauma, and then with time gradually reverts toward normal levels. We have further reported that 18 years after a meniscus injury treated by meniscectomy, SF levels of AGS aggrecan are no
different from uninjured and un-operated individuals, but higher concentrations within these relatively normal levels were associated with less progression of radiographic knee OA (ROA) over the next 4–10 years\textsuperscript{11}. Our proposed hypothesis for this association was an effect of higher levels of aggrecan synthesis in combination with aggrecanase activity, leading to both an increased release of ARGS into SF and a net beneficial incorporation into cartilage\textsuperscript{11}.

The primary aim of the present study was therefore to investigate if changes in SF levels of ARGS–aggrecan over 4–10 years were associated with progression of ROA. We hypothesised that individuals with decreasing levels of SF ARGS over time would have an increased risk of ROA progression. A secondary aim was to investigate whether SF ARGS levels, or change in those levels over time, were associated with changes in patient-reported outcomes, using the Knee Injury and Osteoarthritis Outcome Score (KOOS)\textsuperscript{12,13}.

Materials and methods

Materials

Chemicals were as described\textsuperscript{14}. Human recombinant ADAMTS-4\textsuperscript{15} and monoclonal antibody (MAB) OA-1 were from GlaxoSmithKline (Collegeville, PA, USA). MAB AHP0022 against human aggrecan was from Invitrogen (Carlsbad, CA, USA). High bind MA60096-well microtitre plates (no. L11XB-1), streptavidin with Sulfo-Tag (streptavidin tagged with the reporter molecule ruthenium (II) tris-bipyridyl, no. R32AD), 4× Read Buffer T with surfactant (no. R92TC) and the Sector Imager 6000 with software Discovery Workbench 2006 MSD_3.0_18 were from Meso Scale Discovery (MSD, Gaithersburg, MD, USA).

Subjects

The study was approved by the ethics committee of the Faculty of Medicine at Lund University; informed consent was obtained from all participants. Subjects were from a cohort of 317 patients, retrospectively identified to have undergone isolated meniscectomy at Lund University Hospital in 1973, 1978 or 1983–1985\textsuperscript{16}. The first examinations (A) were performed 1994, 1995 and 2000, respectively, and the second examination (B) in 2004. The mean (range) time from meniscectomy to examination A was 18 (15–22) years and the time between examinations A and B was 7.5 (4–10) years (Fig. 1). As described\textsuperscript{16}, reasons for exclusion were previous knee surgery, meniscectomy in both knee compartments, osteochondritis dissecans, fracture in or adjacent to the knee, septic arthritis, osteonecrosis, any ligament injury, or radiographic signs of knee OA at time of surgery. Out of 859 identified subjects, 456 fulfilled criteria and were invited to participate at examination A, 329 responded and 317 had radiographs taken. Here we further excluded subjects with end-stage OA (defined below) of the index knee at examination A. Out of the 317 available subjects, 141 had complete radiographic and demographic data, and SF at examination A; these were included as the Study cohort \( n = 141 \) (Fig. 2) with characteristics as described\textsuperscript{11}. Of the included 141 subjects with SF at examination A, a subset of 74 had SF available also from examination B in average 7.5 years later and were studied as Subset \( n = 74 \) for investigation of longitudinal change in SF levels of ARGS–aggrecan (Fig. 2). Characteristics of Subset \( n = 74 \) is presented in Table I. Based on the previously noted association between SF ARGS at examination A and loss of joint space between examination A and examination B\textsuperscript{11}, the subjects in Subset \( n = 74 \) were also stratified for increasing or decreasing SF ARGS levels from examination A to examination B (Table I).

Radiographic examination

Radiographs of the tibiofemoral (TF) and patellofemoral (PF) joints were obtained as described\textsuperscript{11}. Joint space narrowing (JSN) and osteophytes in the TF and PF joints were graded on a four point scale (0–3, where 0 = no evidence of JSN or osteophytes) according to the 1995 atlas of Osteoarthritis Research Society International (OARSI)\textsuperscript{17,18}, as described\textsuperscript{11}. We considered progression of JSN, osteophytes, or their sum (progression of ROA) to have occurred with an increase from examination A to examination B of the respective scores by one or more in any of the TF compartments or the PF compartment. This includes both incident JSN and osteophytes at examination B and worsening of already existing changes\textsuperscript{11}.

ROA

A knee was defined as having ROA with any of the following:

1. JSN in any TF compartment or the PF compartment \( \geq \) grade 2
2. Sum of marginal osteophyte grades in the medial or lateral TF compartment or the PF compartment \( \geq \) 2
3. JSN grade 1 and osteophyte grade 1 in the same TF compartment or JSN grade 1 and osteophyte grade 1 in the PF compartment

This cut-off approximates grade 2 TF OA on the Kellgren and Lawrence (K/L) scale\textsuperscript{18}.

End-stage OA

A knee was considered to have end-stage ROA either (1) with JSN grade 3 in any of the TF compartments or in the PF compartment, or (2) when a subject had undergone subsequent tibial osteotomy or arthroplasty for OA.

Self-reported patient-relevant outcomes by KOOS

For assessment of patient-relevant outcomes we used the self-administered questionnaire KOOS\textsuperscript{12,13}. The KOOS questionnaire covers the five patient-relevant dimensions pain, other symptoms, function of daily living, function in sports and recreation, and knee-related quality of life, with scores ranging from 0 (worst) to 100 (best)\textsuperscript{12,13}, and was scored according to the instructions on the KOOS home page\textsuperscript{19}. A decline between examinations by 10 units or more was used as cut-off for a clinically relevant progression in all subscales\textsuperscript{20}. Our primary outcome for patient-relevant outcomes was progression in KOOS pain by 10 units or more, but we also studied progression in the other four KOOS dimensions.

Measurement of ARGS-aggrecan by aggrecan capture OA-1 ARGES electrochemiluminescence (ECL) assay

SF samples were aspirated (without lavage) from the index knee joint of patients with previous meniscectomy. The fluids were at the time of aspiration centrifuged at 3,000 g, with the supernatants stored at \(-80^\circ\)C. SF concentrations of aggrecan fragments...
Table 1

Characteristics and outcome measures in Subset n = 74, subjects with SF at both examination A and B

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subset n = 74</th>
<th>Stratified for longitudinal change in SF ARGS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 74</td>
<td>SF ARGS A &lt; B</td>
<td>SF ARGS A &gt; B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 44</td>
<td>n = 30</td>
</tr>
<tr>
<td>Men</td>
<td>61 (82%)</td>
<td>34 (77%)</td>
<td>27 (90%)</td>
</tr>
<tr>
<td>Age at examination A, years</td>
<td>50 (32–73)</td>
<td>52 (35–73)</td>
<td>49 (32–69)</td>
</tr>
<tr>
<td>BMI at examination A, kg/m²</td>
<td>26 (18–41)</td>
<td>26 (18–33)</td>
<td>27 (22–41)</td>
</tr>
<tr>
<td>Years between index surgery and examination A</td>
<td>18 (15–22)</td>
<td>17 (15–22)</td>
<td>19 (15–22)</td>
</tr>
<tr>
<td>Years between examinations A and B</td>
<td>7.5 (4.0–10.3)</td>
<td>7.1 (4.0–10.1)</td>
<td>8.2 (4.0–10.3)</td>
</tr>
<tr>
<td>SF ARGS concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF ARGS at examination A, pmol ARGS/ml</td>
<td>6.66 (0.15–14.58)</td>
<td>5.59 (0.15–12.79)</td>
<td>8.24 (3.05–14.58)</td>
</tr>
<tr>
<td>SD</td>
<td>3.28</td>
<td>2.82</td>
<td>2.91</td>
</tr>
<tr>
<td>SF ARGS at examination B, pmol ARGS/ml</td>
<td>7.06 (0.12–14.17)</td>
<td>7.68 (1.46–14.71)</td>
<td>6.15 (0.12–10.74)</td>
</tr>
<tr>
<td>SD</td>
<td>2.97</td>
<td>3.11</td>
<td>2.55</td>
</tr>
<tr>
<td>Radiographic status at examinations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROA at examination A</td>
<td>41 (55%)</td>
<td>23 (52%)</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>ROA at examination B</td>
<td>57 (77%)</td>
<td>31 (70%)</td>
<td>26 (87%)</td>
</tr>
<tr>
<td>End-stage OA at examination B</td>
<td>18 (24%)</td>
<td>9 (20%)</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>Radiographic progression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of joint space/Progression of the JSN score</td>
<td>41 (55%)</td>
<td>20 (46%)</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Osteophyte progression</td>
<td>34 (46%)</td>
<td>20 (46%)</td>
<td>14 (47%)</td>
</tr>
<tr>
<td>ROA progression</td>
<td>52 (70%)</td>
<td>27 (61%)</td>
<td>25 (83%)</td>
</tr>
<tr>
<td>KOOS score progression (worsening by ≥10 units)</td>
<td>23 of 69 (33%)</td>
<td>10 of 41 (24%)</td>
<td>13 of 28 (46%)</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other symptoms</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Function of daily living</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Function in sports and recreation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee-related quality of life</td>
<td></td>
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</tbody>
</table>
| Values are numbers (%) or mean (range). P-values are by Pearson’s Chi-square test for comparisons of frequencies, respectively Student’s t test for comparisons of means. ARGS, aggrecan neoepitope amino acid sequence.
containing the ARGS neoepitope were measured using an ELCL technology\textsuperscript{11} on the MSD platform recently developed in our laboratory\textsuperscript{11}. It is an assay that uses an anti-human aggrecan capture antibody (AHP0022, Invitrogen), reactive against the globular domains 1 and 2 (G1 and G2) of human aggrecan, in combination with the MAB OA-1\textsuperscript{22}, specific for the ARGS-neoepitope within the interglobular domain of aggrecan. The assay and its evaluation has been described in detail\textsuperscript{11}. Briefly, 25 µl aliquots of SF samples were, after deglycosylation, analysed for ARGS-content against a standard of ADAMTS-4 digested and deglycosylated human cartilage A1D1 aggrecan. Samples from examinations A and B were analysed separately but directly following each other over a period of 4 months using the same batches of standard, high bind 96-well microtitre MSD plates, and antibodies. Data for SF ARGS at examination A has been previously published in a study using these samples\textsuperscript{11}. Inter and intra assay coefficients of variations were as reported\textsuperscript{11}.

\textbf{Statistical analysis}

We used the Shapiro-Wilk test to assess normal distribution of SF ARGS, Pearson’s correlation (r) for normally distributed continuous variables and Spearman’s rank order correlation (rs) when categorical variables were included. For comparison of SF ARGS between examinations A and B we used a paired samples t test. For group comparison of SF ARGS in males and females we used analysis of covariance (ANCOVA) with or without adjustments for age, body mass index (BMI), and time between meniscectomy and examination A; for other group comparisons of means we used factorial analysis of variance (ANOVA). For comparison of SF ARGS at examination A in men compared with women in the whole Study cohort n = 141\textsuperscript{11}, we adjusted for SF ARGS levels at examination A and loss of joint space from examination A to examination B, those with higher levels at examination A tended to decrease, and those with lower levels at examination A tended to increase [Fig. 3(B)].

As reported\textsuperscript{11}, a weak inverse association between SF ARGS and BMI was higher in men at both examinations (P < 0.001 examination A, P = 0.002 examination B) with mean (range) values for men and women being 26.6 (22.2–41.0) kg/m\textsuperscript{2} and 22.7 (17.9–27.3) kg/m\textsuperscript{2} at examination A, and 28.1 (23.0–40.4) kg/m\textsuperscript{2} and 24.4 (16.9–31.5) kg/m\textsuperscript{2} at examination B. No correlation was, however, observed between SF ARGS and BMI at either examination (not shown). We found no correlation between SF ARGS and individual or summed scores of radiographic features of OA or patient-relevant outcomes in the KOOS instrument at examination A (n = 141) or at examination B (n = 74) (data not shown).

\textbf{SF ARGS-aggrecan: gender differences and correlations}

We found SF ARGS at examinations A and B to be normally distributed (P = 0.65 and 0.85, respectively). In this sample and time frame (range 15–22 years) we found no significant correlation between SF ARGS and BMI at either examination (P = 0.36). In the Subset n = 74 with SF samples available from both examinations, SF ARGS concentrations showed a correlation between examinations [Fig. 3(A)], and the mean concentrations did not differ significantly between examinations (6.66 and 7.06 pmol ARGS/ml at examinations A and B, respectively; P = 0.21; Table I). In longitudinal change in SF ARGS from examination A to examination B, those with higher levels at examination A tended to decrease, and those with lower levels at examination A tended to increase [Fig. 3(B)].

As reported\textsuperscript{11}, a weak inverse association between SF ARGS and BMI was higher in men at both examinations (P < 0.001 examination A, P = 0.002 examination B) with mean (range) values for men and women being 26.6 (22.2–41.0) kg/m\textsuperscript{2} and 22.7 (17.9–27.3) kg/m\textsuperscript{2} at examination A, and 28.1 (23.0–40.4) kg/m\textsuperscript{2} and 24.4 (16.9–31.5) kg/m\textsuperscript{2} at examination B. No correlation was, however, observed between SF ARGS and BMI at either examination (not shown). We found no correlation between SF ARGS and individual or summed scores of radiographic features of OA or patient-relevant outcomes in the KOOS instrument at examination A (n = 141) or at examination B (n = 74) (data not shown).

\textbf{SF ARGS-aggrecan and its association with progression of radiographic features of OA}

\textbf{Examination A levels of SF ARGS}

As reported\textsuperscript{11}, a weak inverse association between SF ARGS concentration at examination A and loss of joint space from examination A to examination B was present in the larger Study cohort n = 141 (Table III). Associations in Subset n = 74 were essentially the same, with ORs 0.90, 0.95, and 0.87 for loss of joint space, osteophyte progression and progression of ROA, respectively.

\textbf{Results}

\textbf{Subject characteristics}

Analyses were made on all subjects with SF available from examination A (Study cohort n = 141), and on the subset of subjects with SF available also from examination B (Subset n = 74) (Fig. 2). Characteristics of Study cohort n = 141 have been described\textsuperscript{11}; characteristics of Subset n = 74 are presented in Table I. Self-reported patient-relevant outcomes (KOOS) for all 141 subjects and for the Subset n = 74 are presented in Table II. No statistically significant differences were noted between the larger Study cohort and the Subset regarding patient characteristics, radiographic status and KOOS scores at either examination, or change in radiographic status and KOOS scores from examination A to examination B, nor between the 67 subjects lacking SF at examination B and the Subset n = 74 (not shown).

\textbf{Table II}

\textbf{Self-reported KOOS data at examinations A and B}

<table>
<thead>
<tr>
<th></th>
<th>Study cohort n = 141</th>
<th>Subset n = 74</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examination A</td>
<td>Examination B</td>
</tr>
<tr>
<td>Pain</td>
<td>88 (17)</td>
<td>82 (19)</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>86 (15)</td>
<td>80 (20)</td>
</tr>
<tr>
<td>Function of daily living</td>
<td>89 (15)</td>
<td>86 (18)</td>
</tr>
<tr>
<td>Function in sports and recreation</td>
<td>67 (30)</td>
<td>62 (32)</td>
</tr>
<tr>
<td>Knee-related quality of life</td>
<td>76 (24)</td>
<td>70 (26)</td>
</tr>
</tbody>
</table>

Values are mean (SD). There were no statistically significant differences in KOOS dimensions between the Study cohort and the Subset.
although the smaller sample size increased the confidence intervals (not shown) and the cross-sectional association with loss of joint space was no longer significant.

**Examination B levels of SF ARGS**

The examination B levels of SF ARGS in Subset \( n = 74 \) were inversely associated with loss of joint space from examination A to examination B (odds ratio (OR) 0.78; Table III), and with progression of ROA from examination A to examination B (OR 0.73; Table III), where higher SF ARGS levels decreased the risk of radiographic progression. Although the logistic regression analysis showed ORs below one also for SF ARGS at examination B and osteophyte progression up to examination B, no significant association was seen (Table III).

**Table III**

Progression of outcomes and their association with levels of SF ARGS-aggrecan, and decreasing levels of SF ARGS-aggrecan between examinations by logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>SF ARGS at A</th>
<th>SF ARGS at B</th>
<th>SF ARGS A &gt; B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P</td>
<td>OR</td>
</tr>
<tr>
<td><strong>Radiographic progression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of joint space/progression of the JSN score</td>
<td>0.89 (0.80–0.99)*</td>
<td>0.029</td>
<td>0.78 (0.65–0.93)</td>
</tr>
<tr>
<td></td>
<td>0.89 (0.79–0.996)*</td>
<td>0.043</td>
<td>0.78 (0.64–0.95)</td>
</tr>
<tr>
<td>Osteophyte progression</td>
<td>0.96 (0.87–1.07)*</td>
<td>0.48</td>
<td>0.89 (0.76–1.05)</td>
</tr>
<tr>
<td></td>
<td>0.97 (0.87–1.08)*</td>
<td>0.59</td>
<td>0.89 (0.74–1.06)</td>
</tr>
<tr>
<td>ROA progression</td>
<td>0.90 (0.80–1.00)*</td>
<td>0.059</td>
<td>0.77 (0.63–0.93)</td>
</tr>
<tr>
<td></td>
<td>0.89 (0.78–1.02)*</td>
<td>0.10</td>
<td>0.73 (0.58–0.93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KOOS progression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>0.94 (0.84–1.06)</td>
<td>0.32</td>
<td>0.89 (0.74–1.07)</td>
</tr>
<tr>
<td></td>
<td>0.94 (0.84–1.06)</td>
<td>0.31</td>
<td>0.88 (0.72–1.08)</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>1.02 (0.91–1.14)</td>
<td>0.75</td>
<td>1.04 (0.88–1.23)</td>
</tr>
<tr>
<td></td>
<td>1.04 (0.92–1.18)</td>
<td>0.53</td>
<td>1.08 (0.88–1.31)</td>
</tr>
<tr>
<td>Function of daily living</td>
<td>1.01 (0.89–1.14)</td>
<td>0.88</td>
<td>1.05 (0.88–1.26)</td>
</tr>
<tr>
<td></td>
<td>1.01 (0.88–1.15)</td>
<td>0.93</td>
<td>1.04 (0.84–1.30)</td>
</tr>
<tr>
<td>Function in sports and recreation</td>
<td>1.06 (0.95–1.18)</td>
<td>0.33</td>
<td>1.04 (0.88–1.23)</td>
</tr>
<tr>
<td></td>
<td>1.07 (0.95–1.20)</td>
<td>0.29</td>
<td>1.05 (0.87–1.27)</td>
</tr>
<tr>
<td>Knee-related quality of life</td>
<td>1.00 (0.90–1.12)</td>
<td>0.94</td>
<td>1.08 (0.92–1.28)</td>
</tr>
<tr>
<td></td>
<td>1.01 (0.90–1.13)</td>
<td>0.85</td>
<td>1.15 (0.95–1.40)</td>
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</tr>
</tbody>
</table>

Radiographic outcomes (loss of joint space, osteophyte progression, and ROA progression) are based on progression from examination A to examination B of the scores of JSN, osteophytes, and either JSN or osteophytes or both, scored according to the OARSI atlas16. Worsening of patient-relevant outcomes was assessed by KOOS12,13; progression was defined as a worsening in the respective score by 10 units or more18. OR and P-values are crude (italics) or adjusted for age, gender, BMI, and time between examinations A and B, and SF ARGS at examination A (bold text), with 95% confidence interval in parentheses.

* Associations between SF ARGS at examination A and progression of radiographic features of OA was previously published in Larsson et al. Arthritis Res Ther 2010;12:R23011.

† n for KOOS scores varies between 132 and 136 for the larger Study cohort, and between 69 and 71 for the Subset.
Changing levels of SF ARGS from examination A to examination B

In subjects who decreased in SF ARGS-aggrecan from examination A to examination B, the likelihood for loss of joint space between examinations was increased 3-fold compared to those increasing in SF ARGS (crude OR 2.80; Table III). Including adjustments for age, gender, BMI and time between visits did not essentially alter this estimate of risk, but including adjustments also for SF ARGS concentration at examination A increased the point estimate further (adjusted OR 5.72; Table III).

With ORs around one, decreasing SF ARGS did not show an association with osteophyte progression (Table III).

In subjects with decreasing SF ARGS, the risk of progression of the combination of loss of joint space and osteophytes (ROA progression) was essentially the same as the risk for loss of joint space alone (Table III).

SF ARGS-aggrecan and its association with KOOS

No significant associations were seen between SF ARGS levels at either examination and progression of any of the five KOOS dimensions (Table III).

In subjects with decreased in SF ARGS-aggrecan from examination A to examination B, the likelihood for KOOS pain worsening between examinations was increased 3.7-fold compared to those increasing in SF ARGS (OR 3.66, P = 0.048; Table III). None of the other four KOOS dimensions associated with decreasing SF ARGS (Table III).

Discussion

We showed for this study cohort that 18 years after meniscectomy SF ARGS concentrations were inversely associated with future loss of joint space in the following 4 – 10 years, where low levels increased the risk of progression and higher levels decreased the risk11. In the present study we corroborate our earlier findings by showing the same inverse association between SF ARGS concentrations, but now between ARGS-aggrecan levels at the second examination 4–10 years later and loss of joint space prior to that examination. Importantly, we here extend these findings to show that, regardless of SF ARGS level at examination A, a decrease in SF ARGS over the following 4–10 years was associated with a six-fold increased likelihood of loss of joint space over the same times period. This estimated risk was not substantially altered by the addition of osteophyte progression to loss of joint space (a combination here termed ROA progression), which is in agreement with our findings that SF ARGS seems to be unrelated to osteophyte progression. Within the range of levels seen in these subjects, having low levels of SF ARGS, or having decreasing levels of SF ARGS over time, thus appears to be associated with a higher risk of loss of joint space. For this patient cohort SF ARGS thus qualifies as marker prognostic of disease in the Burden of disease, Investigative, Prognostic of disease, Efficacy of intervention, Diagnostic of disease, and Safety of intervention (BIPEDS) classification of OA biomarkers3, 11.

These results may seem inconsistent with multiple previous reports placing aggrecan degradation as central in OA pathology, and aggrecanase activity as detrimental to cartilage integrity. Ablation of aggrecanase activity has been shown to be protective of OA development in mouse models of OA 23,24. Since the first evidence emerging of an aggrecanase being involved in human joint pathology 25,26, aggrecanase activity giving rise to the release of ARGS-aggrecan from the cartilage has been shown to be increased above normal under inflammatory conditions in vitro27,28, in inflammation in vivo9,10,29,30 following a knee trauma9,10,25,30 as well as in subjects with knee OA9,10,29. The SF concentrations of ARGS-aggrecan in the acute phases of injury or inflammation in vivo were in some cases more than 100 times increased compared to SF of knee healthy subjects9. The levels of SF ARGS and change in those levels between examinations described here are much lower, and fall within the range of levels found in unoperated matched reference subjects without meniscus injury11. With SF levels of the CS846 epitope, (proposed to be present only on newly synthesised aggrecan21) being elevated two- to three-fold up to 20 years following a knee injury compared to uninjured22 (suggesting an increased de novo aggrecan synthesis) not only aggrecan catabolism but also aggrecan anabolism appears to be affected by knee trauma and arthritis. The relatively small fluctuations in the concentrations of SF ARGS seen in these patients long after meniscectomy are thus likely to be affected by variations in both aggrecan synthesis and aggrecanase activity. In contrast, the extreme elevation in SF ARGS seen in the acute phases of joint injury or inflammation may be due to larger variations in aggrecanase activity where the effect of variations of aggrecan synthesis are likely to be overshadowed9,10,25. In the subjects studied here, on average 18 years after meniscectomy, we hypothesise that the lower risk for loss of joint space seen in subjects that have higher or over time increasing levels of SF ARGS, is a reflection of a tissue repair response involving increased synthesis of aggrecan in combination with aggrecanase activity. Increased de novo synthesis of aggrecan that is then successfully incorporated in the tissue and in part degraded by aggrecanase activity resulting in a net beneficial incorporation, would best explain a lower risk for ROA progression and a simultaneous release of ARGS-aggrecan.

It appears as if SF ARGS within the low levels found in these subjects can be interpreted as an anabolic marker (through its inverse association with loss of joint space). In acute stages of knee injury and in inflammation the marker is elevated many-fold above normal9,10,25, and appears as a marker of catabolism. This apparent duality in the interpretation of SF ARGS as a marker, suggests that a combination of markers of synthesis and degradation is needed for a better understanding of the processes underlying the release of proteolytic fragments into SF.

To the best of our knowledge, this study is the first to show that aggrecan ARGS-fragments in SF are associated with pain in human OA. The correlation between radiographic features of OA and pain is poor16,31. Others have presented evidence suggesting that aggrecan fragments may be involved in pain pathogenesis through their ability to modulate neurite growth34,35. Furthermore, in a mouse model of OA, ADAMTS-5 deficient mice do not develop mechanical allodynia associated with OA36. It is therefore possible that the association between changing SF levels of ARGS-aggrecan and pain seen in this study may be caused by the aggrecan fragments having a direct involvement in pain development, as well as indirectly through loss of joint space (i.e., loss of joint cartilage and meniscus), and development of bone marrow lesions37,38.

When studying the association between changing levels of SF ARGS and progression of our outcomes, we have (in addition to the adjustments for age, gender, BMI and time between examinations) included SF ARGS levels at examination A in the final model. We did this knowing that SF ARGS levels at examination A showed an inverse association with loss of joint space11; a decrease from initially low levels of SF ARGS would have a different starting point than a decrease from higher levels. To study the effect of the change itself, we therefore included SF ARGS at examination A in the final model. For transparency, we show ORs from the final model, as well as for the crude model and the model adjusted for only age, gender, BMI, and time between examinations above.

This study has several limitations. Only about half of the subjects included in the study had SF available at both examinations. The loss of power due to the reduced number of subjects is the reason why we have not made the regression analysis stratified for
absence and presence of ROA in the subset, as we previously did for the larger Study cohort \( n = 141 \). Furthermore, we cannot exclude that variable clearance rates of matrix molecules from the joint cavity might influence the associations noted here, but with marker concentrations measured long after trauma a steady state between markers released into the SF and markers cleared from the SF has likely occurred.

In conclusion we found that in middle-aged subjects with a meniscectomy 18 years earlier, SF ARGS levels were predictive of disease progression, where decreasing levels of SF ARGS over the next 4–10 years were associated with an increased risk for loss of joint space and worsening of knee pain over the same time period.

**Author contributions**

SL developed and ran the ARGs ELCL assay, carried out the statistical analysis and interpretation of data, and drafted the manuscript. ME, one of the principal investigators in the original study of meniscectomy, read and scored the radiographs together with another investigator (Ludvig Dahl) and revised the manuscript. AS contributed in the development of the ARGs ELCL assay, and revised the manuscript. LSL conceived the original study of meniscectomy, collected samples, and revised the manuscript. All authors participated in the design, interpretation of results, and approved the final manuscript.

**Conflict of interest**

None to declare.

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