Plasma proteomics identifies CRTAC1 as a biomarker for osteoarthritis severity and progression

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Abstract

Objectives: The aim of this study was to identify biomarkers for radiographic osteoarthritis severity and progression acting within the inflammation and metabolic pathways.

Methods: For 3,517 Rotterdam Study participants, 184 plasma protein levels were measured using Olink inflammation and cardiometabolic panels. We studied associations with severity and progression of knee, hip, hand osteoarthritis, and a composite overall OA burden-score by multivariable regression models, adjusting for age, sex, cell counts and BMI.

Results: We found 18 significantly associated proteins for overall osteoarthritis burden, of which 5 stayed significant after multiple testing correction: circulating *Cartilage acidic*

protein 1 (CRTAC1), *Cartilage oligomeric matrix protein* (COMP), *Thrombospondin 4* (THBS4), *Interleukin 18 receptor 1* (IL18R1) and *Tumor necrosis factor ligand superfamily member 14* (TNFSF14). These proteins were also associated with progression of knee OA, with the exception of IL18R1. The strongest association was found for the level of CRTAC1 with 1 SD increase in protein level resulting in an increase of 0.09 (95%CI:0.06-0.12) in overall osteoarthritis KLsum-score ($p=2.9x10^{-8}$), in the model adjusted for age, sex, BMI and cell counts. This association was also present with severity of osteoarthritis in all three joints, progression of knee osteoarthritis and was independent of BMI. We observed a stronger association for CRTAC1 with osteoarthritis than for the well-known osteoarthritis-biomarker COMP.

Conclusion: We identified several compelling biomarkers reflecting overall osteoarthritis burden and increased risk for osteoarthritis progression. CRTAC1 was the most compelling and robust biomarker for osteoarthritis severity and progression. Such a biomarker may be used for disease monitoring.

*Keywords**: biomarkers, osteoarthritis, severity, progression, inflammation, metabolic pathway***

Key messages

- CRTAC1, COMP, THBS4, IL18R1 and TNSF14 associated with overall osteoarthritis severity, possibly highlighting diverse etiological pathways.
- CRTAC1 is a strong biomarker reflecting overall osteoarthritis disease severity and progression.
- CRTAC1 protein might be useful for monitoring disease activity during clinical trials and/or osteoarthritis treatment.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and is characterized by alteration of the joint structure, including progressive cartilage destruction, synovial inflammation and changes in the subchondral bone [1]. It is a heterogeneous and complex disorder with several pathways being involved in the etiology of osteoarthritis. Beside genetic and biomechanical mechanisms, altered metabolism [2] and inflammation [3, 4] might have a key role in osteoarthritis etiology. Studies on biomarkers (biochemical markers), i.e. proteins, lipids etc., could provide further insight into the different pathways leading to osteoarthritis.

Thus far, several studies have focused on the search for accurate OA biomarkers, however, factors that hinder this process were the small sample sizes, the focus on one joint and the cross-sectional design [5]. Among the most studied biomarkers, there is strong evidence for urine CTX-II and serum COMP protein levels to be associated with OA-prevalence and -progression on population level [6-8]. However, none of these biomarkers have been implemented in a clinical setting at patient level yet. Despite this knowledge, biomarkers could help further elucidate the different pathological processes linked to OA, and in this way identify different underlying biological mechanisms to personalize treatments [9, 10].

The exploration of the proteome in is an opportunity to find biomarkers acting within specific pathways that could provide more insight into the etiology of OA and represent targets for novel therapies[11]. Recent technological advances now make it possible to measure a large number of proteins in high number of individuals. There are two main techniques that can measure up to 5000 proteins in plasma [12], which include Somascan and Olink technologies. Both techniques have been shown to be successful for identifying novel biomarkers, while comparison of both techniques also showed the synergistic nature of these technologies to better

identify disease mechanisms. A recent study performed a large proteomics screen (using Somascan) to identify biomarkers for osteoarthritis [12]. This study identified CRTAC1 as a promising novel biomarker for advanced osteoarthritis, but lacked replication in an independent dataset.

The aim of this study was to identify biomarkers acting within specific pathways that could provide more insight into the etiology of OA and represent targets for novel therapies. We studied the relationship between protein levels and disease severity to examine whether protein biomarkers are linked to disease activity in a large prospective study. Furthermore, we examined disease severity and progression in multiple joints in a longitudinal design.

Methods

Study population

We selected our study population from the Rotterdam Study (RS) cohort, a population-based prospective study ongoing since 1990 in the city of Rotterdam in the Netherlands. Details of the RS cohort can be found elsewhere [13]. In short, baseline measurements were collected in 3 rounds of inclusion for 3 sub-cohorts (RS-I, RS-II, and RS-III). As of 2008, 14,926 participants aged 45 years and over comprise the RS. The participants are followed for a variety of diseases that are frequent in the elderly with the aim to investigate determinants of disease occurrence and progression. The RS is approved by the medical ethics committee of the Erasmus University Medical Centre and the review board of the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants in the study. For this study we used data available for participants in the RS-III cohort in which proteomics measurements were available.

Measurements

Baseline data was obtained through a home interview and visits to the research center for physical examinations and laboratory assessments. Body mass index (BMI) was computed from measurements of height and weight (kg/m2). Blood samples were drawn, blood cell composition was measured, and plasma was stored at –80°C.

Weight-bearing anteroposterior radiographs of the knee and hip were obtained at baseline and after 5 years of follow-up; for hands anteroposterior radiographs were taken at baseline only. Radiographs were acquired with the knee extended and the patella in a central position. Radiographs of the pelvis were obtained with both feet in 10° internal rotation and the X-ray beam centered on the umbilicus [14]. All radiographs were scored according to the Kellgren and Lawrence (KL)-scoring system as described before [15, 16]. Each radiograph was scored

by one trained reader of in total seven readers. The interrater reliability between two of the seven readers was tested in a random set of 10% of radiographs. The intercorrelation coefficient, κ value (cut-off value: K&L score ≥2) was 0.71 (95% CI 0.66 to 0.76) for the knee. The radiographs at baseline and follow-up were read without knowledge of the clinical status of the participants or without knowledge of the research hypothesis or exposure status of the participants. Left and right radiographs were grouped per subject and read by pairs in chronological order [17]. As there is no consensus on the definition of incidence and progression, we combined both in one definition for the overall progression of osteoarthritis. This was defined as an increase in the K&L score between baseline and follow-up of 1 or more. In the case of a baseline score 0, overall progression was defined as an increase of 2 or more. Patients with scores 4 or 5 at baseline were left out of the analysis. In this study, we included 3,517 RS-III participants who underwent blood measurement for proteomics assessment and radiographic measurements at baseline (RS-III -1) and after a mean follow-up time of 5.5 years (RS-III-2).

Plasma biomarker measurements

Protein levels in plasma were measured using the Olink Proseek® Multiplex Inflammation (v.3021) and Cardiometabolic (v.3602) 96-plex panels at the Olink core laboratory (Olink Proteomics AB, Uppsala, Sweden). The OLINK immunoassays are based on the high-throughput Proximity Extension Assay (PEA) technique [18]. Further processing steps by Olink are described in the **Supplementary Material**, including **Supplementary Table S1, Table S2,** available at *Rheumatology* online. Normalized Protein Expression (NPX) values of the remaining proteins (on the log2 scale) were standardized to unit variance by applying a z-transformation.

Outcome definition

We defined radiographic OA according to the original KL scoring system [15, 16]. The different OA outcomes considered for the present study are described in **Table 1.**

Statistical analyses

We examined the associations of all available protein levels with OA in knee, hip and hand using multivariate regression models. Phenotype-protein associations were estimated cross-sectionally for overall OA burden and for severity of OA in all joints separately. Subsequently, the nominally significant proteins were analyzed longitudinally for OA progression in knee and hip separately. For each of these scenarios, we analyzed the relationships with multivariate regression models using linear models for continuous outcomes and generalized linear models with binomial link function for dichotomous outcomes. We tested two statistical models: in model 1, we adjusted for age, sex and cell counts, in model 2 we additionally adjusted for BMI. For each protein, we reported effect estimate (β) per standard deviation (SD) difference in protein levels with 95% Confidence Interval (CI) and nominal p-value (significance level<0.05). We used false discovery rate (FDR) correction for multiple testing correction (significance level FDR P-value <0.05).

As secondary analyses, we investigated the association between baseline CRTAC1 levels and osteophytes (OST) formation and joint space narrowing (JSN). We explored the relationship between CRTAC1 and COMP by constructing a multivariable model including COMP and CRTAC1 in a model with overall OA burden as outcome and adjusted for age, sex and BMI. Moreover, we investigated the association of CRTAC1 with OA-related pain as outcome through linear regression and adjusted for age, sex, cell counts and BMI. We performed a Receiver Operating Characteristic (ROC) analysis to assess the predictive power of CRTAC1, COMP, and models including also age, sex and BMI as predictors for osteoarthritis.

All statistical analyses were performed in R version 3.5.2 [19].

Results

Proteomics data and baseline characteristics of the study population

After Quality Control (QC) of the proteomics data (see the **Supplementary Material,** available at *Rheumatology* online), there were 3,502 and 3,456 samples left for cross-sectional analyses with the cardiometabolic and the inflammation panels, respectively (**Figure 1**). For longitudinal analyses, there were 3,444 and 3,399 samples included (**Supplementary Figure S1**),

We were able to measure a total of 88 cardiometabolic and 83 inflammation proteins in the plasma samples. We examined the correlation among proteins (**Supplementary Figure S2**) and observed that many of them are correlated to some extent. When we examined the association of proteins with age, sex and BMI, we found that respectively 66 cardiometabolic and 69 inflammation (age), 57 cardiometabolic and 31 inflammation (sex), and 55 cardiometabolic and inflammation (BMI) proteins were associated with these important risk factors for OA.

Characteristics of our study participants are presented in **Table 2**. In total, we examined 3,068 individuals with at least one OA outcome. Our population included slightly more females than males and the mean follow-up time for OA-progression was 5.56 years.

Overall OA burden

We examined the relation between protein levels and overall OA-burden adjusting for age, sex and cell counts. In total, we found 18 proteins to be significantly associated with overall OA burden across the two protein assays (**Figure 2**, **Supplementary Figure S2 and S3, Supplementary Table S3**), of which five passed FDR correction for multiple testing: CRTAC1, COMP, THBS4, IL18R1 and TNFSF14 (model 1, **Table 3**). Additional adjustment for BMI (model 2) slightly attenuated the effect size for most of the proteins, and four proteins stayed nominally significant after BMI adjustment: CRTAC1, COMP, FCN2 and IL18R1.

For the 18 proteins that we found associated with overall burden of OA, we examined their relationship with the hip, hand and knee joints.

Hip OA

For hip OA severity, none of the 18 proteins were associated (model 1, adjusted for age/sex/cell counts, **Supplementary Table S4)**. Also, when we examined radiographic Hip OA progression none of the 18 proteins were found to be significantly associated. As the number of Hip OA cases was very limited (**Table 2**) these results may possibly reflect lack of power for this joint.

Hand OA

For severity of hand OA, we found 12 out of the 18 proteins to be significantly associated (model 1, adjusted for age/sex/cell counts) with the outcome of interest (**Supplementary Table S4**). Additional adjustment for BMI slightly attenuated the effect size for most of the proteins, although four proteins stayed nominally significant after BMI adjustment: COMP, CRTAC1, FCN2, and MMP-10.

Knee OA

For severity of knee OA, 15 out of the 18 proteins were significantly associated (model 1, adjusted for age/sex/cell counts, **Supplementary Table S4**). After additional adjustment for BMI, most of the protein effects were attenuated and lost their significance, with the exception of CRTAC1 that showed a slightly stronger association with knee OA severity (effect = 0.05, 95%CI 0.03-0.07, in model 2, **Supplementary Table S4**). In addition, 11 of the 18 proteins investigated, significantly associated (model 1, adjusted for age/sex/cell counts) with progression of knee OA (**Supplementary Table S5**), 4 proteins from the cardiometabolic and 7 proteins from the inflammation assay. After BMI-adjustment the strength of association was

slightly attenuated and CRTAC1 and MMP10 remained significantly associated (**Supplementary Table S5**). To further investigate the possible role of CRTAC1 in specific OA tissues, we investigated separate radiographic features: joint space narrowing (JSN), as well as osteophytes formation (OST) (n=1440 participants). Baseline CRTAC1 levels significantly associate with increased JSN (effect=0.23, 95%CI 0.07-0.39, $p=4.48x10^{-3}$) and OST (effect=0.18, 95%CI 0.09-0.26, $p=6.46x10^{-5}$), also with additional adjustment for BMI (**Supplementary Table S6**). Among the other promising proteins, COMP, THBS4 and IL18R1 significantly associated with OST and JSN, and stayed significant after Bonferroni correction ($p<0.0042$). In contrast to CRTAC1, these associations were attenuated after BMI adjustment.

CTRAC1 and COMP can predict Knee OA

As COMP is a well-known biomarker for OA, we wanted to investigate the relationship between CRTAC1 and COMP, and their roles in OA burden. We performed a multivariable regression model, including both COMP and CRTAC1 as independent variables while adjusting for age, sex and BMI. CRTAC1 was found to be significantly associated to overall OA burden, and its effect size remained comparable to that in the univariate model (effect-univar=0.20, $p=1.41x10^{-7}$, effect-multivar=0.18, $p=1.02x10^{-5}$), while the effect of COMP almost halved and lost its significance (effect-univar =0.13, $p=8.86x10^{-4}$, effect-multivar =0.07, p=0.09).

We also examined the association between CRTAC1 and OA-related knee pain which is the most common symptom for a patient to visit their general practitioner. We found similar effect size in the model adjusted for age/sex/BMI (RR=1.20, effect=0.18, $p=6.29x10^{-5}$) to the association with radiographic knee OA (RKOA). After adjusting for the presence of RKOA,

the association stayed significant (effect=0.17, p=6.31x10^{-4}). We did not find a significant association with incidence of OA-related pain (effect=0.14, p=0.11).

Finally, we examined whether CRTAC1 has predictive power for RKOA progression in addition to clinical features and found an AUC of 0.57, very similar to COMP alone. However, it did not add much predictive power to the clinical factors, AUC 0.72 (**Supplementary Table S7**).

Sensitivity analyses

The exclusion of participants with other joint diseases (23 rheumatic arthritis and 2 gout cases) did not affect our association results of the top proteins with overall OA burden (**Supplementary Table S8**). Exclusion of progressors to total knee replacement (n=31) did not affect our results for progression of knee OA (**Supplementary Table S9**).

Discussion

In our study, we found a total of five proteins to be associated with overall OA disease severity: CRTAC1, COMP, THBS4, IL18R1 and TNSF14 (**Table 3**). When we examined the joints separately, we observed associations (model 1, adjusted for age/sex/cell counts) of all 5 proteins with severity of OA in knee and hand, but not in hip. Importantly, all five proteins except IL18R1 were associated with progression of knee OA. The most compelling biomarker was CRTAC1, which reflected disease severity and predicted OA progression.

A recent study from Styrkarsdottir *et al*, identified *Cartilage acidic protein 1* (CRTAC1) as a potential novel biomarker for established OA in a large proteomic exploratory study using a case control design [20]. In that study, CRTAC1 was found associated with advanced OA, as well as future total joint replacement. We here, for the first time, replicate CRTAC1 as a promising biomarker for OA. We show, in a population-based setting, that CRTAC1 levels are associated with overall disease severity as well as radiographic progression. CRTAC1 levels seem to drive both bone- and cartilage-driven processes in OA, since we observed association with both osteophyte formation as well as joint space narrowing. Moreover, we also show a similar AUC (0.72) for knee OA progression of our prediction model compared to the above-mentioned study (AUC 0.70 for TKR using the same risk factors). However, we also show that CRTAC1 does not add much predictive information on top of the basic risk factors as age, gender, BMI for knee OA progression in our population. To sum up, CRTAC1 is a compelling biomarker candidate reflecting overall OA burden and might be used as a monitoring tool for disease activity in OA trials.

CRTAC1 is a glycosylated extracellular matrix protein that is found in the inter-territorial matrix of articular deep zone cartilage [21]. CRTAC1 protein can mediate the interaction of chondrocytes with the extracellular matrix of cartilage and CRTAC1 levels have been used to

distinguish between cartilage and osteoblasts in mesenchymal stem cell culture [21]. This suggests that CRTAC1 protein is primarily produced in the cartilage cells. Interestingly, CRTAC1 protein has been linked to skin damage repair [22, 23], suggesting a role in collagen damage, wound healing, therefore possibly linked to fibrosis – a process linked to OA in a recent large-scale genetics study [24]. Future functional studies are needed to understand the exact function of this protein in the pathway leading to OA.

Our findings confirmed the role of *Cartilage Oligomeric Matrix Protein* (COMP) in OA. COMP, also called *Thrombospondin 5*, is well-known in the literature for its diagnostic and prognostic value as a biomarker for knee and hip OA [25]. We here additionally show that COMP reflects overall OA burden. COMP levels reflect the release of COMP from all cartilage and/or bone structures in the body [26] and is therefore a marker of cartilage and bone metabolism. Serum COMP associated with structural change in OA as well as joint pain [27]. However, elevated levels have also been reported in rheumatic arthritis and therefore COMP is not OA-specific [28]. In our study, we showed that CRTAC1 is a stronger predictor for OA compared to COMP, and that most of the predictive power of COMP is captured by CRTAC1. This, together with the apparent OA-specific association of CRTAC1 [20], suggests that CRTAC1 might be a better biomarker for OA.

In addition to CRTAC1 and COMP, we found two other proteins, *Thrombospondin 4* (THBS4, or TSP4) and *Tumor necrosis factor superfamily member 14* (TNFSF14), that reflected overall OA-burden and were associated with knee OA progression (model 1, adjusted for age/sex/cell counts). THBS4 is a close homolog to THBS5 (COMP) and has been shown to be strongly upregulated during chondrogenesis [29]. Expression of THBS4 in knee cartilage was found to be correlated with OA disease severity [30]. A recent report showed that the expression of

THBS4 is restricted to hypertrophic chondrocytes during endochondral bone formation, while COMP was distributed through all layers of cartilage [31]. Chondrocyte hypertrophy is suggested to play a role in the initiation and progression of OA [32], our results (model 1, adjusted for age/sex/cell counts) open the possibility that THBS4 is a specific biomarker for chondrocyte hypertrophy. The last identified protein biomarker TNFSF14, is known to be involved in inducing pro-inflammatory cytokines in macrophages [33]. TNFSF14 has been shown to be elevated in obese individuals and can influence bone metabolism through activation of NFkB and JNK pathways [34]. These are known pathways thought to be involved in osteoarthritis.

Although results were not consistent across all OA-outcomes, the *Matrix Metallopeptidase 10* (MMP-10) protein is another interesting biomarker that associated with progression of knee OA in both models. MMP10 is well-known for its role in cartilage breakdown and its potential to activate collagenases [25]. In contrast, MMP-10 can regulate the generation of 'M2' anti-inflammatory macrophages or their migration into tissue as part of the resolution phase of acute inflammation, which can be problematic in chronic inflammatory settings. Both acute and chronic inflammation can be regulated by MMP activity [35]. Therefore, these possible conflicting roles of MMP-10 warrant further research to disentangle the functional role of this protein in the osteoarthritic disease process.

It is worth noting that a number of studies have implicated MMP-10 [36, 37], CRTAC1 [20, 38], COMP [39, 40], THBS4 [41, 42] and TNFSF14 [43, 44] in disease processes related to atherosclerosis, i.e. vascular calcification. Interestingly, OA and atherosclerosis are two diseases that are mutually associated independently from co-factors [14]. Therefore, the overlap

of proteins implicated in both diseases provide a promising ground for further exploration of the common pathway that may lead to both OA and atherosclerosis.

Our study has several strengths. Firstly, this study was embedded in a large prospective study of a population-based cohort. This enabled us to study the phenotype-protein associations both cross-sectionally and longitudinally. Secondly, we performed the analysis in the three joints that are most often affected by OA: knees, hips and hands. Thirdly, we used a highly sensitive high-throughput method to measure protein concentrations, with two panels, targeting two highly relevant pathways underlying OA pathophysiology. Fourth, due to detailed phenotyping in the Rotterdam Study, we have investigated the possible cartilage- vs bone-driven effect of CRTAC1. In addition, the availability of both structural and symptomatic data available in the study cohort provided insight into the discriminative power of CRTAC1 for the assessment of its clinical relevance. Lastly, we present results from two models - with and without BMI-adjustment – and show that part of the identified associations are (partly) driven by BMI. These biomarkers (including CRTAC1, COMP and THBS4) might be part of metabolic pathways underlying BMI and therefore interesting to examine further, especially in case of knee OA.

As any other study, our study has also limitations. Firstly, due to no assessment of hand OA at follow-up, we were not able to perform analysis for progression of hand OA. Secondly, data on uCTX-II, a well-known biomarker for OA, was not available and we were unable to investigate its relationship with CRTAC1. Thirdly, our study population for progression consisted of participants who were able to come to the research center for radiographic assessment and therefore may represent a healthier group. Finally, other joints that have a high burden to OA, i.e. spine, were not included.

In conclusion, we identified several compelling biomarkers reflecting overall OA burden, OA severity and increased risk for OA progression. Our results indicate that CRTAC1 is a robust and promising biomarker for osteoarthritis severity and progression in our study. Moreover, we showed that CRTAC1 is a stronger predictor for OA than COMP, but only added marginally to already known predictors in our elderly population. Such a biomarker might be useful for targeting the right patients and monitoring disease activity during clinical trials and/or treatment for OA.

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Contributions

All authors contributed substantially to the conception and design of the article. IAS and CLV performed the analysis and drafted the initial manuscript. All authors critically revised it for interpretation of results and important intellectual content. All authors approved the final version of the manuscript.

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Competing interests

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Patient and Public Involvement

Patients and the General Public will be informed of the results through the dedicated website of Artrose Gezond (https://artrosegezond.nl/), ZonMw (https://zonmw.nl), and via the Erasmus MC Rotterdam Osteoarthritis Research (ROAR) twitter account (@roar_NL).

Data Availability Statement

Rotterdam Study data can be made available to interested researchers upon request. Requests can be directed to data manager Frank J.A. van Rooij (f.vanvanrooij@erasmusmc.nl) or visit the following website for more information https://www.ergo-onderzoek.nl/contact. We are unable to place data in a public repository due to legal and ethical restraints. Sharing of individual participant data was not included in the informed consent of the study, and there is potential risk of revealing participants' identities as it is not possible to completely anonymize the data.

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Table 1. OA outcomes considered for the present study

Overall OA burden (continuous)	the sum of the three individual joints (sum of the weighted KLsum scores for the knee, hip and hand); overall KLsum-score range: 0-5.47 (max. 12);
Knee OA severity (continuous)	the sum of KL scores of the left and right knee, excluding total knee replacements, was divided for number of joints (2 joints); knee KLsum-score range: 0-4.00 (max. 4);
Hip OA severity (continuous)	the sum of KL scores of the left and right hip, excluding total hip replacements, was divided for number of joints (2 joints); hip KLsum-score range: 0-3.50 (max. 4);
Hand OA severity (continuous)	the sum of KL scores across all DIP, PIP, MCP, IP and CMC1 joints in both hands (15 joints per hand, 30 joints per individual) was divided for number of joints (15 joints); hand KLsum-score range: 0-3.67 (max. 8);
Knee OA progression	any KL score at baseline (including KL<2), progressor if KL at follow-up was higher than at baseline, non-progressor otherwise. Progressors from KL0 to KL1 were excluded.
Hip OA progression	any KL score at baseline (including KL<2), progressor if KL at follow-up was higher than at baseline, non-progressor otherwise. Progressors from KL0 to KL1 were excluded.
Joint Space Narrowing (JSN)	(semi)-quantitative endophenotype (0–3 scoring); JSN sum score: sum of median and lateral JSN
Osteophytes (OST)	(semi)-quantitative endophenotype (0–3 scoring); OST sum score: sum of median and lateral OST
OA-related pain	Chronic pain defined as pain for more than 3 months

Table 2. Baseline characteristics of the Rotterdam Study participants from cohort RS-III included in the analyses

	Cardiometabolic panel analyses	Inflammation panel analyses
Maximum participants in analysis, N	3103	3065
Females, n(%)	1749 (56.4)	1727 (56.4)
age, mean (SD)	56.71 (6.38)	56.68 (6.33)
BMI, mean (SD)	27.60 (4.45)	27.62 (4.47)
Overall OA burden, N	2273	2240
weighted KLsum score, median (range)	0.4 (0 - 5.47)	0.4 (0 - 5.47)
diagnosed any radiographic OA at baseline, n(%)	426 (18.9)	415 (18.8)
Knee OA severity, N	2961	2922
weighted KLsum score, median (range)	0 (0 - 4)	0 (0 - 4)
diagnosed radiographic OA at baseline, n(%)	257 (8.7)	252 (8.6)
Hip OA severity, N	3103	3065
weighted KLsum score, median (range)	0 (0 - 3.5)	0 (0 - 3.5)
diagnosed radiographic OA at baseline, n(%)	41 (1.3)	38 (1.2)
Hand OA severity, N	2390	2356
weighted KLsum score, median (range)	0.13 (0 - 3.67)	0.13 (0 - 3.67)
diagnosed radiographic OA at baseline, n(%)	582 (24.3)	575 (24.4)
Knee OA progression, N	1965	1949
Knee OA progressors, n(%)	198 (10.1)	201 (8.9)
diagnosed radiographic OA at baseline, n(%)	98 (5.2)	98 (5.2)
Hip OA progression, N	1998	1982
Hip OA progressors, n(%)	127 (6.4)	129 (6.5)
diagnosed radiographic OA at baseline, n(%)	21 (1.0)	20 (1.0)

Table 3. Results of the five proteins that pass False Discovery Rate (FDR) correction out of the 18 significant proteins for overall OA burden

Model 1	**CRTAC1**			**COMP**			**THBS4**			**IL_18R1**			**TNFSF14**		
OA outcome	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**
Overall OA burden	0.09	0.05 – 0.12	2 x10-7	0.07	0.04 – 0.11	2 x10-5	0.06	0.03 – 0.09	3 x10-4	0.08	0.04 – 0.11	4 x10-6	0.06	0.02 – 0.09	8 x10-4
Knee OA severity	0.04	0.02 – 0.06	2 x10-5	0.03	0.01–0.05	0.01	0.03	0.01 – 0.04	0.01	0.04	0.02 – 0.06	4 x10-5	0.03	0.01 – 0.05	1 x10-3
Hip OA severity	0.01	-0.003 – 0.02	0.14	0.003	-0.01 – 0.01	0.64	0.003	-0.01 – 0.01	0.62	0.004	-0.01 – 0.01	0.51	-0.004	-0.02 – 0.01	0.43
Hand OA severity	0.03	0.01 – 0.04	2 x10-3	0.03	0.02 – 0.05	5 x10-5	0.02	0.01 – 0.04	4 x10-3	0.03	0.01 – 0.04	1 x10-3	0.03	0.01 – 0.04	2 x10-3
Knee OA progression	0.19	0.04 – 0.34	0.01	0.23	0.07– 0.39	4 x10-3	0.18	0.03 – 0.33	0.01	0.12	-0.04 – 0.27	0.15	0.21	0.06 – 0.37	7 x10-3
Hip OA progression	0.19	-0.01 – 0.38	0.06	0.08	-0.13 – 0.29	0.44	0.06	-0.15 – 0.26	0.58	0.06	-0.15 – 0.27	0.56	-0.01	-0.23 – 0.19	0.89
Model 2	**CRTAC1**			**COMP**			**THBS4**			**IL_18R1**			**TNFSF14**		
OA outcome	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**	**Beta**	**95% CI**	**P-value**
Overall OA burden	0.09	0.06 – 0.12	3 x10-8	0.05	0.02 – 0.09	1 x10-3	0.03	-0.01 – 0.06	0.99	0.04	0.01 – 0.08	0.01	0.02	-0.01 – 0.06	0.20
Knee OA severity	0.05	0.03 – 0.07	3 x10-6	0.01	-0.01–0.03	0.29	-0.003	-0.02 – 0.02	0.76	0.01	-0.01 – 0.04	0.16	0.004	-0.02 – 0.03	0.73
Hip OA severity	0.01	-0.003 – 0.02	0.17	0.005	-0.01 – 0.02	0.36	0.01	-0.003 – 0.02	0.14	0.01	-0.003 – 0.02	0.13	0.0003	-0.01 – 0.01	0.95
Hand OA severity	0.03	0.01 – 0.04	1 x10-3	0.03	0.01 – 0.04	1 x10-3	0.01	-0.01 – 0.03	0.22	0.01	-0.003 – 0.03	0.11	0.01	-0.005 – 0.03	0.18
Knee OA progression	0.25	0.09 – 0.41	2 x10-3	0.15	-0.01– 0.32	0.06	-0.0001	-0.16 – 0.16	1.00	-0.10	-0.27 – 0.07	0.26	0.01	-0.17 – 0.18	0.93
Hip OA progression	0.19	-0.01 – 0.39	0.06	0.08	-0.13 – 0.29	0.45	0.06	-0.15 – 0.26	0.60	0.06	-0.16 – 0.28	0.61	-0.03	-0.25 – 0.19	0.81

Model 1 was corrected for age, sex and cell counts

Model 2 was additionally corrected for BMI

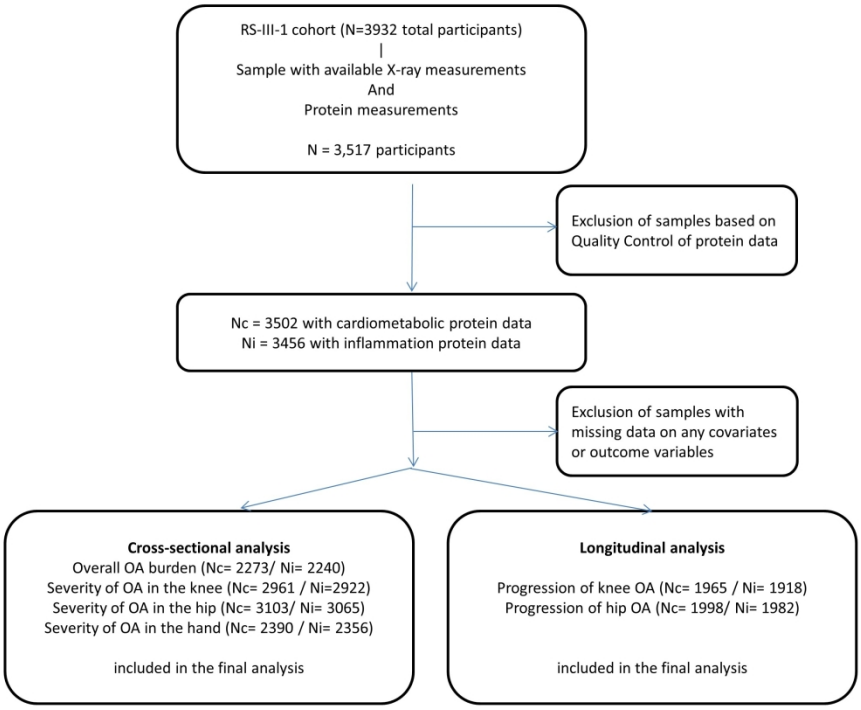
List of Figures

Figure 1. Flowchart of the study population included in analyses.

Legend: Nc = total number of participants included in the analysis of cardiometabolic proteins; Ni = total number of participants included in the analysis of inflammation proteins

Figure 2. Forest plot of the 18 significantly associated proteins with overall OA burden (results from linear regression models)

Legend: Model 1 is adjusted for age, sex and cell counts; Model 2 is additionally adjusted for BMI; The results are ordered from most significant (top) to least significant (bottom) according to model 2.



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Legend: Nc = total number of participants included in the analysis of cardiometabolic proteins; Ni = total number of participants included in the analysis of inflammation proteins

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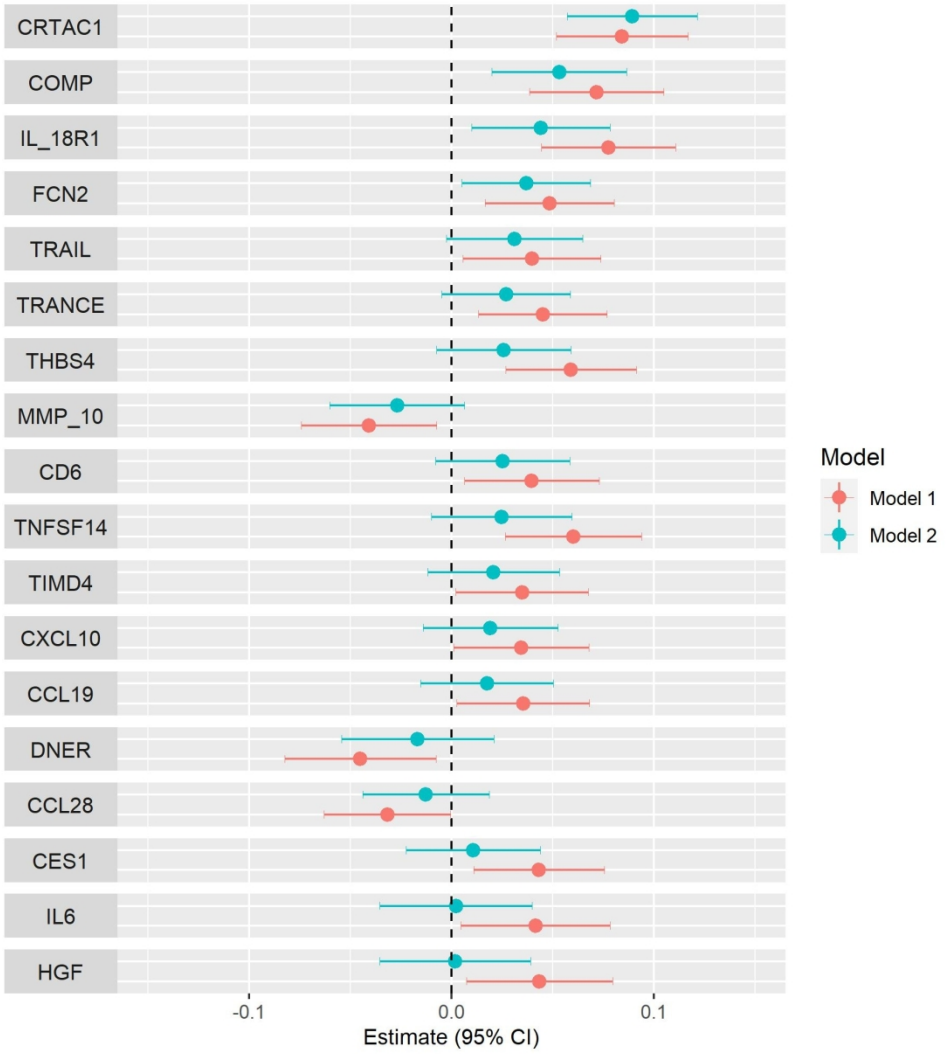

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163x174mm (300 x 300 DPI)