RHEUMATOLOGY

Rheumatology 2023;62:1286–1295
https://doi.org/10.1093/rheumatology/keac415
Advance access publication 4 August 2022

Original article

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 OA severity and progression acting within the inflammation and metabolic pathways.

Methods. For 3517 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 and hand OA 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 OA burden, of which 5 stayed significant after multiple testing correction: circulating cartilage acidic protein 1 (CRTAC1), cartilage oligomeric matrix protein (COMP), thrombospondin 4, IL-18 receptor 1 (IL-18R1) and TNF ligand superfamily member 14. These proteins were also associated with progression of knee OA, with the exception of IL-18R1. 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 the overall OA Kellgren–Lawrence sum score ($P = 2.9 \times 10^{-8}$) in the model adjusted for age, sex, BMI and cell counts. This association was also present with the severity of OA in all three joints and progression of knee OA and was independent of BMI. We observed a stronger association for CRTAC1 with OA than for the well-known OA biomarker COMP.

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

Key words: biomarkers, osteoarthritis, severity, progression, inflammation, metabolic pathway

BASIC SCIENCE

Rheumatology key messages

- CRTAC1, COMP, THBS4, IL-18R1 and TNSF14 associated with overall osteoarthritis severity, possibly highlighting diverse aetiological 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

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 aetiology of OA. Besides genetic and biomechanical mechanisms, altered metabolism [2] and inflammation [3, 4] might play key roles in OA aetiology. Studies on biomarkers (biochemical markers), i.e. proteins, lipids etc., could provide further insights into the different pathways leading to OA.

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

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Submitted 2 February 2022; accepted 7 July 2022

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one joint and the cross-sectional design [5]. Among the most studied biomarkers, there is strong evidence for urine CTX-II and serum cartilage oligomeric matrix protein (COMP) levels to be associated with OA prevalence and progression on a population level [6–8]. However, none of these biomarkers have yet been implemented in a clinical setting at the patient level. These 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].

Exploration of the proteome is an opportunity to find biomarkers acting within specific pathways that could provide more insights into the aetiology of OA and represent targets for novel therapies [11]. Recent technological advances now make it possible to measure a large number of proteins in a large number of individuals. There are two main techniques that can measure up to 5000 proteins in plasma [12]: Somascan and Olink technologies. Both techniques have been shown to be successful for identifying novel biomarkers, while comparison of the 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 OA [12]. This study identified circulating cartilage acidic protein 1 (CRTAC1) as a promising novel biomarker for advanced OA but it lacked replication in an independent dataset.

The aim of this study was to identify biomarkers acting within specific pathways that could provide more insights into the aetiology of OA and represent targets for novel therapies. In a large prospective study we looked at the relationship between protein levels and disease severity to examine whether protein biomarkers are linked to disease activity. 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, The Netherlands. Details of the RS cohort can be found elsewhere [13]. In short, baseline measurements were collected in three rounds of inclusion for three subcohorts (RS-I, RS-II and RS-III). As of 2008, 14 926 participants $\geq$45 years of age comprise the RS. The participants are followed for a variety of diseases that are frequent in the elderly with the aim of investigating 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 were obtained through a home interview and visits to the research centre for physical examinations and laboratory assessments. BMI was computed from measurements of height and weight (kg/m^2). 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 centred on the umbilicus [14]. All radiographs were scored according to the Kellgren and Lawrence (KL) scoring system as described earlier [15, 16]. Each radiograph was scored by one trained reader of in total seven readers. The inter-rater reliability between two of the seven readers was tested in a random set of 10% of radiographs. The intercorrelation coefficient, the κ value (cut-off value was a KL score $\geq$2), was 0.71 (95% CI 0.66, 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 OA. This was defined as an increase in the KL score between baseline and follow-up of $\geq$1. In the case of a baseline score of 0, overall progression was defined as an increase of $\geq$2. Patients with scores of 4 or 5 at baseline were left out of the analysis. In this study we included 3517 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 (version 3021) and Cardiometabolic (version 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 technique [18]. Further processing steps by Olink are described in the Supplementary Material, including Supplementary Tables S1 and S2, available at *Rheumatology* online. Normalized Protein Expression (NPX) values of the remaining proteins (on a log2 scale) were standardized to unit variance by applying a z-transform.

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 the 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 analysed longitudinally for OA progression in the knee and hip separately. For each of these scenarios we analysed 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, while in model 2 we additionally adjusted for BMI. For each protein we reported the effect estimate (β) per S.D. difference in protein levels with 95% 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 osteophyte (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 the outcome and adjusted for age, sex and BMI. Moreover, we investigated the association of CRTAC1 with OA-related pain as the outcome through linear regression and adjusted for age, sex, cell counts and BMI. We performed a receiver operating characteristics (ROC) analysis to assess the predictive power of CRTAC1, COMP and models including age, sex and BMI as predictors for OA.

All statistical analyses were performed in R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) [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 3502 and 3456 samples left for cross-sectional analyses with the cardiometabolic and inflammation panels, respectively (Fig. 1). For longitudinal analyses, there were 3444 and 3399 samples included (Supplementary Fig. S1, available at *Rheumatology* online).

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 Fig. S2, available at *Rheumatology* online) 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 66 cardiometabolic and 69 inflammation (age), 57 cardiometabolic and 31 inflammation (sex) and 55 cardiometabolic and 55 inflammation (BMI) proteins, respectively, were associated with these important risk factors for OA.

Characteristics of our study participants are presented in Table 2. In total, we examined 3068 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.

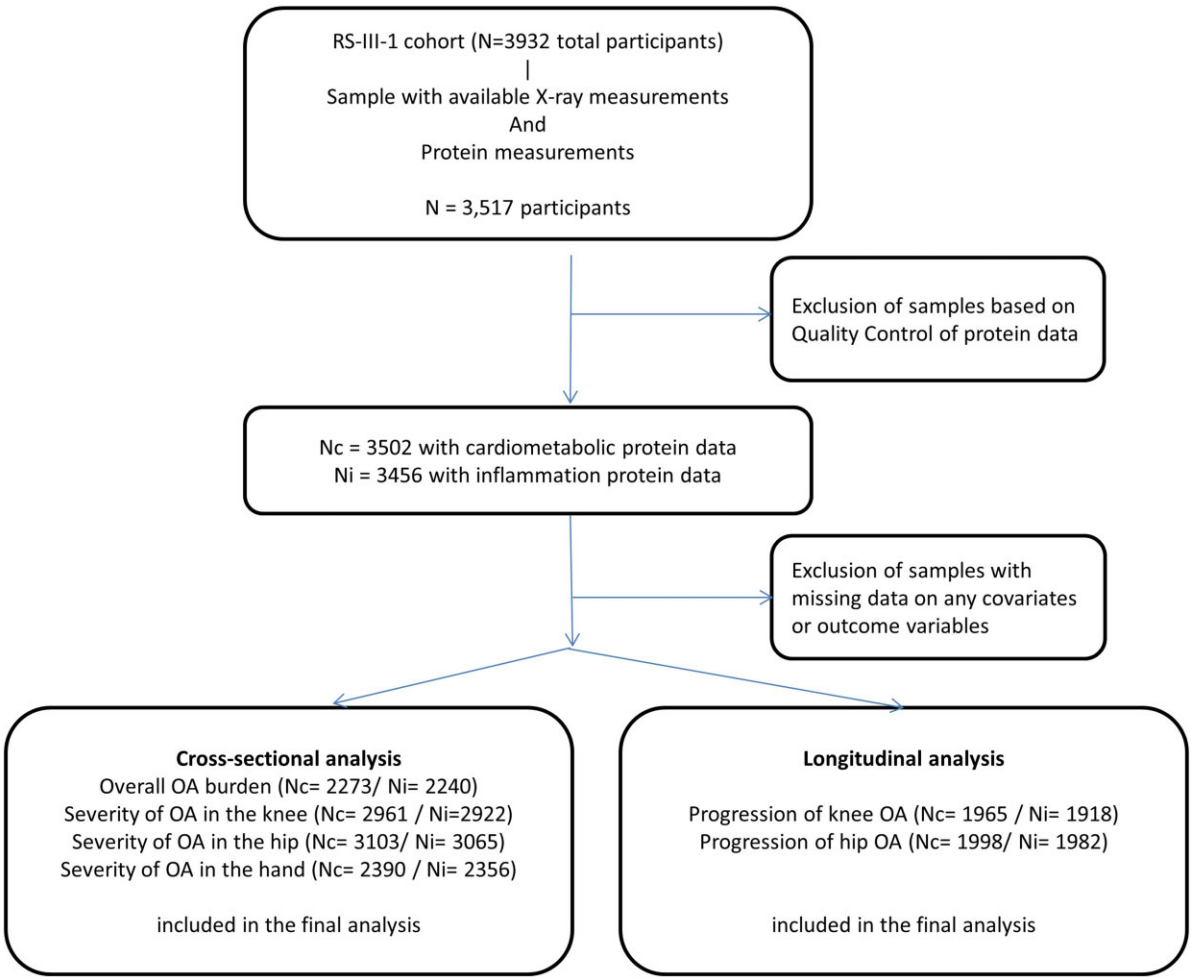
Overall OA burden

We examined the relationship 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 (Fig. 2, Supplementary Figs S2 and S3,

Table 1 OA outcomes considered in the present study

Overall OA burden (continuous)	The sum of the three individual joints (sum of the weighted KL sum scores for the knee, hip and hand); overall KL sum score range: 0–5.47 (maximum 12)
Knee OA severity (continuous)	The sum of KL scores of the left and right knee, excluding total knee replacements, was divided by the number of joints (2 joints); knee KL sum score range: 0–4.00 (maximum 4)
Hip OA severity (continuous)	The sum of KL scores of the left and right hip, excluding total hip replacements, was divided by the number of joints (2 joints); hip KL sum score range: 0–3.50 (maximum 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 by the number of joints (15 joints); hand KL sum score range: 0–3.67 (maximum 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 KL 0 to KL 1 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 KL 0 to KL 1 were excluded
JSN	(Semi)-quantitative endophenotype (0–3 scoring); JSN sum score: sum of median and lateral JSN
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 >3 months

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



Nc, total number of participants included in the analysis of cardiometabolic proteins; Ni, total number of participants included in the analysis of inflammation proteins.

Supplementary Table S3, available at *Rheumatology* online), of which 5 passed FDR correction for multiple testing: CRTAC1, COMP, THBS4, IL-18R1 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 IL-18R1.

For the 18 proteins that we found associated with the 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 and cell counts, Supplementary Table S4, available at *Rheumatology* online). 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 a lack of power for this joint.

Hand OA

For the severity of hand OA, we found 12 of the 18 proteins to be significantly associated (model 1, adjusted for age, sex and cell counts) with the outcome of interest (Supplementary Table S4, available at *Rheumatology* online). 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 the severity of knee OA, 15 of the 18 proteins were significantly associated (model 1, adjusted for age, sex and cell counts; Supplementary Table S4, available at *Rheumatology* online). After additional adjustment for BMI, most of the protein effects were attenuated and lost their significance, with the exception of CRTAC1, which showed a slightly stronger association with knee OA severity [effect = 0.05 (95% CI 0.03, 0.07) in model 2,

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

Characteristics	Cardiometabolic panel analyses	Inflammation panel analyses
Maximum participants in analysis, *N*	3103	3065
Females, *n* (%)	1749 (56.4)	1727 (56.4)
Age, years, mean (S.D.)	56.71 (6.38)	56.68 (6.33)
BMI, mean (S.D.)	27.60 (4.45)	27.62 (4.47)
Overall OA burden, *N*	2273	2240
Weighted KL sum 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 KL sum 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 KL sum 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 KL sum 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)

Supplementary Table S4, available at *Rheumatology* online]. In addition, 11 of the 18 proteins investigated significantly associated (model 1, adjusted for age, sex and cell counts) with progression of knee OA (Supplementary Table S5, available at *Rheumatology* online), 4 proteins from the cardiometabolic and 7 proteins from the inflammation assay. After BMI adjustment, the strength of the association was slightly attenuated and CRTAC1 and MMP-10 remained significantly associated (Supplementary Table S5, available at *Rheumatology* online). To further investigate the possible role of CRTAC1 in specific OA tissues, we investigated separate radiographic features: JSN as well as OST formation ($n = 1440$ participants). Baseline CRTAC1 levels significantly associate with increased JSN [effect = 0.23 (95% CI 0.07, 0.39), $P = 4.48 \times 10^{-3}$] and OST [effect = 0.18 (95% CI 0.09, 0.26), $P = 6.46 \times 10^{-5}$], also with additional adjustment for BMI (Supplementary Table S6, available at *Rheumatology* online). Among the other promising proteins, COMP, THBS4 and IL-18R1 significantly associated with OST and JSN and stayed significant after Bonferroni correction ($P < 0.0042$). In contrast with 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 (univariate effect = 0.20, $P = 1.41 \times 10^{-7}$; multivariate effect = 0.18, $P = 1.02 \times 10^{-5}$), while the effect of COMP almost halved and lost its significance (univariate effect = 0.13, $P = 8.86 \times 10^{-4}$, multivariate effect = 0.07, $P = 0.09$).

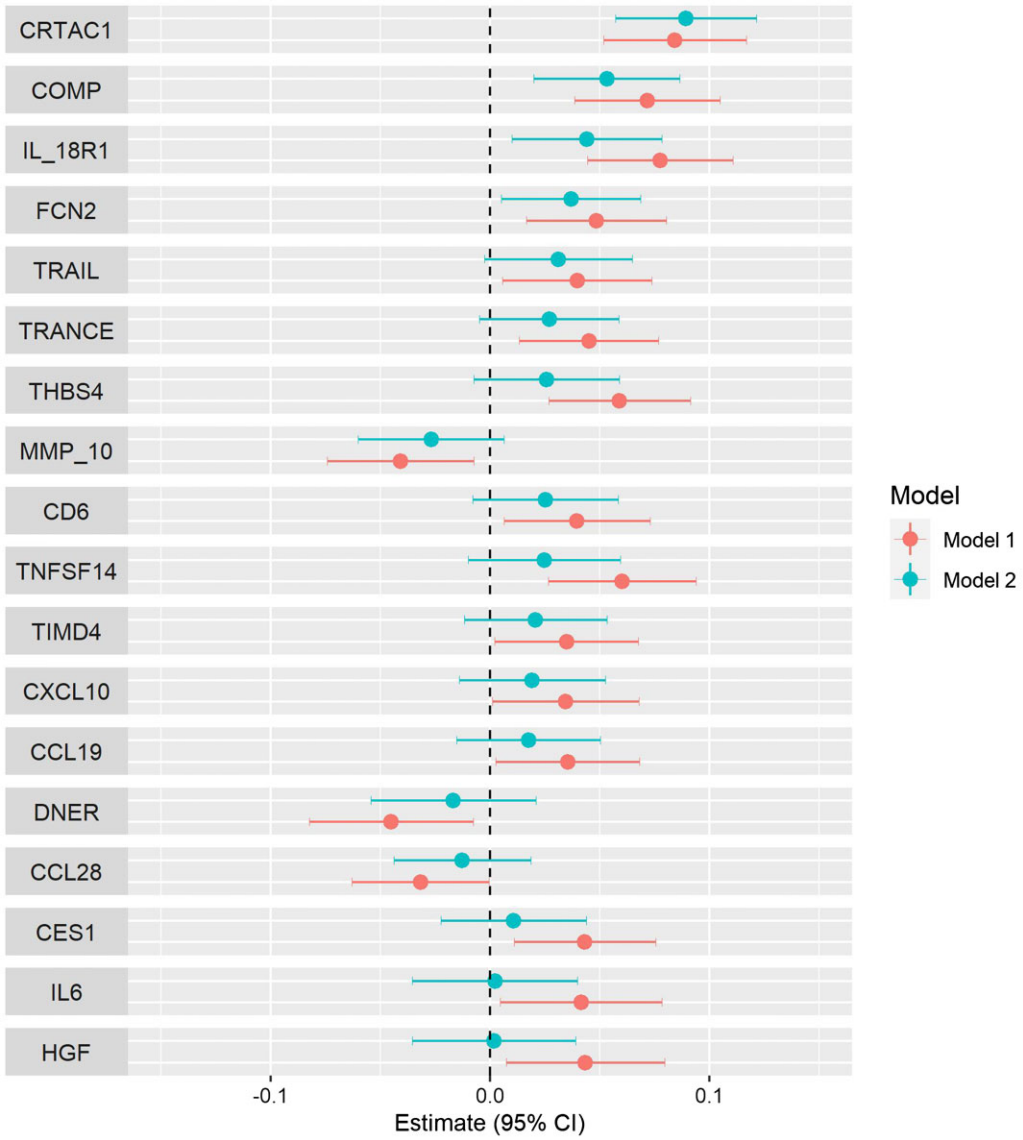
We also examined the association between CRTAC1 and OA-related knee pain, which is the most common symptom for a patient to visit their general practitioner. We found a similar effect size in the model adjusted for age, sex and BMI [relative risk (RR) 1.20, effect = 0.18, $P = 6.29 \times 10^{-5}$) and the association with radiographic knee OA (RKOA). After adjusting for the presence of RKOA, the association stayed significant (effect = 0.17, $P = 6.31 \times 10^{-4}$). We did not find a significant association with the 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 area under the curve (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, available at *Rheumatology* online).

Sensitivity analyses

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

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



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.

OA (Supplementary Table S9, available at *Rheumatology* online).

Discussion

In our study we found a total of five proteins associated with overall OA disease severity: CRTAC1, COMP, THBS4, IL-18R1 and TNSF14 (Table 3). When we examined the joints separately, we observed associations (model 1, adjusted for age, sex and cell counts) of all five proteins with severity of OA in the knee and hand, but not the hip. Importantly, all five proteins except IL-18R1 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.* [20] identified CRTAC1 as a potential novel biomarker for established OA in a large proteomic exploratory study using a case–control design. In that study, CRTAC1 was found to be associated with advanced OA, as well as future total joint replacement. We herein, 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 an association with both OST formation as well as JSN.

Table 3 Results of the five proteins that pass FDR correction of the 18 significant proteins for overall OA burden

Model 1	CRTAC1			COMP			THBS4			IL-18R1			TNFSF14		
OA outcome	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Overall OA burden	0.09	0.05, 0.12	2×10^{-7}	0.07	0.04, 0.11	2×10^{-5}	0.06	0.03, 0.09	3×10^{-4}	0.08	0.04–0.11	4×10^{-6}	0.06	0.02, 0.09	8×10^{-4}
Knee OA severity	0.04	0.02, 0.06	2×10^{-5}	0.03	0.01, 0.05	0.01	0.03	0.01, 0.04	0.01	0.04	0.02, 0.06	4×10^{-5}	0.03	0.01, 0.05	1×10^{-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	2×10^{-3}	0.03	0.02, 0.05	5×10^{-5}	0.02	0.01, 0.04	4×10^{-3}	0.03	0.01, 0.04	1×10^{-3}	0.03	0.01, 0.04	2×10^{-3}
Knee OA progression	0.19	0.04, 0.34	0.01	0.23	0.07, 0.39	4×10^{-3}	0.18	0.03, 0.33	0.01	0.12	−0.04, 0.27	0.15	0.21	0.06, 0.37	7×10^{-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	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
Overall OA burden	0.09	0.06, 0.12	3×10^{-8}	0.05	0.02, 0.09	1×10^{-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	3×10^{-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	1×10^{-3}	0.03	0.01, 0.04	1×10^{-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	2×10^{-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.

Moreover, we also show a similar AUC (0.72) for knee OA progression of our prediction model compared with the above-mentioned study (AUC 0.70 for total knee replacement 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 and 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 interterritorial 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 and 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 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]. Here we 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 RA and therefore COMP is not OA specific [28]. In our study, we showed that CRTAC1 is a stronger predictor for OA compared with 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 TNF necrosis factor superfamily member 14 (TNFSF14), that reflected overall OA burden and were associated with knee OA progression (model 1, adjusted for age, sex and cell counts). THBS4 is a close homologue 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], and our results (model 1, adjusted for age, sex and 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 nuclear factor κB and Janus kinase pathways [34]. These are known pathways thought to be involved in OA.

Although results were not consistent across all OA outcomes, the MMP-10 protein is another interesting biomarker that associated with progression of knee OA in both models. MMP-10 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 provides promising grounds for further exploration of the common pathway that may lead to both OA and atherosclerosis.

Our study has several strengths. First, 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. Second, we performed the analysis in the three joints that are most often affected by OA: knees, hips and hands. Third, 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 in the study cohort provided insights 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 are interesting to examine further, especially in case of knee OA.

As in any other study, our study has also limitations. First, due to no assessment of hand OA at follow-up, we were unable to perform analysis for progression of hand OA. Second, data on uCTX-II, a well-known biomarker for OA, was not available and we were unable to investigate its relationship with CRTAC1. Third, our study population for progression consisted of participants who

were able to come to the research centre for radiographic assessment and therefore may represent a healthier group. Finally, other joints that have a high burden to OA, such as the 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 OA 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.

Acknowledgements

The RS is funded by Erasmus Medical Center and Erasmus University, Rotterdam; the Netherlands Organization for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII) and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff of the RS and the participating general practitioners and pharmacists. All authors contributed substantially to the conception and design of the article. I.A.S. and C.L.V. 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. 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).

Funding: This research was funded by the Netherlands Organisation for Health Research and Development (project no. 849200003). The funding source did not have any influence on study design, collection, analysis and interpretation of data, the writing of the manuscript or the decision to submit the manuscript for publication.

Disclosure statement: S.M.A.B.-Z. declares consultancy work for Pfizer (tanezumab) and reports grants from the Netherlands Organization for Health Research and Development, the Dutch Arthritis Association and Foreum. The remaining authors declare no competing financial interests. All authors declare no non-financial conflicts of interest.

Data availability statement

The RS data can be made available to interested researchers upon request. Requests can be directed to data manager Frank J. A. van Rooij (f.vanrooij@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 constraints. 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.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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