Arthritis & Rheumatology
Vol. 75, No. 4, April 2023, pp 544–552
DOI 10.1002/art.42376

American College of Rheumatology
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Cartilage Acidic Protein 1 in Plasma Associates With Prevalent Osteoarthritis and Predicts Future Risk as Well as Progression to Joint Replacements: Results From the UK Biobank Resource

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Objective. The level of cartilage acidic protein 1 (CRTAC1) in plasma was recently discovered to be associated with osteoarthritis (OA) risk and progression to joint replacement in Iceland. This study was undertaken to validate these findings in an independent population.

Methods. In this study, 1,462 plasma proteins were measured in 54,265 participants from the UK Biobank on the Olink Explore platform. We analyzed the association of plasma proteins with prevalent OA, incident OA, and progression to joint replacement. We assessed the specificity of OA association through comparison of associations with inflammatory joint diseases and with previous joint replacement.

Results. The CRTAC1 protein showed the strongest association with prevalent knee OA (odds ratio [OR] 1.34 [95% confidence interval (95% CI) 1.27, 1.41]) and was associated with hip OA (OR 1.19 [95% CI 1.11, 1.28]). It predicted incident diagnosis of OA in the knee (hazard ratio [HR] 1.40 [95% CI 1.35, 1.46]) and hip (HR 1.25 [95% CI 1.19, 1.31]), as well as progression to joint replacement (HR 1.20 [95% CI 1.08, 1.33] for the knee and HR 1.22 [95% CI 1.08, 1.38] for the hip), while no association was found with inflammatory joint diseases. Individuals in the highest quintile of risk based on CRTAC1 level, age, sex, and body mass index had a 10-fold risk of knee or hip OA within 5 years compared to those in the lowest quintile. Adding aggrecan core protein (ACAN) and neurocan core protein (NCAN) to the model improved the prediction of OA but not joint replacement. Furthermore, we replicated the association of CUB domain–containing protein 1 with prior joint replacement.

Conclusion. Plasma CRTAC1 is a specific biomarker for OA and a predictor of OA risk and progression to joint replacement. Adding ACAN and NCAN protein levels to the CRTAC1 model improved the prediction of OA.

INTRODUCTION

The lack of a biomarker for osteoarthritis (OA) has hindered development of effective therapies for this common disease. No measures are presently available for early diagnosis of OA, the phase before destructive changes are observable on radiographs, and there are no disease-modifying drugs marketed for OA. A biomarker that is associated with disease occurrence and/or progression would help to identify cases earlier and monitor the disease course.

Recently, we identified plasma levels of cartilage acidic protein 1 (CRTAC1) as a promising biomarker for OA, where we showed that levels of CRTAC1 in plasma were associated with OA and predicted progression to joint replacement in a large non–hypothesis-driven study of 4,700 plasma proteins measured using the SomaScan V4 platform in 37,278 Icelanders (1).

Supported by deCODE genetics/Amgen Inc.

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Author disclosures are available at https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fart.42376&file=art42376-sup-0001-Disclosureform.pdf.

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Submitted for publication June 20, 2022; accepted in revised form September 29, 2022.

We found CRTAC1 levels to capture OA risk better than levels of any of the other proteins, including that of cartilage oligomeric matrix protein (COMP), the most extensively studied biomarker for OA (2–5). We also showed that CRTAC1 is specific to OA, as it was not associated with other joint disorders. CRTAC1 levels did not correlate with sex or body mass index (BMI), the classical risk factors for OA, but increased slightly with age, indicating that CRTAC1 is an independent risk factor for OA.

Lack of replication in an independent sample set was a limitation of our previous study (1), but replications in other populations of various ethnicities as well as further technical validations are needed before further implementation of a disease biomarker. Recently, a study in the Rotterdam cohort confirmed CRTAC1 as a biomarker for OA severity and progression (6).

Here, we report on the association of plasma protein levels of participants in the UK Biobank, a large-scale prospective study, using a subset of the Olink Explore 3072 proteome platform, measuring 1,462 proteins. We replicated CRTAC1 as the lead biomarker for prevalent knee OA and hip OA, as well as future OA risk and progression to joint replacement. Furthermore, we replicated the association of the plasma CUB domain–containing protein 1 (CDCP1) levels with prior joint replacement.

PATIENTS AND METHODS

Study population. The UK Biobank resource is a large-scale prospective study that includes data from 500,000 volunteers who were recruited at ages 40–69 years in 2006–2010 across the UK (https://www.ukbiobank.ac.uk/). For all participants, inpatient and outpatient health care records and health-related information were collected and regularly updated. The hospital inpatient records (HESIN) should cover all participants who have visited public hospitals, while the primary care/general practitioner (GP) records are only available for ~45% of the participants. The HESIN records start in 1997, with sporadic registration before that. The GP records start in 1950, although registration increased substantially after 1990. Thus, the records are far from complete for all individuals and, as always when relying on International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes, these diagnoses are sometimes misclassified. Plasma samples were collected at the baseline visit, and for a subset of 54,265 individuals, the levels of 1,462 proteins were measured with 4 Olink Explore 384-plex panels as a part of the UK Biobank – Pharma Proteomics Project (UK Biobank application no. 65851) (7). A large majority of the samples were randomly selected from the UK Biobank. The participants are predominantly of White ethnicity (81.3%).

OA cases were captured from the HESIN and GP records using the following ICD-10 codes: M15.1, M15.2, and M19.04 defined hand OA cases; M16.0, M16.1, and M16.9 defined hip OA cases; and M17.0, M17.1, and M17.9 defined knee OA cases. To address the specificity of CRTAC1 for OA, we also assessed associations with rheumatoid arthritis (RA; ICD-10 codes M05.9, M06.0, and M06.9), psoriatic arthritis (PsA; ICD-10 codes L40.5 and M07.3), and gout (ICD-10 codes M10.0 and M10.9). Individuals receiving prescriptions of drugs used to treat gout (allopurinol, febuxostat, or probenecid) were also defined as having gout. Hip joint replacement was defined by the Office of Population, Censuses and Surveys: Classification of Interventions and Procedures operational codes W37.1 and W38.1, and knee joint replacement by operational codes W40.1 and W41.1.

We designed 3 main studies analyzing the association of plasma protein levels with: 1) prevalent OA, 2) incident OA diagnosis, and 3) progression of knee OA or hip OA to knee or hip joint replacement, respectively (Figure 1). In study 1, we also analyzed the association of proteins in plasma with prior joint replacement, since we previously found several proteins to be strongly associated with prior joint replacement but not with OA diagnosis. In study 1, we included all 54,265 individuals with protein measurements. In study 2, we included 50,935 individuals who did not have an OA, RA, gout, or PsA diagnosis when the plasma was collected, and gathered information on the first record of these diseases in health care records during follow-up. In study 3, we included those who had been diagnosed as having knee OA (n = 892) or hip OA (n = 392) for the first time within 2 years of the date when the plasma sample was taken, excluding those who had undergone previous joint replacement or had RA, gout, or PsA. We then gathered information on joint replacement operation during follow-up. Table 1 outlines the main characteristics of the study participants in studies 1 and 2, and Table 2 outlines the main characteristics of the study participants in study 3.

All participants gave informed consent and UK Biobank's scientific protocol and operational procedures were reviewed and approved by the North West Research Ethics Committee (reference no. 06/MRE08/65). This research has been conducted using the UK Biobank Resource under application numbers 23359 and 65851.

Protein assessment. We measured 1,462 distinct proteins in plasma using the Cardiometabolic, Inflammation, Neurology, and Oncology 384-plex panels on the Olink Explore 3072 platform at Olink's facilities in Uppsala, Sweden. All samples were randomized and plated by the UK Biobank laboratory team prior to delivery. Samples were processed across 3 NovaSeq 6000 Sequencing Systems, leveraging Proximity Extension Assay technology. Extensive quality control measures and normalization of protein concentration was performed at Olink's facilities, producing Normalized Protein eXpression (NPX) values for each protein per participant. NPX is Olink's relative protein quantification unit on $\log_2$ scale. A detailed description of quality measures of the UK Biobank proteomics data set was recently submitted (7).

Statistical analysis. Plasma protein levels were adjusted for the age of the individual at the time of plasma collection and rank-transformed onto the standard normal distribution with a mean of 0 and an SD of 1, for each sex separately. All modeling was completed with standardized protein levels, whereas raw

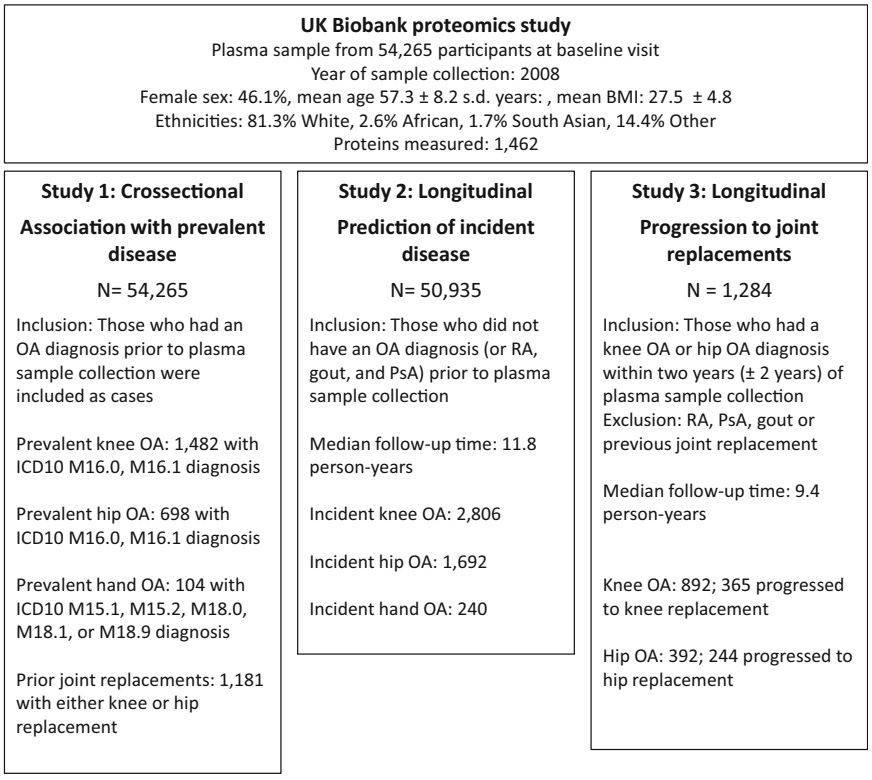

Figure 1. Schematic overview of the osteoarthritis (OA) biomarker study with inclusion criteria for the 3 main studies. BMI = body mass index; ICD-10 = International Statistical Classification of Diseases and Related Health Problems, Tenth Revision; RA = rheumatoid arthritis; PsA = psoriatic arthritis.

unadjusted levels of proteins were used in visual representations (Kaplan-Meier curves). The association of plasma proteins with prevalent OA, RA, gout, or PsA diagnosis or prior joint replacement was estimated using logistic regression, adjusting for age and sex, and visualized with box plots.

The risk of OA diagnosis and the risk of hip and knee replacement from the time of sample collection was estimated using Cox proportional hazards regression, adjusting for age, sex, and BMI, when significant, and visualized with Kaplan-Meier curves. The proportional hazards assumption was assessed both visually and with statistical test with the cox.zph function in R. We chose to report the risk at 5 years to be consistent with the approach in our previous paper (1); however, each time interval is shown until 12+ years.

The difference in CDCP1 and CRTAC1 protein levels in samples from individuals who underwent joint replacement either prior to or following plasma collection was estimated with linear regression and visualized with a regression line on a scatterplot, with an interaction term indicating whether the replacement was before or after plasma collection. The difference in increment per year before and after arthroplasty was estimated by including an interaction term in the regression model and tested with an F test.

For hip and knee OA, we built a single model including the 3 proteins that predicted both hip and knee OA (CRTAC1, aggrecan core protein [ACAN], and neurocan core protein [NCAN]) as well as age, sex, and BMI. We reduced the model with a backward selection based on a likelihood ratio test such that only proteins with P values less than 0.05 in the likelihood ratio test remained in the model. We used three-fourths of the data to fit the models and the remaining one-fourth to test their performance, using area under the curve (AUC) as the statistical measure. We resampled the test and training sets 1,000 times with replacement and reported the average of the AUC over the test sets. We then compared a model including CRTAC1, age, sex, and BMI to the model including CRTAC1, ACAN, NCAN, age, sex, and BMI, using a likelihood ratio test.

The association analysis of CRTAC1, ACAN, and NCAN with age, sex, and BMI was conducted for the entire cohort that had available protein measurements, a total of 54,265 individuals who were ages 39–71 years when plasma sample was collected. Odds ratios (ORs), hazard ratios (HRs), and 95% confidence intervals (95% CIs) were calculated.

Specificity. To ensure specificity of the proteins for OA diagnosis we excluded those 91 proteins that were also associated with either prevalent inflammatory joint diseases (RA [n = 336 cases], gout [n = 694 cases], and PsA [n = 35 cases]) or incident inflammatory joint diseases (RA [n = 606 cases], gout [n = 1,248 cases], and PsA [n = 101 cases]) (at $P < 3.4 \times 10^{-5}$,

Table 1. Characteristics of the subjects in studies 1 (prevalent disease) and 2 (incident disease)*

Variable	Prevalent disease (n = 3,330)	Progression to disease (n = 6,619)	No progression to disease (n = 44,316)
CRTAC1	0.17 ± 1.1	0.16 ± 1.0	−0.04 ± 1.0
Age at time of sample collection, years	61.7 ± 6.6	60.3 ± 7.2	56.6 ± 8.3
Year of sample collection	2,008.7 ± 0.9	2,008.5 ± 0.9	2,008.6 ± 0.9
Follow-up time, years	−5.9 ± 5.4	5.7 ± 3.5	11.8 ± 1.8
Follow-up range, years	−52.0, −0.01	0.0, −13.6	0.02, −15.0
Age at follow-up	73.1 ± 6.6	72.2 ± 7.2	68.4 ± 8.2
Age at follow-up range, years	47.3–83.6	40.4–84.8	49.5–83.8
BMI, kg/m^2	29.7 ± 5.3	29.3 ± 5.3	27.0 ± 4.6
Female sex, no. (%)	1,835 (55.1)	3,072 (46.4)	20,086 (45.3)
Knee OA, no.	1,482	2,806	0
Age at knee OA diagnosis, years	58.0 ± 8.3	66.2 ± 7.8	NA
Hip OA, no.	698	1,692	0
Age at hip OA diagnosis, years	60.1 ± 8.2	67.7 ± 7.2	NA
Hand OA, no.	104	240	0
Age at hand OA diagnosis, years	60.6 ± 7.3	65.6 ± 6.8	NA
Gout, no.	694	1,248	0
Age at gout diagnosis, years	55.9 ± 10.2	64.9 ± 8.3	NA
PsA, no.	35	101	0
Age at PsA diagnosis, years	55.5 ± 10.0	63.3 ± 8.7	NA
RA, no.	336	606	0
Age at RA diagnosis, years	56.8 ± 11.1	66.3 ± 8.0	NA
Prior joint replacement, no.	1,181	NA	NA
Age at prior joint replacement	63.3 ± 6.6	NA	NA
Ethnicity, no.			
African	46	115	1,246
White	2,776	5,564	35,789
South Asian	65	100	746
Other	443	840	6,535

* Except where indicated otherwise, values are the mean ± SD. Prevalent disease includes those who had knee osteoarthritis (OA), hip OA, hand OA, rheumatoid arthritis (RA), gout, or psoriatic arthritis (PsA) diagnosis at the time of plasma collection (based on International Statistical Classification of Diseases and Related Health Problems, Tenth Revision codes and/or drug use in case of gout) or had a prior joint replacement (based on Office of Population, Censuses and Surveys: Classification of Interventions and Procedures operation codes). Progression to disease and no progression to disease groups include all others, separated into progressors and nonprogressors. CRTAC1 = cartilage acidic protein 1; BMI = body mass index; NA = not applicable.

correcting for multiple testing). In addition, we excluded 1 protein that was primarily associated with prior joint replacement (n = 1,181 cases), assessed by strength of association with prior joint replacement compared to prevalent disease, and by visual inspection of association in samples from different patients at different time points before or after joint replacement, indicating an association with consequences of the joint replacement rather than of OA disease itself (1) (Supplementary Figure 1A and Supplementary Table 1, https://onlinelibrary.wiley.com/doi/10.1002/art.42376). A total of 92 proteins were excluded based on association with these other conditions (Supplementary Table 2).

RESULTS

Association of plasma protein levels with prevalent OA. To investigate the association of plasma protein levels with prevalent disease, we compared patients with a knee OA (n = 1,482), hip OA (n = 698), or hand OA (n = 104) diagnosis at the time of plasma sampling to those who did not have an OA diagnosis (Table 1). We adjusted each analysis for age, sex, and BMI when significant and set the significance threshold at $P < 1.14 \times 10^{-5}$, accounting for multiple testing with Bonferroni correction (1,462 proteins and 3 OA phenotypes). We then excluded 92 nonspecific proteins that were associated with other joint diseases or a history of joint replacements (Supplementary Table 2, https://onlinelibrary.wiley.com/doi/10.1002/art.42376).

Four proteins were associated with knee OA, and 6 were associated with hip OA (Table 3) after exclusion of the nonspecific proteins, whereas no proteins were significantly associated with hand OA. The strongest association with knee OA was with levels of CRTAC1 (OR 1.34 per 1-SD increase of the standardized protein level, $P = 5.5 \times 10^{-29}$). CRTAC1 levels were also associated with hip OA (OR 1.19, $P = 2.1 \times 10^{-6}$), but the strongest association with hip OA was with ACAN (OR 1.22, $P = 1.8 \times 10^{-7}$), which was also associated with knee OA (OR 1.22, $P = 4.3 \times 10^{-14}$). CRTAC1 was not significantly associated with hand OA in the small sample set of 104 cases (OR 1.18 [95% CI 0.98, 1.42],

Table 2. Characteristics of the hip or knee OA patients in study 3 (joint replacement)*

Variable	No joint replacement (n = 671)	Joint replacement (n = 607)
CRTAC1	0.16 ± 1.1	0.37 ± 1.0
Age at time of sample collection, years	59.7 ± 7.4	63.1 ± 5.2
Year of sample collection, years	2,008.6 ± 0.9	2,008.6 ± 0.9
Follow-up time, years	11.5 ± 2.3	2.3 ± 3.1
Follow-up range, years	0.2–15.4	0.0–13.4
Age at follow-up	71.2 ± 7.3	74.7 ± 5.3
Age at follow-up range, years	51.4, 83.4	45.8, 81.0
BMI, kg/m^2	29.4 ± 5.5	30.0 ± 5.3
Female sex, no.	338	243
Knee OA, no.	527	365
Age at knee OA diagnosis, years	59.5 ± 7.6	63.9 ± 5.2
Age at knee replacement, years	NA	66.7 ± 5.3
Hip OA, no.	148	244
Age at hip OA diagnosis, years	61.9 ± 7.2	63.7 ± 6.1
Age at hip replacement, years	NA	64.7 ± 5.8
Ethnicity, no.		
African	12	9
White	547	515
South Asian	15	8
Other	97	75

* Except where indicated otherwise, values are the mean ± SD. Only patients who received their first hip OA or knee OA diagnosis within 2 years of when the plasma sample was obtained were included in the study. Those who had undergone joint replacements or had RA, gout, or PsA were excluded. See Table 1 for definitions.

P = 0.089) (Supplementary Table 3, https://onlinelibrary.wiley.com/doi/10.1002/art.42376).

Protein levels predicted knee and hip OA risk. To analyze whether protein levels predicted incident OA diagnosis during the follow-up period or other joint diseases for evaluation of specificity, we included 50,936 individuals who did not have OA or other joint diseases (RA, gout, or PsA) and who had not undergone joint replacement at baseline; we followed them until March 23, 2021. During a total follow-up of 559,406.5 person-years, with a median follow-up time of 11.8 person-years, we determined whether any of the 1,462 proteins in plasma could predict incident diagnosis of OA, RA, gout, or PsA, using Cox proportional hazards regression and adjusting for age, sex, and BMI when significant. There were 2,806 individuals who were diagnosed as having knee OA during follow-up, 1,692 with hip OA, 240 with hand OA, 606 with RA, 1,248 with gout, and 101 with PsA (Table 1). In all 6 regression models, individuals were censored at the first diagnosis of any of the other 5 diseases and/or at death. We set a threshold of significance at $P < 1.14 \times 10^{-5}$, accounting for multiple testing using Bonferroni correction (1,462 proteins and 3 OA phenotypes).

After exclusion of the 92 nonspecific proteins, 8 proteins predicted future risk of knee OA diagnosis during follow-up, and 3 proteins predicted future risk of hip OA, whereas none predicted hand OA diagnosis. The 3 proteins that predicted hip OA also predicted knee OA: CRTAC1, ACAN, and NCAN (Table 4).

CRTAC1 was the strongest predictor of both knee OA and hip OA and was several orders of magnitude stronger than any of the other OA specific proteins measured (HR 1.40, $P = 1.08 \times 10^{-69}$ for knee OA and HR 1.25, $P = 2.6 \times 10^{-19}$ for hip OA) (Table 4). CRTAC1 did not significantly predict hand OA in this data set (HR 1.11 [95% CI 0.97, 1.27], P = 0.12) (Supplementary Table 3, https://onlinelibrary.wiley.com/doi/10.1002/art.42376).

Next, we assessed the likelihood of knee OA or hip OA diagnoses over time using the Kaplan-Meier estimator based on plasma CRTAC1 levels, and age, sex, and BMI. Individuals who were in the highest quintile of risk were 9.8 times more likely to develop knee OA within 5 years of plasma sample collection than those who were in the lowest quintile, and 9.7 times more likely to

Table 3. Association of plasma proteins with prevalent OA*

Protein	Knee OA (n = 1,482 patients and 49,453 controls)		Hip OA (n = 698 patients and 50,237 controls)	
	OR (95% CI)	P	OR (95% CI)	P
CRTAC1	1.34 (1.27, 1.41)	5.5×10^{-29}†	1.19 (1.11, 1.28)	2.1×10^{-6}†
ACAN	1.22 (1.16, 1.29)	4.3×10^{-14}†	1.22 (1.13, 1.31)	1.8×10^{-7}†
NELL2	0.87 (0.83, 0.92)	2.5×10^{-7}†	0.83 (0.77, 0.89)	5.2×10^{-7}†
IGFBP1	1.15 (1.09, 1.22)	4.6×10^{-7}†	1.12 (1.04, 1.21)	4.8×10^{-3}
NCAN	0.89 (0.85, 0.94)	4.6×10^{-5}	0.83 (0.77, 0.89)	6.5×10^{-7}†
DCXR	0.94 (0.89, 0.99)	0.018	0.82 (0.76, 0.89)	2.8×10^{-7}†
MASP1	1.00 (0.95, 1.05)	0.87	0.84 (0.78, 0.91)	4.1×10^{-6}†

* Proteins that were significantly associated with knee osteoarthritis (OA) or hip OA, and not with other inflammatory joint diseases or specifically with prior joint replacements, are shown. Odd ratios (ORs) are per SD increase in protein level. 95% CI = 95% confidence interval; CRTAC1 = cartilage acidic protein 1; ACAN = aggrecan core protein; NELL2 = protein kinase C binding protein NELL2; IGFBP1 = insulin-like growth factor binding protein 1; NCAN = neurocan core protein, DCXR = dicarbonyl and L-xylulose reductase; MASP1 = MBL-associated serine protease 1.
† Significant ($P < 1.14 \times 10^{-5}$) association.

Table 4. Plasma proteins that predict future risk of knee OA or hip OA*

	Knee OA (n = 2,806 progressors and 553,211 follow-up years)		Hip OA (n = 1,692 progressors and 553,045 follow-up years)	
Protein	HR (95% CI)	*P*	HR (95% CI)	*P*
CRTAC1	1.40 (1.35, 1.46)	1.08×10^{-69}†	1.25 (1.19, 1.31)	2.61×10^{-19}†
CNTN2	0.89 (0.85, 0.92)	4.46×10^{-10}†	0.94 (0.90, 0.99)	0.013
DPP10	0.89 (0.86, 0.93)	1.56×10^{-9}†	0.92 (0.88, 0.97)	9.65×10^{-4}
ACAN	1.12 (1.08, 1.17)	2.91×10^{-9}†	1.20 (1.15, 1.27)	1.70×10^{-13}†
NELL2	0.90 (0.87, 0.94)	1.20×10^{-7}†	0.92 (0.88, 0.97)	0.0011
NCAN	0.91 (0.88, 0.95)	2.58×10^{-6}†	0.87 (0.83, 0.91)	3.90×10^{-8}†
SFTPD	0.92 (0.88, 0.95)	3.95×10^{-6}†	0.98 (0.94, 1.03)	0.49
AOC3	0.92 (0.88, 0.95)	4.27×10^{-6}†	1.03 (0.98, 1.08)	0.24

* Proteins that significantly predict progression to knee OA or hip OA, but do not predict or associate with other inflammatory joint diseases or associate with specifically with prior joint replacement, are shown. Hazard ratios (HRs) are per SD increase in protein level. Progression to OA and other joint diseases was assessed among individuals who did not have a prior diagnosis of any of these diseases; subjects were censored at first diagnosis of any of the other diseases. CNTN2 = contactin 2; DPP10 = dipeptidyl peptidase-like 10; SFTPD = surfactant protein D; AOC3 = amine oxidase copper-containing 3 (see Table 3 for other definitions).
† Significant ($P < 1.14 \times 10^{-5}$) association.

develop hip OA (Figure 2). Using unadjusted CRTAC1 levels in the absence of established risk factors, those in the highest quintile of risk were 3.8 times more likely to develop knee OA and 3.3 times more likely to develop hip OA within 5 years of plasma sample collection than those in the lowest quintile.

Plasma CRTAC1 levels predicted joint replacement in patients with hip or knee OA. We determined whether those proteins that predicted knee or hip OA could also predict progression to joint replacement. We limited the study to those who were diagnosed as having knee or hip OA within 2 years (±2 years) of plasma sample collection to represent clinical settings, rather than to focus on all with prevalent disease, as this could introduce biases in the study. Of the 892 patients who had knee OA, 365 progressed to knee joint replacement within the study period, and of the 392 patients who had hip OA, 244 progressed to hip joint replacement (Table 2). We set the significance threshold at $P < 0.0031$, accounting for the 8 proteins and 2 phenotypes that were tested.

Of the 8 proteins, only CRTAC1 levels significantly predicted progression to knee replacement (HR 1.20, $P = 5.3 \times 10^{-4}$) or to hip replacement (HR 1.23, $P = 1.2 \times 10^{-3}$) in a Cox proportional hazards regression model (Supplementary Table 4, https://onlinelibrary.wiley.com/doi/10.1002/art.42376), with adjustment for significant established risk factors (age, sex, and BMI for knee replacement; age and sex for hip replacement).

Using the Kaplan-Meier estimator, patients who were in the highest quintile of risk based on CRTAC1 levels, age, sex, and BMI, were 5.5 times more likely to progress to knee replacement within 5 years of plasma sample collection than those in the lowest quintile, and 2.2 times more likely to progress to hip replacement (Figure 1). Without adjustment for age, sex, and BMI, knee OA patients in the highest quintile of CRTAC1 levels were 2.5 times more likely to progress to knee replacement, and hip OA patients were 2.0 times more likely to progress to hip replacement within 5 years of plasma sample collection.

Combining CRTAC1, ACAN, and NCAN. Three proteins were associated with both prevalent knee and hip OA and also predicted a risk of both knee and hip OA: CRTAC1, ACAN, and NCAN. The plasma levels of these proteins were not highly correlated (Supplementary Table 5, https://onlinelibrary.wiley.com/doi/10.1002/art.42376). We therefore tested a model that included all 3 proteins in addition to age, sex, and BMI.

A comparison of the full model (CRTAC1, ACAN, and NCAN) to a model including CRTAC1 as the only protein revealed improvement in the prediction of both prevalent OA diagnoses ($P = 1.19 \times 10^{-14}$ for prevalent knee OA and $P = 4.77 \times 10^{-17}$ for prevalent hip OA) and future risk of disease ($P = 7.3 \times 10^{-14}$ for knee OA and $P = 3.3 \times 10^{-22}$ for hip OA) but not progression to joint replacement (Supplementary Table 6, https://onlinelibrary.wiley.com/doi/10.1002/art.42376).

Association of CRTAC1, ACAN, and NCAN levels with age, sex, and BMI. To address the generalizability of CRTAC1, ACAN, and NCAN as biomarkers, we assessed their association with age, sex, and BMI (adjusted for time from sample collection and source of sample collection), using the raw Normalized Protein eXpression (NPX) values of the Olink Explore assay for the entire cohort of individuals with plasma protein measurements (N = 54,265; age range 39–71 years). CRTAC1 levels increased slightly with age, by 0.012 NPX values per year ($P < 1 \times 10^{-300}$), and decreased slightly by 0.001 per NPX unit with BMI ($P = 0.00014$) (Supplementary Figure 2, https://onlinelibrary.wiley.com/doi/10.1002/art.42376). CRTAC1 and NCAN levels were lower in women than in men (mean difference 0.12 NPX units [$P = 2.6 \times 10^{-300}$] and mean difference 0.28 NPX units [$P < 1 \times 10^{-300}$], respectively), whereas ACAN levels were slightly

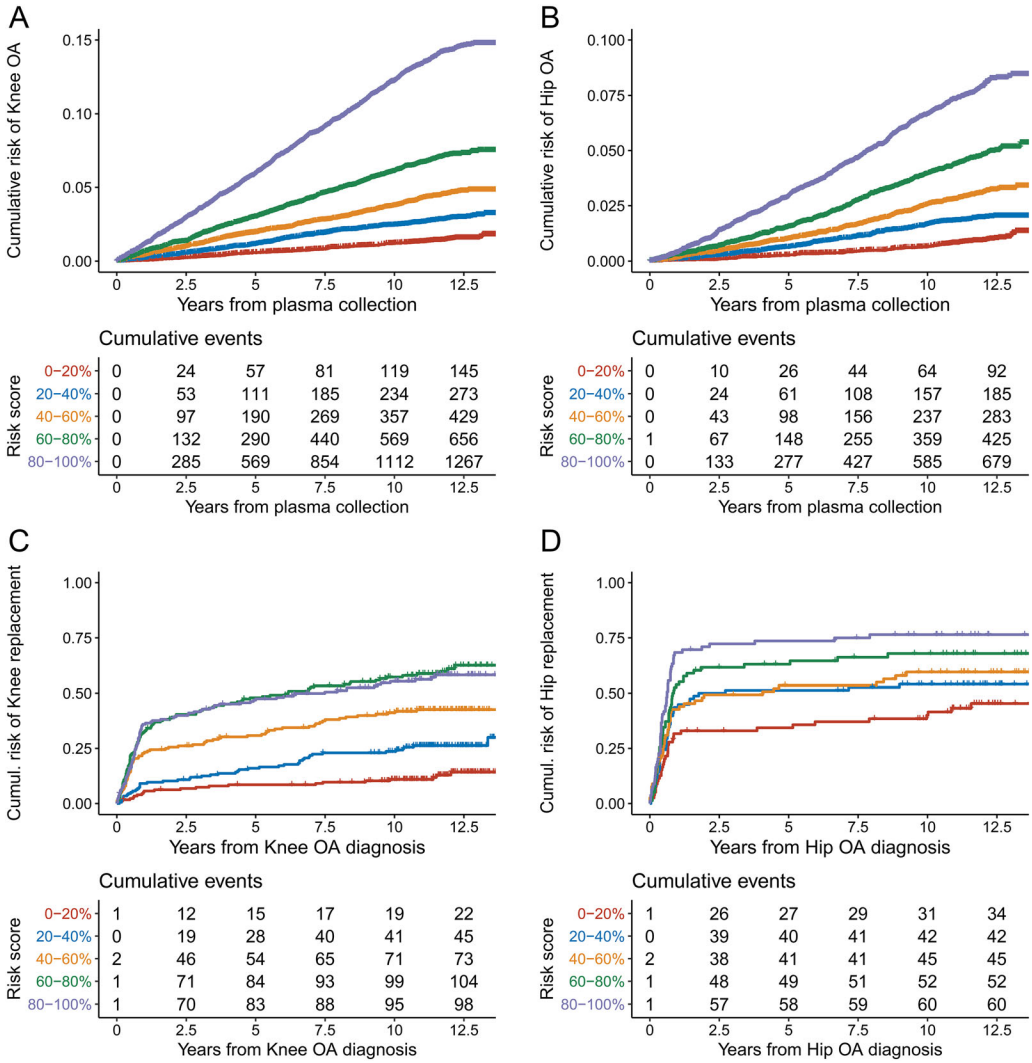

Figure 2. Kaplan-Meier estimator of risk of osteoarthritis (OA) or joint replacement. Kaplan-Meier estimates of the cumulative risk of knee OA (**A**), hip OA (**B**), knee replacement among knee OA patients (**C**), or hip replacement among hip OA patients (**D**), from the time of plasma collection, based on plasma cartilage acidic protein 1 (CRTAC1) levels, and age, sex, and body mass index (**A**–**C**), or age and sex (**D**) (top portion of each panel). Cumulative number of OA patients or joint replacement events over time according to quintiles of risk score among participants (bottom portion of each panel). In this study, 2,806 OA-free individuals developed knee OA, and 1,692 developed hip OA; 365 knee OA patients progressed to knee replacement, and 244 hip OA patients progressed to hip replacement.

lower in men (–0.11 NPX units [$P < 1 \times 10^{-300}$]). NCAN and ACAN levels in women did not change with age, while ACAN levels in men increased slightly with age (0.017 NPX units by year). ACAN and NCAN levels both decreased significantly with BMI (–0.014 NPX units [$P < 1 \times 10^{-300}$] and –0.028 NPX units [$P < 1 \times 10^{-300}$], respectively).

Association of protein levels with prior joint replacement. Previously, we observed a striking difference in protein levels between OA patients who had undergone a joint replacement before plasma collection and those who had not (1). Therefore, we also tested association of proteins with prior joint replacements in the UK Biobank data set of 1,181 patients and 53,084 controls. As in the Icelandic data set, we found that of the 1,462 proteins, the CDCP1 was most significantly associated with prior joint replacement (OR 2.3, $P = 7.5 \times 10^{-102}$; OR 2.67, $P = 1.1 \times 10^{-198}$ in the Icelandic data set).

Furthermore, based on samples from those individuals who underwent joint replacement either, prior to or after plasma collection, we found that CDCP1 levels varied very little between individuals during the 12 years preceding the joint replacement ($\beta_1 = 0.01$ SD per year [95% CI 0.00, 0.02]), whereas an increase of 0.416 SD was observed at the time of arthroplasty ($P = 5.77 \times 10^{-13}$), with a steady increase of 0.100 SD per year thereafter (95% CI 0.077, 0.123; $P_{interaction} = 4.05 \times 10^{-17}$). In contrast, CRTAC1 levels were lower in individuals who had plasma collected after arthroplasty ($\beta = 0.223$ SD, $P = 0.0015$)

(Supplementary Figures 2B and C, https://onlinelibrary.wiley.com/doi/10.1002/art.42376). These data align with our previous findings in the Icelandic study population.

DISCUSSION

In this study of a population from UK, we confirm and extend our previous findings of the CRTAC1 plasma protein as a biomarker for OA in an Icelandic data set, profiled using the SomaScan platform (1). Here we show, using plasma proteomic data of 1,462 proteins measured with the Olink Explore platform, that CRTAC1 is also the lead biomarker and is associated with both prevalent and future OA risk, as well as with joint replacement. Thus, these results replicate our previous findings in a different population (1). Contrary to the Icelandic study, CRTAC1 did not associate significantly with hand OA in UK, likely due to underdiagnosis and a small sample size that was purely based on ICD-10 codes for hand OA, while an extensive clinical evaluation of hand OA had been performed in the Icelandic study population.

The strong replication of the association of CRTAC1 with OA shows that the Olink Explore and SomaScan affinity-based platforms measure CRTAC1 in a similar manner. We had previously also shown a high correlation between CRTAC1 levels in the SomaScan and Olink Target assays (1), and recently, CRTAC1 was confirmed as a biomarker for OA severity and progression using the Olink Target assay (6). Furthermore, CRTAC1 was recently found to be a potential OA biomarker candidate using a mass spectrometry platform (8). Thus, several different protein assay platforms seem to capture CRTAC1 protein levels in plasma with similar results.

We found 2 other proteins, ACAN and NCAN, that improved association with prevalent and incident disease over just CRTAC1 alone, but not joint replacement. ACAN was also associated with prevalent OA in our previous work in which we used the SomaScan platform in Iceland, albeit not as strongly as CRTAC1, but was not selected into prediction models of disease or joint replacements (1). In Icelandic samples that were profiled using both SomaScan and Olink Explore (n = 1,000), the correlation of ACAN levels between platforms was only 0.34 and could explain these differences. ACAN is the main proteoglycan of articular cartilage and important for cartilage strength and biology. Mutations in *ACAN* have been shown to cause spondyloepiphyseal dysplasia, short stature, and early-onset OA (OMIM ID155760). As such an important structural protein in cartilage, ACAN is a strong candidate biomarker, with support from this study. Markers of ACANs degradation/remodeling products have been developed (9). NCAN, like ACAN, is a chondroitin sulphate proteoglycan but with an unknown function. Although the correlation of NCAN between the Olink and SomaScan platforms was high (0.74), NCAN was not significantly associated with OA in our Icelandic study, nor did any of the other proteins, apart from CRTAC1 and ACAN (1). The lack of association in the Icelandic samples could reflect a difference between the 2 populations studied. Further validation studies of these biomarkers of OA in other populations of various ancestries are warranted.

We assessed levels of CRTAC1, ACAN, and NCAN in relation to established risk factors for OA (age, sex, and BMI) to address the generalizability of these proteins as biomarkers. Here, CRTAC1 levels decreased slightly with an increase in BMI, particularly in women, whereas CRTAC1 did not correlate with BMI in Iceland. As in the Iceland-based study, CRTAC1 levels increased slightly with age. ACAN and NCAN levels did not change with age but decreased significantly with higher BMI. These data demonstrate that all 3 proteins are independent risk factors of OA.

Several studies have examined candidate biomarkers of OA (10–12), with serum COMP and urinary C-terminal telopeptide degradation product of type II collagen (CTX-II) as the 2 most extensively studied biomarkers (2–5,13). However, none of these markers are routinely used in the clinic. In this study, we found that COMP was associated with RA and therefore excluded it as an unspecific biomarker for further analysis, and we did not have urinary CTX-II measurements to test. Neither CRTAC1, ACAN, nor NCAN was associated with other common types of joint diseases (inflammatory arthritis) in this study or in the Icelandic study, highlighting all 3 as promising biomarkers that are specific for OA.

This study has several limitations. Due to the registry nature of the data, the possibility of misclassification cannot be excluded, and there is limited information about disease severity (e.g., Kellgren/Lawrence grades), except for the need for joint replacement. Furthermore, information on hand OA is limited. Health-related information about the participants is not complete either, with 55% of the participants having no information available about diagnoses made by general practitioners. In particular, missing information on joint replacements limits analyses of both the progression of OA to joint replacement and of the association with prior joint replacement. Finally, the study participants are predominantly of European descent, calling for studies in populations that primarily comprise other ancestries.

In conclusion, we have shown in a hypothesis-free proteomic analysis that plasma CRTAC1 levels of UK Biobank participants are associated with prevalent OA, OA risk, and progression to joint replacement, and it is independent from established risk factors. This confirms and extends our previous findings in Iceland using a different protein assay. Therefore, CRTAC1 is validated as a biomarker for OA and carries with it a promise to facilitate an earlier diagnosis of OA, which is currently captured only when destruction is evident. We additionally identified 2 other proteins, ACAN and NCAN, as possible biomarkers that could improve the prediction of OA when used in conjunction with CRTAC1.

ACKNOWLEDGMENT

We thank the study subjects for their valuable participation. This research has been conducted using the UK Biobank Resource under application numbers 23359 and 65851.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Styrkarsdottir had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Styrkarsdottir, Lund, Gudbjartsson.
Acquisition of data. Stefansson.
Analysis and interpretation of data. Styrkarsdottir, Lund, Thorleifsson, Saevarsdottir, Gudbjartsson, Thorsteinsdottir.

ROLE OF THE STUDY SPONSOR

deCODE genetics/Amgen Inc. had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by deCODE genetics/Amgen Inc.

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