Arthritis & Rheumatology
Vol. 73, No. 11, November 2021, pp 2025–2034
DOI 10.1002/art.41793

American College of Rheumatology
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The CRTAC1 Protein in Plasma Is Associated With Osteoarthritis and Predicts Progression to Joint Replacement: A Large-Scale Proteomics Scan in Iceland

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Objective. Biomarkers for diagnosis and progression of osteoarthritis (OA) are lacking. This study was undertaken to identify circulating biomarkers for OA that could predict disease occurrence and/or progression to joint replacement.

Methods. Using the SomaScan platform, we measured 4,792 proteins in plasma from 37,278 individuals, of whom 12,178 individuals had OA and 2,524 had undergone joint replacement. We performed a case–control study for identification of potential protein biomarkers for hip, knee, and/or hand OA, and a prospective study for identification of biomarkers for joint replacement.

Results. Among the large panel of plasma proteins assessed, cartilage acidic protein 1 (CRTAC1) was the most strongly associated with both OA diagnosis (odds ratio 1.46 [95% confidence interval 1.41–1.52] for knee OA, odds ratio 1.36 [95% confidence interval 1.29–1.43] for hip OA, and odds ratio 1.33 [95% confidence interval 1.26–1.40] for hand OA) and progression to joint replacement (hazard ratio 1.40 [95% confidence interval 1.30–1.51] for knee replacement and hazard ratio 1.31 [95% confidence interval 1.19–1.45] for hip replacement). Patients with OA who were in the highest quintile of risk of joint replacement, based on known risk factors (i.e., age, sex, and body mass index) and plasma CRTAC1 level, were 16 times more likely to undergo knee replacement within 5 years of plasma sample collection than those in the lowest quintile, and 6.5 times more likely to undergo hip replacement. CRTAC1 was not associated with other types of inflammatory arthritis. A specific protein profile was identified in those patients who had undergone joint replacement prior to plasma sample collection.

Conclusion. Through a hypothesis-free approach, we identified CRTAC1 in plasma as a novel promising candidate biomarker for OA that is both associated with occurrence of OA and predictive of progression to joint replacement. This biomarker might also be useful in the selection of suitable patients for clinical trial enrollment.

INTRODUCTION

Osteoarthritis (OA) is a major global health burden affecting >300 million people worldwide (1). Prevalence of OA is increasing since the prevalence of its main risk factors, obesity and older age, continue to rise (2–4). The disease can have a great impact on quality of life due to pain and loss of joint function, mostly affecting the knees, hips, and hands. OA is a heterogeneous disease with variable radiographic and clinical features (5), and there are presently no measures available for early diagnosis before destructive

Supported by deCODE genetics, Inc./Amgen Inc. A portion of the study was conducted using the UK Biobank Resource under application number 23359.

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Drs. Styrkarsdottir, Lund, Saevarsdottir, Norddahl, Frigge, Ivarsdottir, Bjornsdottir, Holm, Thorgeirsson, Rafnar, Jonsdottir, Sulem, Thorsteinsdottir, Gudbjartsson, and Stefansson and Mr. Magnusson and Ms Gunnarsdottir are employed by deCODE genetics, Inc./Amgen Inc. No other disclosures relevant to this article were reported.

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Submitted for publication December 8, 2020; accepted in revised form April 27, 2021.

changes are observable on radiographs. The current therapeutic approach consists of pain medications, lifestyle changes with weight reduction and exercise, and joint arthroplasty for severe disease (3), since no disease-modifying drugs are available. Thus, there is an unmet need for treatment that could effectively slow or halt OA progression.

A biomarker that is associated with OA disease occurrence and/or progression would help to identify cases earlier or monitor the disease course. Lack of such biomarkers has hindered development of effective therapy for this common disease. There are several studies on candidate biomarkers for OA (6–8), with somewhat inconclusive findings, possibly reflecting heterogeneity in the disease definition, study design, or size. However, meta-analyses of the 2 most extensively studied biomarkers for OA, serum cartilage oligomeric matrix protein (COMP) and urinary C-terminal telopeptide of type II collagen (CTX-II), show correlation with both disease occurrence and progression (9–13). These biomarkers are, however, not currently used in clinical settings.

In this study, we conducted a large-scale screen of biomarkers for OA, examining 4,792 human proteins in plasma from 39,155 individuals. We aimed to find a biomarker that is specific for OA development and/or disease progression, preferably a single biomarker since this is most feasible in the clinical setting, but also a more comprehensive tool that could yield better prediction. We included plasma proteins and known risk factors for OA (i.e., age, sex, and body mass index [BMI]) in the association and prediction models. To improve the models, we also included polygenic risk scores for OA, accounting for the role of genetics, as >80 independent sequence variants have been associated with the risk of OA to date (14,15).

SUBJECTS AND METHODS

Study population. We generated proteomics data from plasma collected from 39,155 Icelanders between August 2000 and January 2019, using 4,963 slow off-rate modified aptamers (SOMAmers) (SomaLogic; https://somalogic.com/) that measured 4,792 individual human plasma proteins (Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). We gathered information on OA diagnosis (International Statistical Classification of Diseases and Related Health Problems, Tenth Revision codes M16.0, M16.1, M17.0, M17.1, M15.1, M15.2, M18.0, M18.1, or M18.9, and clinical hand OA) and history of joint replacement (Nordic Medico-Statistical Committee Classification of Surgical Procedures [NCSP] codes NFB or NGB) from hospitals, health care providers, and clinicians (through March 2020). Detailed descriptions of the proteomics data, the OA phenotype definition, and study procedures are provided in Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

We designed 3 main studies from these 39,155 samples: 1) case–control association studies of OA, including 6,136 subjects with primary OA of the hips, knees, or hands (excluding those with prior joint replacement) and 13,789–16,001 controls without OA, 2) a prospective study of progression to joint replacement, including 10,701 subjects with OA, and 3) a case–control association study of prior joint replacement, including 1,452 cases and 35,826 controls. Subjects with missing information regarding age, sex, or BMI were excluded from all analyses, as were those who had rheumatoid arthritis (RA) or were age <40 years at the time of plasma sampling from analyses 1 and 2 (Figure 1 and Supplementary Methods).

In a secondary analysis of hand OA severity, we used 2 data sets. One included patients with hand OA for whom the severity of disease was evaluated by radiography and clinical findings. The second data set included individuals who participated in the population-based deCODE Health study (16) and had available high-quality hand photographs for analysis of hand OA (17,18). Subjects who participated in the deCODE Health study also answered a simple question regarding pain. Details of these analyses are available in Supplementary Methods.

The study was approved by the National Bioethics Committee of Iceland (VSN_14-015v8, VSN_14-148, and VSN_15-214), and was conducted in accordance with requirements issued by the Data Protection Authority of Iceland. All participants were of Icelandic descent.

Comparison of SOMAmer measurements with Olink assay. To test the specificity of the SomaScan measurements, we measured proteins using a proximity extension assay method (Olink Bioscience) (19). Proteins were measured using a cardiometabolic panel of 92 unique proteins, including cartilage acidic protein 1 (CRTAC1) and COMP. Of 200 samples, all of which had been assayed by SomaScan, 199 passed quality control. The median correlation between proteins was 0.757, ranging from –0.128 to 0.949, with an interquartile range of 0.541 to 0.829.

Polygenic risk scores. A polygenic risk score integrates a large fraction of the genetics contributing to a disease. We generated the polygenic risk scores based on effect estimates from OA genome-wide association study (GWAS) data in the UK Biobank (www.ukbiobank.ac.uk) (14) and calculated the risk scores for genotyped Icelanders, using 600,000 common variants and LDpred software, essentially as previously described (20). Details are provided in the Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Genome-wide association of plasma proteins (protein quantitative trait locus [QTL] study). To identify a set of independent DNA sequence variants that are associated with each protein, we performed a genome-wide association analysis (significance threshold $P < 5 \times 10^{-8}$) with a subsequent recursive

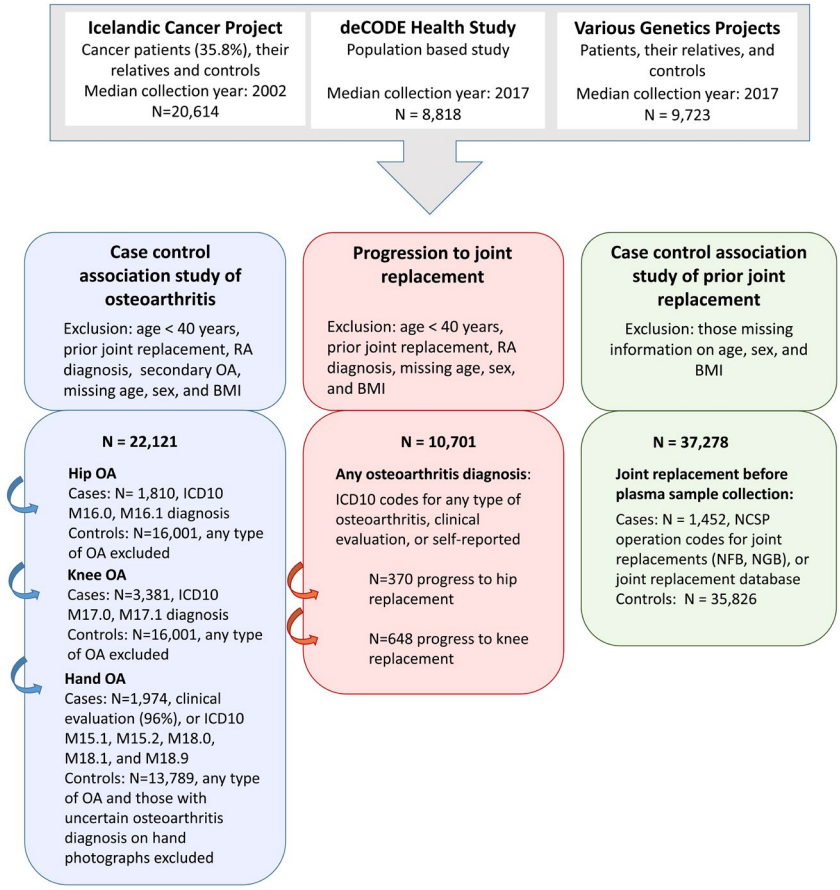

Figure 1. Schematic overview of the osteoarthritis (OA) biomarker study, with exclusion and inclusion criteria for the 3 main studies from which patients were derived. RA = rheumatoid arthritis; BMI = body mass index; ICD-10 = International Statistical Classification of Diseases and Related Health Problems, Tenth Revision; NCSP = Nordic Medico-Statistical Committee Classification of Surgical Procedures. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract.

conditional analysis, resulting in a list of independent protein QTL sequence variants that were associated with proteins (see Supplementary Methods and Supplementary Note, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Statistical analysis. Plasma protein levels were adjusted for the age of the individual at the time of plasma collection, sex, collection site, and the storage age of the sample. After adjustment, the plasma protein levels were rank-transformed onto the standard normal distribution with a mean of 0 and SD of 1. All modeling was completed with standardized protein levels, whereas raw unadjusted levels of CRTAC1 were used in visual representations (e.g., correlation with hand OA severity in Figure 2 and Kaplan-Meier curves in Figure 3).

Differences in plasma protein levels after joint replacement as well as their association with hip, knee, and hand OA were estimated with logistic regression, and area under the curve (AUC) was estimated using the bootstrap method. The risk of hip and knee replacement from the time of sample collection was estimated with Cox proportional hazards regression and visualized with Kaplan-Meier curves. Association of plasma protein levels with hand OA severity was estimated by linear regression. We used Bonferroni correction to adjust for multiple testing, yielding the following P values for each model: 1) $P \leq 3.3 \times 10^{-6}$ in analyses of association with OA, accounting for 3 phenotypes and 4,983 aptamers (which capture 4,792 proteins); 2) $P \leq 1.0 \times 10^{-5}$ in analyses of association with joint replacement, accounting for 1 phenotype and 4,983 aptamers; and 3) $P \leq 5.0 \times 10^{-6}$ in analyses of risk of progression to joint replacement, accounting for 2 phenotypes and 4,983 aptamers. A detailed description of the statistical methods is provided in the Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Data availability. Data supporting the findings of this study are presented herein and in the supplementary data files, and also can be provided upon request from the corresponding author.

RESULTS

Association of plasma proteins with OA diagnosis. We tested whether any of the measured plasma proteins were associated with OA, adjusting the analysis for known disease risk factors (i.e., older age, female sex, and higher BMI). This adjustment is critical

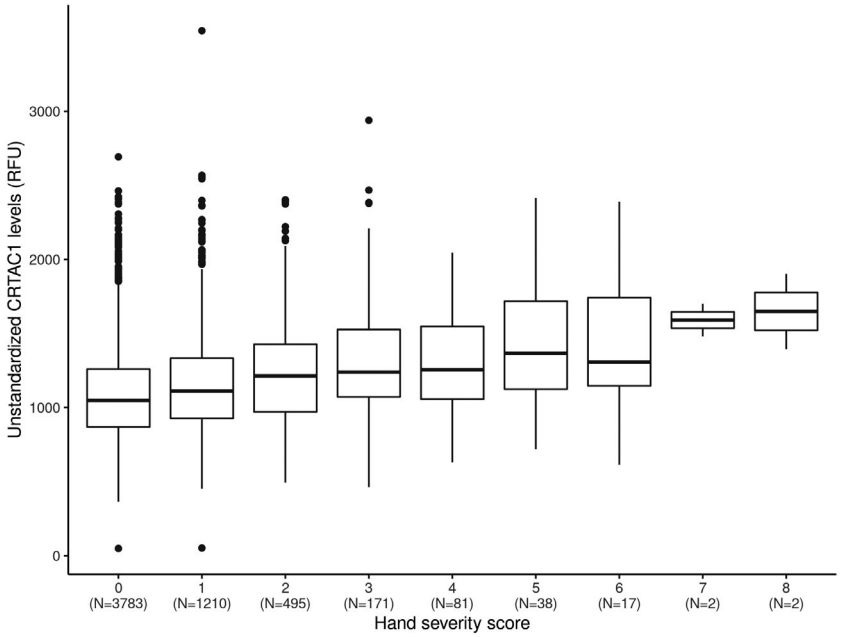

Figure 2. Correlation between cartilage acidic protein 1 (CRTAC1) levels in plasma and hand osteoarthritis severity scores. Correlation between unstandardized levels of CRTAC1 in plasma and hand osteoarthritis severity scores was estimated based on digital photographs (n = 5,445). Photographs were taken at the same time plasma samples were collected from participants in the deCODE Health study (18) and scored for the presence and severity of osteoarthritis, resulting in an aggregate score. Patients with rheumatoid arthritis were excluded. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th to 90th percentiles. Circles represent outliers. All patients were age >40 years. RFU = relative fluorescence units.

to exclude confounders, as a vast number of the 4,792 measured proteins are associated with these traits (e.g., 96% of the proteins tested were significantly associated with BMI in our data set).

We identified 45 proteins associated with knee OA, 7 proteins associated with hip OA, and 44 proteins associated with hand OA, that met the threshold for significance when accounting for multiple testing ($P \leq 3.3 \times 10^{-6}$; accounting for 3 phenotypes and 4,983 protein aptamers) and after excluding proteins that were also associated with inflammatory arthritis (Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.

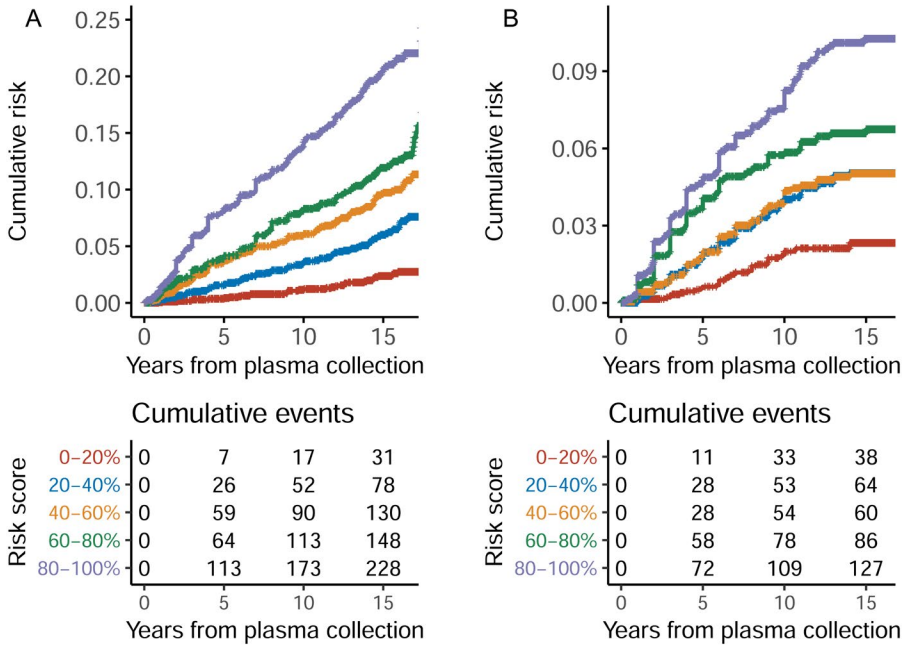

Figure 3. Kaplan-Meier estimates of the cumulative risk of knee replacement (**A**) or hip replacement (**B**) from the time of plasma collection, based on plasma cartilage acidic protein 1 levels and age, sex, and body mass index (top). Cumulative number of joint replacement events over time according to quintiles of risk score among participants (bottom). OA progressed to hip replacement in 376 patients, and to knee replacement in 672 patients.

Table 1. Association of plasma CRTAC1 levels with OA, other joint disorders, joint pain, hand OA severity, and joint replacement*

Phenotype	OR or HR (95% CI)†	*P*	No. cases/ controls
Hand OA	1.33 (1.26–1.40)	4.6×10^{-27}	1,712/15,242
Hip OA	1.36 (1.29–1.43)	2.1×10^{-35}	1,903/17,644
Knee OA	1.46 (1.41–1.52)	1.2×10^{-86}	3,578/17,644
Joint pain	1.15 (1.04–1.27)	0.0043	724/1,832
RA	0.96 (1.05–0.89)	0.39	525/25,764
Gout	0.94 (1.03–0.87)	0.17	512/24,476
Psoriatic arthritis	0.83 (0.98–0.71)	0.024	158/24,830
Thumb severity score	1.09 (1.01–1.17)	0.022	2,350/0
Finger severity score	1.12 (1.04–1.21)	0.0030	2,350/0
Hand severity score	1.08 (1.05–1.11)	7.6×10^{-10}	5,445/0
Hip replacement	1.31 (1.19–1.45)	8.4×10^{-08}	10,701/0
Knee replacement	1.40 (1.30–1.51)	1.0×10^{-18}	10,701/0

* Subjects were age >40 years at the time of plasma collection. For joint pain and hand severity score, plasma samples were obtained at the time the information on joint pain or hand severity score was collected. For all other phenotypes, the plasma samples were obtained at various time points in relation to osteoarthritis (OA) diagnosis. CRTAC1 = cartilage acidic protein 1; 95% CI = 95% confidence interval; RA = rheumatoid arthritis.
† Hazard ratios (HRs) are shown for risk of hip replacement and knee replacement; for all other variables, odds ratios (ORs) are shown. ORs and HRs are per SD increase in the protein level.

com/doi/10.1002/art.41793/abstract). Of these, CRTAC1, a marker of chondrocyte development, was the protein that showed the most strongly significant association with all 3 OA subtypes (odds ratio [OR] for knee OA 1.46 per SD increase of the standardized protein level [$P = 1.2 \times 10^{-86}$], OR 1.36 for hip OA [$P = 2.1 \times 10^{-35}$], and OR 1.33 for hand OA [$P = 4.6 \times 10^{-27}$]) (Table 1 and Supplementary Tables 1 and 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). The plasma CRTAC1 level was more strongly associated with OA by several orders of magnitude compared to any of the other proteins measured. COMP, an investigative biomarker for OA, was the second most significantly associated protein (OR 1.31 for knee OA [$P = 8.5 \times 10^{-43}$], 1.26 for hip OA [$P = 4.5 \times 10^{-20}$], and 1.19 for hand OA [$P = 2.3 \times 10^{-10}$]). COMP was somewhat correlated with CRTAC1 in our data ($r^2 = 0.362$ [95% confidence interval 0.355–0.370]). In models conditioning on the CRTAC1 levels, the association of COMP with OA was no longer significant ($P = 0.013$, $P = 0.029$, and $P = 0.97$ for knee, hip, and hand OA, respectively), whereas the association of CRTAC1 with OA remained significant in models conditioning on the COMP levels ($P = 5.3 \times 10^{-47}$, $P = 5.3 \times 10^{-18}$, and $P = 3.0 \times 10^{-18}$ for knee, hip, and hand OA, respectively).

The majority of the associated proteins correlated nominally with all 3 forms of OA. For 5 of these proteins, CRTAC1, COMP, cartilage intermediate-layer protein, retinoblastoma-like 2, and cytokine-like 1, the correlations with all 3 forms of OA were significant (Supplementary Table 1).

Approximately half of our samples were derived from a cancer study (The Icelandic Cancer Project, see Figure 1 and Supplementary Methods), which may have affected the results of our association analysis. However, when we tested the association of the proteins with any type of cancer, CRTAC1 levels ranked as 1,337. Furthermore, adjusting the association analysis for cancer did not significantly change the results for CRTAC1 (OR 1.46 for knee OA, 1.36 for hip OA, and 1.32 for hand OA) compared to not adjusting for cancer (OR 1.46 for knee OA, 1.33 for hip OA, and 1.26 for hand OA). Likewise, although other comorbidities were more common in OA cases compared to controls (Table 2), these conditions were associated with lower levels of CRTAC1, whereas OA was associated with increased levels of CRTAC1 (Supplementary Table 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). This highlights that CRTAC1 is a biomarker for OA and this association is not influenced by underlying comorbidities.

Correlation of plasma CRTAC1 levels with joint pain and OA disease severity. We further analyzed whether plasma levels of CRTAC1, the most significantly associated protein in OA, correlated with joint pain (a characteristic of OA) or with OA disease severity in the deCODE Health study, where the OA-related information was gathered at the time of plasma collection. Information on joint pain was gathered through a simple yes/no question on pain (answered "yes" by 649 of 2,708 patients), and hand OA severity was scored based on high-quality digital photographs (17,18) (n = 5,445; Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). We also evaluated whether CRTAC1 levels correlated with the number of each joint type affected.

CRTAC1 levels were associated with both joint pain (OR 1.14, $P = 0.0046$) and the hand OA severity score (OR 1.08, $P = 8.7 \times 10^{-11}$), with a direction of effects consistent with that of the association with OA overall. Figure 2 shows an increase in raw, unstandardized CRTAC1 levels (not adjusted for age or sex) with the hand OA severity score. With each increase in score, CRTAC1 levels were increased by 5.7% ($P = 2.2 \times 10^{-48}$).

We also assessed whether CRTAC1 levels correlated with thumb and finger OA severity scores, defined by radiography and clinical assessment in OA patients (n = 2,347) (Supplementary Table 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). We observed that CRTAC1 levels were associated with severity scores in the finger joints (OR 1.12, $P = 0.0030$), specifically the thumb carpometacarpal joint (OR 1.09, $P = 0.022$) of these patients (Table 1). We noted that CRTAC1 was the only protein associated with both OA joint pain and severity of hand OA (Supplementary Table 1).

We evaluated whether CRTAC1 levels increased with the number of OA-affected joint types. In these analyses, we observed that the CRTAC1 plasma levels increased by 0.22 SD with each incremental increase in the number of OA-affected joint types ($P = 2.2 \times 10^{-82}$) (Supplementary Figure 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Plasma CRTAC1 levels as a predictor of joint replacement in patients with OA. Joint replacement is a treatment for severe OA symptoms. We used Cox proportional hazards regression to investigate whether any of the 4,792 proteins measured

Table 2. Characteristics of the study subjects*

Variable	Case–control association study†			Progression to joint replacement study‡	
	Excluded (n = 17,777)	Controls (n = 15,242)	OA (n = 6,136)	No joint replacement (n = 9,654)	Joint replacement (n = 1,047)
Age at time of sample collection, mean ± SD years	48.6 ± 18.9	59.9 ± 13.4	64.0 ± 11.2	63.2 ± 11.5	63.4 ± 10.0
Female sex	10,559 (59.4)	7,847 (51.5)	3,956 (64.5)	6,352 (65.8)	633 (60.5)
Year of sample collection, mean ± SD	2,009.5 ± 7.5	2,007.3 ± 7.0	2,006.8 ± 6.9	2,007.8 ± 7.2	2,003.57 ± 4.3
BMI, mean ± SD kg/m^2	27.1 ± 5.2	26.8 ± 4.7	28.4 ± 5.1	28.0 ± 5.0	29.1 ± 4.8
Hip OA	79	0	1,903	1,420	483
Hip replacement	711	0	376	0	376
Age at time of hip replacement, mean ± SD years	65.0 ± 9.3	NA	71.3 ± 9.5	72.7 ± 11.7	70.2 ± 8.9
Knee OA	211	0	3,578	2,801	777
Knee replacement	559	0	672	0	672
Age at time of knee replacement, mean ± SD years	66.3 ± 8.4	NA	69.7 ± 8.5	72.7 ± 11.7	70.3 ± 8.9
Hand OA	97	0	1,721	1,599	122
Hand severity score, mean ± SD	0.52 ± 0.79	0	1.57 ± 1.65	1.07 ± 1.39	1.47 ± 1.56
Follow-up time, median (IQR) years	2.3 (0.9–16.2)	7.6 (1.8–16.0)	10.3 (1.9–16.1)	12.5 (1.8–16.4)	6.0 (2.6–10.6)
Prior joint replacement	1,452	0	0	0	0
RA	691	0	0	0	0
Age <40 years	7,776	0	0	0	0
Any OA diagnosis	6,329	0	6,136	9,654	1,047
Missing BMI	1,877	0	0	0	0
Missing genotype§	2,909	1,418	379	737	23
Comorbidities					
Coronary artery disease	2,415 (15.8)	3,558 (23.3)	1,788 (29.1)	2,622 (27.2)	345 (33.0)
Metabolic syndrome	2,597 (17.0)	2,457 (16.1)	1,429 (23.3)	2,171 (22.5)	270 (25.8)
Type 2 diabetes mellitus	1,491 (9.8)	1,648 (10.8)	867 (14.1)	1,278 (13.2)	176 (16.8)
Hypertension	6,525 (42.8)	6,770 (44.4)	4,029 (65.7)	6,093 (63.1)	726 (69.3)
Depression	1,442 (9.5)	921 (6.0)	442 (7.2)	735 (7.6)	79 (7.5)
Cancer	2,714 (15.3)	4,682 (30.7)	1,835 (29.9)	2,658 (27.5)	386 (36.9)

* Except where indicated otherwise, values are the number (%). All subjects in both studies were age >40 years at the time of plasma collection, had information on body mass index (BMI) within 5 years of plasma collection, had not undergone joint replacement, were not diagnosed as having rheumatoid arthritis (RA), and had protein levels measured using SOMAScan. NA = not applicable; IQR = interquartile range.
† In the case–control association study, patients were required to have a primary diagnosis of hand, knee, or hip osteoarthritis (OA) for inclusion.
‡ In the progression to joint replacement study, a diagnosis of any OA among the individuals undergoing surgery was sufficient for inclusion.
§ Missing genotype is only an exclusion criterion in the full models.

in plasma could predict the time until joint replacement among patients with OA (n = 10,701), limiting the study group to those whose plasma samples were collected before the joint replacement (described below). Since in many cases the specific diagnosis of knee OA or hip OA was not recorded in the electronic health record registries used in this study until the time of joint replacement surgery, we included any patients diagnosed as having OA in this analysis and not only those who were coded as M16.0, M16.1, M17.0, and M17.1 as was the case in the knee and hip association analysis (Figure 1). CRTAC1 was 1 of only 2 proteins that could significantly predict hip replacement, whereas 8 proteins could predict knee replacement (Supplementary Table 6, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). It was the strongest predictor of both hip replacement (hazard ratio [HR] 1.31, $P = 8.4 \times 10^{-8}$) and knee replacement (HR 1.40, $P = 1.0 \times 10^{-18}$). Therefore, of all the proteins tested, CRTAC1 was most significantly associated with OA diagnosis and was the best predictor of progression to joint replacement.

We assessed the likelihood of joint replacement over time using plasma CRTAC1 levels and classic risk factors for OA (i.e., age, sex, and BMI), stratified into quintiles of risk scores, and compared the risk of joint replacement for each quintile of the predicted risk using the Kaplan-Meier estimator. Patients with OA who were in the highest quintile of risk were 16 times more likely to undergo knee replacement within 5 years of plasma sample collection[‡] than those in the lowest quintile, and 6.5 times more likely to undergo hip replacement (Figure 3). Using unadjusted CRTAC1 levels in the absence of classic risk factors, those in the highest quintile of risk were 3 times more likely to undergo knee replacement and 2.5 times more likely to undergo hip replacement within 5 years of plasma sample collection than those who were in the lowest quintile, which demonstrated that independent of age, sex, or BMI, the CRTAC1 levels alone substantially increased the likelihood of joint replacement (Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

[‡]Correction added after online publication 18 October 2021: the text was changed from "within 5 years of diagnosis" to "within 5 years of plasma sample collection."

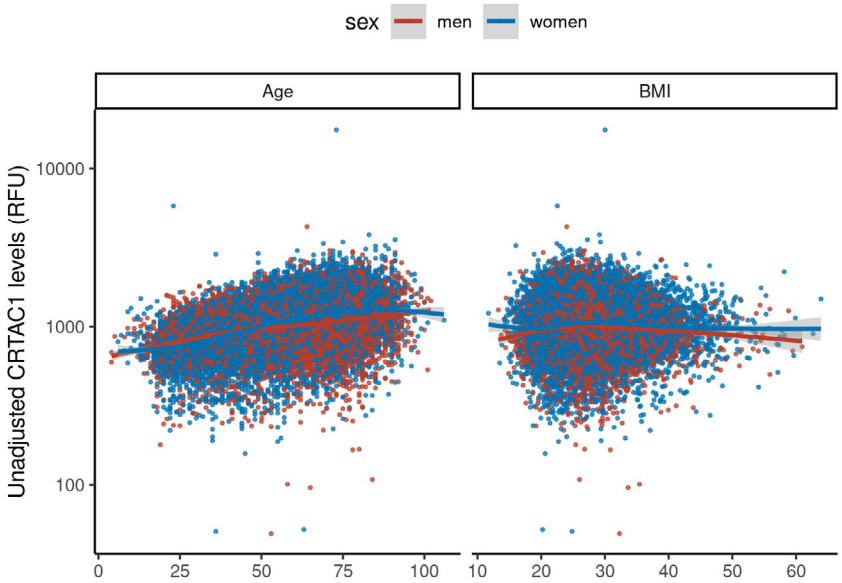

Figure 4. Correlation of unadjusted plasma cartilage acidic protein 1 (CRTAC1) levels with age and body mass index (BMI) in men and women. Results are from the entire data set of 37,278 subjects who had available information on age, sex, and BMI. Each symbol represents an individual subject. RFU = relative fluorescence units. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.41792/abstract.

Generalizability of CRTAC1 as a biomarker. To address the stability of CRTAC1 levels as a biomarker for OA, we assessed its association with age, sex, and BMI (adjusted for time from sample collection, and source of sample collection). CRTAC1 levels did not correlate with BMI ($P = 0.71$), but they did increase slightly with age (8.24 relative fluorescence units per year, $P < 1 \times 10^{-300}$) (Figure 4). In an association analysis stratified by sex, age, and BMI, we showed that the effect of CRTAC1 on OA risk did not differentiate significantly between any of the groups tested (Supplementary Table 7, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract), demonstrating that CRTAC1 was an independent risk factor for OA.

We also evaluated whether the measurement of CRTAC1 using SOMAmer was consistent with other types of protein measurements using the Olink protein immunoassay (n = 200; https://www.olink.com). The correlation between CRTAC1 SOMAmer measurements and CRTAC1 Olink measurements was 0.86 (95% confidence interval 0.80–0.90), and the association of CRTAC1 level with OA was also significant using the Olink method (Supplementary Figure 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Potential role of CRTAC1 in the pathogenesis of OA. To determine whether CRTAC1 levels in plasma contributed to the pathogenesis of OA, we performed a GWAS of DNA sequence variants associated with CRTAC1 levels (protein QTL study) and identified 8 CRTAC1 protein QTL variants, one of which was at the CRTAC1 locus. We then tested those protein QTL variants for association with OA in a combined study population from Iceland and the UK Biobank (14), with a total of 17,151 patients with hip OA, 23,877 with knee OA, and 9,773 with hand OA, compared to >560,000 controls. None of these sequence variants were associated with OA (Supplementary Table 8, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). Furthermore, in large meta-analyses of OA, no association with sequence variants at the CRTAC1 locus has been described (14,15). This indicates that the observed increase in CRTAC1 in plasma was unlikely to cause OA. Moreover, adjusting the CRTAC1 association for the OA polygenic risk scores, which integrated a large fraction of the contribution of genetics to the disease, had little impact on CRTAC1 association with OA (Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). This suggests that CRTAC1 captured a different component of the disease.

Models for OA association and prediction of progression to joint replacement. Although a single, easily interpreted biomarker is currently most feasible to use in clinical settings, a model incorporating other factors besides CRTAC1 alone may provide more accurate association/prediction of OA development and progression to joint replacement. We therefore aimed to build the best available association model for OA diagnosis and for prediction of progression to joint replacement by including all independently associated proteins and the polygenic risk scores for OA in the model, in addition to age, sex, and BMI. Exclusion/inclusion criteria were the same as before, with the exception that those without genotypes were also excluded. To avoid overfitting of the models, we split the data set in two and used three-fourths of the data to build the model and one-fourth to test it.

We performed backward stepwise selection of associated variables (i.e., proteins, polygenic risk score, age, sex, and BMI) in a single logistic regression model and likewise, to determine a set of proteins that predict OA progression to joint replacement, we used Cox proportional hazards regression. We demonstrated both a good fit between the training and test sets and adequate power of our study to detect association with OA (Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

To evaluate the discriminatory power of our models, we applied a receiver operating characteristic curve and calculated the AUC. In the full model, the AUC was 0.762 for hip OA, 0.770 for knee OA, and 0.800 for hand OA, and the cumulative AUC estimate for joint replacement was 0.661 for hip replacement and 0.734 for knee replacement (Supplementary Table 9, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

Of all the proteins in the models (between 1 and 27), CRTAC1 contributed the most to all 5 models (Supplementary Figures 7 and 8, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). The polygenic risk score and the classic risk factors, age and sex, contributed to all of the models. BMI, however, did not contribute to the hip replacement model. As expected, given the composition of the sample set, classic risk factors contributed the most in all of the models. However, adding the proteins and polygenic risk scores significantly improved the association of the models with OA ($P = 1.5 \times 10^{-101}$ for hand OA, $P = 3.1 \times 10^{-75}$ for hip OA, and $P = 3.8 \times 10^{-203}$ for knee OA) and prediction of joint replacement ($P = 4.8 \times 10^{-111}$ for knee replacement and $P = 9.3 \times 10^{-71}$ for hip replacement) (Supplementary Table 9).

These OA risk profiles were strongly associated with joint pain, and the hand risk score was strongly associated with hand OA severity (Supplementary Table 10 and Supplementary Figure 9, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). Patients who were in the highest quintile of joint replacement score were 25 times more likely to undergo knee replacement and 9 times more likely to undergo hip replacement than those in the lowest quintile (Supplementary Figure 10, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). However, in the full models, additional proteins conferred significant risks of other joint diseases (i.e., RA, gout, and psoriatic arthritis [PsA]) (Supplementary Table 11, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract) and were, therefore, not specific for OA. Furthermore, use of the full models required measuring up to 35 proteins for both OA association and prediction of joint replacement, and genotypes for the polygenic risk score, which would be challenging in clinical settings.

As described above regarding CRTAC1, we also analyzed whether the levels of the plasma proteins selected in the association and prediction models could cause OA by testing protein QTL sequence variants (n = 267) (Supplementary Note, Supplementary Tables 12 and 13, and Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract). As with CRTAC1, our results indicated that plasma levels of these proteins are unlikely to cause OA, but their altered levels are consequences of the disease.

Induction of a specific protein profile by joint replacement. We observed a striking difference in levels of OA-associated proteins between patients who had undergone a joint replacement before plasma collection (n = 1,452) and those who had not (n = 4,229). In our data, a prior joint replacement was associated with 137 plasma proteins (Supplementary Table 14, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract), a minority of which were associated with OA in patients who had not undergone joint replacement (n = 16 proteins). This indicated that the joint replacement itself was associated with a plasma protein profile that was a consequence of the joint implant rather than the disease. Therefore, we excluded patients who had undergone joint replacement prior to plasma collection from all the OA association studies described above. The protein with the strongest association with prior joint replacement, CUB domain-containing protein 1 (CDCP1) ($P = 1.1 \times 10^{-198}$, OR 2.67), was not associated with OA without joint replacement. Like most proteins associated with joint replacement but not with OA, CDCP1 levels changed very little during the 20 years preceding joint replacement, followed by an increase in levels at the time of arthroplasty ($\beta = 0.866$, $P = 5.7 \times 10^{-16}$) and a continued steady increase thereafter ($\beta = 0.072$ per year, $P = 8.7 \times 10^{-43}$), as demonstrated in data from different individuals at different time points after joint replacement. In contrast, the proteins that were also associated with OA demonstrated an increase in levels prior to joint replacement that either continued (e.g., ASB9) or decreased (e.g., CRTAC1) after surgery (Supplementary Figure 11, http://onlinelibrary.wiley.com/doi/10.1002/art.41793/abstract).

DISCUSSION

In this study of a large panel of plasma proteins (4,792 measured), we determined that plasma CRTAC1 levels were the most strongly associated with OA diagnosis, and CRTAC1 was the best predictor of OA progression to joint replacement. Patients with OA who were in the highest quintile of risk based on known risk factors (i.e., age, sex, and BMI) and plasma CRTAC1 levels were 16 times more likely to undergo knee replacement within 5 years, and 6.5 times more likely to undergo hip replacement than those in the lowest quintile. Furthermore, CRTAC1 levels in plasma were also associated with joint pain and hand OA severity. Importantly, CRTAC1 levels are not associated with other inflammatory joint diseases such as RA, gout, or PsA. We determined that the protein panels of up to 27 proteins, together with the OA polygenic risk scores (in addition to age, sex, and BMI) in the models, performed better than CRTAC1 alone (in combination with age, sex, and BMI). However, these expanded associations and prediction models were not specific to OA. This highlights CRTAC1 as a promising biomarker for OA, since we found that, among all of the disease subphenotypes studied, this protein was not only strongly associated with OA, but also specific for OA with no association with other inflammatory joint diseases. We also

demonstrated that CRTAC1 is a risk factor for OA independent of age, sex, and BMI. Based on CRTAC1 QTL genetic analysis, we demonstrated that CRTAC1 levels in plasma are unlikely to cause OA.

The function of CRTAC1 is not fully understood. It is a glycosylated, calcium-binding extracellular matrix protein that is highly conserved from bacteria to humans (21), that was initially identified as a marker of chondrocyte development from mesenchymal stem cells (22). This protein is found in greater concentrations in knee synovial fluid from OA patients compared to controls (23), and also found at higher levels in OA cartilage (24). Furthermore, CRTAC1 is also involved in apoptosis and pyroptosis of human lens epithelial cells (25,26).

In addition to these findings, we observed that joint replacement was associated with a protein profile in plasma that lacks association with OA and persists long after joint replacement surgery. This profile may therefore reflect the body's reaction to the joint implant. The plasma levels of these proteins could be candidates for prediction of prosthesis survival time or early prosthesis failure.

To our knowledge, this study comprises the largest biomarker study of OA to date. We simultaneously investigated 4,792 plasma proteins in 39,155 individuals of whom 12,178 had OA. This study thus has greatly increased power over previous studies to identify circulating biomarkers for OA. We also demonstrated that plasma levels of COMP, one of the most investigated biomarkers for OA to date (9–12), were associated with hand, hip, and knee OA and predicted knee replacement. Thus, our study supports previous findings indicating that COMP is a likely biomarker for OA. However, we showed that COMP was a substantially weaker predictor than CRTAC1, and because it is also associated with weight, it is a less suitable biomarker. We did not have access to measurements of the other much-studied biomarker for OA, urinary CTX-II, which is a degradation product of type II collagen found in urine. We therefore could not test whether urinary CTX-II correlated with CRTAC1 or whether it was independently associated with OA.

Limitations of this study include the lack of information on age at the onset of OA symptoms or age at first confirmed diagnosis, which reflected the registry nature of the data. Thus, we were unable to determine whether the CRTAC1 levels were already altered at a very early stage of the disease. Furthermore, in the association analysis, we included all patients diagnosed as having OA by March 2020, irrespective of when their plasma sample was collected. This group is therefore heterogeneous, i.e., at the time the plasma sample was collected, some patients had advanced disease whereas others were symptom-free. Moreover, we did not have access to other population studies for replication of our findings. Follow-up studies in other populations of various ancestries, in addition to studies addressing technical issues, are extremely important for validation of CRTAC1 as a general biomarker for OA.

In this large hypothesis-free study on OA and joint replacement, we identified a novel biomarker, CRTAC1, with a strong, and specific, effect on both disease risk and disease severity. It had a stronger effect than biomarkers that were previously established as having the best predictive abilities. If validated in further studies, CRTAC1 will be a promising biomarker both for clinical use and for therapeutic development, where there is currently a large unmet need.

ACKNOWLEDGMENT

We thank the study subjects for their valuable participation.

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.

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ROLE OF THE STUDY SPONSOR

The study is sponsored by deCODE genetics, Inc./Amgen Inc. The authors independently collected the data, interpreted the results, and had the final decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Amgen.

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