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Association between cartilage and bone biomarkers and incidence of radiographic knee osteoarthritis (RKOA) in UK females: a prospective study

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SUMMARY

Objective: There is a need to find biochemical markers that would identify people with increased risk of developing radiographic knee osteoarthritis (RKOA). The aim of this study was to evaluate the ability of cartilage and bone biomarkers (cartilage oligomeric matrix protein (COMP), aggrecan, cellular inhibitor of apoptosis protein (cIAP), N-telopeptide-to-helix (NTx)) to predict RKOA incidence in a 10-year follow-up of UK females from the Chingford community study.
Method: Joint space narrowing (JSN), osteophytes (OSP) and Kellgren–Lawrence (K/L) grades were scored from radiographs of both knees at study baseline and 10 years later in 1,003 women aged 45–64. Circulating levels of biomarkers and demographic variables were measured at baseline. Statistical association analysis was conducted between the potential predictor factors measured at baseline and documentation of RKOA at 10-year follow-up.
Results: Age and body mass index (BMI), were significant predictors of incidence of RKOA as assessed by K/L and OSP. Considering biomarkers, independent significant association was found between COMP circulating levels and K/L scores (OR = 2.87, 95% CI = 1.19–6.89, $P = 0.018$). Significant negative association was detected between aggrecan plasma concentrations and JSN, with OR = 0.37 (95% CI 0.15 –0.89), $P = 0.026$.
Conclusions: Aggrecan and COMP circulating levels contribute to identification of phenotype-specific RKOA incidence. These data suggest potentially protective role of aggrecan in cartilage loss, as measured by JSN. High COMP levels are risk factors for development of RKOA, as assessed by K/L scores.
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Introduction

Knee osteoarthritis (KOA) is a highly prevalent age-related degenerative disease of synovial joint[1]. The knee joint is a complex organ, and the main pathologic changes seen in KOA include inflammation of the joint lining synovial membrane (synovitis), fibrillation and loss of the articular cartilage, accompanied by thickening and remodeling of the subchondral bone. Ultimately, it results in full thickness loss of cartilage which is seen on radiograph as loss of joint space[2,3]. The involvement of a variety of tissues results in the appearance and interactions of many types of molecules, both locally and in circulation[4–6]. The identification of blood and urine biomarkers or combination of biomarkers that might be useful and reliable indicators of the disease incidence and progression remains an important challenge[6–8]. Until now there is no wide agreement on best and most effective circulating biomarkers[9,10] but there are number of molecules, whose circulating levels appear to be associated with OA. Among these molecules, are those related to cartilage metabolism, namely, aggrecan, cartilage oligomeric matrix protein (COMP), cellular inhibitor of apoptosis protein (cIAP) and molecule related to bone metabolism, N-telopeptide-to-helix (NTx), secreted following bone resorption. The association of the circulating levels of the aforementioned molecules with manifestation and rate of progression of joint destruction in patients with KOA have been tested in several studies[11–18], with conflicting results. For example, there are a series of studies reporting more rapid knee joint destruction in patients with lower levels of aggrecan in synovial fluid and/or in serum in comparison to patients with higher levels[19,20]. Other studies, however, found an

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opposite trend[12,21,22] or even no association at all[13,23]. Another potential marker of OA progression is a non-collagenous protein of articular cartilage matrix COMP, which may protect chondrocytes cells against death[24]. Several studies demonstrated consistently elevated COMP levels in synovial fluid and/or serum during periods of arthritic changes in knee joint[15,21,25]. Yet, other studies found no such correlation[26,27]. There are also data concerning the NTx and cIAP association with OA are controversial and inconsistent[26,28–32]. Thus, there is uncertainty with respect of usefulness of the aforementioned markers of osteoarthritis in general and for KOA, in particular. One potential reason for such uncertainty could be the limited statistical power of many of these studies due to their relatively small sample size, ranging between a few dozens to a couple of hundreds individuals.

We have collected radiographic knee osteoarthritis (RKOA) data over 10-years in a large community-based sample of middle-aged British females. The goal of this study was to investigate whether circulating levels of COMP, aggrecan, NTx and cIAP predict appearance of RKOA-associated phenotypes followed prospectively during 10 years.

Material and methods

Study subjects

A detailed description of the sample, its recruitment, radiographic and clinical assessment has been published previously elsewhere[33]. Briefly, the Chingford Study population was established in 1989 as a case–control study to determine prevalence rates of OA in middle-aged women in the general population, but since become a prospective population-based longitudinal cohort of women seen annually and described in detail by Spector *et al.*[34]. The original response rate of the sample was 78%. The cohort consisted initially of 1,003 middle-aged women aged 45–64 from a general practice in Chingford, northeast London. All participants gave written informed consent before entering the study and the St. Thomas' Hospital research ethics committee approved the project.

Radiographic assessment

Plain AP radiographs were scored by single trained reader who was blinded to the pairing or clinical information. The status of each RKOA was determined based on two characteristics, and following Altman atlas[35]: 1. The Kellgren/Lawrence (K/L) scores ranging from 0 (no evidence of bony changes or joint space narrowing (JSN)) to 4 (definite osteophytes (OSP) and increasing diminution of the joint space). 2. JSN and OSP score, graded on four-point scale (where 0 = OSP (or JSN) are not observable, and 3 = most severe status). The assessment of JSN and OSP was done for lateral and medial compartments on each knee separately. An individual was considered "affected" if at least one knee had K/L $\geq$2, or if OSP and/or JSN $\geq$1. RKOA was assessed twice, first at entrance examination and then 10 years later on films which were not paired.

Biomarkers measurements

Blood and urine samples were obtained at baseline examination and methodology was described in details elsewhere[30]. In short, sera were stored at −80°C after being obtained from venous blood. The circulating levels of all biomarkers were assayed using ELISA kits and following manufacturer instructions. Urine NTx levels were measured using an Osteomark NTx kit (Inverness Medial Innovations, Princeton NJ, USA) and the measured values were corrected renal function. Blood serum samples were used to assay circulating levels of aggrecan, COMP, and cIAP, using kits manufactured by Medgenix Diagnostics (Belgium), AnaMar Medical (Uppsala, Sweden), and Bio-Rad protein Assay kit (Hercules, CA), respectively.

Statistical analysis

This was performed using SPSS package version 19 (SPSS Inc, Chicago, IL, USA), to identify variables, which potentially could be able to predict incidence of primary RKOA, 10 years after the biomarkers assessment. All individuals with K/L grading >1 and/or OSP and/or JSN grading >0 at baseline, and those who underwent total knee replacement during the 10-year follow-up, were excluded from the analysis (N = 136 individuals). We then conducted a series of univariate analyses to determine the relationship of RKOA with body mass index (BMI), and circulating levels of biomarkers, without adjusting for age. RKOA phenotypes, in these analyses, were treated as categorical variables in which individuals not affected at either entrance examination or 10-year follow-up (control) were contrasted with the individuals not affected at baseline, but affected after 10 years (case). Since distributions of the biochemical variables exhibited statistically significant skewness ($P \leq 0.01$), we implemented non-parametric Mann–Whitney tests to compare cases and controls. Next, we conducted a binary logistic regression analysis in which dependent RKOA variables were treated as categorical traits: case vs control. Age, BMI and biomarkers were used as independent predictors in this analysis and as confounders for adjustment.

Results

The basic characteristics of the study sample used in prediction analysis are given in Table I. At baseline examination, 1,003 participants were recruited. Of these, 909 had complete radiographic data, of which 865 had complete blood and urine biomarkers data. For prediction analysis the following sample sizes were obtained:742,784,780,694,788 participants for K/L, OSP_md, OSP_lt, JSN_md, JSN_lt, respectively. These individuals were unaffected at baseline, and had full data at baseline and 10 years after (Appendix 1). Individuals who were not included in the analysis tended to be older (57 ± 0.40 vs 54 ± 0.21) and heavier (BMI 27 ± 0.33 vs 25 ± 0.14) than those included in the study. Univariate analyses showed the controls were significantly ($P \leq 0.002$) younger than cases defined by K/L and OSP, but not JSN. BMI and COMP tended to be significantly higher in the cases for all phenotypes, except JSN_md. Mean aggrecan serum concentrations were consistently lower in the cases, but the difference was not significant for JSN_lt. cIAP and NTx levels showed no clear trend in relation to any of the RKOA phenotypes. Table II summarizes the main results of binary logistic regression. As expected, age and BMI were significant predictors for K/L and OSP at both compartments. In addition, BMI was significantly associated with JSN_lt. Considering biochemical factors; COMP was a significant predictor for K/L only. Its independent effect was quite substantial, as measured by relative risk, RR = 2.87 (95% CI 1.19–6.89), P = 0.018. Fig. 1 shows that risk probability of developing RKOA (K/L $\geq$ 2) increases with the elevation of age, BMI and COMP concentration. The aggrecan circulating levels were statistically significantly lower in affected participants with the OA phenotype as assessed by JSN_md $\geq$ 1 (P = 0.026) and its elevated levels were associated with reduced relative risk, RR = 0.37 (95% CI 0.15–0.89), Table II. These data thus suggest that aggrecan levels in some way act as a protective factor against cartilage destruction and narrowing of joint space. The two other biomarkers cIAP and NTx were not associated significantly with any of the RKOA phenotypes.

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Table I
Basic descriptive statistics of Chingford sample used to examine the risk factors for OA-related phenotypes incidence during 10 years of follow-up. The sample divided into cohorts: (a) Individuals with "not affected" radiographic imaging at baseline and after 10 years (control), and (b) individuals with "not affected" radiographic imaging at baseline and "affected" after 10 years (case). Man–Whitney test was used to compare distribution of quantitative covariates between the case and control cohorts. OSP_lt and OSP_md –OSP from lateral and medial compartments of tibiofemoral joint, JSN_ lat and JSN_md – are JSN of lateral and medial compartments at visit_10 (10 years after baseline)

Predictors	Individuals with "not affected" radiographic imaging at baseline and after 10 years. Control.			Individuals with "not affected" radiographic imaging at baseline and "affected" after 10 years. Case.			Man–Whitney test	Incidence/1,000
	N	Mean (SD)	Range	*N*	Mean (SD)	Range	*P*-value	
K/L scores								
Age_ (years)	565	53.5 (5.8)	44.6–66.0	208	55.5 (5.8)	45.1–66.7	0.001	269
BMI (kg/m^2)	565	24.7 (3.7)	18.8–47.3	208	26.4 (4.4)	18.4–44.5	0.001	269
COMP (μg/ml)	539	1583.8 (330.6)	745–3,101	203	1680.5 (364.2)	943–3,436	0.001	273.6
Aggrecan (μg/ml)	539	2947.6 (789.1)	1,235–6,230	203	2823.0 (774.5)	1,471–6,160	0.014	273.6
cIAP (ng/mmol)	539	1637.7 (1012.1)	100–6,846	203	1750.6 (1059.6)	100–6,507	0.204	273.6
NTx (nmol/mmol)	538	16.9 (4.7)	8–58.4	203	17.5 (5.6)	7.3–66.4	0.110	273.6
OSP_ md_								
Age_ (years)	641	53.7 (5.9)	44.6–66.7	178	55.9 (5.7)	45.3–66.2	0.001	217.3
BMI_ (kg/m^2)	641	24.9 (3.9)	16.8–47.3	178	26.8 (4.4)	18.4–43.3	0.001	217.3
COMP_ (μg/ml)	612	1598.8 (338.7)	745–3,101	172	1702.0 (375.8)	943–3,436	0.001	219.4
Aggrecan_ (μg/ml)	612	2942.5 (787.6)	1,235–6,230	172	2834.3 (768.1)	1,471–5,740	0.055	219.4
cIAP_ (ng/mmol)	612	1640.8 (1011.6)	100–6,846	172	1757.8 (1035.0)	100–5,332	0.155	219.4
NTx_ (nmol/mmol)	610	17.0 (4.7)	8.0–58.4	172	17.5 (4.8)	7.3–38.2	0.100	219.9
OSP_lt_								
Age_ (years)	684	53.8 (5.8)	44.6–66.3	128	55.6 (6.0)	45.3–66.7	0.002	157.6
BMI (kg/m^2)	684	24.9 (3.7)	16.8–47.3	128	27.1 (4.7)	18.6–44.5	0.001	157.6
COMP (μg/ml)	654	1595.9 (334.3)	745–3,101	126	1,677.0 (366.4)	984–3,436	0.027	161.5
Aggrecan (μg/ml)	654	2931.6 (792.8)	1,235–6,230	126	2806.0 (783.1)	1,628–5,740	0.043	161.5
cIAP (ng/mmol)	654	1671.2 (1006.6)	100–6,846	126	1595.3 (978.3)	100–4,900	0.374	161.5
NTx (nmol/mmol)	653	17.0 (4.7)	8.0–58.4	126	16.6 (4.1)	7.3–35.7	0.668	161.7
JSN_md_								
Age_ (years)	638	54.4 (6.0)	44.6–66.2	90	54.7 (6.0)	45.5–65.8	0.674	123.6
BMI_ (kg/m^2)	638	25.4 (4.0)	16.8–47.2	90	26.3 (5.2)	17.6–44.5	0.268	123.6
COMP_ (μg/ml)	606	1620.8 (354.4)	725–3,436	88	1621.9 (317.5)	965–2,827	0.808	126.8
Aggrecan_ (μg/ml)	606	2947.6 (799.3)	1,235–6,240	88	2762.5 (776.1)	1,471–5,230	0.035	126.8
cIAP_ (ng/mmol)	606	1648.4 (993.2)	100–6,846	88	1646.9 (1029.1)	150–4,900	0.877	126.8
NTx_ (nmol/mmol)	604	17.0 (4.4)	7.3–38.2	88	16.8 (3.9)	10.4–32.8	0.846	127.1
JSN_lt_								
Age_ (years)	792	54.4 (5.9)	44.6–66.3	34	55.6 (6.0)	45.3–64.8	0.229	41.2
BMI_ (kg/m^2)	792	25.4 (4.2)	16.8–47.3	34	27.3 (5.1)	20.8–44.5	0.024	41.2
COMP_ (μg/ml)	755	1619.5 (349.5)	725–3,436	33	1722.7 (345.4)	918–2,764	0.028	41.9
Aggrecan_ (μg/ml)	755	2915.9 (793.9)	1,235–6,240	33	2908.6 (819.4)	1,709–4,912	0.843	41.9
cIAP_ (ng/mmol)	755	1664.5 (1005.1)	100–6,846	33	1607.2 (1093.0)	464–4,900	0.524	41.9
NTx_ (nmol/mmol)	753	17.0 (4.7)	7.3–58.4	33	16.4 (4.3)	10–29.2	0.486	42.0

Incidence/1,000 – means the risk of new cases development over 10 years, per 1,000 individuals in the study population.

Discussion

The main goal of this study was to examine the extent to which four study biomarkers (COMP, aggrecan, NTx, cIAP) predict incidence of the RKOA in general population In future, disease-modifying therapy selection in OA will be based on biomarkers and will slow the progression of KOA. To identify biomarkers we used the Chingford cohort – Caucasians females from northeast London, assessed for RKOA at baseline and 10 years later. After excluding women affected at baseline, we compared potential predictors in those who developed RKOA after 10-year follow-up (case) with women who remained unaffected (control). We examined prediction testing specifically in each RKOA phenotype, i.e., K/L, JSN and OSP grades. Our main findings were: (1) Higher serum concentrations of COMP at baseline predicted increased risk of K/L $\geq$ 2 appearance after 10 years; (2) Serum concentrations of aggrecan at baseline were found inversely associated with JSN in medial compartment after 10 years of follow-up. That is, low levels of aggrecan were associated with increased risk of RKOA incidence, caused probably by loss of cartilage. (3) Age and BMI as expected were significant predictors of several RKOA phenotypes, specifically, K/L, OSP in both medial and lateral compartments, and BMI was a reliable predictor of JSN_lt (Table II). Thus our study confirms the association of age and BMI with RKOA. However, the most remarkable findings of the present study were an ability of aggrecan and COMP circulating levels to predict incidence of some RKOA phenotypes, 10 years after the assays were taken (Fig. 1 & Table II). Aggrecan is one of the first matrix components to undergo measurable loss in arthritic disease. Degradation of articular cartilage is a central feature in KOA involving the catabolism of the two major constituents of cartilage matrix, type II collagen (CII) and aggrecan[13,32]. Proteolytic destruction of type II collagen and aggrecan by MMPs (matrix metalloproteases) and by ADAM-TS (a disintegrin and metalloproteinase with thrombospondin motifs)[36] leads to the cartilage loss observes in KOA. Previous studies where aggrecan concentration was taken from synovial fluid found a dose response association between aggrecan concentration and OA grading[12,21,22]. Other studies, however, found a strong[19,20] or weak[37] negative association between synovial fluid aggrecan concentration and loss of joint space. However, no data are so far available on the levels of aggrecan serum concentration as prognostic biomarker of RKOA. There may be several reasons for these discrepancies. The first is the origin of the aggrecan fragments (synovial or serum or urine), the second is the stage of OA (early or

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Table II
Parameter estimates in binary logistic regression analysis testing association between the incidence of RKOA-related phenotypes during 10 years and circulating biomarkers, measured at entrance examination. Comments. Only participants that at baseline and after 10 years follow-up were with "no affected" radiographic imaging (control) vs unaffected at baseline but affected after 10 years (case) were examined in this analysis.

Predictors	B(SE)	RR	95% CI	*P*
K/L_(742)				
Constant	−8.64 (3.600)			0.016
Age_ (years)	0.053 (0.015)	1.055	1.024–1.086	0.001
BMI_ (kg/m^2)	0.094 (0.022)	1.099	1.052–1.147	0.001
COMP_ (μg/ml)	1.055 (0.447)	2.871	1.195–6.894	0.018
Aggrecan	−0.682 (0.353)	0.505	0.253–1.10	0.053
OSP_ md_(819)				
Constant	12.34 (3.154)			0.001
Age_ (years)	0.057 (0.015)	1.059	1.027–1.091	0.001
BMI_ (kg/m^2)	0.099 (0.021)	1.104	1.058–1.151	0.001
COMP_ (μg/ml)	0.001 (0.001)	1.000	1.000–1.001	0.090
OSP_ lt_(812)				
Constant	−8.49 (4.236)			0.045
Age_ (years)	0.045 (0.018)	1.046	1.011–1.083	0.010
BMI_ (kg/m^2)	0.122 (0.024)	1.130	1.078–1.184	0.001
COMP_ (μg/ml)	0.001 (0.001)	1.000	1.000–1.001	0.139
Aggrecan	0.001 (0.001)	1.000	1.000–1.000	0.195
JSN_md_(694)				
Constant	5.81 (3.475)			0.095
Aggrecan	−0.977 (0.440)	0.376	0.159–0.891	0.026
JSN_lt_ (826)				
Constant	−13.53 (6.307)			0.032
BMI_ (kg/m^2)	0.083 (0.036)	1.086	1.012–1.166	0.022
COMP_ (μg/ml)	0.001 (0.001)	1.001	1.000–1.002	0.214

B(SE) and RR – represent regression coefficient estimates, their corresponding standard errors and relative risk, for each significant covariate and/or predictor variable.

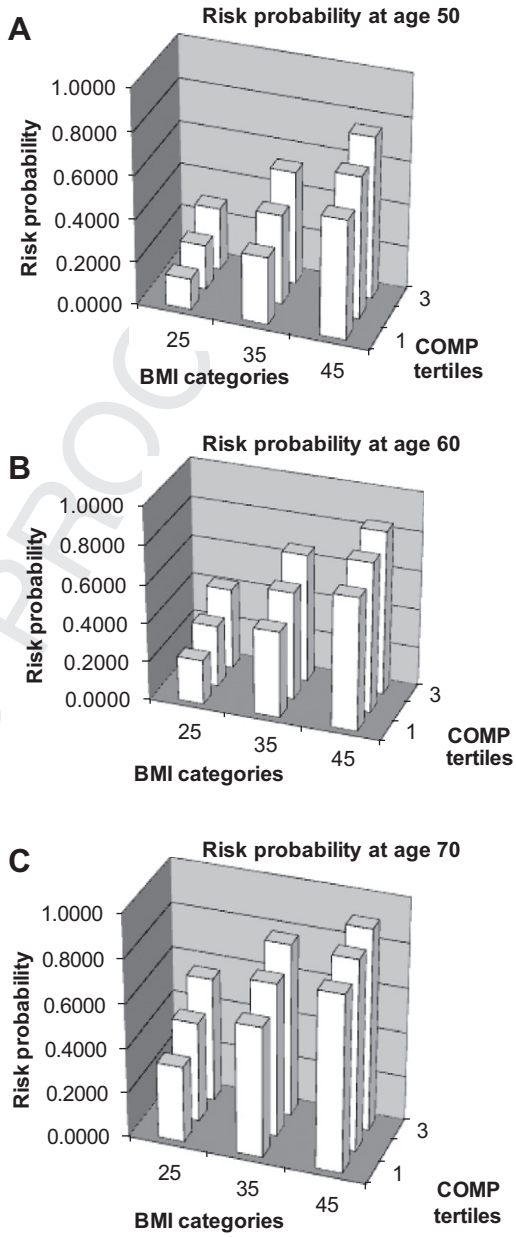

Fig. 1. Risk probability for RKOA (K/L grade ≥2) in Chingford study at age 50 years (A), 60 years (B) and 70 years (C) as a function of BMI and circulating levels of COMP. Values are the mixed-effects logistic model parameter estimates (see Table II) for three mean categories of BMI (25 kg/m^2, 35 kg/m^2, 45 kg/m^2) and for tertiles of COMP (1 = 933 pg/ml, 2 = 1,551 pg/ml, 3 = 2649.5 pg/ml). All COMP data were logarithm-transformed.

progressive), the third is the time after injury, the forth is the amount of inflammation in the joint and the fifth is the disease' rate of progression[6,20,36,38].

COMP is an important non-collagenous protein synthesized by chondrocytes and is abundant in OA cartilage[9]. Its synthesis is increased when chondrocytes and synovial cells are activated by pro-inflammatory cytokines[39]. Previous studies found that increased amounts of COMP fragments were released into the joint fluid and serum in KOA patients and were associated with diagnosis of OA and prognosis of cartilage loss[24,40,41]. Also, serum levels of COMP are heritable[42] showing that they are under genetic control. Important, the COMP prediction of cartilage loss remained statistically significant even after adjustment for age, gender and BMI[23,25,43]. Although, the data are not consistent with respect to the predicted phenotype, Ling *et al.*[44] found in Baltimore longitudinal study that COMP is a predictive biomarker of RKOA as is manifested by K/L grading, 7–10 years later after assay. Golightly and colleagues[45] reported that COMP was highly predictive of incident OSP and JSN, but did not find statistically significant association with K/L grading. Our findings suggest that COMP may be a useful indicator for identifying new RKOA and it might be associated with a combination of OSP and joint space as both are measured by K/L grading, and not to each of them separately. Finally, as seen in Fig. 1, the strength of association of COMP with risk of RKOA reflects also its interplay with age and BMI of the individual.

One of the potential limitations of this study is that biological markers were derived from blood serum or urine and not from synovial fluid of knee joint; the information on knee joint status in particular could only be estimated, however these will be the preferred methods in routine clinical practice. The second limitation is that the radiographs were taken with patients' knees fully extended in weight bearing position, a position that is less appropriate to evaluate JSN, and therefore some cases may have been missed, leading to an underestimate of the association between markers and JSN. However, the strength of this study in fact that it used a relatively large sample at entrance and 10 years later. Thus the present longitudinal study is probably one of the most reliable projects in this field.

In conclusion, KOA is a disease characterized by dynamic changes in tissue macromolecule turnover, which is reflected by measurable biomarkers' serum concentration, COMP and aggrecan, which can predict to some extent who will (and will not) go on to develop RKOA. It will be important to keep on exploring for biochemical markers and understanding the chemical relations during the joint arthritic changes will help us to accelerate toward therapeutic development that will slow the progress of the disease.

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Authors' contributions

GL, OB, designed the study. OB and GL performed statistical analysis and prepared the first draft of the manuscript. TS and NA designed and supervised the entire Chingford project, including follow-up. TS, FW and DH organized and supervised present study data collection, including acquisition of RKOA, assessment of corresponding RKOA-related phenotypes, as well as measurement of circulating levels of study molecules. All authors contributed to manuscript preparation.

Competing interests

The authors certify that there is no conflict of interest related to work presented.

Role of the funding source

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Appendix 1

Sample sizes used in present study at baseline and 10 years after baseline examination. The data are shown before and after exclusion of the "affected" individuals observed at baseline examination, and by each study variable separately Q3

RKOA-phenotype	Baseline			After 10 years		
	Total	Affected	Not affected	Total	Affected	Not affected (*N*)
K/L	909	134	775	907	340	567
K/L + Comp	865	123	742	865	324	541
K/L + Aggrecan	865	123	742	865	324	541
K/L + CIAP	865	123	742	865	324	541
K/L + NTx	863	122	741	863	323	540
OSP_md	909	88	821	907	260	647
OSP_md + Comp	865	81	784	865	247	618
OSP_md + Aggrecan	865	81	784	865	247	618
OSP_md + CIAP	865	81	784	865	247	618
OSP_md + NTx	863	81	782	863	247	616
OSP_lt	909	94	815	906	220	686
OSP_lt t + Comp	865	84	781	864	208	656
OSP_lt + Aggrecan	865	84	781	864	208	656
OSP_lt + CIAP	865	84	781	864	208	656
OSP_lt + NTx	863	83	780	862	207	655
JSN_md	909	180	729	907	209	698
JSN_md + Comp	865	171	694	865	201	664
JSN_md + Aggrecan	865	171	694	865	201	664
JSN_md + CIAP	865	171	694	865	201	664
JSN_md + NTx	863	171	692	863	201	662
JSN_lt	909	82	827	907	69	838
JSN_lt + Comp	865	78	787	865	67	798
JSN_lt + Aggrecan	865	78	787	865	67	798
JSN_lt + CIAP	865	78	787	865	67	798
JSN_lt + NTx	863	83	780	863	67	796
Prediction study (after exclusion of the effected individuals from baseline sample)						
K/L	773	0	773	773	208	565
K/L + Comp	742	0	742	742	203	539
K/L + Aggrecan	742	0	742	742	203	539
K/L + CIAP	742	0	742	742	203	539
K/L + NTx	741	0	741	741	203	538
OSP_md	819	0	819	819	178	641
OSP_md + Comp	784	0	784	784	172	612
OSP_md + Aggrecan	784	0	784	784	172	612
OSP_md + CIAP	784	0	784	784	172	612
OSP_md + NTx	782	0	782	782	172	610
OSP_lt	812	0	812	812	128	684
OSP_lt t + Comp	780	0	780	780	126	654
OSP_lt + Aggrecan	780	0	780	780	126	654
OSP_lt + CIAP	780	0	780	780	126	654
OSP_lt + NTx	779	0	779	779	126	653
JSN_md	728	0	728	728	90	638
JSN_md + Comp	694	0	694	694	88	606
JSN_md + Aggrecan	694	0	694	694	88	606
JSN_md + CIAP	694	0	694	694	88	606
JSN_md + NTx	692	0	692	692	88	604
JSN_lt	826	0	826	826	34	792
JSN_lt + Comp	788	0	788	788	33	755
JSN_lt + Aggrecan	788	0	788	788	33	755
JSN_lt + CIAP	788	0	788	788	33	755
JSN_lt + NTx	786	0	786	786	33	753

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