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Clusters of biochemical markers are associated with radiographic subtypes of osteoarthritis (OA) in subject with familial OA at multiple sites. The GARP study

I. Meulenbelt Ph.D.†*, M. Kloppenburg Ph.D.‡§, H. M. Kroon Ph.D.‖, J. J. Houwing-Duistermaat Ph.D.¶, P. Garnero Ph.D.#, M.-P. Hellio- Le Graverand Ph.D.††, J. DeGroot Ph.D.‡‡ and P. E. Slagboom Ph.D.†

† *Department of Molecular Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands*
‡ *Department of Rheumatology, Leiden University Medical Centre, Leiden, The Netherlands*
§ *Department of Clinical Epidemiology and Haematology, Leiden University Medical Centre, Leiden, The Netherlands*
‖ *Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands*
¶ *Department of Medical Statistics, Leiden University Medical Centre, Leiden, The Netherlands*
# INSERM Research Unit 664 and Synarc, Molecular Markers, Lyon, France
†† *Pfizer Global Research & Development, Ann Arbor, MI, USA*
‡‡ *Business Unit Biomedical Research, TNO Quality of Life, Leiden, The Netherlands*

Summary

Objective: To assess the relationship of biochemical markers and radiographic signs of osteoarthritis (ROA) in the subjects with symptomatic osteoarthritis (OA) at multiple sites of the Genetics osteoARthritis and Progression (GARP) study.

Methods: We have measured eight biochemical markers, representing tissue turnover of cartilage, bone, synovium, and inflammation. ROA was assessed in the knees, hips, hands, vertebral facet joints and spinal disc degeneration (DD) by using the Kellgren score. A proportionate score was subsequently made for each joint location based on the number of joints with ROA. Principal component and linear mixed model analyses were applied to analyze the data.

Results: Three different clusters of markers were identified that may reflect different pathophysiological processes of OA. The first component appeared to be reflected by structural markers of cartilage and bone turnover and associated especially in subjects with hip ROA. The second component was reflected by a marker of inflammation and was associated with knee ROA, high Western Ontario and McMaster Universities (WOMAC) scores and body mass index (BMI). The third component included markers of cartilage turnover and was associated with ROA at hands, spine as well as age. High familial aggregation was observed for serum cartilage oligomeric matrix protein (S-COMP) (70%) and serum N-propeptide of collagen type IIA (S-PIIANP) (62%).

Conclusion: Using a large well-characterized study and eight biochemical markers, we were able to observe three components that may reflect different molecular mechanisms (bone, cartilage, synovium turnover and inflammation). Our data suggested that these components contribute differently to ROA at different joint sites.

Keywords: Osteoarthritis, GARP study, Biochemical markers.

Abbreviations: ACR American College of Rheumatology, BMI body mass index, CIL confidence interval limits, CMC1 first carpometacarpal, COMP serum cartilage oligomeric matrix protein, CTX-I C-terminal cross linking telopeptide of type I collagen, CTX-II C-terminal cross linking telopeptide of type II collagen, DD disc degeneration, DIP distal interphalangeal, GARP Genetics osteoARthritis and Progression, Glc–Gal-PYD urinary glucosyl–galactosyl PYriDinoline, HRT hormone replacement therapy, HsCRP serum high sensitive C-reactive protein, MCP metacarpophalangeal, OA osteoarthritis, OC osteocalcin, PA posterior–anterior, PIIANP serum N-propeptide of collagen type IIA, PIP proximal interphalangeal, ROA radiographic signs of osteoarthritis, S serum, TIINE collagen type II neoepitope, U urinary, WOMAC Western Ontario and McMaster Universities.

Introduction

Osteoarthritis (OA) is a joint disease characterized by degeneration of articular cartilage and bone remodeling that clinically results in pain and joint stiffness. OA is recognized as a complex disease for which different definitions exist. Such definitions may be considered as distinct entities of the disease, potentially caused by different underlying pathophysiological processes[1].

Structural damage of the cartilage is usually assessed by radiographic characteristics which, however, lack sensitivity for monitoring the disease activity and progression of the OA[2]. Biochemical markers have been identified that reflect bone, cartilage and synovium turnover which may have the

*Address correspondence and reprint requests to: Ingrid Meulenbelt, Ph.D., Section Molecular Epidemiology, Leiden University Medical Center, Postzone S-05-P, PO Box 9600, 2300 RC Leiden, The Netherlands. Tel: 31-71-526-9734; Fax: 31-71-526-8280; E-mail: i.meulenbelt@lumc.nl
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ability to monitor quantitative and dynamic variation in joint tissue remodeling and inflammatory responses. Such markers may be useful in identifying more specifically and sensitively, those subjects at risk for incidence of OA and its progression. Furthermore, since some of these markers reflect different aspects of the OA process by measuring separately cartilage, bone and synovial tissue turnover, they may discriminate patients not only on the basis of the established OA definitions but also on the underlying pathophysiology of the different OA subtypes. Other markers may be correlated to such an extent that they may provide redundant data. Among the markers measured, we[3] and others[4–7] have previously reported that urinary C-terminal cross linking telopeptide of type II collagen (U-CTX-II) levels are associated to cartilage damage. To date, U-CTX-II appears as one of the most interesting biological of joint damage as assessed on X-rays in OA, U-CTX-II, however, lacked specificity to reflect OA at specific joint locations and different pathophysiological processes involved in OA[3,8]. In the present study, we investigated the relationship between the levels of eight biochemical markers and OA in different joints in sibling pairs of the Genetics osteoARthritis and Progression (GARP) study. The classification of OA patients by multiple biochemical markers that reflect different but also correlated pathophysiologic processes, may gain power by categorizing the biochemical markers into distinct components, based on their pattern of occurrence within subjects. In order to determine which clusters of biochemical markers occur within subjects with OA at multiple joint sites, we performed factor analysis. Using this information we performed mixed model regression analysis to determine whether these factors coincide with total and joint site specific radiographic signs of OA (ROA) scores. Since we studied sibling pairs, we had the opportunity to estimate the heritability of the marker levels.

Patients and methods

THE GARP STUDY

The primary objective of the ongoing GARP study, which consists of Caucasian sib pairs of Dutch ancestry affected predominantly with symptomatic OA at multiple sites, is to identify genetic determinants of OA susceptibility and progression. Details of the ascertainment have been described elsewhere[9]. Symptomatic OA of a joint within the study was defined as described below. Informed consent was obtained from all patients.

Probands (aged 40–70 years) and their siblings were included in the GARP study with OA at multiple joint sites of the hand, or with symptomatic OA in two or more of the following joint sites: hand, spine (cervical or lumbar), knee or hip[9]. Subjects with symptomatic OA in just one joint site were required to have structural abnormalities in at least one other joint site, defined by the presence of radiographic features in either hip, knee, hand or spine or the presence of two or more Heberden nodes, Bouchard nodes or squaring of at least one carpometacarpal joint on physical examination. Symptomatic OA in the knee and hip was defined according to the American College of Rheumatology (ACR) recommendations for knee and hip OA[10,11]. Knee OA was defined as pain or stiffness for most days of the preceding month and osteophytes at the joint margins of the tibiofemoral joint (X-ray spurs). Hip OA was defined as pain or stiffness in the groin and hip region on most days of the preceding month in addition to femoral or acetabular osteophytes or axial joint space narrowing on radiography. Spine OA (cervical and lumbar) was defined as pain or stiffness in the spine on most days of the preceding month, in addition to a Kellgren–Lawrence score of 2 in at least one disc or one apophyseal joint.

OA in hand joints was defined according to the ACR criteria[12] as pain or stiffness on most days of the preceding month in addition to three of the following four criteria: bony swelling of $\geq$2 of the 10 selected joints (bilateral distal interphalangeal [DIP] joints 2 + 3, bilateral proximal interphalangeal [PIP] joints 2 + 3, and first carpometacarpal [CMC1] joints), bony swelling of $\geq$2 DIP joints, $<$3 swollen metacarpophalangeal (MCP) joints, and deformity of at least one of the 10 selected joints.

Conventional radiographs of the hands (dorso-volar), knees (posterior–anterior [PA] fixed-flexion and lateral), hips (PA), lumbar (PA and lateral) and cervical spine (anterior–posterior, lateral and transbuccal) were obtained for all participants. This was performed in a standardized manner, by a single experienced radiology technician, with a fixed film-focus distance and a fixed joint position. Radiographs were scored by a single experienced musculoskeletal radiologist for osteophytes in the knees and hips, and joint space narrowing in the hips. In addition to the hands (DIPs, PIPs, and CMC1), the discs and apophyseal joints of the cervical and lumbar spine, the hips and the tibiofemoral joints of the knee were also scored according to the Kellgren–Lawrence scale with the help of the original atlas[13]. ROA were defined according to Kellgren scoring system.

Intrareader variability for the different joint sites, scored by the Kellgren–Lawrence method, was assessed: the intraclass correlation coefficient (ICC, with 95% confidence interval) was for the hands, 0.95 (0.92–0.96); for the knees (tibiofemoral), 0.92 (0.86–0.96); for the hips, 0.95 (0.92–0.98); for the cervical spine (apophyseal and disc), 0.71 (0.52–0.84); and for the lumbar spine (apophyseal and disc), 0.67 (0.46–0.81). Intrareader variability was based on an examination of 40 radiographs that were selected randomly throughout the duration of the study period and were blinded for any patient characteristics.

Definite ROA at a particular joint site was defined as a Kellgren score of two or higher. The Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) was used to assess pain and physical functioning of the lower extremes. This questionnaire contains questions on pain, stiffness, and disability in the lower extremities resulting from day to day activities. Radiographic and symptomatic OA scoring, in addition to intrareader variability assessed as ICCs, has been described in detail elsewhere[9].

In the present paper we defined a proportionate score for each joint location based on the number of joints with ROA identical as described previously[3]. The specific ROA scores used may represent a score proportional to cartilage abnormalities at each joint location. Cartilage or bone turnover products are not expected from arthroplastic joints except for recent joint replacements[14]. Therefore, subjects with recent joint replacements ($<$1 year) were considered as ROA present whereas all other joints with replacements as ROA absent. In the GARP study seven and one subjects had undergone, respectively, uni- and bilateral knee replacements and 23 and 15 for, respectively, uni- and bilateral hip replacements. Of them five had a recent hip replacement and one a recent knee replacement.

The GARP study consists of 382 subjects (312 women and 70 men). Of the women 260 (83%) were postmenopausal, including eight women who currently used hormone

replacement therapy (HRT). Since menopause and HRT have recently been shown to influence some biochemical markers[15] postmenopausal women not on HRT were selected ($N = 252$). The present study was performed in 222 postmenopausal women not on HRT, and 67 men from whom we had levels available of all biochemical markers.

BIOCHEMICAL ANALYSIS

For each participant of the GARP study, we have collected at the same time non-fasted second void morning urine and serum samples. Samples were stored within 4 h at -80°C until measurement was performed. All biochemical markers were measured by a specialized laboratory (Synarc Lyon, France), except for urinary glucosyl–galactosyl pyridinoline (U-Glc–Gal-PYD) (INSERM research unit 403, Lyon, France) and urinary collagen type II neoepitope (U-TIINE) (Pfizer Global Research & Development, Ann Arbor, USA).

Markers of bone turnover

Serum total osteocalcin (S-total OC), a specific marker of bone formation, was measured by a two-site assay measuring both intact and N-mid-peptide using an automatic system (Elecsys, Roche Diagnostic, Manheim, Germany). Measuring N-mid-peptide the main proteolytic fragment of OC allows correction for the potential degradation of OC *in vitro* and the determination of precise measurements. Intra- and inter-assay coefficients of variation (CVs) are lower 2.5% and 3%, respectively.

Urinary excretion of β isomerized C-terminal cross linking telopeptide of type I collagen (U-CTX-I) was measured by the Crosslaps enzyme-linked immunosorbent assay (ELISA) (Nordic Biosciences, Herlev, Denmark)[16]. This assay uses a polyclonal antiserum raised against the β isomerized EKAH β DGGR sequence of the C-telopeptide of α1 chains of human type I collagen. Intra- and inter-assay CVs are below 3% and 10%, respectively.

Markers of cartilage turnover

Urinary CTX-II was measured using an ELISA based on a monoclonal antibody raised against the EKGPDP linear 6-amino acid epitope of the CII C-telopeptide (CartiLaps: Nordic Bioscience, Herlev, Denmark). Intra- and inter-assay variations were lower than 9% and 11%, respectively.

Serum N-propeptide of collagen type IIA (S-PIIANP) was measured using a newly developed ELISA using a polyclonal antibody raised against recombinant glutathioneine-S-transferase-human type II procollagen exon 2 fusion protein[17]. This antibody is specific for the type IIA of the N-propeptide of type II collagen and Western-blot analysis of human OA serum showed that it does not cross-react with circulating proteins which share homologies with N-propeptide of collagen type IIA (PIIANP) sequence such as thrombospondin and von-Willebrand factor[18]. The ELISA used in the current study employed the same antibody and standard than the one used previously[19], but in a different assay format. This technical modification which did not alter the specificity of the assay resulted in improved precision and increase in the absolute serum levels of PIIANP. Intra- and inter-assay CVs were lower than 10%.

Serum cartilage oligomeric matrix protein (S-COMP) was measured by a two-site immunoassay (COMP™ ELISA kit, AnaMar Medical, Lund, Sweden). Intra- and inter-assay CVs (%) are below 7% and 8%, respectively.

Quantitation of urinary collagen type II neoepitope (U-TIINE)-containing 45-mer peptides with 5-hydroxyproline (HyP) was performed by 2D LC–MS/MS, utilizing an HP1100 high performance liquid chromatography (HPLC) system (Agilent, Palo Alto, CA) composed of a quaternary pump, a CTC Analytics HTS PAL autosampler (LEAP Technologies, Carrboro, NC), and switching valve plumbed inline with another HP1100 pump and interfaced to an API 4000 triple quadrupole mass spectrometer (MDS-Sciex, Toronto, Canada) operated in the positive ion electrospray and multiple-reaction-monitoring (MRM) modes. A 45-mer peptide containing 5-Hyp that was deuterated (d5) at the C-terminal glutamine residue was used as an internal standard. For the immunoaffinity/reversed-phase LC–MS/MS analyses, urine samples diluted in 50 mM ammonium acetate were injected onto an immunoaffinity column prepared by cross linking 5109 antibody, which specifically recognizes a unique sequence of type II collagen (GEPGDDGPS) adjacent to the neoepitope on the 3/4-length fragment[20], to protein G-Poros material. The column was then washed for 3 min with 50 mM NH_4OAc, pH 7, at 1 mL/min, following which the valve was switched and the captured peptides were eluted off the immunoaffinity column with a 1% formic acid solution onto a C-18 peptide macro trapping column (Michrom BioResources, Auburn, CA) for another 3 min. Peptides were then eluted off the trapping column onto a 2.1×100 mm C-18 analytical column (Keystone Scientific, Bellefonte, PA) with a 10-min gradient of 95% H_2O, 1% HCOOH to 65% CH_3CN, 1% HCOOH.

Selected 45-mer peptides in urine (referred to hereafter U-TIINE) were specifically detected by monitoring HPLC elution times and ion pairs corresponding to the parent and specific fragment ion mass/charge values of 1038.8/568.3 and 1040.3/573.3 for 45-mer TIINE and for its deuterated internal standard, respectively. Analyte concentrations were determined by comparing the LC–MS/MS peak areas to that of deuterated internal standard. Standard analyte curves were analyzed prior to, and following, assay of the study samples to ensure equivalent instrument responses for both the analyte and internal standard. The LC–MS/MS assay was performed by Quest Pharmaceutical Services, LLC (Newark, DL). The inter-assay precision, determined as the CV, ranged from 4.0% to 8.7%, increasing to 12.3% at the LLOQ. Inter-run accuracy ranged from 0.6% to 2.7% relative error, with 14.2% at the LLOQ. Concentrations of U-TIINE (ng/mL) were normalized for the urinary creatinine (Cr) concentration (mM/mL), and the units reported for the corrected U-TIINE concentrations are ng/mM Cr.

Markers of synovium and inflammation

U-Glc–Gal-PYD a non-reducible cross link of collagen molecules which is present in human synovial tissue and is released during its destruction was measured as previously described[21]. Intra- and inter-assay CVs are below 8% and 11%, respectively. Serum high sensitive C-reactive protein (S-HsCRP) was assayed using an ultrasensitive immunonephelometry method (N Latex CRP mono, Behringwerke AG, Marburg, Germany) on a BNA Behring nephelometer. The intra- and inter-assay variations are lower than 5% and the detection limit is of 0.2 mg/Lon non-hydrolyzed samples by HPLC.

STATISTICAL ANALYSIS

Familial aggregation (heritability) of the biochemical marker levels was estimated by comparing twice the

between sibling variance divided by the total variance. The heritability estimates indicate the fraction of the total variance that is explained by shared genetic and environmental factors. Estimates were adjusted for the presence of total ROA score, body mass index (BMI), age and sex.

Principal component analysis (PCA) was to reduce the data of the biochemical markers that are correlated. The eight biochemical markers were entered in the PCA in addition to age and BMI. We used both empirical criteria (percentage of variance explained by factors and scree plots) and interpretability in determining the number of factors. Using Eigen values >1, three factors were suggested. We explored the interpretability of these factors after applying a Varimax rotation with Kaiser Normalization. For each factor, a score was computed as the average of joint measures that loaded significantly on that factor with a factor loading of at least 0.4. A factor loading represents the linear relationship (Pearson correlation under Varimax rotation) between a variable and a factor. Factor loadings >0.4 are frequently considered to be significant[22]. In interpreting the rotated factor pattern, in our study an item was said to load on a given factor if the factor loading was ≥0.4 for that factor and <0.4 for the others.

In order to assess the relationships between OA characteristics and the clusters of biochemical markers, a mixed model regression analyses were performed using SPSS version 11 (SPSS, Chicago, IL, USA). The extracted clusters of the biochemical marker levels were used as dependent variable and as co-variables clinical symptoms and sex in addition to either the total ROA score (0–10) or the specific ROA scores as defined above in knee (0–2), hip (0–2), hand (0–2), facet joints (0–2), and spinal disc degeneration (DD) (0–2). In the mixed model analysis, we included family identity numbers (representing family relations) as random variables in order to model the familial dependencies that might occur for the biochemical marker levels. Results of the mixed model analyses are expressed as estimates (β) that represent the association between increasing ROA grades and clinical symptoms with clusters of biochemical marker levels including BMI and age. The estimates, however, should be interpreted relative to the ranges of the scores that are equal for knee, facet, hip and hand and DD (0–2), however, larger for the total ROA score (0–10) and clinical symptoms (0–100). Because CTX-I, CTX-II, PIIANP, and high sensitive C-reactive protein (HsCRP) levels were not normally distributed, data were logarithmically transformed in these analyses.

Results

Table I shows the characteristics of sample of the GARP study used in the current analysis. Of the 289 patients 77% were female with a large prevalence of spinal DD and facet ROA and consisted of 144 pairs. Although the mean age of this study group (61.4 years) was slightly higher as compared to the total group (60.3 years) characteristics were comparable. Table II shows for each marker the mean with the standard deviation (SD), and the median with the interquartile range (IQR). As shown by the differences in mean and median levels, the CTX-I, CTX-II, PIIANP, TIINE and HsCRP levels were not normally distributed. In the analyses the logarithm of these levels was used. Observed levels were similar as detected in previous studies[4]. Familial aggregation of the biochemical marker levels was estimated in the sibling pairs. Especially cartilage oligomeric matrix protein (COMP) and PIIANP show high and significant heritability estimates of 0.70, 95% confidence interval limits (CIL) 0.39–1.00 and 0.62, 95% CIL 0.25–0.99, respectively (Table II).

Table I
*Characteristics and frequencies of ROA of the sample of the GARP study (*N = *289)*

Characteristics	GARP study*	ROA score†		
		0	1	2
Total number of subjects	289 (1.0)			
Hip (%)	86 (30)‡	222 (77)	42 (15)	25 (9)
Knee (%)	121 (42)‡	172 (60)	69 (24)	48 (17)
Hand (%)	237 (82)‡	120 (42)	89 (31)	80 (28)
Facet (%)	287 (99)‡	103 (36)	134 (46)	52 (18)
DD (%)	264 (91)‡	84 (29)	139 (48)	66 (23)
WOMAC median (range)	22.1 (95)			
Age mean (SD)	61.4 (7.0)			
BMI mean (SD)	27.0 (4.5)			
Women (%)	222 (77)			

*Studied sample consists of postmenopausal women not on HTR and men for whom ROA scores and biochemical marker levels were available.

†Indicated are the number of individuals with, respectively, ROA scores of 0–2 for hip, knee, hand, facet and discus degeneration of the spine. ROA scores of joint replacements which occurred >1 year ago were scored as ROA absent (see also Materials and methods).

‡Numbers represent patients within GARP with uni- or bilateral ROA at the specific joint location including subjects with uni- and/or bilateral joint replacement ($N = 31$ for hip and $N = 6$ for knee for this sample).

ASSOCIATION OF BIOCHEMICAL MARKERS

As can be expected from markers that reflect turnover of cartilage, bone, and synovial tissue within subjects with OA the levels measured were correlated (results not shown). High correlations (coefficients between 0.4 and 0.7) were observed for CTX-II with TIINE, CTX-I and glucosyl–galactosyl pyridinoline (Glc–Gal-PYD) whereas moderate (coefficients 0.3–0.2) between CTX-II and total OC, CTX-I and BMI, age and Glc–Gal-PYD and BMI and HsCRP. For the remaining markers correlations were low, between 0.1 and 0.2 (results not shown).

To reduce the correlated biochemical marker data to independent components in which these variables cluster, PCA including all biochemical marker levels, age, and BMI was performed. Table III shows the three components that were extracted. The coefficients depicted in Table III explain how well each individual marker is represented within the clusters. The marker levels of CTX-I, CTX-II, TIINE, total OC and Glc–Gal-PYD levels loaded together on the first component, explaining 27% of the variance (Eigen value = 2.7). The second component, explaining 18% of the variance (Eigen value = 1.8), is represented by subjects with high BMI, systemic inflammation (HsCRP). The third component, explaining 12% of the variance (Eigen value = 1.2), is represented by subjects with high PIIANP (collagen synthesis), COMP (cartilage turnover), and age.

Subsequently the relationship between the three components and the presence of OA characteristics was investigated by mixed model regression analysis. The first part of Table IV shows that the total ROA score (0–10) contributed mainly to components 1 and 2 (P-value = 0.001 and

Table II
Biochemical marker levels of the sample of the GARP study (N = 289)

	Process	Mean (SD)	Median (IQR)	Heritability*(95% CIL)
Cartilage				
U-CTX-II (ng/mmol creat)	Degradation	221 (180)	265 (168)	0.35 (−0.04–0.74)
U-TIINE (ng/ml)	Degradation	135 (104)	115 (82)	0.31 (−0.05–0.68)
S-COMP (U/L)	Turnover	11.4 (4.1)	11.9 (3.3)	0.70 (0.39–1.00)
S-PIIANP (ng/ml)	Synthesis	182 (121)	207 (98)	0.62 (0.25–0.99)
Bone				
U-CTX-I (μg/mmol creat)	Resorption	161 (116)	177 (101)	0.54 (0.19–0.89)
S-total OC (μg/L)	Formation	21.7 (10.1)	22.7 (9.0)	0.34 (0.01–0.67)
Synovial				
U-Glc–Gal-PYD (nmol/mmol creat)	Synovial tissue turnover	4.8 (2.4)	5.4 (2.1)	0.51 (0.15–0.87)
Inflammation				
S-HsCRP (mg/L)	Systemic	1.8 (2.8)	3.3 (5.0)	0.22 (−0.14–0.58)

*Familial aggregation of levels was estimated by comparing the within and between covariance of the sibling pairs. Estimates were adjusted for presence of total ROA score, BMI, age and sex.

0.001, respectively). These two components may be influenced by ROA at a combination of multiple joint sites. In order to investigate whether ROA at specific joint sites cluster to these components, the separate joint site specific ROA scores (0–2) were added together as co-variables in the analysis. Especially subjects with hip, hand and high WOMAC scores independently load onto the first component, subjects with especially knee ROA and high WOMAC scores load on the second component whereas, subjects with facet and hand ROA and DD ROA load onto the third component. The estimates in Table IV should be interpreted relative to the ranges of the scores that are equal for knee, facet, hip and hand and DD (0–2), however, larger for the total ROA score (0–10) and clinical symptoms (0–100).

Discussion

In the present study, it was investigated whether OA patients may be discriminated on the basis of biochemical marker patterns and whether the pattern may reflect different aspects of the pathophysiology of OA. The GARP study provided a unique study population since radiographs and symptoms were registered for knee, hip, hand, facet joints as well as intervertebral discs. The principal component analyses of eight different markers showed that the data may be reduced to three independent underlying dimensions (components) that may reflect the most predominant pathophysiological processes of OA. The coefficients in Table III show how well each individual marker is able to represent a particular component and may enable a more suitable choice in measuring markers for different processes. We found that the CTX-II levels clustered with TIINE, CTX-I, total OC, and Glu–Gal-PYD, especially in subjects with hip, facet, hand and knee ROA (Table IV), indicating that ROA in these joint locations coincide with alterations of the cartilage, bone and synovial tissue metabolism. This result was almost identical with the previously observed association of the CTX-II within the GARP study[3] and the findings recently reported by Garnero *et al.* in patients with hip OA[4].

Table III
PCA of biochemical marker levels, BMI and age of the sample of the GARP study (N = 289)

Process	Marker	Component		
		1	2	3
Cartilage degradation	CTX-II	0.85		
Cartilage degradation	TIINE	0.80		
Synovial tissue turnover	Glc–Gal-PYD	0.72		
Bone resorption	CTX-I	0.64		
Bone formation	Total OC	0.48		
	BMI		0.76	
Inflammation	HsCRP		0.69	
Collagen synthesis	PIIANP			0.59
Cartilage turnover	COMP			0.70
	Age			0.60
Variance explained (%)		27	18	12

PCA using a Varimax rotation with Kaiser Normalization. Components with Eigen values >1 are extracted, coefficients with values >0.4 are displayed.

We also found that HsCRP, a well established non-specific molecular marker of systemic inflammation, consistently co-occurs together with BMI. We show here that this relation may be specifically for subjects with knee ROA and high WOMAC scores. Given the fact that the level of HsCRP, in general, is influenced by many factors we cannot exclude the presence of additional confounders. The WOMAC score consists of three subscales: pain (five items), stiffness (two items), and function (17 items) that may be used as separate scales. When the subscales are entered as co-variables in mixed model regression analysis especially the function scale appears to associate to the second component. Together these results may indicate a pathophysiological process for knee ROA involving BMI and an inflammatory process. In the current cross sectional analysis we are, however, not able to assess a causal relationship and needs to be investigated in a follow-up design.

The third component was represented by COMP and PIIANP levels, clustered with age and occurs especially among subjects with facet, hand ROA and spinal DD. Although COMP has initially been proposed as a cartilage-specific molecule, it has subsequently been shown to be synthesized also by ligament, tendon, and synovial fibroblasts, and osteoblasts and has recently been suggested to reflect cartilage breakdown and/or inflammation of the synovial membrane[4]. In our study we did not find evidence for an inflammatory component of COMP. However, we observed that COMP together with PIIANP may be under genetic influence also reflected by a heritable influence of the third component (results not shown). In the heritability

Table IV
*Linear relationship of extracted principal components (1–3) and OA characteristics (ROA score, clinical symptoms, sex) of the subjects of the GARP study sample (*N = *289)*

OA characteristics	Components*					
	1		2		3	
	Estimate	*P*	Estimate	*P*	Estimate	*P*
Total ROA (0–10)	0.16	0.0001	0.06	0.054	0.16	0.0001
Sex	−0.32	0.014	0.12	0.337	0.18	0.142
WOMAC (0–100)†	0.01	0.002	0.01	0.0001	−0.00	0.214
Knee (0–2)	0.18	0.014	0.19	0.008	0.09	0.194
Facet (0–2)	0.20	0.016	0.05	0.554	0.26	0.001
Hip (0–2)	0.38	0.0001	−0.14	0.113	−0.03	0.697
Hand (0–2)	0.17	0.015	0.01	0.919	0.14	0.033
DD (0–2)	−0.03	0.713	0.11	0.179	0.24	0.002
Sex	−0.31	0.020	0.15	0.264	0.19	0.124
WOMAC (0–100)†	0.01	0.002	0.01	0.0001	−0.03	0.239

*Component 1 represents subjects with high UCTX-I and -II, total OC, Glc–Gal-PYD; Component 2 represents subjects with high levels of HsCRP and high BMI; component 3 represents subjects with high PIIANP, COMP and age.

†Total WOMAC score. Data were analyzed using mixed model regression analyses with the components as dependent variable and as covariables the presence of clinical symptoms and sex in addition to either the total ROA score or the joint site specific ROA score of the knee, facet, hip, hand and DD.

analyses for COMP adjustments were necessary for the presence of ROA, and age. Given these results we propose that among subjects of the GARP study, COMP and PIIANP levels may be influenced independently by ROA, age and heritable factors. Due to the high correlation between ROA and age in our study group we are not able to distinguish to what extent there is also a genetic predisposition to age related COMP levels irrespective of the presence of OA and *vice versa*. These age related aspects of COMP and PIIANP need to be investigated further among subjects without ROA. Genetic studies may reveal genes influencing the variation in these levels.

To compensate, at least in part, for the amount of cartilage of the small joints of the hands as compared to for example the hip joints we have used the proportioned ROA score for each joint location. Furthermore the estimates provided in Table IV were assessed independently of the effect of ROA at other joint locations. Given the cartilage volumes of the large joints (knees and hips) as compared to the small joints (hand, facets and discus degeneration of the spine), however, estimates for the small joints may be underestimated and for the large joints (especially the knee with different compartments) overestimated.

In a study of Sharif *et al.*[14] it was shown that COMP levels remain elevated in the period (up until 1 year) following replacement of the knee. We have, therefore, considered subjects with recent joint replacements as having ROA at that respective joint. When data were analyzed with all arthroplastic joints as ROA absent or missing, the model appears to fit to a lesser extent, especially for component 1 and hip ROA. These results may indicate that also biochemical markers levels of cartilage and bone turnover remain elevated after joint replacement surgery. Furthermore, it has previously been shown that joint space narrowing may provide more valid correlation of biochemical marker responses. When joint space narrowing and osteophyte scores for either knee or hip were analyzed separately, we did not observe such a relationship.

It should be noted that, because the dynamics of each biochemical marker differ between separate body compartments, i.e., serum and urine, the results of our principal component analyses should be interpreted with caution and should be confirmed by other studies using measurements of all markers in the same biological fluid.

Since our study has OA data available from most prevalent ROA joint locations, i.e., hips, knees, facet, hands and DD of the spine, our results are not likely to be confounded by ROA at joint locations for which radiographic data were lacking. The absence of radiographic data for example of shoulders and diarthroidial joints may, however, have caused some bias. This paper concerned cross sectional data, we could, therefore, not assess the potential predictive value of the biochemical markers to predict disease progression. Furthermore, the women in our study consist mainly of postmenopausal women, some of our findings may therefore not apply to younger women or postmenopausal women receiving HRT.

Based on the results of the present study, we propose that classification of OA patients by biochemical marker levels may indicate three specific molecular mechanisms primarily involving (1) structural markers of bone turnover, cartilage degradation and synovial involvement; (2) inflammation; and (3) age related changes. Our data also suggest that some of these processes may have a genetic component and especially contribute to OA development at specific joints. Further research is necessary to establish the association of these markers with progression of OA.

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