

The Heritable Determinants of Cartilage Oligomeric Matrix Protein

Frances M. K. Williams,¹ Toby Andrew,¹ Tore Saxne,² Dick Heinegard,² Tim D. Spector,¹
and Alex J. MacGregor³

Objective. Cartilage oligomeric matrix protein (COMP) is a cartilage matrix macromolecule. The protein is detectable in serum and has been investigated as a biomarker of osteoarthritis (OA). An association between COMP and OA has been shown, yet the precise factors governing serum levels of COMP remain unclear. The aim of this study was to determine whether genetic factors influence serum levels of COMP.

Methods. A classic twin study was conducted using COMP levels in serum obtained from healthy female twin volunteers. COMP levels were determined by an inhibition enzyme-linked immunosorbent assay method. The heritability of COMP was determined by comparing correlation among 160 monozygotic and 349 dizygotic twin pairs. Data on potential confounding factors, including age, body mass index, and the presence of OA as assessed by hand, hip, and knee radiographs, were included in the analysis.

Results. Serum levels of COMP showed a correlation of 0.72 (95% confidence interval [95% CI] 0.65–0.80) among monozygotic twin pairs and 0.47 (95% CI

0.39–0.55) in dizygotic pairs. This equated to an estimated heritability for COMP of 40% (95% CI 20–60%). Although age and body mass index were found to be significantly associated with COMP in regression analysis, taking the effects of these factors into account did not influence the estimate of heritability.

Conclusion. This study showed that heritable factors influence serum levels of the cartilage matrix biomarker COMP. Together with other published data, the results suggest that genetic factors operate at an early stage in the etiologic pathways that influence the development of radiographically discernible OA.

Osteoarthritis (OA) has a significant heritable component, with genetic factors accounting for 39–65% of the variation in prevalence of the disease (1). However, the individual genes responsible remain elusive. One potential explanation is that genetic studies of OA focus exclusively on clinical or radiographic end points. Such studies may lack the subtlety required to detect the genetic factors determining the initiation and early progression of OA at the level of articular cartilage. Alteration in cartilage metabolism is recognized as taking place long before radiographic changes become apparent.

Cartilage oligomeric matrix protein (COMP) clearly plays some role in OA: it is known to be expressed in abundance in OA cartilage (2), and prospective studies have shown serum levels to be elevated early in patients experiencing progression from chronic knee pain without radiographic OA to radiographic disease (3). An association of serum COMP (alone and in combination with other serum markers) with prevalent OA has been reported (3). Elevated levels of COMP may be a marker of rapid radiographic progression (4), and alterations in serum COMP levels may reflect the cyclic nature of the progression of OA (5). Studies of

Supported by the Arthritis Research Campaign, the Swedish Medical Research Council, the NIH (grant U01-AR050926 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases), the King Gustaf V 80-Year Foundation, the Swedish Rheumatism Association, the Österlund Foundation, and the Kock Foundation.

¹Frances M. K. Williams, MRCP, PhD, Toby Andrew, PhD, Tim D. Spector, MD, MSc, FRCP: Twin Research & Genetic Epidemiology Unit, St. Thomas' Hospital, London, UK; ²Tore Saxne, MD, PhD, Dick Heinegard, MD, PhD: Lund University, Lund, Sweden; ³Alex J. MacGregor, MRCP, MD: Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, London, UK, and Institute of Health, University of East Anglia, Norwich, UK.

Drs. Saxne and Heinegard own stock in AnaMar Medical, Lund, Sweden.

Address correspondence and reprint requests to Frances M. K. Williams, MD, Twin Research & Genetic Epidemiology Unit, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK. E-mail: Frances.Williams@gstt.nhs.uk.

Submitted for publication November 16, 2005; accepted in revised form March 20, 2006.

cartilage COMP provide one route to understanding the molecular processes underlying the early development of OA.

COMP is a 435,000-dalton pentameric member of the thrombospondin protein family. It was isolated initially from cartilage, is synthesized by chondrocytes, and is present in small amounts in synovium and tendon as well as being detectable in serum. Little attention has been given to the genetic factors that influence markers of cartilage damage, such as COMP. In this study, we used a classic twin design to assess whether genetic factors determine levels of COMP in a volunteer population.

SUBJECTS AND METHODS

Study sample. The study subjects were female monozygotic and dizygotic twins enlisted in the volunteer-based national Twins UK Registry (6). Subjects have been recruited to the Registry since 1992, through successive media campaigns. Between 1997 and 2000, a number of twins were recruited to participate in a series of radiographic studies of OA in the hands, hip, and knees. The twins were selected at random from the Registry and had not been enlisted specifically for the assessment of joint disease. Subjects eligible for radiography were older than age 35 years. The group included in this study comprised 718 twins (from 102 monozygotic and 257 dizygotic pairs) for whom radiographic data were available, as well as 300 subjects (58 monozygotic and 92 dizygotic pairs) selected at random from a Registry group with a similar age range.

Twin pairs attended the unit in the morning. The twins independently completed questionnaires relating to baseline demographic information. Measurements of height and weight were obtained, and fasting venous blood was drawn prior to radiography. Twins enlisted on the Twins UK Registry are representative of the adult UK population with respect to the prevalence of joint symptoms, individual radiographic features of OA, and a range of other variables (7).

Serum COMP measurements. Serum COMP levels were determined by an inhibition enzyme-linked immunosorbent assay, which is described in detail elsewhere (8). Wells of microtiter plates were coated with COMP, and the binding of anti-COMP antibodies was inhibited by standard dilutions of either human COMP or serum samples. Bound antibodies were detected using alkaline phosphatase-conjugated anti-Ig antibody, and enzyme activity was measured using *p*-nitrophenyl phosphate as substrate. Samples were analyzed in triplicate. The intraassay variation was <5%, and the interassay variation was <8%.

Assessment of radiographic OA. Standard radiographs of the hands, pelvis, and knees were obtained. The radiographs were graded by a single observer, using standard atlases. The presence of hand OA was defined as a Kellgren/Lawrence (K/L) score (9) of ≥ 2 in 2 or more of the 8 distal interphalangeal joints of the finger or in 2 interphalangeal joints of the thumb. Knee OA was defined as a K/L score of ≥ 2 in either

tibiofemoral joint. Hip OA was defined as the presence of a K/L score of ≥ 2 in either joint.

Statistical analysis. Monozygotic twins are genetically identical whereas dizygotic twins share, on average, half of their genetic material. In the classic twin model, an excess trait correlation between monozygotic twins as compared with dizygotic twins indicates a genetic influence, if it assumed that the shared family environment has an equal influence in both types of twin. The extent to which variation in a trait is attributable to genetic variation (heritability) can be estimated quantitatively through variance components analysis. According to this approach, phenotypic variation in a trait among twins is attributed to additive (A) and nonadditive (dominance [D]) variation and to environmental variation that may be both individual-specific (unique environmental variance [E]) or shared among family members (common environmental variance [C]). The additive genetic effects (A) result from single-gene effects added over multiple loci, while nonadditive genetic effects (D) reflect genetic interaction at the same locus. By contrasting the phenotypic trait covariance in monozygotic and dizygotic twins, it is possible to infer through modeling the relative contribution of the individual variance components (10).

The significance of individual components of variation is assessed according to a standard procedure that examines fit in a sequence of models containing combinations of the variance components (A, D, C, and E) in a stepwise deletion process. In this procedure, E is retained in all models, and a model containing all 4 variance components is not permissible.

Modeling was carried out using Mx software (11). The relative suitability of competing models was estimated by comparing Akaike's information criterion (AIC), a score that represents the balance between model fit and the number of parameters (parsimony). Lower values for the AIC indicate more suitable models. The relative genetic contribution to the most suitable model was obtained in order to provide an estimate of heritability (10).

In the present analysis, we examined variation in serum COMP levels in monozygotic and dizygotic twin pairs. We also assessed the influence on serum COMP of age, body mass index (BMI), and a range of other potential confounding factors, together with the presence of OA itself, using regression methods. To take into account the potential influence of these factors on the estimate of heritability, quantitative modeling was repeated on the residual variables of the regression analysis, in which confounders significantly associated with COMP were included.

RESULTS

The characteristics of 160 monozygotic and 349 dizygotic twin pairs in whom serum COMP levels were measured are shown in Table 1. The monozygotic twins were older than the dizygotic twins and had lower overall BMI. Radiographs were available for 719 individuals (71%), 31% of whom had evidence of OA in at least 1 joint area. The prevalence of OA at all sites was

Table 1. Characteristics of the twins*

Characteristic	Monozygotic	Dizygotic
Age, mean (range) years	55.7 (40.2–70.8)	52.5 (37.1–72.7)
BMI, mean (range) kg/m ²	24.5 (16.5–38.7)	25.1 (17.1–45.4)
Smoker	15.3	19.9
Former smoker	31.2	29.1
Postmenopausal	79.9	63.9
Currently taking HRT	19.0	26.8
Former user of HRT	15.2	12.1
COMP, mean \pm SD units/liter	14.3 \pm 2.4	14.3 \pm 2.2
Hand OA	16.8	12.0
Knee OA	22.1	16.9
Hip OA	9.3	7.6
OA at any site	37.8	29.3

* Except where indicated otherwise, values are the percent. BMI = body mass index; HRT = hormone replacement therapy; COMP = cartilage oligomeric matrix protein; OA = osteoarthritis.

similar in monozygotic and dizygotic twins after taking into account differences in age.

In univariate regression analyses, COMP levels in the individual twins were significantly associated with age (for age younger than 53.5 years and age older than 53.5 years, 13.92 units/liter and 14.71 units/liter, respectively; $P < 0.001$), BMI (for BMI < 24.5 kg/m² and BMI > 24.5 kg/m², 13.96 units/liter and 14.72 units/liter, respectively; $P < 0.001$), and smoking (for current smokers versus never smokers and former smokers, 13.83 units/liter and 14.38 units/liter, respectively; $P = 0.003$), as well as use of hormone replacement therapy (for current users versus never users and former users, 13.85 units/liter versus 14.45 units/liter; $P < 0.001$). Multivariate

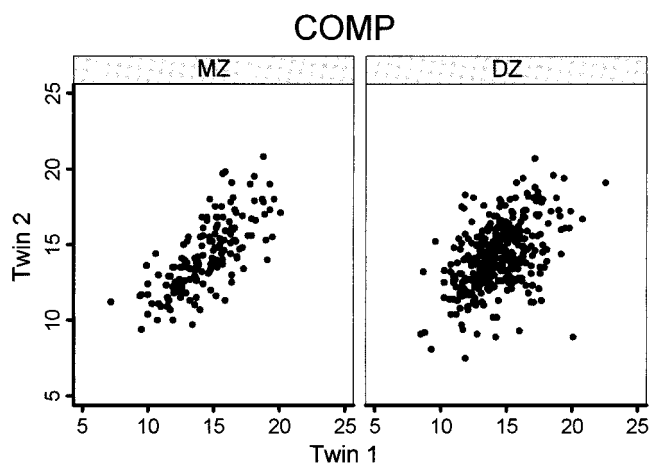


Figure 1. Scatter plots showing the distribution of serum cartilage oligomeric matrix protein (COMP) levels (units/liter) in 160 monozygotic (MZ) and 349 dizygotic (DZ) twin pairs.

Table 2. Correlation of COMP in monozygotic and dizygotic twin pairs*

Twin pair	COMP	COMP, adjusted†
Monozygotic	0.72 (0.65–0.80)	0.72 (0.64–0.80)
Dizygotic	0.47 (0.39–0.55)	0.46 (0.37–0.54)

* Values are the intraclass correlation coefficient (95% confidence interval). COMP = cartilage oligomeric matrix protein.

† Adjusted for age, body mass index, current smoking, use of alcohol, and use of hormone replacement therapy.

regression analyses revealed that the association of serum COMP levels with OA was accounted for entirely by the influence of increasing age on the serum COMP level.

Figure 1 shows the distribution of COMP in the monozygotic and dizygotic twin pairs. As shown in Table 2, the intraclass correlation for values in monozygotic twins was significantly higher than that in dizygotic twins (0.72 and 0.47, respectively). The most suitable model for the data (Table 3) contained contributions from additive genetic variance, common environmental variance, and the unique environment (ACE model). In this model, the heritability of COMP was estimated as 40% (95% confidence interval 20–60%). This estimate was not altered after taking into account the influence of age, BMI, smoking status, alcohol use, and hormone replacement therapy.

DISCUSSION

This study is the first to assess the influence of genetic factors on biomarkers of OA. We have shown that genetic factors account for 40% of the variation in COMP levels in an adult population. Our study examined twins who are healthy volunteers and are known to be representative of the UK population (7); relatively few of these subjects exhibit mild radiographic OA. This, and the fact that our measurement was from a single

Table 3. Results of variance components modeling*

Model	ACE	ACE, adjusted†
A	0.40 (0.20–0.60)	0.40 (0.20–0.61)
C	0.29 (0.11–0.45)	0.28 (0.10–0.44)

* Values are the estimated proportion (95% confidence interval). The model deemed most suitable for the data (see Subjects and Methods) is shown. ACE = additive genetic variance, common environmental variance, and unique environment.

† Adjusted for age, body mass index, current smoking, use of alcohol, and use of hormone replacement therapy.

time point, may account for the fact that we did not find an association between serum COMP levels and radiographic OA, which has been widely reported. Furthermore, we did not have spine radiographs to examine for OA, so a relationship between serum COMP levels and the burden of OA overall may have been missed.

Radiographic OA is likely to indicate established disease. It is possible that preclinical OA without detectable radiographic alterations is widespread and detected by the "COMP molecular indicator." In our data, the genetic influence on COMP is not explained by genes influencing age or BMI (12). These findings indicate that the level of serum COMP is independently genetically determined and is a potential target for future genetic studies aimed at identifying an increased risk of disease development.

OA is a common disabling condition that has been the subject of intensive research for several decades. Despite recognition of the fact that genetic factors play an important role in the etiology of OA (~50% of the variation in susceptibility to disease) (1), defining the genes responsible has proven difficult. Such difficulty is, perhaps, not surprising when considering that OA is a disease in which individual gene effects are likely to be small, and that the actions of the genes need to be interpreted against a background of considerable phenotypic complexity. OA affects more than one type of tissue (both bone and cartilage are involved in peripheral joint OA, and intervertebral disk in spine OA), and the range of potential candidate genes is large. As currently defined, OA is a widely heterogeneous condition. Not only does the genetic influence on OA differ according to sex and the anatomic site affected, but also the clinical manifestations vary, with some patients mainly experiencing bone formation, while in other patients cartilage loss predominates. Genetic influences determining early joint development and joint shape may also need to be taken into account when attempting to understand this disease.

The genetic influence on serum COMP may operate through allelic variation, through factors determining gene expression, or through effects on the biologic pathways influencing cartilage metabolism and degradation. Mutations in the COMP gene are responsible for several heritable skeletal disorders that are characterized by severe early-onset OA. However, the extent to which variation in the COMP gene itself accounts for disease in the population is much less certain. Mabuchi et al observed no difference in allele frequency between Japanese patients with OA and

controls in a study of 6 polymorphisms spanning the entire gene (13). In a recent single-nucleotide polymorphism-based association study of 24 candidate genes in the UK population (2), the COMP gene failed to emerge as one of 8 candidate genes associated with OA susceptibility or progression.

One interesting aspect of this analysis is the factors in the shared environment of the twins that influence serum COMP levels. This is not the result of the association with age or any other measured lifestyle factor. One can speculate on a range of environmental factors shared by a pair of twins that might determine COMP levels and the subsequent risk of OA. Possible influences include the antenatal shared maternal environment of twins and subsequent shared dietary patterns.

It is known that changes in bone and cartilage metabolism take place early in the course of OA (14). The finding that serum levels of COMP at baseline are predictive of subsequent OA progression (4) suggests that serum COMP levels reflect early events in the pathogenesis of OA. Our demonstration that serum levels of COMP are independently heritable suggests that genes that control the serum levels of COMP, including but not confined to the COMP gene, should be sought. Through this approach, important insight into the genetic influence on the pathogenesis of OA may be gained, leading ultimately to targeted therapies.

ACKNOWLEDGMENT

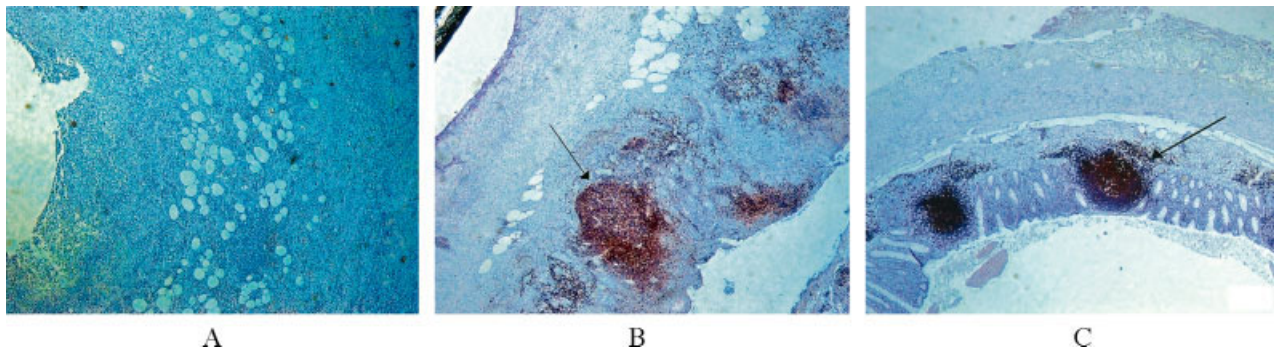
We appreciate the skillful technical assistance of Mrs. Mette Lindell.

REFERENCES

1. MacGregor AJ, Spector TD. Twins and the genetic architecture of osteoarthritis. *Rheumatology (Oxford)* 1999;38:583-8.
2. Valdes AM, Hart DJ, Jones KA, Surdulescu G, Swarbrick P, Doyle DV, et al. Association study of candidate genes for the prevalence and progression of knee osteoarthritis. *Arthritis Rheum* 2004;50:2497-507.
3. Petersson IF, Boegard T, Svensson B, Heinegard D, Saxnes T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *Br J Rheumatol* 1998;37:46-50.
4. Vilim V, Olejarova M, Machacek S, Gatterova J, Kraus VB, Pavelka K. Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2002;10:7-13.
5. Sharif M, Kirwan J, Elson C, Granell R, Clarke S. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurement of serum cartilage oligomeric matrix protein levels over five years. *Arthritis Rheum* 2004;50:2479-88.
6. Spector TD, MacGregor AJ. The St. Thomas' UK Adult Twin Registry [review]. *Twin Res* 2002;5:440-3.

7. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res* 2001;4:464–77.
8. Saxne T, Heinegard D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. *Br J Rheumatol* 1992;31:583–91.
9. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis* 1957;16:494–502.
10. Neale MC, Cardon LR. *Methodology for genetic studies of twins and families*. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1992.
11. Neale MC. *Mx: statistical modeling*. 4th ed. Department of Psychiatry, Virginia Commonwealth University, Richmond, VA; 1997.
12. Jordan JM, Luta G, Stabler T, Renner JB, Dragomir AD, Vilim V, et al. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. *Arthritis Rheum* 2003;48:675–81.
13. Mabuchi A, Ikeda T, Fukuda A, Koshizuka Y, Hiraoka H, Miyoshi K, et al. Identification of sequence polymorphisms of the COMP (cartilage oligomeric matrix protein) gene and association study in osteoarthritis of the knee and hip joints. *J Hum Genet* 2001;46:456–62.
14. Huebner JL, Hanes MA, Altenburg E, Beemkan B, Tekoppele JM, Kraus VB. A comparative analysis of bone and cartilage metabolism in two strains of guinea-pig with varying degrees of naturally occurring osteoarthritis. *Osteoarthritis Cartilage* 2002;10:758–67.

DOI 10.1002/art.21871

Clinical Images: B cell depletion in the appendix following rituximab treatment

The patient, a 28-year-old man with systemic lupus erythematosus (SLE) and class IV lupus nephritis, had been treated with prednisone and monthly intravenous (IV) cyclophosphamide (CYC) pulses, with poor response. Despite this treatment, he also developed severe symptomatic thrombocytopenia which did not respond to high-dose prednisone (1 mg/kg), oral pulse dexamethasone (40 mg/day for 3 days), or IV immunoglobulin (2 gm/kg). Due to refractory thrombocytopenia, he was treated with IV rituximab 375 mg/m² per week for 4 weeks, with a rapid and long-lasting response. Marked reduction in proteinuria and normalization of complement levels and anti-DNA antibodies were also noted. Two months after completing rituximab treatment and after receiving 6 pulses of CYC, he developed acute appendicitis and underwent an uneventful appendectomy. The appendix was stained for CD20+ cells and found to be totally depleted of B cells (A). CD20+ staining (arrows) in acute appendicitis (B) and in a normal appendix (C) are shown for comparison. Rituximab has been widely used for the treatment of B cell lymphomas and recently for idiopathic thrombocytopenic purpura and other systemic autoimmune diseases as well as SLE (1–4). Full-dose rituximab treatment leads to high rates of peripheral blood B cell depletion, but its effect on B cells in normal lymphoid tissue has been difficult to assess. Schroder et al have reported B cell depletion in blood and lymphatic tissue of cynomolgus monkeys treated with rituximab (5). Kneitz et al have reported complete depletion of B cells in the bone marrow and spleen of an SLE patient with thrombocytopenia treated with rituximab (6). Depletion of B cells in the appendix following rituximab treatment has not been previously described.

1. Plosker GL, Figgitt DP. Rituximab: a review of its use in non-Hodgkins lymphoma and chronic lymphocytic leukemia. *Drugs* 2003;63:803–43.
2. Braendstrup P, Bjerrum OW, Nielsen OJ, Jensen BA, Clausen NT, Hansen PB, et al. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adult refractory idiopathic thrombocytopenic purpura. *Am J Hematol* 2005;78:275–80.
3. Silverman GJ, Weisman S. Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy [review]. *Arthritis Rheum* 2003;48:1484–92.
4. Silverman GJ. Anti-CD20 therapy in systemic lupus erythematosus: a step closer to the clinic [editorial]. *Arthritis Rheum* 2005;52:371–7.
5. Schroder C, Azimzadeh AM, Wu G, Price JO, Atkinson JB, Pierson RN. Anti-CD20 treatment depletes B-cells in blood and lymphatic tissue of cynomolgus monkeys. *Transpl Immunol* 2003;12:19–28.
6. Kneitz C, Wilhelm M, Tony HP. Effective B cell depletion with rituximab in the treatment of autoimmune diseases. *Immunobiology* 2002;206:519–27.

Daphna Paran, MD
 Leonor Trej'ó, MD
 Dan Caspi, MD
 Tel Aviv University
 Tel Aviv, Israel