ORIGINAL ARTICLE

Meta-analysis identifies loci affecting levels of the potential osteoarthritis biomarkers sCOMP and uCTX-II with genome wide significance

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ABSTRACT

Background Research for the use of biomarkers in osteoarthritis (OA) is promising, however, adequate discrimination between patients and controls may be hampered due to innate differences. We set out to identify loci influencing levels of serum cartilage oligomeric protein (sCOMP) and urinary C-telopeptide of type II collagen (uCTX-II).

Methods Meta-analysis of genome-wide association studies was applied to standardised residuals of sCOMP and uCTX-II levels available in 6 and 7 studies, respectively, from TreatOA. Effects were estimated using a fixed-effects model. Six promising signals were followed up by de novo genotyping in the Cohort Hip and Cohort Knee study (N=964). Subsequently, their role in OA susceptibility was investigated in large-scale genome-wide association studies meta-analyses for OA. Differential expression of annotated genes was assessed in cartilage.

Results Genome-wide significant association with sCOMP levels was found for a SNP within MRC1 (rs691461, p=1.7×10−12) and a SNP within CSMO1 associated with variation in uCTX-II levels with borderline genome-wide significance (rs1983474, p=8.5×10−6). Indication for association with sCOMP levels was also found for a locus close to the COMP gene itself (rs10038, p=7.1×10−6). The latter SNP was subsequently found to be associated with hip OA whereas COMP expression appeared responsive to the OA pathophysiology in cartilage.

Conclusions We have identified genetic loci affecting either uCTX-II or sCOMP levels. The genome wide significant association of MRC1 with sCOMP levels was found likely to act independent of OA subtypes. Increased sensitivity of biomarkers with OA may be accomplished by taking genetic variation into account.

INTRODUCTION

Osteoarthritis (OA) is a prevalent, complex, disabling disease affecting articular joints. Age and unfavourable metabolic states, for example high body mass index (BMI), are strong risk factors of OA,1 however, development of OA is also determined by a considerable genetic component of a polygenic nature.2 Commonly, OA is diagnosed clinically and radiographically at a stage in which joint damage is already significant, and non-surgical therapies to cure or halt progress of OA are not yet available. Research directed to identify clinical biochemical markers to sensitively monitor OA disease activity in an individual over time or to assess quantitative joint tissue remodelling is currently in progress.3,4 So far, these studies have suggested that serum cartilage oligomeric protein (sCOMP), and urinary C-telopeptide of type II collagen (uCTX-II) are promising candidates and they are listed among the biomarkers that were recommended to focus on in future research efforts.4

Initially, sCOMP and uCTX-II were considered as markers for cartilage degradation: sCOMP would be representative of cartilage turnover, and uCTX-II is a collagen type II cleavage product representative of hyaline (articular) cartilage degradation. However, some reports indicate that uCTX-II may not merely be a cartilage specific marker but rather a reflection of joint tissue remodelling including calcified cartilage and subchondral bone.7 8 Moreover, Meulenbelt et al9 showed that in patients with generalised OA uCTX-II is a sensitive marker of whole body cartilage degeneration as reflected by radiographic signs of OA but association with a specific joint site could not be demonstrated. This was recently confirmed in a large-scale meta-analyses showing that uCTX-II associated with risk for hand, knee and hip OA.10 In addition, the analyses showed that sCOMP as well as uCTX-II can be useful to monitor OA disease activity. However, the study also highlighted that the use of sCOMP and uCTX-II as biomarkers in the clinic still remains largely unsatisfactory.

Importantly, investigations have shown that in subjects with familial OA at multiple joint sites besides clustering of sCOMP together with hand OA and with age, sCOMP has a high familial aggregation.8 11 At present, little is known about
Genetic variants affecting basal levels of sCOMP and uCTX-II. Identification of genes that contribute to these levels may provide mechanistic insight into factors that influence cartilage tissue turnover, dependent or independent of the OA pathophysiology, which in the latter case would thus perturb the use of these molecules as biochemical markers of OA. The objective of the current work is, therefore, to identify genetic variants involved in the variation in basal levels of sCOMP and uCTX-II by means of a large-scale hypothesis-free meta-analysis of genome-wide association studies (GWAS) across three English and four Dutch Caucasian studies. Additional replication occurred in the Dutch Cohort Hip and Cohort Knee (CHECK) study and signals were further tested for association with hip and knee OA. Finally, given that functional follow-up studies of susceptibility loci have demonstrated that they frequently result in expression differences of positional genes within the relevant diseased tissue, we explored the behaviour of mRNA expression of the annotated genes in preserved and osteoarthritic articular cartilage.

MATERIALS AND METHODS

Studies
A full detailed description of each study cohort is presented in the online supplementary methods section. Baseline characteristics and sample sizes are shown in table 1. We included studies from the Netherlands: The Genetics Osteoarthritis and Progression Study (GARP), Leiden Longevity Study (LLS), Rotterdam Study I and II (RS-I and RS-II), and the UK: TwinsUK, Chingford, Vitamin D evaluation in osteoarthritis (VIDEO). All studies were approved by their institutional ethics review committees and all participants provided written informed consent.

Study design
To identify genes associated with uCTX-II and sCOMP levels a two-stage design was applied (figure 1 shows a schematic representation of the workflow). In the first (discovery) stage, a meta-analysis of GWAS was performed by combining the summary statistics of seven independent studies (four Caucasian studies in the Netherlands and three in UK) for genome-wide association studies (GWAS) for OA associated loci among six and seven studies, respectively, and OA-related expression changes of annotated genes in cartilage were derived from an available data set generated within the ongoing Research Arthritis and Articular Cartilage study.

An overview of the populations and available measurements is shown in table 1. Detailed description of the populations included in this study is provided in online supplementary methods, and online supplementary table S1 shows quality control (QC) thresholds applied in each study.

Urinary CTX-II and serum COMP measurements
All measurements for uCTX-II in samples of Chingford, Rotterdam (studies I and II), TwinsUK and VIDEO were performed as part of the TreatOA consortium with the use of an ELISA based on a monoclonal antibody raised against the linear 6-amino acid EKGPDP epitope of the CII C-telopeptide (CartiLaps; Immunodiagnostics systems, Paris, France). Intra-assay and inter-assay variations were lower than 9% and 11%, respectively. Serum COMP levels for Chingford, RS-II, Twins-UK and VIDEO were measured using a two-site immunoassay (COMP ELISA kit, AnaMar Medical, Lund, Sweden). Intra-assay and interassay variations were below 10%.

Assessment of uCTX-II and sCOMP levels in samples of the GARP study and LLS was performed previously by Synarc Laboratories Lyon, France and has been described in detail elsewhere. Measurement of the biomarkers in the CHECK cohort was done with commercially available ELISA kits according to the manufacturers protocol as described previously.

Genotyping, quality controls and imputation
Genotyping of the cohorts was done by Illumina Infinium HumanHap550 Beadchip (RS-I and RS-II), the Illumina Infinium HumanHap610 (GARP, LLS and TwinsUK) or the Illumina Infinium HumanHap300 (VIDEO and CHINGFORD). Criteria for QC are shown in online supplementary table S2. For all studies, normalised intensity data of the GWAS were pooled, and genotypes were called on the basis of the Illuminus 1D assay.

Table 1 Overview of the discovery and replication studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Ref.</th>
<th>Study design</th>
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<th>uCTX-II</th>
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<tr>
<td></td>
<td></td>
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<td># samples</td>
<td># SNPs</td>
</tr>
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<td>536 984</td>
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<td>Sibling pair study</td>
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<td>2 162 385</td>
</tr>
<tr>
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<td>Offspring of long-lived subjects and partners</td>
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<td>2 267 101</td>
</tr>
<tr>
<td>RS-I</td>
<td>15</td>
<td>Population based cohort</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RS-II</td>
<td>16</td>
<td>Population based cohort</td>
<td>1161</td>
<td>2 543 887</td>
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<td>Twins based cohort</td>
<td>754</td>
<td>3 044 064</td>
</tr>
<tr>
<td>VIDEO</td>
<td>19</td>
<td>Vitamin D evaluation in OA</td>
<td>271</td>
<td>537 298</td>
</tr>
<tr>
<td>Total # samples:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHECK</td>
<td>25</td>
<td>Early OA cohort</td>
<td>964</td>
<td>NA</td>
</tr>
</tbody>
</table>

Avg (SD), average of the log10-transformed values with the SD; CHECK, Cohort Hip and Cohort Knee; Chingford, Chingford 1000 Women Study; GARP, Genetics Osteoarthritis and Progression Study Leiden; LLS, Leiden Longevity Study; RS-I and RS-II, Rotterdam study I and II, respectively; sCOMP, serum cartilage oligomeric protein; Twins UK, the UK Adult Twin Registry; uCTX-II, urinary C-telopeptide of type II collagen; VIDEO, Vitamin D evaluation in osteoarthritis.
Stage I

Discovery Meta-analysis

uCTX-II sCOMP
N = 4654 N = 3316

threshold P ≤ 1 x 10⁻⁶

Stage II

Replication

2 SNPs 4 SNPs

uCTX-II sCOMP
N = 964 N = 964

OA phenotype check in GWAS

uCTX-II 2 SNPs sCOMP 4 SNPs

Hip (4349 cases vs 46903 controls) Knee (5755 cases vs 48578 controls)

Cartilage expression annotated genes

N = 33 OA vs P

Analysis of GWAS
After selection and QC, data of sCOMP and uCTX-II were analysed separately for each study. For both traits log₁₀ transformations were performed and standardised residuals from regression were extracted with mean=0 and SD=1. All studies performed regression analysis, adjusting for age, age squared, sex and population specific covariables such as familial dependencies or population stratification. Multiplicative (Cochran-Armitage)/additive allele-dose were carried out for imputed SNPs where possible using a linear regression framework as implemented in MACH2DAT, QTASSOC (inhouse analysis program, developed to correct for familial dependence) and PLINK (see online supplementary table S3). The estimated inflation factors in the sCOMP analyses were 1.002, 0.986, 1.033, 1.01, 0.987 and 0.977 for Chingford, GARP, LLS, RS-II, TwinsUK and VIDEO, respectively. The estimated inflation factors in the uCTX-II analyses were 1.003, 1.008, 1.026, 1.020, 0.997, 1.008 and 1.008 for Chingford, GARP, LLS, RS-I, RS-II, TwinsUK and VIDEO, respectively (table 1).

Meta-analyses
Summary statistics of the individual GWAS were combined by means of a fixed-effects inverse variance-weighted meta-analysis, as implemented in METAL across 1 961 964 autosomal SNPs. In the meta-analysis the genomic control (GC) method was used as implemented in METAL which corrects for any residual population stratification or relatedness that is not accounted for by the four most important principal component (PCs). The GC inflation factor of the metadata was than calculated again to assess resulting inflation of the test statistics in R statistical programming language using the GenABEL-package.

A value of p<5×10⁻⁶ was considered genome-wide significant. Variants with a p≤1×10⁻⁶ in the meta-analyses were selected for follow-up in the replication stage of this study, and for these SNPs meta-analyses were performed in R (http://www.r-project.org) using the meta package. Here, summary regression coefficients (effect sizes, betas) were estimated using the fixed-effects model of DerSimonian and Laird. Heterogeneity was quantified using the I² statistic for inconsistency and its statistical significance was tested with the χ² distributed Cochran Q statistic. I² describes the proportion of variation due to heterogeneity that is unlikely to be due to chance and is considered large for values over 50%. Regional association plots of the meta-analysis results were obtained with LocusZoom.

Exploratory OA phenotype check
To explore whether the identified variants in our study may share common pathophysiological paths and are associated with OA we tested for potential associations in previously performed meta-analyses of GWAS for hip OA (a total of 4349 cases vs 46 903 controls, including the following studies: arcOGEN stage 1, deCODE, EGcut, GARP, RS-I, RS-II and TwinsUK) and knee OA (a total of 5755 cases vs 48 578 controls, including the same studies as for hip OA and in addition the Framingham study). Specifically, associations were assessed using a fixed-effects inverse variance meta-analysis of GWAS for the aforementioned phenotypes to obtain the summary effect size and the magnitude of the association along with the direction of the effect. A full detailed description of included studies on recruitment with radiographic and clinical assessment has been published previously.

Gene expression analyses
OA-related changes in expression levels of positional genes were assessed in the Research Arthritis and Articular Cartilage study aimed at the biobanking of joint materials (cartilage, bone and where available ligaments), mesenchymal stem cells and primary chondrocytes of patients and controls in the Leiden University...
Medical Center and collaborating outpatient clinics in the Leiden area. A gene expression data set of 33 paired samples of OA-affected versus preserved cartilage of the same joint derived from patients that underwent joint replacement due to end-stage OA was used (described previously22 36 and explained in more detail in online supplementary methods). In short, a total of 0.5 μg of RNA was used as template. Subsequently, first-strand and second-strand reverse transcription steps and labelling with biotin-labeled nucleotides was performed using an Ambion RNA amplification kit (TotalPrep RNA Amplification Kit). Microarray analysis (Illumina HT-12 V3) was performed and analysed in R statistical programming language using the Limma package to compare gene expression in the 33 sample pairs with a paired t test. Mean expression of all genes analysed was 7.4 (6.6–14.9), expressed as the overall relative light intensity signals in the preserved and OA affected cartilage, respectively, as provided by Illumina.

RESULTS

A meta-analysis of GWAS was performed across Caucasian samples to identify loci associated with serum levels of COMP available in 3316 subjects across six studies and of uCTX-II available in 4654 subjects across seven studies (figure 1). Of these, two studies had only genotyped SNPs (N~530 000) whereas the other studies used additional imputed SNPs (N~2.5 million; table 1 and online supplementary methods).

GWAS meta-analysis for sCOMP

In the sCOMP meta-analysis a total of 1 961 964 SNPs (directly genotyped or imputed in at least four studies) were tested for association. The overall GC λ (λGC) was 1.0131, indicating no significant population stratification (see online supplementary figure S1). A genome wide significant signal was found for SNP rs691461 (p=1.9×10^{-12}), located at chromosome 10 (17.9 Mb) within intron 7 of the Mannose receptor C type 1

![Figure 2](https://example.com/figure2.png)

**Figure 2** Association of serum cartilage oligomeric protein (sCOMP) levels with rs691461 localised within the MRC1 gene. (A) Forest plot of sCOMP meta-analysis for rs691461 in the MRC1 gene (p=1.7×10^{-12}). EA, effect allele; Chingford, Chingford 1000 Women Study; GARP, Genetics OsteoArthritis and Progression study Leiden; LLS, Leiden Longevity Study; RS-II, Rotterdam study cohort 2; VIDEO, Vitamin D evaluation in osteoarthritis; TwinsUK, the UK Adult Twin Registry; CHECK, Cohort Hip and Cohort Knee. (B) LocusZoom plot showing positional genes of the sCOMP SNP rs691461. BETA refers to standardised effect.
(MRC1) gene (figure 2). Two other signals that reached the pre-specified follow-up threshold, were rs10429475 with $p=7.6 \times 10^{-8}$ and rs17635227 with $p=1.4 \times 10^{-6}$ located, respectively, at chromosome 9 (120.2 Mb) between the Deleted in breast cancer 1 (DBC1) and Toll-like receptor 4 (TLR4) genes and at chromosome 14 (83.3 Mb) in a gene desert (see online supplementary figure S2 and table S4A). Furthermore, of note was a SNP identified within the top 15 of independent loci of the meta-analyses, located at chromosome 19 (18.8 Mb), annotated 1 kb downstream of the COMP gene (rs10038; $p=2.3 \times 10^{-5}$) that was as positional candidate included in the replication stage of our study.

Replication of the four identified SNPs within the Dutch early OA study CHECK (N=964) indicated suggestive evidence for independent association with sCOMP levels only for SNP rs691461 ($p=0.066$; see online supplementary table S4B). When we added this result to the meta-analysis the association for the G allele (allele frequency: 0.502) of rs691461 with lower sCOMP levels became stronger ($\beta=-0.16$, $p=1.7 \times 10^{-12}$) without evidence for significant heterogeneity ($I^2=23.8$%; $p=0.3$; figure 2A). Although the independent effect of rs10038, located close to the COMP gene, was not significant in the CHECK cohort ($p=0.106$), when we added this result to the meta-analysis the association for the A allele (allele frequency: 0.334) with increased levels of sCOMP became stronger ($\beta=0.11$; $p=7.1 \times 10^{-6}$).

**GWAS meta-analysis for uCTX-II**

In the uCTX-II meta-analysis a total of 1 836 485 SNPs (directly genotyped or imputed in at least four studies) were tested for association. The GC inflation factor showed no systematic inflation ($\lambda_{GC}=1.0132$; see online supplementary figure S3). Two independent variants reached the prespecified threshold for replication, respectively, rs1983474 ($p=1.0 \times 10^{-6}$) located at chromosome 8 (4.4 Mb) within the CUB and sushi domain-containing protein 1 (CSMD1; figure 3B) and rs4518370.

**Figure 3** Suggestive evidence for association of rs1983474 with urinary C-telopeptide of type II collagen (uCTX-II) levels. (A) Forest plot of uCTX-II meta-analysis for rs1983474 within the CSMD1 gene ($p=8.5 \times 10^{-8}$). EA, effect allele; Chingford, Chingford 1000 Women Study; GARP, Genetics OsteoArthritis and Progression study Leiden; LLS, Leiden Longevity Study; RS-I, Rotterdam study cohort 1; RS-II, Rotterdam study cohort 2; VIDEO, Vitamin D evaluation in osteoarthritis; TwinsUK, the UK Adult Twin Registry; CHECK, Cohort Hip and Cohort Knee. (B) LocusZoom plot showing positional genes of the uCTX-II SNP rs1983474. BETA refers to standardised effect.
(p=1.3×10^{-6}) located at chromosome 5 (82.2 Mb) close to three different genes (see online supplementary figure S4). In CHECK we observed significant independent association for rs1983474, (p=0.032; see online supplementary table S4B). When combining discovery and replication results of rs1983474, the association with uCTX-II became borderline genome-wide significant (β=0.13; p=8.5×10^{-8}) without evidence for heterogeneity (I²=0%; p value=0.5; figure 3A).

Analysis for association with OA phenotypes and expression of annotated genes in cartilage

We explored whether the six loci identified in this study by virtue of their association with either sCOMP or uCTX-II (table 2) also associated with OA. For this, the TreatOA genome-wide association scan data set including all participants stratified for hip and knee OA were examined. As shown in table 3, evidence for nominal significance association (p < 0.05) was only found for rs10038, within the COMP gene, for hip OA (p=8.9×10^{-3}; OR=1.07 per allele, 95% CI 1.02 to 1.13).

Expression of the identified annotated genes of table 2 in articular cartilage and their responsiveness to OA pathophysiology was examined in a microarray mRNA expression data set of human matched OA and preserved articular cartilage obtained from 33 individuals that underwent joint replacement surgery due to end-stage OA. As expected, very high expression of COMP was observed in cartilage tissues (relative levels of expression are within the highest quartile of the data set; table 3) reflecting its relative importance here. Furthermore, expression of COMP appeared subtle but significantly increased (1.1-fold) in cartilage harvested near the OA lesion (OA-affected cartilage) as compared with cartilage distal to the OA lesion (preserved cartilage; p=3.6×10^{-5}). In contrast, low overall expression in articular cartilage and no differential expression between OA-affected and preserved cartilage was detected for the other genes (table 3).

DISCUSSION

In the current study, we have established novel genetic variants influencing levels of the well-known OA biochemical markers sCOMP and uCTX-II by means of a large-scale GWAS meta-analysis design. A genome-wide significant locus within the MRC1 gene (rs691461) was associated with variation in the levels of sCOMP (p=1.7×10^{-12}) in the combined analysis of the discovery and follow-up stage. The effect allele G with a frequency of 0.502 was associated with a decrease of sCOMP in the discovery and replication stage (p=8.5×10^{-8}). The C-allele, with a frequency of 0.770, was associated with increased uCTX-II (β=0.13). We did not find association between rs1983474 and hip or knee OA. A previous study identified association between OA and another SNP in CSMD1, rs9657371, with negligible linkage disequilibrium with rs1983474 (r²=0.03). However, exploration of the TreatOA GWAS data set for hip and knee OA showed no association for rs9657371 (data not shown). The rat homologue of CSMD1 was identified in a search for proteins of the ‘regulators of complement activation’ family, and it was shown that CSMD1 blocked the classical complement pathway activation. It may therefore be speculated that innate dysregulated expression of CSMD1 results in a (slight) activation of the complement cascade in articular cartilage independent of OA, leading to changes in the release of CTX-II. It should be noted, however, that in articular cartilage CSMD1 gene expression was relatively low (table 3). Altogether, thorough additional investigation is necessary to obtain mechanistic insight in this locus with respect to uCTX2 levels.

Our study has some limitation. Despite the large number of samples that we have assembled, small genetic effects could well have been missed in our analyses with the biomarker levels or OA phenotypes. Power may have eroded due to measurement

<table>
<thead>
<tr>
<th>SNP</th>
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<th>EAF</th>
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<td>0.071</td>
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<td>1.35×10^{-7}</td>
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*In CHECK rs8 104 411 was genotyped (pairwise LD (r²) between rs8104411 and rs10038=0.9).

BETA, effect of the indicated effect allele; CHR, chromosome; Dir. direction of the effect for the indicated effect allele in the respective studies (Chingford, GARP, LLS, RS-I (only for uCTX-II), RS-II, TwinsUK, VIED and CHECK); EA, effect allele; EAF, effect allele frequency; sCOMP, serum cartilage oligomeric protein; POS, chromosomal position; uCTX-II, urinary C-telopeptide of type II collagen.

**Table 3** Association of identified loci with hip and knee osteoarthritis (OA) and expression of annotated genes in cartilage

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP Chr</th>
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<tbody>
<tr>
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<td>rs10038</td>
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<td>A</td>
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<td>1.02 to 1.13</td>
<td>8.92×10⁻¹⁰</td>
<td>+</td>
<td>1.08</td>
<td>0.98 to 1.08</td>
<td>3.57×10⁻¹⁰</td>
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<td>0.92 to 1.02</td>
<td>2.22×10⁻⁶</td>
<td>+</td>
<td>1.04</td>
<td>0.99 to 1.09</td>
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<td>0.96 to 1.07</td>
<td>6.17×10⁻¹⁰</td>
<td>7.4</td>
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**Abbreviations:** AveExpr, Average expression in cartilage (mean=7.4 (6.6–14.9)); CHR, chromosome; Dir, Direction of the effect for the indicated effect allele; FC, fold change in OA affected cartilage respective to preserved; NA, not available; PVS, positional single nucleotide polymorphisms; OR, Odds Ratio; p valexpr, p value for differential expression between OA affected and preserved cartilage; SNP, single nucleotide polymorphism.

In conclusion, by applying meta-analysis of GWAS we are the first to identify a locus within the CSMD1 gene influencing innate levels of uCTX-II with borderline genome-wide significance. Moreover, we identified two loci influencing the sCOMP levels, located within respectively the MRC1 and the COMP gene. The data and function of MRC1 indicate that, apart from OA pathophysiology, OA independent processes are also likely to affect sCOMP levels. Taking such effects into account when applying sCOMP as OA biomarker increased sensitivity of sCOMP may be accomplished. In this respect, we advocate that the recently published meta-analysis on uCTX-II and sCOMP as OA biomarkers should now be analysed by taking into account the identified genetic factors.
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