The role of plasma cytokine levels, CRP and Selenoprotein S gene variation in OA

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Summary

Objective: Investigating the association between plasma levels of cytokines and chemokines, Selenoprotein S (SELS) gene variation and osteoarthritis (OA) subtypes.

Methods: The genetics of osteoarthritis and progression (GARP) study consists of 191 sibling pairs with symptomatic OA at multiple joint sites. We have measured plasma levels of 17 cytokines and chemokines and genetic variation at the SELS gene.

Results: Nine out of 17 serum markers could be assessed quantitatively, whereas eight markers were assessed qualitatively. Principal component analysis (PCA) on the quantitatively assessed markers and serum high sensitive C-reactive protein (S-HsCRP) revealed that three components underlie 61% of the total plasma variation. Three single nucleotide polymorphisms (SNPs) in the SELS gene revealed four common haplotypes, one of which, GAG (frequency 3.5%) showed significant association to an anti-inflammatory (P = 0.019) and acute phase related (P = 0.036) component. OA subtype analysis showed that one component (mainly representing chemokine variation) was significantly associated to hand OA and disc degeneration (P = 0.029 and P = 0.010 respectively) as well as a physical component score (PCS) (P = 0.042). The CRP related component also showed a strong association to the PCS (P = 0.007). SELS haplotypes showed no association to OA subtypes in the GARP study.

Conclusion: Genetic variation in the SELS gene associates to components representing inflammatory signaling. Another component, representing chemokine variation, showed association to hand OA and disc degeneration in the GARP study indicating chemokines may contribute to OA pathogenesis.

Key words: Osteoarthritis, Inflammation, Selenoprotein S, CRP, Genetic.

Introduction

Osteoarthritis (OA) is a common joint disease and an important cause of pain and disability in the general population. Elucidation of common pathways that are involved in the onset and progression of the disease will assist in the development of new drug targets and provide a better management of this disabling condition in the future. Several studies have shown that genetic factors play an important role in OA etiology1,2. Although OA is not regarded to be an inflammatory disease, there is increasing evidence for the involvement of an innate low grade systemic inflammatory component which may partly explain the genetic susceptibility3–5. More specifically, support for the hypothesis that local variation in cytokine levels in the joint may influence OA onset and progression is found in the catabolic effects of pro-inflammatory cytokines, and the protective effects of anti-inflammatory cytokines in articular cartilage6,7. Gene expression studies have shown that in OA cartilage several cytokines and chemokines are highly expressed10–12. The recent introduction of multiplexed cytokine assays facilitates sensitive measurements of different cytokines using small amounts of valuable sample material from different origins. This sensitive technique allows investigation of the basal levels of chemokines and cytokines in plasma in relation to the ongoing disease processes for diseases where, as in OA, no pronounced and obvious inflammatory component is present.

In a previous study, Curran et al. showed that subjects can be characterized by a high (proinflammatory) or low (noninflammatory) plasma cytokine profile depending on a common promoter single nucleotide polymorphism (SNP) −105G > A (rs28665122) in the Selenoprotein S (SELS) gene. A significant association of this SELS SNP was observed specifically with higher plasma levels of tumor necrosis factor alpha (TNFα), interleukin beta (IL1β) and interleukin 6 (IL6)13. SELS is a widely expressed protein involved in maintaining the functional integrity of the endoplasmatic reticulum (ER) by participating in the removal of misfolded proteins and regulating the cellular redox balance. SELS inhibition by small interfering RNA (siRNA) revealed that a functionally impaired ER leads to activation of numerous pro-inflammatory cytokines mediated by
nuclear factor-κB (NF-κB) activation. In a cohort of patients with intestinal inflammation, there was no association to the SELS locus, however, the pro-inflammatory allele associated to a high serum CRP levels in Crohn Disease patients with active disease. Finally, SELS polymorphisms associated with coronary heart disease and ischemic stroke. These studies trigger the question whether SELS gene variation also influences inflammatory responses and the etiology of OA. Hence, we set off to map the plasma levels of 17 cytokines and chemokines by use of a multiplexed bead array system in subjects with symptomatic OA at multiple joint sites of the GARP study. We tested whether SELS gene variation influenced these inflammatory plasma mediators and OA, and whether these markers associated to OA subtypes and severity.

Materials and methods

THE GARP STUDY

The GARP study consists of 191 sibling pairs. All participants have symptomatic OA at multiple sites in the hand or OA at two or more joint sites of four joint sites examined. Symptomatic OA was determined following the American College of Rheumatology recommendations whereas radiographic OA (ROA) was scored according to Kellgren/Lawrence. Details on the GARP OA phenotype and inclusion criteria can be found in previous publications. Physical functioning was assessed with the physical component score (PCS), a subscore of the Dutch validated RAND 36-item Health Survey. This questionnaire covers health related aspects including social functioning, role limitations, mental health and vitality. A higher score on the PCS indicates a better physical functioning. For the current study we used the proportionate ROA score based on the presence of ROA at each joint location and on the number of joints with ROA identical as described previously. In short, scores 0, 1 and 2 represent respectively no, uni- and bilateral hip and knee OA for these joint sites. The hand ROA score (0–2) represents subjects with, respectively, 0–2, 3–6, and >7 hand joints affected out of 20 scored. For spinal disc degeneration (DD) score (0–2) represents subjects with DD at respectively 0–2, 3–5 and >6 levels out of 11 levels scored. Hand OA following the American College of Rheumatology (ACR) criteria was analyzed in addition to the ROA criteria. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. Written informed consent as approved by the ethical committee was obtained from all subjects in the GARP study.

SERUM AND PLASMA COLLECTION AND MEASUREMENTS

For each participant of the GARP study a morning serum and EDTA plasma sample was collected. Samples were processed within 4 h upon collection and stored at −80 C until measurement. Serum levels of high sensitive C-reactive protein (SHsCRP) was assayed earlier. A 17-plex bead assay provided by Bio-RAD was used to measure plasma levels of cytokines and chemokines on a Luminex platform. Intra assay variation was estimated at 6.2%. The standard protocol was adjusted using twice the indicated amounts of plasma sample in half the amount of sample buffer to increase signal in the measurements. On each plate 10% of samples were in duplicate, in 15% of samples were in triplicate, in which no inconsistencies were observed. For calculation of z-scores the average value of duplos was used as a single value. For cytokines and chemokines with less than 60% of the fluorescence levels above background noise, z-scores of fluorescence levels were calculated per plate (quantitative analysis, QT). In the principal component analysis (PCA) individuals with missing values in five or more out of nine cytokine/chemokines measurements were excluded (N = 13). For the analyzed individuals remaining missing values were given the specific marker mean score. Sample distribution was random per plate on a total of eight plates.

GENOTYPING

In the current study three SNPs of the SELS were genotypes selected from the original paper of Curran et al. SNP positions relative to translation start of the SELS gene are: −105 (G > A, rs28665122), +3705 (G > A, rs4965814), and −6218 (A > G, rs98794). SNP were measured using hME™ chemistry on a mass array platform and scoring of the dye-coupled mass spectrometry MassARRAY Mass Spectrometer (Sequenom Inc., San Diego, CA, USA). Assays were designed using the Sequenom MassARRAY Assay Design software (version 3.1). Assay conditions were standard conditions as described earlier. In addition to the genotypes obtained by the Sequenom for rs28665122, a Taqman assay (Applied Biosystems, Foster City, California, United States) was performed to deal with low genotyping success rates this SNP using the Sequenom technique. Primers used for the ABI-genotyping were: forward primer 5‘-GGTTGCGTGTCG-3‘ and reverse primer 5‘-CTTCGCGTGCCGGCCGTCTTA-3‘, probes were 5‘-TGCGGCGGACCAAC labeled with VIC and 5‘-TTGCGCAGGACACCAC labeled with FAM. Assays were run on a 7900HT (Applied Biosystems) according to the manufacturers specifications. Genotypes of both techniques were used in addition to each other where necessary and were used as a control where both techniques provided reliable genotypes.

STATISTICAL ANALYSIS

PCA was applied to reduce the correlated data of the cytokines and chemokines plasma levels. Subjects (N = 341) with available levels of S-HsCRP and with >4 out of nine quantifiable markers were entered in the PCA analysis. In PCA analyses random missing data (see Table II) was replaced by mean values. Both empirical criteria (percentage of variance explained by factors and Eigen values > 1) and interpretability were used to determine the number of factors. We explored the interpretability of these factors after applying a Varimax rotation with Kaiser Normalization. The loading score of each variable onto the individual factors represents the contribution of that variable to the variance observed in the resulting factor. For analysis only individual variable contributions of >0.4 qualified for loading a component. A factor loading represents the linear relationship (Pearson correlation under Varimax rotation) between a variable and a factor.

In order to assess the relationships between OA characteristics, genetic variation at the SELS gene and the clusters of cytokines and chemokines, a mixed model regression analyses was performed. To investigate the individual associations, subject specific regression scores of each extracted cluster were used as dependent variable and age, sex, BMI (Body Mass Index) and all OA subtypes as co-variables. OA features and subtypes tested were specific ROA scores as defined previously; knee (0–2), hip (0–2), hand (0–2) and spinal DD (0–2) and the PCS derived from the SF36 questionnaire. Furthermore, for each linear mixed model analyses family identity numbers (representing family relations) as random variables in order to model the familial dependencies that might occur for the levels.

Genotype distributions of SELS SNPs were checked by use of the HWE program available at http://linkage.rockefeller.edu. Thesias V3.1 was used to assess linkage disequilibrium between the SNPs and to assess the expected haplotype contribution to a mean quantitative measure in carriers of a specific haplotype. Quantitative measures analyzed by use of Thesias are e.g., the subject specific regression scores as determined by the PCA analyses. In individuals the expected quantitative measure is determined by the contribution of the two carried haplotypes. The Thesias program allows for adjustments of co-variables but not for familial relationship. Chi square analyses were performed to test for association of individual SNPs or proportionate ROA scores to the qualitatively analyzed cytokines and chemokines. P-values are unadjusted for multiple testing. Analyses were done in SPSS version 14 (SPSS, Chicago, IL, USA) unless mentioned otherwise.

Results

CYTOKINE AND CHEMOKINE MEASUREMENTS

Characteristics of the GARP study are displayed in Table I. For all participants of the GARP study cytokine and chemokine levels were analyzed in a plasma sample by use of a 17-plex bead array system. Table II shows cytokines and chemokines measured and the percentage of samples with levels above detection limit. In nine instances (see Table II) we were able to assess a semi quantitative measure by the use of z-scores reflecting relative plasma levels. For the remaining eight instances (see Table II), plasma levels were analyzed in a qualitative matter (detectable vs not detectable).

ASSOCIATION ANALYSIS OF CYTOKINE/CHEMOKINE LEVELS WITH OA FEATURES

Nine cytokines and chemokines and S-HsCRP were analyzed in a quantitative manner, the remaining eight cytokines and chemokines were qualitatively analyzed. In the
quantitatively analyzed cytokine and chemokine z-scores we observed substantial correlations (Supplementary Table 1). In addition to the association between S-HsCRP and BMI shown previously (24), Supplementary Table 1 also shows the frequently observed association between BMI and plasma IL6 levels. GARP subjects in the highest BMI quartile (BMI > 29.1 kg/m²) had an OR of 1.7 (95% C.I. 1.01–2.82) to reside in the highest quartile of IL6 plasma levels (P = 0.042) when corrected for age, sex and familial relationship.

To reduce the redundancy between the markers to more independent components in which these variables cluster, a PCA including all inflammatory markers for which a quantitative measure was available (see Table II) and S-HsCRP 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 IL2, IL6, Granulocyte CSF (GCSF) and IL10 loaded together on the first component, explaining 34.1% of the total variation in the GARP study. Component 2 is determined by three chemokines MCP (Monocyte Chemotactic Protein), IL8 and MIP (Macrophage Inflammatory Protein), explaining 15.7% of the variation, whereas the third component is determined by S-HsCRP, IL5 and IL7 explaining 11.6% of the variation. It should be noted that S-HsCRP has a negative value in the third component (Table III), indicating that on average, within subjects there is an inverse relation between S-HsCRP levels and IL7 and IL5.

Subsequently the relationship between the three components as dependent variables and the presence of OA characteristics (Table I) as co-variables was investigated by mixed model regression analysis. The upper section of Table IV shows that component 2, consisting of chemokines IL8, MIP and MCP, has significant negative associations to hand ROA score (beta = −0.14;  P = 0.039) and to disc degeneration ROA score (beta = −0.22;  P = 0.005), independent of age, sex and BMI. This implies that subjects with high chemokine levels have lower hand ROA and DD scores. In addition, component 2 showed a similar association to subjects that had hand OA according to the ACR criteria (beta = −0.26;  P = 0.024, data not shown). When

### Table I

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
<th>Mean (sd)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>312 (81.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>382 (100)</td>
<td>60.27 (7.54)</td>
<td>46.12–79.44</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>379 (99.2)</td>
<td>27.00 (4.67)</td>
<td>19.10–46.48</td>
</tr>
<tr>
<td>Clinical hand OA</td>
<td>354 (92.7)</td>
<td>3.63 (6.43)</td>
<td>0.21–56.80</td>
</tr>
<tr>
<td>PCS SF36</td>
<td>375 (98.4)</td>
<td>54.02 (21.10)</td>
<td>8.75–98.75</td>
</tr>
<tr>
<td>ROA score*</td>
<td>0</td>
<td>1</td>
<td>2</td>
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### Table II

<table>
<thead>
<tr>
<th>Cytokine/Chemokine</th>
<th>N Detectable (%)</th>
<th>Analysis</th>
</tr>
</thead>
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<tr>
<td>Interleukin 1β</td>
<td>107 (28.3)</td>
<td>QL</td>
</tr>
<tr>
<td>Interleukin 2</td>
<td>26 (6.9)</td>
<td>QL</td>
</tr>
<tr>
<td>Interleukin 12</td>
<td>90 (23.8)</td>
<td>QL</td>
</tr>
<tr>
<td>Interleukin 13</td>
<td>172 (45.5)</td>
<td>QL</td>
</tr>
<tr>
<td>Interleukin 17</td>
<td>25 (7.1)</td>
<td>QL</td>
</tr>
<tr>
<td>Interferon γ</td>
<td>179 (47.4)</td>
<td>QL</td>
</tr>
<tr>
<td>TNFα</td>
<td>165 (43.4)</td>
<td>QL</td>
</tr>
<tr>
<td>Granulocyte Monocyte CSF</td>
<td>144 (37.8)</td>
<td>QL</td>
</tr>
<tr>
<td>Interleukin 2</td>
<td>234 (61.6)</td>
<td>QT</td>
</tr>
<tr>
<td>Interleukin 5</td>
<td>222 (58.4)</td>
<td>QT</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>374 (98.4)</td>
<td>QT</td>
</tr>
<tr>
<td>Interleukin 7</td>
<td>274 (72.1)</td>
<td>QT</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>359 (94.5)</td>
<td>QT</td>
</tr>
<tr>
<td>Interleukin 10</td>
<td>327 (86.1)</td>
<td>QT</td>
</tr>
<tr>
<td>GCSF*</td>
<td>248 (65.3)</td>
<td>QT</td>
</tr>
<tr>
<td>MCP-1</td>
<td>377 (98.9)</td>
<td>QT</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>375 (98.4)</td>
<td>QT</td>
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### Table III

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<th>Component</th>
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<tr>
<td>IL2</td>
<td>0.839</td>
<td></td>
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</tr>
<tr>
<td>IL6</td>
<td>0.815</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCSF</td>
<td>0.764</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL7</td>
<td>0.594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.799</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8</td>
<td>0.799</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP-1β</td>
<td>0.765</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-HsCRP</td>
<td>-0.747</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL5</td>
<td>0.585</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total variation explained</td>
<td>34.1%</td>
<td>15.7%</td>
<td>11.6%</td>
</tr>
</tbody>
</table>

*Extraction Method: PCA in which missing values were replaced by mean levels.

### Table IV

<table>
<thead>
<tr>
<th>OA characteristics</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Radiographic OA</td>
<td>0.079</td>
</tr>
<tr>
<td>Knee ROA score</td>
<td>-0.079</td>
</tr>
<tr>
<td>Hand ROA score</td>
<td>0.030</td>
</tr>
<tr>
<td>DD ROA score</td>
<td>0.002</td>
</tr>
<tr>
<td>SF36 outcome PCS</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* P < 0.05 ** P < 0.01.

Components 1 contains IL2, GCSF, IL6, IL10 and IL7. Component 2 contains MCP, IL8, MIP and component 3 contains S-HsCRP (negative), IL5 and IL7. Data was analyzed using mixed model regression analyses with the components as dependent variable and as co-variables the joint specific ROA scores (top) or the PCS (bottom) in addition to age, sex and BMI.
analyzing the relationship of the components to the PCS derived from the SF36 a significant negative association of component 2 ($P = 0.035$) and a positive association to component 3 ($P = 0.004$) was observed, independent of sex, age and BMI (Table IV, lower section). This indicates that subjects with high chemokine levels experience more functional impairment whereas subjects with high IL7, IL5 and low S-HsCRP levels experience less functional impairment. The association of PCS to component 2, however, appeared not independent of the hand ROA scores (data not shown). In each of the mixed models significant associations with BMI were observed for component 1 (positive, $P = 0.049$), for component 2 (negative, $P < 1 \times 10^{-5}$) and component 3 (negative, $P < 1 \times 10^{-5}$). In the qualitatively analysis of the cytokines no significant associations were observed for OA subtypes (data not shown).

**SEL S gene variation, inflammatory parameters and OA**

In the GARP study we could not confirm the previously reported association of rs28665122 with TNF-$\alpha$, IL1-b and IL6 plasma levels by Curran et al.\textsuperscript{13}

The influence of the SELS SNPs on the cytokine and/or chemokine levels, as expressed by the three components, was investigated by haplotype analysis since high linkage disequilibrium was observed between the three SELS SNPs ($D^2 > 0.8$). As shown in Table V, four common haplotypes with frequencies over one percent were observed similar to a Finnish population\textsuperscript{15}. A significant association was observed between haplotype GAG (frequency 3.5%) and component 1 ($P = 0.019$). Since component 1 reflects variation in IL2, IL6, GCSF and IL10 levels this association indicates that carriers of this haplotype have higher levels of the cytokines in this component. Upon further investigation, this association appeared to be mainly driven by IL10 variation (univariate analysis $P = 0.001$). In addition, the GAG haplotype shows association to component 3 ($P = 0.036$) containing IL5, IL7 and inversely S-HsCRP. This association appeared to be mainly attributable to S-HsCRP levels in the component (univariate analysis $P = 0.002$). These associations were independent of BMI, age and sex. The fact that both components associate to the GAG haplotype indicates some interrelation between the haplotype and these components. We were not able to assess association between SELS SNPs or haplotypes and OA subtypes.

**Discussion and conclusion**

In inflammation driven diseases high circulating plasma levels of pro-inflammatory cytokines and S-HsCRP are present well above detection limits of current methods and readily used for diagnostic and prognostic purposes\textsuperscript{28}. Cytokines, however, are known to exert their wide ranged actions also in very low concentrations. The recent introduction of multiplexed cytokine assays facilitates simultaneous measurements of multiple cytokines. However, the described absence of a large scale upregulation or strong association of any of the measured cytokines or chemokines in the plasma of subjects with familial OA at multiple joint sites indicates that, in blood plasma, these markers are not sufficiently suitable to monitor the ongoing OA process. Synovial fluid measurements might better reflect the ongoing disease process since it better reflects the cytokine activities near the site where the disease is mainly active\textsuperscript{29}. PCA analyses of cytokine/chemokines measurements revealed 3 components. Component 1 reflects variation at IL2, IL6, GCSF and IL10 levels, component 2 reflects variation at chemokines MCP, IL8 and MIP and component 3 S-HsCRP (negative), IL5 and IL7. The components seem to reflect different ongoing (patho) physiological processes identified by subjects underlying the components. Component 1 may be classified as a marker of ongoing anti-inflammatory signaling based on the strong involvement of IL10, whereas component 2 shows chemokine signaling and component 3 reflects more acute phase related signaling. The observed (Supplementary Table 1) and known correlation of IL6 to S-HsCRP is, with the current setting in the PCA (Eigen values $> 1$), not reflected in the components since together they do not explain sufficient amount of the variation.

We found a significant negative association for component 2 to hand OA and DD as well as to the SF36 derived PCS. This indicated that especially the subjects that exhibit high functional impairment and have low hand ROA scores have high chemokine levels. It should be noted that, by definition of the GARP selection criteria, subjects of the GARP study with low hand or disc ROA scores have OA at other joint sites. We could not, however, attribute the negative association of hand and disc ROA to positive effects caused by these other joint sites. Given these results it appears the higher levels of chemokines act protective in hand and disc OA among subjects of the GARP study. The strong association of the PCS to component 3 including S-HsCRP

*Scores displayed are the expected haplotypic contribution (independent of the BMI effects) to the mean “principle component regression score” of subjects calculated by the Thesias program. In individuals the expected level is determined by the contribution of the 2 carried haplotypes. The THESIAS program does not allow correction for familial relationship. Alleles are in the following order of SNPs in the SELS gene: -105 (rs28665122G > A), +3705 (rs4965814G > A), and +6218 (rs9874A > G).

Component 1 reflects variation of IL2, IL6, GCSF, IL10 and IL7 levels, component 2 reflects variation of chemokines MCP, IL8 and MIP and component 3 reflects variation of HsCRP (negative), IL5 and IL7.

BMI adjusted $P$-value < 0.05 for contribution of the haplotype GAG to the component score as compared to the other haplotypes as determined by THESIAS. C1 component 1, C2 component 2, C3 component 3.

### Table V

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Number (%)</th>
<th>C1$^\dagger$ mean (95% C.I.)</th>
<th>C2$^\dagger$ mean (95% C.I.)</th>
<th>C3$^\dagger$ mean (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGA</td>
<td>638 (83.3)</td>
<td>-0.25 (-0.61 to -0.11)</td>
<td>-0.27 (-0.58 to -0.05)</td>
<td>0.85 (0.56 to 1.15)</td>
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<tr>
<td>AAG</td>
<td>81 (10.6)</td>
<td>-0.22 (-0.65 to -0.20)</td>
<td>-0.39 (-0.79 to 0.00)</td>
<td>0.83 (0.48 to 1.18)</td>
</tr>
<tr>
<td>GAG</td>
<td>27 (3.5)</td>
<td>0.68 (0.24 to 1.10)$^\ddagger$</td>
<td>-0.23 (-1.06 to 0.61)</td>
<td>1.98 (1.35 to 2.61)$^*$</td>
</tr>
<tr>
<td>GAA</td>
<td>10 (1.3)</td>
<td>-0.68 (-2.57 to -1.21)</td>
<td>-0.37 (-1.38 to 0.64)</td>
<td>0.92 (0.29 to 1.54)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (1.1)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Total</td>
<td>764 (100)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
might reflect impairment of physical functioning mediated or reflected by the individual markers in this component, the observed effects are independent of BMI and ROA status. Due to the relative large amount of missing values in the IL5, IL7 and GCSF data we may have missed specific associations with these markers.

Previously, it has been shown that there is a major upregulation of chemokines in human OA affected cartilage. Our analysis show that, amongst subjects of the GARP study, this upregulation might be less pronounced in subjects with hand OA and disc degeneration of the spine as compared to the other subjects, when correcting for all involved joint sites. This may reflect a different pathophysiological process underlying hand OA and disc degeneration as compared to knee and hip OA. This needs to be further explored in other cohorts of OA patients and especially using control samples.

Given the earlier found associations of SELS SNPs to inflammatory factors measured in blood, we expected to find associations of cytokines and chemokines especially to rs28665122, however, no direct associations of these levels or presence of these cytokines and chemokines were observed for variation at the SELS gene. A recent paper of Seiderer et al. also showed no confirmation of the association for rs28665122 to cytokine levels in a study including patients with intestinal inflammation. As compared to the study in intestinal inflammation patients, however, Curran et al. used a more sensitive method of measuring cytokines. Seiderer et al. did observe an association of the pro-inflammatory allele to higher serum CRP levels in a subgroup of Crohn disease patients with higher signs of the pro-inflammatory allele to higher serum CRP levels in et al.

did not affect formation-maximization (EM) algorithm did not affect formation-multiplication using a winMICE implemented expectation-maximization (EM) algorithm did not affect formation of components or subsequent associations (data not shown). The components reported should be considered robust. The effect of the familial dependencies on the component formation is considered to be minimal as the total number of pairs in the dataset cancels out possible intra sibling pair correlations. This is strengthened by the results of an analysis of the data using only one member per sibling pair (data not shown) which shows highly similar component and scores.

Moreover, by excluding individuals with over four out of nine missing values we may have excluded individuals with a particular low-inflammatory profile. Finally, missing values in the qualitatively analyzed cytokines may likewise have been subject to possible misclassification of individuals due to bad sample quality, assay errors or measurement problems.

Since OA patients are likely to use drugs which alter the immune system we explored whether the use of NSAIDs significantly influenced our results. In a split analysis for use vs no use of NSAIDs the formation of the components in the PCA showed no major changes, except for the third component in NSAID users where IL5 and IL7 disappeared. The described subsequent associations remained present in both separate datasets (data not shown).

In earlier investigations of the GARP study, we have found that S-HsCRP levels are independently associated to a CRP haplotype and by the occurrence of the closely related factors knee ROA, BMI and high Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. In the current study we show that a SELS haplotype is additionally influencing the S-HsCRP level. When we fitted a mixed model with S-HsCRP as dependent variable and as co-variables the CRP haplotype H7/8, SELS haplotype GAG, and the factor representing knee ROA, BMI and WOMAC, it was shown that all three consistently and independently influence the S-HsCRP level. In this model, the SELS haplotype (GAG) significantly decreased (beta = –0.42; P = 1.9 x 10^-3) S-HsCRP levels in carriers whereas the S-HsCRP haplotype H7/8 (beta = 0.14; P = 0.08) and the PCA component (beta = 0.14; P = 1.6 x 10^-6) increased S-HsCRP level.

The strength of the GARP study lies in the availability of extended clinical and radiological data of OA features for four joint sites. Furthermore, demographic data for the participants is available, as well as additional familial information, a range of biological fluids and DNA. A downside for this study is, however, that the sample size is relatively small for genetic studies and no synovial fluid samples and control samples of healthy individuals are available for the cytokine measurements in this study.

The analysis of a range of cytokines as measured in blood has not shown any strong associations of one single cytokine to OA features; however, a component comprised of several chemokines did show association to OA in smaller joints and PCS. Another component which has strong involvement of S-HsCRP shows a highly significant association to this PCS, indicating physical impairment might be reflected or mediated by the markers in this component, independent of presence of specific ROA subtypes. Future measurements of chemokines and related signaling proteins in synovial fluid may shed more light on the origin of this association. Long term effects of lower circulating S-HsCRP, cytokine or chemokine levels might be reflected by OA progression. Upcoming follow up data in the GARP study will reveal whether these inflammatory parameters associate to further active progression of OA.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank all participants of the GARP study. For the GARP study, the Dutch Arthritis Association, the Netherlands Organization for Scientific Research and Pfizer Inc., Groton, CT, USA, provided generous support. In addition, we
acknowledge the support of the cooperating hospitals and referring rheumatologists, orthopedic surgeons and general practitioners. The work described in this paper was supported by the Netherlands Organization of Scientific Research (MW 904-61-095, 911-03-016, 917 66344 and 911-03-012), Leiden University Medical Centre, the Centre for Medical Systems Biology (CMSB) in the framework of the Netherlands Genomics Initiative (NGI). Furthermore, we thank Dennis Kremer for work and support to the genotyping.

Supplementary material


References