Genes and mediators of inflammation and development in osteoarthritis

Bos, S.T.

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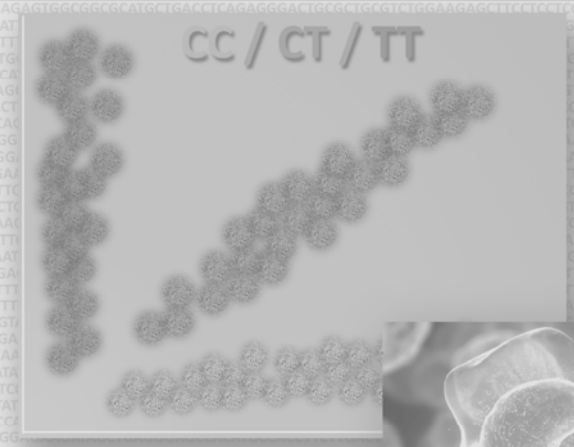

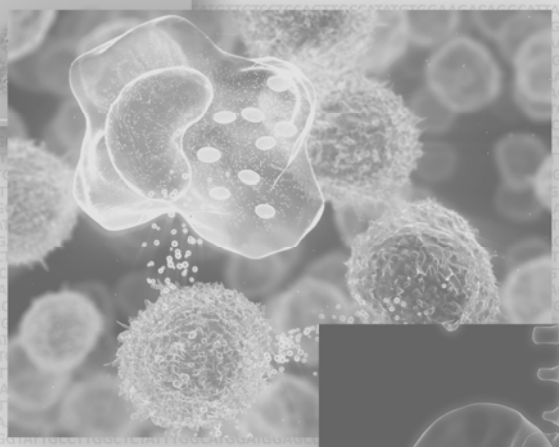

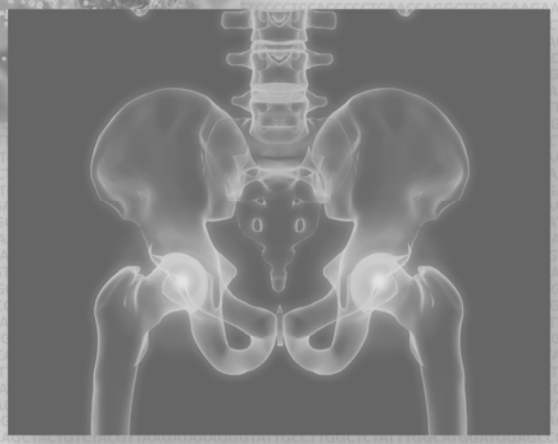

Allelic variation at the C-reactive protein gene associates to both hand osteoarthritis severity and serum high sensitive CRP levels in the GARP study.

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Abstract

Objective: To gain more insight into the role of genetic variation of the C-reactive protein (*CRP*) gene in serum CRP levels and osteoarthritis (OA).
Methods: Serum high sensitive CRP (S-HsCRP) levels were measured in the Genetics of osteoARthritis and Progression (GARP) study. Furthermore, to assess genetic variation of the *CRP* gene, genotypes of five tagging single nucleotide polymorphisms were assessed in the GARP study and a random control sample.
Results: A significant and consistent relation between S-HsCRP levels and observed haplotypes was identified. Additionally, a *CRP* haplotype, which also associated to a significantly higher expected phenotypic mean S-HsCRP level, was associated to severe hand OA. This haplotype was tagged by a single nucleotide polymorphism (rs3091244). Carriers of this allele have an increased risk for the presence of severe hand OA with an OR of 2.3 (95% confidence interval 1.2 to 4.3, p=0.009).
Conclusions: A haplotype of the *CRP* gene, associated to high basal S-HsCRP level, is also associated to severity of hand OA, indicating that innate high basal S-HsCRP levels may influence OA onset.

Introduction

Osteoarthritis (OA) is characterised by degeneration of articular cartilage and remodelling of bone. Heritability estimates range from 30% to approximately 80%, depending on the specific joint affected or the number of joint sites involved. Although OA pathophysiology lacks a large-scale inflammatory process, there may be a low-grade systemic inflammatory component[1,2]. Chondrocytes are known to respond to pro-inflammatory stimuli by decreasing synthesis of extracellular matrix components and increasing synthesis of metalloproteinases. As such, an innate low-grade pro-inflammatory state of the body may affect susceptibility to the onset of OA[2], or may exacerbate progression once the OA disease process is initiated[3].
S-HsCRP is a sensitive marker of both low-grade[4] and acute phase systemic inflammation[5]. Previously, *CRP* haplotypes (locus 1q23.2) were identified that may partly explain the heritability of S-HsCRP levels (52%)[6,7]. Furthermore, S-HsCRP level, as a marker of low-grade inflammation, has been associated to a range of OA features[4,8-10]. In the current study we would like to investigate whether the innate inflammatory state, as expressed by the S-HsCRP level and variation at the *CRP* gene, contributes to the presence of OA in the Genetics of osteoARthritis and Progression (GARP) study.

Material and methods

The GARP study

The ongoing GARP study consists of 191 (n=382) Caucasian sibling pairs affected with symptomatic OA at multiple sites. For the current paper genotypic information was available for 381 individuals and S-HsCRP levels for 353 individuals. Detailed descriptions of the phenotypes and inclusion criteria can be found elsewhere[11]. In the current paper "quantitative hand OA" was defined by the number of hand joints (out of 20 scored) with radiographic OA (ROA). "Severe hand OA", as a qualitative measure, was defined by

presence of seven or more ROA affected hand joints, equalling 27% of subjects. Partners of the offspring in the Leiden longevity study were used as a random control population (n=739)[12].

Statistical analysis

Haplotypic means were assessed using Thesias V3.1[13]. Haplotypic associations were analysed by testing the particular haplotype to the remaining haplotypes. To assess the strength of association to severe hand OA a logistic regression analysis was performed in STATA. In this analysis robust standard errors were estimated from the variance between sibling pairs to compensate for familial relationships within GARP[14]. A linear mixed model was tested to assess association between S-HsCRP levels and OA phenotypes, with family numbers included as random variables to model possible familial effects. Differences in allele frequencies between subjects with and without severe hand ROA were calculated by Pearson's χ^2. Analyses were done in SPSS14.0 unless mentioned otherwise.

Results

Study characteristics

For 381 GARP subjects and 739 controls genotypes were completed. Baseline characteristics of these are shown in table 1.

Table 1. Characteristics of the GARP study and the random control population.

	GARP	Control
Total, no.	382[1]	739
Women, no. (%)	311 (81.4)	429 (58.1)
Age, median (range) years	59.7 (42.7-79.4)	58.3 (30.0-79.0)
BMI[2], median (range)	26.0 (19.1-46.5)	
S-HsCRP[3], mean (SE of mean)	3.63 (0.29)	
S-HsCRP[3], median (range)	1.83 (0.21-56.8)	
Mean number of affected hand joints (range)	4.62 (0-20)	
Subjects with severe hand OA (%)	103 (27)	

[1] GARP study sample consists 191 sibling pairs, for 381 subjects DNA was available.
[2] BMI stands for Body Mass Index in kgm^{-2}
[3] S-HsCRP stands for Serum High Sensitive CRP level in mgl^{-1}, numbers are calculated for subjects with S-HsCRP levels available (N=353). In all analysis logarithmic transformed values of S-HsCRP were used.

C-reactive protein gene haplotype frequencies

As is shown in table 2, six common haplotypes were resolved with frequencies ranging from 0.01 to 0.33. The frequencies in GARP and the control population were comparable with the frequencies observed by Carlson *et al*[6]. No significant differences in haplotype frequencies were found between the GARP study and the control population.

Association of C-reactive protein haplotypes with high sensitive C-reactive protein serum levels

Figure 1A shows the mean log(S-HsCRP) level for each haplotype within the GARP sample (n=353). Haplotype 1 (H1) has a significant lower (p=0.009), whereas haplotype 7/8 (H7/8) has a significant higher (p=0.02) contribution to the mean log(S-HsCRP) level.

Table 2. Assigned haplotype frequencies, expected phenotypic means of log(S-HsCRP) for GARP and the control sample haplotype frequencies.

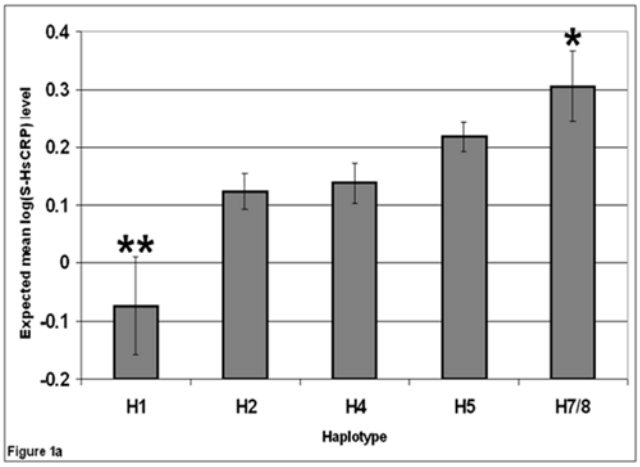
Haplotype[1]	*Study*	*N*	*frequency*	*Log(HsCRP)*[2]	*Se(log(HsCRP))*[2]
Other[3]	GARP	7	0.01	-	-
	Control	7	0	-	-
Haplotype 1	GARP	46	0.07	-0.074	0.084
CACAA	Control	97	0.07	-	-
Haplotype 2	GARP	199	0.28	0.123	0.030
CAGAA	Control	389	0.26	-	-
Haplotype 3	GARP	7	0.01	0.	0.10
CAGGA	Control	10	0.01	-	-
Haplotype 4	GARP	188	0.27	0.137	0.034
CAGGG	Control	423	0.29	-	-
Haplotype 5	GARP	217	0.31	0.217	0.026
TTGGA	Control	482	0.33	-	-
Haplotype 7/8	GARP	42	0.06	0.306	0.062
AAGGA	Control	70	0.05	-	-
TOTAL	GARP	706	1	-	-
	Control	1478	1	-	-

[1] Genotyping was done on a Sequenom™ platform with slightly modified protocols. SNPs used to resolve haplotypes with gene positions relative to AFF449713 and minor allele frequencies were rs3091244 , 1440 (C>T>A, 0.315/0.057), rs1417938 1919 (A>T, 0.248), rs1800947 2667 (G>C, 0.063), rs2808630 5237 (A>G, 0.268) and rs2808628 (A>G, 0.336). The latter SNP is in close LD to SNP rs1205 used in the original study by Carlson *et al.*[6], of which the haplotype nomenclature used was adapted.
[2] Levels displayed are the expected haplotypic contribution to the mean log(S-HsCRP) level of carriers as calculated by the Thesias program. In individuals the expected S-HsCRP level is determined by the contribution of the 2 carried haplotypes. The Thesias program does not allow correction for familial relationship.
[3] Rare haplotypes with frequencies below 0.01 were pooled as "other".

Association of C-reactive protein haplotypes with osteoarthritis subtypes

A significant positive association of H7/8 was observed for increasing number of ROA affected hand joints. As shown in fig 1(B), quantitatively a significantly higher (p=0.04) expected mean number of affected hand joints was observed for H7/8 (mean 3.88, SE 0.66) as compared with remaining haplotypes (mean 2.23, SE 0.13).

Subsequently it was investigated whether H7/8 associated to GARP subjects with severe hand ROA (n=103). The frequency of H7/8 in severe hand ROA cases (frequency 0.096) was significantly higher as compared with the other subjects of GARP (frequency 0.04, p=0.038) and as compared with a random control sample (n=739, frequency 0.046, p=0.016). H7/8 is discriminated by the rarer allele of single nucleotide polymorphism rs3091244. Carriers of the A allele have an increased risk (p=0.009) of severe hand OA as compared with the random controls with a crude OR of 2.3, 95% CI 1.2 to 4.3. The frequency of the A allele does not allow robust recessive model testing. Adjusting for age and/or body mass index in the logistic regression did not change the extent or significance of the genotypic risk.



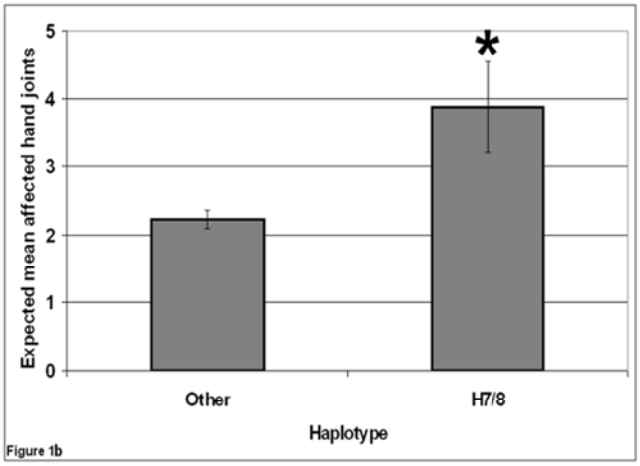


Figure 1 (**a**) Expected mean log(S-HsCRP) levels of common (frequency >0.05) *CRP* haplotypes in the GARP sample for which both genotype and S-HsCRP levels were available (N=353). H1 to H7/8 represent clades of the phylogenetic tree of the SNPs in the *CRP* gene, where H1 is the most recent and H7/8 represent the most ancient split (See Carlson *et al.*[6]). (**b**) Expected mean number of affected hand joints of common (frequency >0.05) *CRP* haplotypes in the GARP study (N=381).
Error bars represent standard error of the mean, * $P < 0.05$ ** $P < 0.01$

There were no significant differences in *CRP* haplotype frequencies between subjects with and without involvement of knee, hip, spine or extent of clinical features of OA expressed by WOMAC (Western Ontario MacMaster osteoarthritis questionnaire) scores.

Association of serum high sensitive C-reactive protein levels and osteoarthritis
Moderate positive associations were observed between S-HsCRP levels and both knee ROA (p=0.06) and WOMAC scores for pain and stiffness (p=0.08). Both these associations, however, were merely due to their association with high body mass index. We could not asses direct association between S-HsCRP levels and hand OA.

Discussion

S-HsCRP serum levels and *CRP* gene haplotypes were assessed in the GARP study to investigate the role and extent of low inflammatory processes in the development of symptomatic OA at multiple joint sites. We show that mean and median basal S-HsCRP levels observed in the GARP study as a whole are not within acute phase ranges[6], confirming that OA is not a large-scale inflammatory disorder.
Furthermore, *CRP* haplotypes, with frequencies ranging from 0.01 to 0.31, showed a specific pattern of mean S-HsCRP level. An increasing S-HsCRP level from H1 to H7/8 was observed, which coincides with the phylogenetic clades of the *CRP* gene[6]. This may indicate an evolutionary development towards low innate S-HsCRP levels. Although the mean S-HsCRP level in GARP was slightly higher (approximately 1 mg/l) the specific haplotypic pattern was strikingly similar to the one identified in the study of Carlson *et al.* in healthy individuals[6]. Of these haplotypes, H1 had a significantly lower and H7/8 had a significant higher expected mean S-HsCRP level as compared with other haplotypes. Furthermore, an allele that discriminates H7/8 associated to the mean number of affected hand joints with an OR of 2.3 for the presence of severe hand OA. The low-grade pro-inflammatory profile brought about by this single nucleotide polymorphism may affect cartilage homeostasis and may ultimately lead to a systemic form of OA. As in many genetic studies we cannot exclude the possibility of false positive findings due to multiple testing; however, this is the first report of an association of a *CRP* gene polymorphism to OA. Punzi *et al*[9] showed an association of erosive hand OA and high serum CRP levels. Despite the association between H7/8 of both S-HsCRP levels and hand OA, no direct association between S-HsCRP levels and hand ROA could be established in this study. Initial associations observed between, S-HsCRP levels and knee ROA and WOMAC scores in the GARP study were merely confounded by body mass index[15]. Our study may either not provide enough power to show associations between S-HsCRP profiles and other OA features, or acute phase responses, by, for example, obesity, may obscure association of disease and innate ongoing low-grade inflammatory effects. Furthermore, it is known that S-HsCRP may not cover the whole spectrum of inflammatory processes, therefore, future studies may focus also on other inflammatory mediators in relation to OA. To show absence of familial effects in our data the analyses were repeated in unrelated individuals of the GARP study yielding similar results (supplemental figure 1).
Together the current study confirms that genetic contribution of the low-grade basal CRP levels may be attributed to haplotypes of the *CRP* gene. Furthermore, it is shown that a specific systemic low-grade pro inflammatory profile may predispose to severe hand ROA among subjects of the GARP study as compared with healthy individuals. To investigate further the role of CRP in OA of the hand, upcoming progression data in this study may provide more insights into the prognostic effect of *CRP* haplotypes and in baseline CRP levels.

Acknowledgments

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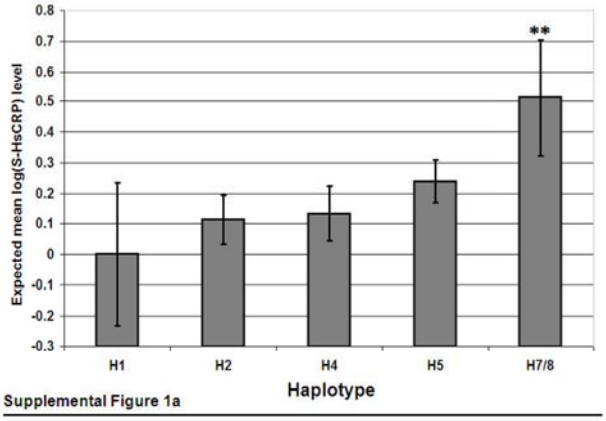
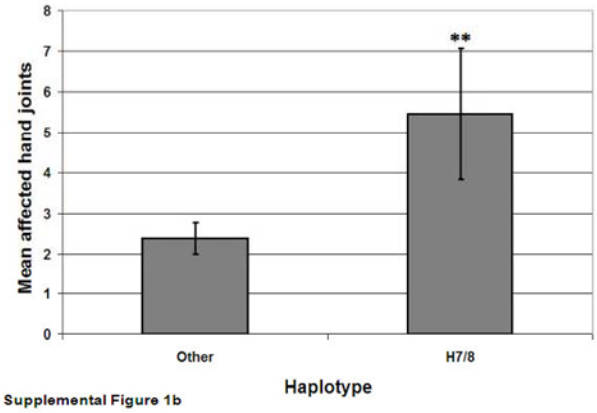


Supplemental Figure 1a



Supplemental Figure 1b

Supplemental Figure 1. (**a**) Expected mean log(S-HsCRP) levels of common (frequency >0.05) *CRP* haplotypes in the unrelated GARP sample for which genotype and S-HsCRP levels were available (N=187). (**b**) Expected mean number of affected hand joints of common (frequency >0.05) *CRP* haplotypes in the unrelated GARP sample (N=191).
Error bars represent standard error of the mean, ** $P < 0.01$