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Genetic Effects on Baseline Values of C-Reactive Protein and Serum Amyloid A Protein: A Comparison of Monozygotic and Dizygotic Twins

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Background: **C-Reactive protein (CRP) and serum amyloid A protein (SAA) are exquisitely sensitive acute-phase reactants, but their baseline values are surprisingly constant in individuals in the general population. These values, especially of CRP, are associated with future atherothrombotic events, and the determinants of baseline CRP and SAA concentration are therefore of considerable interest.**

Methods: **CRP and SAA concentrations were measured by well-validated automated microparticle capture enzyme immunoassays, standardized on the respective WHO International Reference Standards, in serum from 146 monozygotic and 164 dizygotic healthy female UK twin pairs from the general population, with mean (range) ages of 58.0 (40–69.6) and 55.7 (40–70.3) years, respectively, who were also very closely matched for height, weight, body mass index, blood pressure, and lifestyle variables. Statistical modeling based on variance components analysis was used to estimate the genetic contribution to the observed values.**

Results: **As reported previously, CRP values were associated with body mass index, smoking, and hormone replacement therapy. After exclusion of the few samples with CRP concentrations >10 mg/L, which indicate an ongoing acute-phase response rather than baseline values, and inclusion of adjustments for all known confounding variables, there was significantly higher correlation of CRP and SAA results among monozygotic than among dizygotic twins. The estimated hereditability (95% confidence interval) of baseline values was 52% (40–62%) for CRP and 59% (49–67%) for SAA.**

Conclusion: **There is a substantial genetic contribution to baseline serum concentrations of CRP and SAA.**

Serum concentrations of C-reactive protein (CRP),[3] the classic acute-phase protein, cover a remarkable dynamic range and may exceed 500 mg/L at the peak of the acute-phase response *(1)*. The median baseline concentration in ostensibly healthy adults is very low, ~0.8 mg/L in carefully screened individuals *(2)* and 1–2 mg/L in unscreened general populations *(3–5)*. However, the recent application of sensitive immunoassay procedures has shown that modest increases in CRP concentrations, well within the previously adopted "normal range" of up to 10 mg/L, significantly predict coronary events in patients with angina and, remarkably, in asymptomatic adult populations *(6, 7)*.

Serum amyloid A protein (SAA) is the other major human acute-phase protein with a very wide dynamic range, ~1–2000 mg/L. It has been much less extensively studied than CRP in relation to cardiovascular disease, but SAA concentrations in severe unstable angina behaved similarly to those of CRP *(8)*. These findings suggest an association between inflammation and atherothrombotic events, but the stimuli for and the effects of the acute-phase response are not known.

Studies of individual variation in the baseline and acute-phase circulating concentrations of these proteins have been limited. The typical low baseline concentrations of both CRP and SAA are rather constant for each individual, with occasional higher values probably reflecting responses to intercurrent subclinical events *(9, 10)*. Indeed, in the general population, the regression dilution ratio for pairs of CRP measurements made up to

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[3] Nonstandard abbreviations: CRP, C-reactive protein; SAA, serum amyloid A protein; MZ, monozygotic; DZ, dizygotic; and BMI, body mass index.

several years apart is ~0.6 *(11–14)*. Acute-phase responses of CRP and SAA generally closely reflect the extent and activity of the underlying disease *(1)*, but studies of rheumatoid arthritis and other diseases indicate that even individuals with apparently identical disease activities may mount widely varying acute-phase responses *(15, 16)*.

In the absence of an ethically acceptable standardized experimental stimulus, it is not possible to study individual acute-phase responsiveness in humans. However, the extent to which baseline concentrations of CRP and SAA are genetically determined is of considerable interest, and we report here, for the first time, values in large well-matched cohorts of monozygotic (MZ) and dizygotic twins (DZ). This extends recent observations of CRP values among MZ twins alone *(17)*.

Materials and Methods

TWIN ASCERTAINMENT

The study was conducted in female twins who were ascertained through a media campaign that targeted healthy volunteers. Details of the recruitment are given elsewhere *(18)*. The twins had volunteered to take part in a study on aging but were unaware of any specific hypothesis under test. The characteristics of the twins who volunteered were similar to those of a population of apparently healthy individuals sampled from a general practice *(19)*. All twins were interviewed by a nurse practitioner and completed questionnaires concerning their physical health and drug treatment; height, weight, and blood pressure were measured. Venous blood samples were obtained at a similar time of day at the start of the assessment, and separated serum was stored frozen at −45 °C until the time of assay 6–36 months later. Zygosity was determined by questionnaire supplemented by DNA fingerprinting in cases with disputed or uncertain zygosity.

MEASUREMENT OF CRP AND SAA CONCENTRATIONS

Serum concentrations of CRP and SAA were measured by automated microparticle capture enzyme immunoassay, precisely as reported previously *(9, 10)*. The CRP assay provides mg/L concentrations to one decimal place, and the SAA assay provides mg/L values to whole integers only. Both assays were standardized on the respective WHO International Reference Standards *(20, 21)*.

ANALYTICAL APPROACH

MZ twins are genetically identical. DZ twins share, on average, one-half their genetic material. Both groups can be assumed to share cultural and family environments to an equal extent. Hence, demonstrating greater phenotypic correlation in MZ compared with DZ pairs provides an indication that genetic factors are involved in determining a trait. The relative contribution of genetic and environmental variation to a trait can be assessed quantitatively by variance components analysis based on the pattern of correlation among the twins *(22)*. The approach considers the observed variation in CRP and SAA concentrations in the population to be attributed to genetic and environmental components. The genetic contribution to variation has a potential contribution from additive (*A*) and nonadditive or dominance (*D*) variation. Environmental variation has a potential contribution from variation in the common family environment of the twins (*C*) and variation that is unique to individual twins (*E*). The twin model stipulates that the phenotypic covariance (Cov) among twins can be expressed in terms of these variance components such that:

$$\text{Cov (MZ)} = A + D + C$$

$$\text{Cov (DZ)} = 0.5A + 0.25D + C$$

The extent to which the observed patterns of variation and covariation among traits measured in MZ and DZ twin pairs can be accounted for by contributions from *A*, *D*, *C*, and *E* can be assessed by comparing the fit of a set of nested models from which variance components are sequentially removed. The significance of the contribution of individual variance components is assessed by the change in model χ^2 statistics. The parameter estimates of the model that provides the best balance of fit and parsimony give a quantitative measurement of the relative contribution of the size of each variance component. The analysis was confined to pairs in which both twins had a CRP value ≤10 mg/L, this being the 99th centile of the CRP distribution in carefully screened adult volunteer blood donors in the UK *(2)*. Values of SAA and CRP were log-transformed to achieve a gaussian distribution. CRP and SAA concentrations are known to be influenced by age, body mass index (BMI), smoking history, and use of hormone replacement therapy. To take their potential effects into account, the variance components analysis was carried out on the residuals of a regression analysis in which all these confounding variables were included.

Both baseline and acute-phase CRP and SAA values are known to be correlated in individuals. The extent to which shared genetic and environmental factors might explain this correlation was investigated here by considering a set of bivariate models, constructed as a Cholesky factorization *(22)*, which included genetic and environmental variance components that were both unique to CRP and SAA and shared between them. Parameter estimates from the most appropriate bivariate models provide an estimate of the extent to which genetic and environmental factors contribute to the phenotypic correlation between CRP and SAA in individuals.

Results

Results were available for 146 MZ and 164 DZ twin pairs, well matched for age, BMI, and lifestyle variables, and none of whom had either chronic disease or significant intercurrent illness (Table 1).

The distributions of CRP and SAA in the MZ and DZ

Table 1. Characteristics of the twins.

	MZ twins (292 individuals)	DZ twins (328 individuals)
Age, mean (range), years	58.0 (40–69.6)	55.7 (40–70.3)
Height, mean (SD), cm	161.3 (5.8)	162.2 (6.0)
Weight, mean (SD), kg	63.0 (10.1)	64.6 (11.1)
BMI, mean (SD), kg/m^2	24.2 (3.2)	24.6 (4.2)
Current smoking, %	16	20
Current HRT[a] use, %	25	22
Postmenopausal, %	90	80
Systolic BP, mean (SD), mmHg	137 (22)	135 (22)
Diastolic BP, mean (SD), mmHg	83 (12)	82 (11)
Antihypertensive drugs, %	10	18

[a] HRT, hormone replacement therapy; BP, blood pressure.

twins presented as singletons are shown in Table 2. The range and distribution of values of both analytes were comparable to those reported for other healthy general populations *(3–5, 23)*. The slightly lower CRP values in these twins reflect the more rigorous screening for genuinely healthy individuals than is the case in the very much larger surveys of general populations, and the present CRP values thus correspond more closely to those seen in volunteer blood donors *(2)*. In the group as a whole, CRP values were associated with BMI, with smoking, and with the use of hormone replacement therapy, all consistent with other published studies *(7)*, with a trend toward higher CRP concentrations in smokers and those with high BMI. As expected in healthy individuals, values in the great majority of individuals fell below the generally accepted upper limit of the reference interval (upper limit of normal) of 10 mg/L for both CRP and SAA *(2, 9)*. CRP exceeded 10 mg/L with similar frequencies in the two groups, 7 MZ and 14 DZ pairs, and these were excluded from further analysis. However, inclusion of these higher values did not significantly affect the conclusions (analysis not shown).

Shown in Fig. 1 are the distributions of CRP and SAA in the MZ and DZ twins among pairs in which both twins had CRP values ≤10 mg/L, with values adjusted for age, BMI, smoking status, and the use of hormone replacement therapy. Both showed a higher correlation in MZ compared with DZ twins, indicating a genetic contribution.

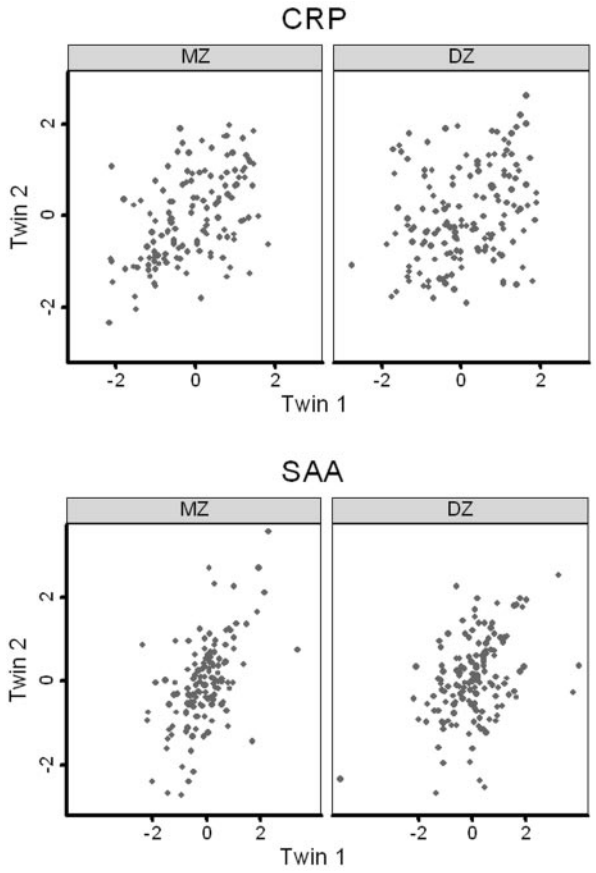

Fig. 1. Scatterplots showing pairwise distributions of the standardized residuals (SD from the mean) of the regression analysis in which log-transformed CRP (*top*) and SAA (*bottom*) values were regressed against age, BMI, systolic blood pressure, smoking status, and the use of hormone replacement therapy.

The results of statistical modeling are shown in Table 3. For both CRP and SAA, the pattern of variances and covariance in the twins was best explained by a model that contained only additive genetic and unique environ-

Table 2. Distribution of baseline CRP and SAA values in MZ and DZ twins.[a]

	CRP, mg/L		SAA, mg/L	
	MZ	DZ	MZ	DZ
n (individuals)	292	328	292	328
Minimum	0.1	0.1	2	1
25th percentile	0.3	0.3	4	4
Median	0.6	1	5	5
75th percentile	2.5	2.9	7	7
Maximum	41.8	75.3	91	88

[a] There were no significant differences in distribution between MZ and DZ twins (CRP, $P = 0.17$; SAA, $P = 0.38$).

Table 3. Correlation among twins and variance component analysis.

	CRP	SAA
r*MZ*r*mz*[a] (95% confidence interval)	0.49 (0.36–0.62)	0.55 (0.43–0.67)
r*DZ*r*dz* (95% confidence interval)	0.28 (0.14–0.43)	0.38 (0.24–0.52)
Model[b]	AE	AE
Heritability (95% confidence interval)	0.52 (0.40–0.62)	0.59 (0.49–0.67)

[a] *R*, intraclass correlation coefficient.

[b] Model, combination of variance components that represent the best balance of fit and parsimony in variance component analysis. A, additive genetic variance component; E, unique environmental variation. Modeling was conducted on the residuals of a regression analysis in which age, BMI, systolic blood pressure, and the use of hormone replacement therapy were included.

mental variance components (AE model). The relative contribution of additive genetic variance (which equates to heritability) was estimated in the model to be 52% for CRP and 59% for SAA.

Baseline serum concentrations of CRP and SAA among the individuals studied here showed a significant correlation coefficient of 0.4, as expected from previous observations among unrelated individuals *(23)*. When the modeling was extended to consider the full distribution of variances and covariance for CRP and SAA in the twins simultaneously, a bivariate AE model provided the most appropriate fit for data. In this model, 20% of the phenotypic covariance was accounted for by shared genetic factors ($P = 0.05$). Thus, genetic factors shared between CRP and SAA make a significant contribution to the correlation in baseline concentrations in individuals.

Discussion

The significantly higher degree of correlation in baseline serum CRP and SAA concentrations among healthy female MZ compared with DZ twins is consistent with an important contribution of genetic factors to these values. Our results show that approximately one-half of the variation in baseline values of CRP and SAA can be accounted for by genetic variation in the population. These estimates could not be accounted for by differences in physical or lifestyle characteristics of the MZ and DZ twins. The short half-lives of the acute-phase proteins obviously preclude a possible direct influence of differences in the shared environment between MZ and DZ twins in earlier life, which has been raised as a concern in assessing the results of twin studies for other variables *(24)*, but it does not exclude potential early life exposure to, e.g., a persistent pathogen.

Our results provide evidence that common genetic factors are involved in determining the baseline values of CRP and SAA. This is consistent with several possible mechanisms, for example, genetically determined polymorphisms in the proinflammatory cytokine cascade that regulates hepatic acute-phase protein gene expression, and some candidates with respect to CRP, which could also affect SAA production, have been suggested *(25, 26)*. No amino acid sequence polymorphism of human CRP has been reported, but there may be otherwise silent intronic or regulatory genetic polymorphisms in the CRP gene that affect CRP production *(27–29)*. In contrast, the acute-phase component of SAA, which was measured here, is a polymorphic family of proteins, and no studies have been reported of associations between particular isoforms and different baseline values. The present study did not include any genetic analysis of either CRP or SAA genes in these twin populations.

It is important to note that the correlations in the present study were sought only after exclusion of values from twin pairs in which one or more values exceeded the reference interval. Both CRP and SAA are exquisitely sensitive and extremely rapidly responsive systemic markers of inflammation and tissue damage, with enormous dynamic ranges, so that any acute-phase response value of either protein must completely obscure genetic determinants of the very low baseline values that are the subject of this study.

Inflammation is a very important pathogenetic component of both atherosclerosis and atherothrombotic events. There is clearly inflammation locally within atherosclerotic plaques. There are also systemic signs of inflammation, indicated most notably by the extremely sensitive circulating acute-phase proteins, although the source and identity of the triggers for their low-level up-regulation that predicts atherothrombotic events are not known. Thus it is not clear whether the modest increases in baseline CRP values that predict future coronary events reflect inflammation in atherosclerotic vascular lesions or low-grade inflammation elsewhere in the body that is either associated with or promotes atherogenesis and atherothrombosis. A further key unknown factor is whether acute-phase proteins, in particular CRP, which binds LDLs and can activate complement, and SAA, which is an apoprotein of HDL, may themselves contribute to the pathogenesis of atherothrombosis. Although the present demonstration that baseline CRP and SAA values are significantly genetically determined is of interest and potentially important in this regard, it still does not enable epidemiologic studies to establish or refute causal relationships between these proteins and cardiovascular disease. This will require specific drug or other interventions that selectively target the individual proteins and the demonstration that such maneuvers can affect cardiovascular disease outcomes.

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References

1. Pepys MB. The acute phase response and C-reactive protein. In: Warrell DA, Cox TM, Firth JD, Benz EJ Jr, eds. Oxford textbook of medicine, 4th ed., Vol. 2. Oxford: Oxford University Press, 2003: 150–6.
2. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clin Chim Acta 1981;117:13–23.
3. Hutchinson WL, Koenig W, Fröhlich M, Sund M, Lowe GDO, Pepys MB. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clin Chem 2000;46:934–8.
4. Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. Clin Chem 2003;49:666–9.
5. Imhof A, Fröhlich M, Loewel H, Helbecque N, Woodward M,

Amouyel P, et al. Distributions of C-reactive protein measured by high-sensitivity assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669–72.
6. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199–204.
7. Pepys MB, Hirschfield GM. C-Reactive protein: a critical update. J Clin Invest 2003;111:1805–12.
8. Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, et al. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. N Engl J Med 1994;331:417–24.
9. Wilkins J, Gallimore JR, Tennent GA, Hawkins PN, Limburg PC, van Rijswijk MH, et al. Rapid automated enzyme immunoassay of serum amyloid A. Clin Chem 1994;40:1284–90.
10. Wilkins J, Gallimore JR, Moore EG, Pepys MB. Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. Clin Chem 1998;44:1358–61.
11. Ridker PM. Inflammation, atherosclerosis, and cardiovascular risk: an epidemiologic view. Blood Coagul Fibrinolysis 1999;10: S9–12.
12. Kayaba K, Ishikawa S, Gotoh T, Nago N, Kajii E, Nakamura Y, et al. Five-year intra-individual variability in C-reactive protein levels in a Japanese population-based study. The Jichi Medical School Cohort Study at Yamato, 1993–1998. Jpn Circ J 2000;64:303–8.
13. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem 2001;47:444–50.
14. Koenig W, Sund M, Fröhlich M, Löwel H, Hutchinson WL, Pepys MB. Refinement of the association of serum C-reactive protein concentration and coronary heart disease risk by correction for within-subject variation over time. The MONICA Augsburg Studies, 1984 and 1987. Am J Epidemiol 2003;158:357–64.
15. van Leeuwen MA, van Rijswijk MH, Sluiter WJ, van Riel PLCM, Kuper IH, van de Putte LBA, et al. Individual relationship between progression of radiological damage and the acute phase response in early rheumatoid arthritis. Towards development of a decision support system. J Rheumatol 1997;24:20–7.
16. Fagan EA, Dyck RF, Maton PN, Hodgson HJF, Chadwick VS, Pepys MB. Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. Eur J Clin Invest 1982;12:351–60.
17. Retterstol L, Eikvar L, Berg K. A twin study of C-reactive protein compared to other risk factors for coronary heart disease. Atherosclerosis 2003;169:279–82.
18. Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D. Genetic influences on osteoarthritis in women: a twin study. BMJ 1996; 312:940–3.
19. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin Res 2001;4:464–77.
20. WHO Expert Committee on Biological Standardization, 37th Report. WHO Technical Report Series 760. Geneva, Swwitzerland: WHO, 1987;21–2.
21. Poole S, Walker D, Gaines Das RE, Gallimore JR, Pepys MB. The first international standard for serum amyloid A protein (SAA). Evaluation in an international collaborative study. J Immunol Methods 1998;214:1–10.
22. Neale MC, Cardon LR. Methodology for genetic studies of twins and families. Dordrecht, The Netherlands: Kluwer Academic, 1992:496pp.
23. Danesh J, Muir J, Wong Y-K, Ward M, Gallimore JR, Pepys MB. Risk factors for coronary heart disease and acute-phase proteins. A population-based study. Eur Heart J 1999;20:954–9.
24. Phillips DIW. Twin studies in medical research: can they tell us whether diseases are genetically determined? Lancet 1993;341: 1008–9.
25. Berger P, McConnell JP, Nunn M, Kornman KS, Sorrell J, Stephenson K, et al. C-Reactive protein levels are influenced by common IL-1 gene variations. Cytokine 2002;17:171–4.
26. Vickers MA, Green FR, Terry C, Mayosi BM, Julier C, Lathrop M, et al. Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res 2002;53:1029–34.
27. Zee RY, Ridker PM. Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. Atherosclerosis 2002;162:217–9.
28. Szalai AJ, McCrory MA, Cooper GS, Wu J, Kimberly RP. Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. Genes Immun 2002;3:14–9.
29. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, et al. A polymorphism in the human CRP gene influences basal and stimulated CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Atherosclerosis 2003;168: 192.