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Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study

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Abstract

Inflammation is thought to play a central role in the etiology and outcome of atherosclerosis. Animal studies as well as in vitro and in vivo human studies suggest that host factors modulate the magnitude and extent of inflammatory responses. We investigated familial aggregation of three systemic markers of inflammation (C-reactive protein (CRP), white blood cell count (WBC), and albumin) in a large, cross-sectional study conducted in four US communities. We found evidence of substantial heritability (35–40%) for CRP levels as well as for WBC and albumin levels. Negligible spouse correlations suggested little influence of shared household environment on these traits. The combination of sociodemographic factors (age, center, education), behavioral and lifestyle factors (cigarette smoking, alcohol intake, hormone replacement therapy), obesity and fat patterning, and prevalent diabetes explained 13–30% the interindividual variability of these traits. There was no evidence that these inflammation phenotypes were linked to a microsatellite marker in the interleukin-1 gene cluster on chromosome 2q, a region that includes several candidate genes for chronic inflammatory diseases. Our findings suggest that CRP levels, albumin levels, and WBC are determined at least partially by genetic factors. Further efforts to identify gene loci affecting these traits are warranted. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Inflammation; C-reactive protein; Albumin; White blood cell count; Interleukin-1

1. Introduction

Inflammation is thought to play an important role in the initiation, progression, and clinical outcome of atherosclerosis [1]. A number of prospective epidemiological studies have reported associations between acute-phase proteins and other systemic markers of inflammation and cardiovascular (CVD) events, including coronary heart disease (CHD), stroke, and peripheral arterial disease [2–12]. As reviewed in a recent meta-analysis [13], the associations between inflammation markers such as C-reactive protein (CRP), albumin, white blood cell count (WBC), or fibrinogen and CVD outcomes are remarkably consistent in magnitude despite varying study designs, target populations, ages of participants, lengths of follow-up, and case ascertainment methods. Findings from these observational epidemiological studies, coupled with those emerging from basic science [14,15] and clinical interventions [16], have sparked renewed interest in inflammation as a contributing factor to atherosclerosis and its clinical manifestations.

In general, prospective studies have indicated that individuals with mild elevations in inflammation factors

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within the normal range are at increased risk of CVD events. However, the population determinants of normal variation in acute-phase proteins and other systemic markers of inflammation are not well established. Observational studies or clinical trials have reported that a mild acute-phase response is associated with older age [9,17,18], female sex [18,19], lower socioeconomic status [9,17–19], cigarette smoking [8,9,12,17–19], obesity [8,17,19–21], use of female hormones [22,23], greater alcohol consumption [9], and chronic diseases such as periodontitis [18], chronic bronchitis [18], arthritis [18], diabetes mellitus [8,9,12], and atherosclerotic cardiovascular diseases [8,12,17,20]. Genetic factors likely modify the magnitude and extent of the systemic inflammatory response to these and other stimuli. Genes encoding critical pro-inflammatory cytokines, such as the interleukin-1 gene cluster on chromosome 2q, the interleukin-6 gene on chromosome 7p, and the tumor necrosis factor-α gene on chromosome 6q are now recognized as potential candidate loci for diseases with an inflammatory basis [24,25], including atherosclerotic cardiovascular diseases [26].

Previously, we reported substantial familial and genetic influences on the plasma concentrations of two hemostatic factors, plasminogen activator inhibitor-1, a mild acute-phase protein associated strongly with body fat and insulin levels, and fibrinogen, a major acute-phase protein [27]. In the present analysis, we report on the familial aggregation of three additional systemic markers of inflammation (CRP, WBC, and albumin) and results of variance-component genetic linkage analyses of these factors with a microsatellite marker on chromosome 2q, a region that includes several candidate genes for chronic inflammatory diseases.

2. Methods

2.1. Study population

The NHLBI Family Heart Study (FHS) is an investigation of genetic and nongenetic determinants of coronary heart disease, preclinical atherosclerosis, and cardiovascular risk factors. The design and methods of the NHLBI FHS have been described in detail elsewhere [28]. Briefly, unrelated individuals (probands) were selected from population-based cohort studies in four US communities. In two of the communities (Forsyth County, NC and suburban Minneapolis, MN, USA), probands were participants in the Atherosclerosis Risk in Communities Study. In Salt Lake City, UT, USA probands were participants in the Utah Health Family Tree Study, and in Framingham, MA, USA probands were offspring of members of the original Framingham Cohort Study.

Probands and their relatives were mailed a self-administered medical history questionnaire in phase I of the study. The participation rate for probands was 67%; response rates varied from 63 to 82% across centers. Approximately 86% of eligible relatives completed the questionnaire; response rates varied from 78 to 94% across centers. In phase II of the study, selected probands ages 45 years and older and their immediate family members (i.e. parents, siblings, children, and spouse) ages 25 years and older were invited for a comprehensive physical examination at a local clinic. A total of 5975 individuals from 1151 families completed either a full or abbreviated examination. A total of 541 of these families were ascertained randomly and 610 families were ascertained as high-risk pedigrees because of greater than expected familial burden of CHD. The study was approved by an institutional review committee at each site and the subjects gave informed consent.

For the present analysis, we excluded non-Whites ($n = 59$) because there were insufficient numbers of individuals with IL1A marker data to conduct genetic linkage analyses in other ethnic groups. White blood cell count (WBC) was determined on a total of 5043 individuals and albumin levels were determined on a total of 5627 individuals. Serum CRP levels were determined on a total of 2163 individuals, including, (1), a subsample of unrelated probands from randomly ascertained families ($n = 454$ individuals) and several other (non-mutually exclusive) subsamples selected for genetic linkage studies; (2), a subsample of sibships with at least two siblings with elevated carotid IMT (i.e. above the gender-specific 90th percentile of carotid IMT in two or more arterial segments) ($n = 246$ individuals from 82 sibships); (3), a subsample of sibships in which at least two of the siblings had CHD, defined as self-reported history of myocardial infarction, coronary artery bypass surgery, or coronary angioplasty ($n = 341$ individuals from 82 sibships); (4), a subsample of sibships with at least one sibling above the age-gender specific 80th percentile of the Individual Risk Score (IRS), a score derived for each individual using values from gender-specific proportional hazards models predicting the age-of-onset of CHD from carotid IMT thickness, lipids, body-mass index, blood pressure, hypertension, diabetes, and a CHD family risk score ($n = 1224$ individuals from 353 sibships); and (5), a subsample of the largest pedigrees ($n = 1032$ individuals from 101 pedigrees). IL1A genotypes were determined on a total of 1208 individuals from subsamples 1, 2, and 3 described above and two additional sub-samples, (6), a subsample of sibships in which at least two siblings had familial combined hyperlipidemia ($n = 170$ individuals from 71 sibships); and (7), a subsample of sibships in which at least two siblings had hypertension (i.e. blood pressure $\geq 140/90$ or current use of an antihypertensive medication) ($n = 697$ individuals from 278 sibships).

2.2. Laboratory methods

Participants were asked to fast at least 12 h before arrival at the clinic. Blood was drawn from an antecubital vein of seated participants with free blood flow and minimal trauma. Samples were placed in a −70°C freezer no more than 90 min after venipuncture. Frozen samples were packaged in dry ice, shipped to the FHS Central Laboratory at the Department of Laboratory Medicine and Pathology, University of Minnesota, and stored at −70°C. Serum glucose was measured on the Kodak EKTACHEM Clinical Chemistry Slide (Eastman Kodak, Rochester, NY). White blood cell count (WBC) was determined by standard impedance counting techniques using a Coulter STKS hematology analyzer (Coulter Electronics Inc., Hialeah, FL). Serum albumin was measured by a thin film adaptation of a bromcresol green colorimetric procedure (Corcoran, 1977) using the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY). The coefficient of variation (CV) was 2 and 4% at 2.1 and 4.2 g/dl, respectively. Serum CRP was measured at the Laboratory for Clinical Biochemistry Research, University of Vermont, using a high sensitivity ELISA calibrated with WHO reference material [29]. The intra- and inter-assay CVs were 3 and 6%, respectively.

2.3. Other measurements

Interviewers ascertained current and former cigarette smoking habits, alcohol consumption, and use of female hormones by questionnaire. Technicians measured standing height, rounded down to the nearest centimeter, using a wall-mounted vertical metal ruler. Technicians measured bodyweight, recorded to the nearest pound, using a balance scale. Technicians measured waist and hip circumferences, rounded to the nearest centimeter, at the level of the umbilicus and at the maximum protrusion of the gluteal muscles, respectively. We computed body-mass index (BMI, kg/m^2) and waist-to-hip ratio (WHR). Interviewers obtained information by questionnaire on history of myocardial infarction and coronary revascularization procedures, history of diabetes, and current use of insulin or hypoglycemic medications. An electrocardiogram (ECG) was obtained to assess known and silent myocardial infarction. Carotid B-mode ultrasound examinations were performed with a Biosound 2000IIsa and a common scanning protocol at each of field centers; readings were done by trained and certified readers at a central reading center. Measurements of intimal media thickness (IMT) were derived in the far wall of the right and left extracranial carotid arteries. For this analysis, we used the mean wall thickness in the 1-cm segment of the right and left common carotid artery proximal to the dilatation of the carotid bulb. A DINAMAP 1846 SX automated oscillometric device was used to measure resting blood pressures in both the ankle and the arm. The ankle-brachial index (ABI) was calculated as resting ankle systolic pressure divided by resting brachial systolic pressure.

We classified educational attainment into one of five categories, less than high school, high school graduate/vocational training, some college, college graduate, or graduate/professional school. We defined diabetes as self-reported history of diabetes, nonfasting glucose ≥ 200 mg/dl, fasting glucose ≥ 126 mg/dl, or current pharmacological treatment for diabetes. We defined prevalent CHD as self-reported personal history of myocardial infarction, coronary angioplasty, coronary artery bypass surgery, or ECG evidence of a major Q-wave or minor Q-wave and ischemic ST-T changes as determined by Minnesota coding. We defined subclinical atherosclerotic disease as a mean common carotid IMT ≥ 1 mm or ABI ≤ 0.9.

2.4. Genotyping

The dinucleotide (AC_n) repeat polymorphism in intron 5 of the interleukin-1 alpha gene (IL1A) was determined at the FHS Molecular Laboratory at the University of Utah based on PCR amplification of genomic DNA [30]. PCR reactions were carried out in 96-well trays in a MJ Research PTC-225 thermal cycler instrument (Watertown, MA). The final volume for each reaction was 25 μl. Each reaction contained 100 ng of template DNA, 0.5 Unit of *Taq* polymerase (Boehringer Mannheim, Indianapolis, IN), 10 pmol of both forward and reverse primers, 0.25 pmol of ^{25}P labeled primer A end-labeled with T4 polynucleotide kinase (Molecular Biology Resources, Inc., Milwaukee, WI), 200 μm of dNTPs, 5% DMSO, and PCR buffer (final concentration of 100-mM Tris pH 8.4, 40-mM NaCl, 1.5-mM $MgCl_2$, 0.25-mM spermidine). The PCR temperature conditions were as follows, after an initial denaturation at 94°C, five PCR cycles were carried out with a denaturing phase of 94°C for 20 s, an annealing phase of 58°C for 20 s, and an extension phase at 72°C for 40 s. This was followed by an additional 30 cycles at similar conditions with the exception of the annealing phase which was done at 54°C for 20 s. PCR products were then loaded on a 7% acrylamide gel containing 6.7-M urea and 32% formamide. Electrophoresis of the gel was carried out at a constant 80 W for 4–6 h. The gels were exposed to X-ray film (Hyperfilm, Amersham Life Science, Buckinghamshire, UK) for 12–16 h. Genotypes were scored relative to an M13mp18 sequence ladder according to their size in base pairs. We observed a total of 11 different alleles for the IL1A microsatellite marker among the 1208 participants who were genotyped. The observed heterozygosity of the marker was 68%. IL1A

genotypes were determined on 55 replicate samples. Replicate results were identical on 53 of these samples (96%).

2.5. Statistical methods

We adjusted the phenotypes for the effect of covariates in SAS® by multiple linear regression. We fitted regression models separately for women and men and included covariates in these models regardless of their statistical significance. We used standardized residual values obtained from linear regression in familial correlation and linkage analyses. Because of significant skewness, we logarithmically transformed CRP values prior to the analysis. We split three-generation kindreds into a maximum of two nuclear families for the familial correlation and variance-component linkage analyses. One member of each identical twin pair was excluded randomly from these analyses.

We used the SEGPATH program [31] to estimate familial correlations for serum CRP, WBC, and albumin using maximum-likelihood methods. For each trait, we estimated spouse, sibling, and parent–child correlations after adjusting for age and gender (phenotype 1) and after further adjusting for field center, education, cigarette smoking status and pack-years, alcohol intake, body-mass index, waist-to-hip ratio, diabetes, and in women, hormone replacement therapy (phenotype 2). Results for phenotypes 1 and 2 were compared to assess the extent to which sociodemographic, lifestyle, anthropometric, and metabolic factors could account for age- and gender-adjusted familial correlations. As described above, familial correlations for one of the inflammation markers (CRP) could not be estimated in a true random sample of families because this analyte was measured only in non-random subsets of relatives selected post hoc for more focused and cost-efficient studies of novel risk factors and candidate gene polymorphisms. We conducted variance-components linkage analysis using SEGPATH [32]. In this program, the expected genetic covariance between relatives is modeled as a function of the identity-by-descent (IBD) at a given test locus and the kinship coefficient. The phenotypic heritability (h^2) is partitioned into a component reflecting heritability attributable to a latent trait locus (h_g^2) and residual heritability (h_r^2) attributable to polygenes and other sources of familial resemblance. The null hypothesis, that there is no effect of the test locus on the trait heritability ($h_g^2 = 0$), is evaluated by a likelihood ratio test. The computer package MAPMAKER/SIBS [33] was used to estimate the allele-sharing proportions among siblings. Population marker allele frequencies were estimated from a sample of 181 probands drawn from randomly-ascertained families. We included relatives from both random and high-risk families in linkage analyses.

3. Results

3.1. Participant characteristics and distribution of inflammation markers

The mean age of participants was 52.5 years (range, 25–93 years). Nearly half of the participants (46%) reported a history of cigarette smoking, 13% had prevalent CHD, and 6% had mean common carotid IMT ≥ 1 mm and/or ABI ≤ 0.9. The mean WBC was 6.2 ($\pm$ 1.8) $\times 10^3$/mm^3 and the mean albumin level was 4.10 (± 0.28) g/dl. The median CRP concentration was 1.7 mg/l. There was a moderately strong positive correlation between WBC and log-transformed CRP concentration (Pearson correlation coefficient, $r = 0.31$; $P < 0.01$). Correlations between (ln)CRP and albumin ($r = -0.20$; $P < 0.01$) and between WBC and albumin ($r = -0.04$; $P < 0.01$) were weaker in magnitude.

In multiple regression analyses, the model R^2 indicated that the combination of sociodemographic factors (age, center, education), behavioral and lifestyle factors (cigarette smoking status and pack-years, alcohol intake, hormone replacement therapy), obesity and fat patterning (BMI, WHR), and prevalent diabetes explained 30 and 22% of the interindividual variability in CRP levels in women and men, respectively. In women, these factors explained 20% of the variance in WBC and 13% of the variance in albumin. In men, these factors explained 13 and 20% of the variance in WBC and albumin, respectively.

3.2. Familial correlation analysis

Spouse correlations for CRP were not statistically significantly different from zero (Table 1). By contrast, gender- and age-adjusted correlations for first-degree relatives (i.e. sibling and parent–child correlations) were positive and moderate in size. Familial correlations for CRP remained virtually unchanged when participants with a personal history of smoking, prevalent CHD, or indices of atherosclerosis were excluded and were only slightly attenuated after adjustment for BMI, WHR, and other factors that may aggregate within families (phenotype 2).

Familial correlation patterns for WBC (Table 2) were similar to those for CRP, with spouse correlations near zero and sibling and parent–child correlations ranging from 0.16–0.24. Sibling and parent–child correlations for albumin (Table 3) were positive but somewhat smaller in magnitude than sibling and parent–child correlations for CRP and WBC.

3.3. Variance-components linkage analysis

There was no evidence that the gender- and age-adjusted CRP, WBC, or albumin phenotypes were linked

Table 1
Familial correlations (± standard errors) for serum C-reactive protein (CRP)

Relative pair	All participants	Never smokers	No prevalent CHD	No evidence of subclinical athero
Spouse (pairs)	120	36	64	94
Phenotype 1[a]	−0.06 ± 0.09	−0.01 ± 0.14	0.00 ± 0.11	−0.02 ± 0.10
Phenotype 2[b]	0.02 ± 0.09	0.03 ± 0.12	0.07 ± 0.11	0.11 ± 0.10
Sibling (pairs)	2654	782	1782	2329
Phenotype 1[a]	0.21 ± 0.03	0.22 ± 0.05	0.18 ± 0.03	0.21 ± 0.03
Phenotype 2[b]	0.20 ± 0.03	0.18 ± 0.04	0.19 ± 0.03	0.19 ± 0.03
Parent–child (pairs)	929	266	654	794
Phenotype 1[a]	0.18 ± 0.04	0.22 ± 0.07	0.16 ± 0.05	0.18 ± 0.04
Phenotype 2[b]	0.15 ± 0.04	0.16 ± 0.06	0.13 ± 0.05	0.16 ± 0.05

[a] Phenotype 1, CRP adjusted for gender, age and age^2.
[b] Phenotype 2, CRP adjusted for gender, age, age^2, field center, education, cigarette smoking status and pack-years, alcohol intake, hormone replacement therapy (women), body-mass index, waist-to-hip ratio, and diabetes.

Table 2
Familial correlations (± standard errors) for white blood cell count (WBC)

Relative pair	Random and high-risk families	Random families only			
		All participants	Never smokers	No prevalent CHD	No evidence of subclinical athero
Spouse (pairs)	638	373	131	293	322
Phenotype 1[a]	0.05 ± 0.04	0.03 ± 0.05	0.04 ± 0.08	0.09 ± 0.06	0.02 ± 0.05
Phenotype 2[b]	0.04 ± 0.04	0.04 ± 0.06	0.04 ± 0.09	0.08 ± 0.06	0.03 ± 0.06
Sibling (pairs)	3980	2008	960	1778	1852
Phenotype 1[a]	0.18 ± 0.02	0.20 ± 0.03	0.21 ± 0.04	0.19 ± 0.03	0.21 ± 0.03
Phenotype 2[b]	0.15 ± 0.02	0.18 ± 0.03	0.18 ± 0.04	0.17 ± 0.03	0.19 ± 0.03
Parent–child (pairs)	2607	1463	541	1250	1293
Phenotype 1[a]	0.18 ± 0.02	0.19 ± 0.03	0.24 ± 0.04	0.18 ± 0.03	0.17 ± 0.03
Phenotype 2[b]	0.16 ± 0.02	0.17 ± 0.03	0.23 ± 0.04	0.16 ± 0.03	0.16 ± 0.03

[a] Phenotype 1, WBC adjusted for gender, age and age^2.
[b] Phenotype 2, WBC adjusted for gender, age, age^2, field center, education, cigarette smoking status and pack-years, alcohol intake, hormone replacement therapy (women), body-mass index, waist-to-hip ratio, and diabetes.

Table 3
Familial correlations (± standard errors) for serum albumin

Relative pair	Random and high-risk families	Random families only			
		All participants	Never smokers	No prevalent CHD	No evidence of subclinical athero
Spouse (pairs)	613	384	127	303	333
Phenotype 1[a]	0.12 ± 0.04	0.04 ± 0.05	0.08 ± 0.09	−0.01 ± 0.06	0.05 ± 0.06
Phenotype 2[b]	0.12 ± 0.04	0.07 ± 0.06	0.09 ± 0.09	0.04 ± 0.06	0.07 ± 0.06
Sibling (pairs)	4056	2459	976	2192	2278
Phenotype 1[a]	0.18 ± 0.02	0.18 ± 0.03	0.13 ± 0.04	0.18 ± 0.03	0.19 ± 0.03
Phenotype 2[b]	0.16 ± 0.02	0.14 ± 0.03	0.12 ± 0.04	0.14 ± 0.03	0.15 ± 0.03
Parent–child (pairs)	2679	1663	551	1415	1476
Phenotype 1[a]	0.16 ± 0.02	0.12 ± 0.03	0.18 ± 0.05	0.13 ± 0.03	0.13 ± 0.03
Phenotype 2[b]	0.13 ± 0.02	0.10 ± 0.03	0.17 ± 0.05	0.13 ± 0.04	0.11 ± 0.04

[a] Phenotype 1, albumin adjusted for gender, age and age^2.
[b] Phenotype 2, albumin adjusted for gender, age, age^2, field center, education, cigarette smoking status and pack-years, alcohol intake, hormone replacement therapy (women), body-mass index, waist-to-hip ratio, and diabetes.

Table 4
Variance-components linkage analysis of systemic markers of inflammation to a microsatellite marker in the interleukin-1 alpha gene (IL1A)

Phenotype[a]	Sibling pairs[b]	LOD score	Trait gene heritability ($h_g^2 \pm$ S.E.)	Polygenic heritability ($h_r^2 \pm$ S.E.)
C-reactive protein	924	0.00	0.00 ± 0.16	0.40 ± 0.06
White blood cell count	1037	0.01	0.02 ± 0.16	0.35 ± 0.09
Albumin	1165	0.00	0.00 ± 0.11	0.36 ± 0.03

[a] All phenotypes were adjusted for gender, age, and age^2.
[b] Number with complete phenotype and IL1A marker data.

to the IL1A microsatellite marker on chromosome 2q (Table 4). However, the residual heritability estimates ($h_r^2 = 0.40$ for CRP, 0.35 for WBC, and 0.36 for albu min) were statistically significantly greater than zero, indicating that major genes in other chromosomal regions, polygenes, and other familial factors may account for up to 35–40% of the variance in these traits.

4. Discussion

Recent prospective studies have reported that acute-phase proteins and other systemic markers of inflammation predict CVD morbidity and mortality [2–12]. In this context, circulating concentrations of these inflammation markers are consistent with mild, subclinical inflammation, rather than a clinically significant episode of acute inflammation. The determinants of such a low-grade inflammatory response are not understood completely but are likely to be heterogeneous [8,9,12,17–23]. While the source of this low-grade inflammation has been suggested to be the vascular wall, there is no conclusive evidence in support of this hypothesis.

Several lines of evidence suggest that host factors modulate the magnitude and extent of inflammatory responses. Mouse models have demonstrated substantial differences between different inbred strains in the inflammatory response to an atherogenic diet, suggesting that this response is modified by genetic factors [34]. In humans, studies with repeated blood sampling on the same individuals over the course of several months report relatively tight regulation of CRP and IL-6 concentrations [29,35–38], even though they are integral to the clinical acute-phase response. Finally, experiments on human peripheral blood mononuclear cells indicate large and stable inter-individual differences in the capacity of these cells to produce pro-inflammatory cytokines upon stimulation [39,40].

Before embarking on molecular genetic studies of a quantitative trait, it is important to obtain indirect evidence that genetic factors contribute to the variation in that trait. Family studies such as our own can be used to make inferences regarding the relative influence of genetic factors and shared family environment on the trait by evaluating patterns of familial correlation. For example, stronger correlations between first-degree relatives than between spouse pairs would suggest important genetic influences on the trait because, on average, first-degree relatives also share half of their genes in addition to environmental effects common to the family. Family studies can also be used to obtain an estimate of heritability, often defined as the proportion of the total variation of the trait that can be attributed to unobserved genetic effects. A high heritability for a trait would provide further justification for molecular genetic studies of that trait, such as gene mapping studies to identify quantitative trait loci of modest to large effect.

Our findings suggest substantial familial and genetic influences on three non-specific markers of inflammation, CRP, WBC, and albumin. To our knowledge, no other studies have reported familial correlations or heritability estimates for C-reactive protein concentrations. For serum albumin, classical heritability estimates from twin studies range from 36 to 77% [41–43]. The heritability estimate for albumin in our study of US nuclear families (36%) was near the lower end of this range. By contrast, the heritability estimate for WBC in our study (35%) was somewhat higher than an estimate of 20% reported in a study of Japanese twin pairs [44].

Several observations suggest that shared environment cannot explain entirely the familial aggregation of these inflammation markers. First, spouse correlations for CRP, WBC, and albumin were negligible, indicating little, if any, influence of shared household environment on these traits. Second, gender- and age-adjusted familial correlation estimates in biological relatives were only slightly attenuated after further adjustment for other variables (e.g. BMI, WHR) which are associated with inflammation factors and also aggregate within families [45]. Recent studies determined that adipose tissue is an important source of several pro-inflammatory cytokines such as IL-6 [46] and TNF-α [47] that may lead to chronic elevations in CRP and other acute-phase proteins. Intra-abdominal fat appears to play the greatest role in this process [48–50]. It is possible that the familial correlations for CRP reported herein may be attenuated to a greater extent if adjusted for measures

of fat mass or fat patterning more sensitive and specific than BMI or WHR.

Interindividual variability in acute-phase proteins and other systemic markers of inflammation may be attributable partly to underlying vascular disease, especially in older adults with a significant burden of atherosclerosis [51]. It is possible that the association of inflammation markers with atherosclerosis may vary at different stages of the natural history of the disease [52]. However, CRP levels were only weakly associated with prevalent CVD and subclinical measures of atherosclerosis in this (AR Folsom, personal communication) and other studies [12], suggesting that clinical or subclinical atherosclerotic disease is not a major determinant of low-grade inflammation. Excluding participants with clinical CHD or indices of atherosclerosis had very little effect on the familial correlations for CRP, WBC, and albumin.

We did not detect linkage between the systemic markers of inflammation and a dinucleotide repeat polymorphism in intron 5 of the IL1A gene. Francis et al. [26] recently reported a positive association between a variant (VNTR allele 2) in intron 2 of the interleukin-1 receptor antagonist gene (IL1RN) and single-vessel coronary artery disease in a hospital-based case-control study of Caucasians from Britain. IL1RN is located on chromosome 2q within 430 kb of the other two members of the IL-1 gene family, IL1B and IL1A [53]. Individuals with specific IL-1 genotypes may be predisposed to a low-grade systemic inflammatory response because of higher endogenous production of IL-1α and IL-1β [40] and/or lower production of IL-1 receptor antagonist [54]. Absence of linkage in our sample of sibling-pairs should only be interpreted as a lack of a strong effect of the genes in the IL-1 cluster on CRP, WBC, or albumin concentrations because current linkage analysis techniques have limited statistical power to detect genes accounting for less than 10% of the phenotypic variance, even in large, extended pedigrees [55]. Our results do not exclude the possibility of major genes for these traits in other chromosomal regions. There are numerous other candidate genes, including those encoding TNF-α and IL-6, that may influence these phenotypes.

In conclusion, we found substantial heritabilities for CRP levels, WBC, and albumin levels in the NHLBI FHS. Familial correlations for these systemic markers of inflammation remained significant after adjustment for other genetic and non-genetic factors that may influence these traits, suggesting that unmeasured genes or environmental factors account for the aggregation of these traits within families. The heritability estimates of 35–40% for these traits indicate that they are reasonable phenotypes for gene mapping studies. If CRP acts directly in the promotion of inflammation and thrombosis, as recently hypothesized [56], then further research may be able to identify individuals at greater risk of CVD because of genetic differences in the CRP response. The NHLBI FHS investigators are planning genome-wide linkage analyses in the largest and most informative families to provide further clues regarding novel quantitative trait loci that regulate levels of acute-phase proteins and other systemic markers of inflammation.

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