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The Relation of Genetic and Environmental Factors to Systemic Inflammatory Biomarker Concentrations

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Abstract

Background—Environmental and genetic correlates of inflammatory marker variability are incompletely understood. In the family-based Framingham Heart Study, we investigated heritability and candidate gene associations of systemic inflammatory biomarkers.

Methods and Results—In Offspring participants (n=3710), we examined 11 inflammatory biomarkers [CD40 ligand, C-reactive protein, intercellular adhesion molecule-1 (ICAM1), interleukin-6, urinary isoprostanes, monocyte chemoattractant protein-1, myeloperoxidase, P-selectin, tumor necrosis factor-alpha, tumor necrosis factor receptor II, fibrinogen]. Heritability and bivariate genetic and environmental correlations were assessed by Sequential Oligogenic Linkage Analysis routines (SOLAR) in 1012 family members. We examined 1943 tagging SNPs in 233 inflammatory pathway genes with ≥5 minor allele carriers using a general genetic linear model.

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Clinical correlates explained 2.4% (CD40 ligand) to 28.5% (C-reactive protein) of the variability in inflammatory biomarkers. Estimated heritability ranged from 10.9% (isoprostanes) to 44.8% (P-selectin). Most correlations between biomarkers were weak although statistically significant. A total of 45 SNP-biomarker associations met the q-value threshold of 0.25. Novel top SNPs were observed in *ICAM1* gene in relation to ICAM1 concentrations (rs1799969, $p=1.32\times10^{-8}$) and *MPO* in relation to myeloperoxidase (rs28730837, $p=1.9\times10^{-5}$).

Lowest p-values for *trans*-acting SNPs were observed for *APCS* with monocyte chemoattractant protein-1 concentrations (rs1374486, $p=1.01\times10^{-7}$) and confirmed for *IL6R* with interleukin-6 concentrations (rs8192284, $p=3.36\times10^{-5}$). Novel potential candidates (*APCS, MPO*) need to be replicated.

Conclusions—Our community-based data support the relevance of clinical and genetic factors for explaining variation in inflammatory biomarker traits.

Keywords

single nucleotide polymorphism; heritability; systemic inflammation; biomarker; cohort study; environmental factors

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Chronic inflammation predisposes to long-term morbidity and mortality from cardiovascular disease, chronic pulmonary disease, chronic kidney disease, osteoporosis, dementia and the aging process.[1–6] Chronic inflammation is associated with abdominal obesity, smoking, and physical inactivity.[7,8] Furthermore, the modern epidemic of metabolic syndrome, and its sequelae insulin resistance and type 2 diabetes, have been attributed to an elevated proinflammatory state, with adipose tissue being considered the main source of pro-inflammatory cytokines.[9] Environmental and lifestyle factors are likely to contribute to increased low-grade inflammatory activity, as well as an individual's genetic predisposition.

Systemically measurable inflammatory mediators provide a link between genetics and risk of disease. Inflammatory biomarker concentrations are heritable phenotypes.[10,11] For instance, estimated C-reactive protein (CRP) concentration heritability is at least 20 per cent.[12] Several single nucleotide polymorphisms (SNPs) are associated with adjusted CRP concentrations and the degree of chronic low-grade inflammation.[13–15] However, the genetic contribution to systemic concentrations of most inflammatory markers remains incompletely understood.

We hypothesized that in a community-based cohort, enrolled irrespective of phenotype, SNPs in inflammatory gene regions are associated with concentrations of pro-inflammatory biomarkers. The well-characterized Framingham Heart Study provides a unique opportunity to examine the association of genetic and environmental factors with inflammatory biomarkers.

Methods

Study Sample

Participants were eligible if they attended the seventh examination cycle (1998–2001, n=5124) of the Framingham Heart Study Offspring, a white, community-based cohort of European ancestry enrolled in 1971.[16,17] Reasons for exclusion from analyses were off-site visits (n=207), none of the 11 biomarkers available (n=10), and missing covariate data (n=17). Heritability and correlations were estimated in 1843 phenotyped individuals in 567 families, in addition to 1468 unrelated participants. To be resource effective and to maximize statistical power, we focused on unrelated individuals and hence genotyped 1565 randomly selected individuals on the standard Offspring unrelated plate set. This plate set is one of several standard Framingham plate sets that is publicly available to researchers.

According to protocol, all participants underwent routine medical history, physical examination, and laboratory testing at the Framingham Heart Study (see Supplement for details). The study was approved by Boston University Medical Center Institutional Review Board; participants signed informed consent.

Determination of inflammatory Biomarkers

Fasting biomarkers, selected to represent various phases and functions in the inflammation process (Supplement Table 1) included: CD40 ligand, CRP, fibrinogen, intercellular adhesion molecule-1 [ICAM1], interleukin-6, urinary isoprostanes indexed to urinary creatinine (isoprostanes), monocyte chemoattractant protein-1, myeloperoxidase, P-selectin, tumor necrosis factor receptor II, and tumor necrosis factor-alpha. Biomarkers methods have been detailed previously.[18] The mean inflammatory biomarkers' intra-assay coefficients of variation were <10%.

Genotyping

Genotyping (2942 SNPs in 233 candidate inflammation genes) was conducted by Perlegen Sciences, Inc. (Mountain View, CA) using high-density oligonucleotide, photolithographic microarrays (DNA chips). Common SNPs were chosen from a genome-wide compilation discovered by Perlegen Sciences and supplemented with others from the HapMap project (build 35), if they had >4% (or unknown) minor allele frequency in the HapMap CEU samples or were coding SNPs. To obtain maximum information, a binning procedure was used to identify tagging SNPs with a criterion of $r^2>0.8$ to create bins; one or two SNPs were selected from each bin depending on bin size. Candidate gene selection details have been reported earlier. [18] Because SNPs with low call rates showed excess departure from HWE equilibrium, we restricted our analyses to a subset of 1834 SNPs with call rate ≥98% and HWE p>0.01. Only SNPs with at least 5 minor allele carriers in the Framingham sample were evaluated for association. An additional 109 SNPs in 9 candidate inflammatory genes previously genotyped by the CardioGenomics project (http://cardiogenomics.med.harvard.edu/genes/gene-list) on the Sequenom MassARRAY platform, with call rate ≥90% and HWE p≥0.01 were included in the present report, for a total of 1943 SNPs in 233 genes.

Statistical Analysis

Multiple regression analysis was performed on the log-transformed biomarker phenotypes to obtain residuals adjusted for age, sex, cohort (Omni), current smoking, systolic and diastolic blood pressure, hypertension treatment, body mass index, waist circumference, total/high-density lipoprotein cholesterol, triglycerides, lipid lowering medication, glucose, diabetes, aspirin (≥3 days per week), hormone replacement therapy and prevalent cardiovascular disease. For genetic analyses, we adjusted for the same covariates across markers for simplification. The residuals of the log-transformed biomarker phenotypes were rescaled to mean 0,SD 1.

Genetic analyses

The statistical methods for assessing heritability for biomarkers was described previously.[10] Sequential Oligogenic Linkage Analysis (SOLAR, (www.sfbr.org/pages/genetics_projects.php?p=37)) was used to estimate residual log-biomarker concentration heritability for age- and sex-adjusted and multivariable-adjusted models and to calculate correlations. The correlation coefficient between any two covariate-adjusted natural log-transformed inflammatory biomarker concentrations was decomposed into genetic and environmental components.

Analysis of variance (ANOVA) was performed to compare means of log-biomarker residuals (model1: age and sex; model2: age, sex and multiple variables) among inflammatory SNP

genotypes using a general genetic model (2 degrees of freedom). ANOVA is not robust for SNPs with low MAF; we used the nonparametric Kruskal-Wallis test instead for SNPs with fewer than 10 individuals in the lowest frequency genotype category. Within each biomarker phenotype, the q-value method,[19] a variation of the false discovery rate method, to adjust for multiple testing. We used a threshold of $q<0.25$ to identify potentially important findings, meaning that the expected proportion of false positive tests among the tests we report within each phenotype is 25%.

Secondary analyses

We assessed potential interactions of 10 SNPs with the smallest p-value for each biomarker with sex, age, smoking status, and body mass index using linear regression. The full set of covariates were included in the model, as well as the SNP (coded with 2 degrees of freedom), and a 2 parameter SNP by covariate interaction term.

Replication from the literature

We searched PubMed for English-language literature that reported SNP-biomarker associations with the inflammatory phenotypes characterized in our sample in studies comprising at least 500 individuals that reached statistical significance level of $p\leq0.05$ and provided the direction of association. We identified SNPs reported in the publications or allowed for proxies with an LD r^2 of ≥0.5 in our database and provided the association p-value. We omitted CRP and P-selectin associations in *cis*-acting *SELP* and *CRP* genes because we have previously reported on both.[20,21] In the online supplement we provide the comprehensive results on SNP-circulating biomarker association studies that were available for the search terms inflammation, inflammatory biomarkers, and single nucleotide polymorphisms, or genetics (August 2008). Phenotype residuals were created using SAS version 8.1 (Cary, NC, http://www.sas.com/presscenter/guidelines.html). Genetic analyses were performed with R (www.r-project.org). All authors had full access to the data, take responsibility for its integrity, and have read and agree to the manuscript as written.

External replication

External replication was attempted in the previously described Athero*Gene* cohort[22] in up to 1752 patients with documented coronary artery disease and 430 controls free of manifest cardiovascular disease. We confined replication to top findings in the current study to limit the number of tests performed. SNPs were selected if they had not been reported in the literature in comparable studies, were in trans-acting genes and were in low linkage disequilibrium ($r^2<0.5$) with other top SNPs. Residuals were created using age, sex, case-control status, smoking status, body mass index, total/HDL cholesterol, triglycerides, serum glucose, diabetes, hypertension, and lipid treatment.

Results

Participant Characteristics

The clinical and laboratory characteristics of the study cohort have been reported before.[20] The heritability and genotype samples' characteristics are outlined in Supplement Table 2. Briefly, the mean age of the genotyped sample was 62±9 years, 51% were women, and the cardiovascular disease prevalence was 13%. Clinical variables explained between 2.4 (CD40 ligand) to 28.5% (CRP) of inflammatory biomarker variability (Supplement Table 3).

Heritability

All inflammatory traits were heritable ($p<0.05$; Table 1, second/third column); estimated multivariable-adjusted heritability values ranged from 10.9% (isoprostanes) to 44.8% (P-

selectin); age- and sex-adjusted results were generally slightly higher. The Pearson correlation coefficients and portion of correlation due to genetic factors also are displayed. Significant environmental correlations were observed for 24 biomarker combinations. Strongest overall pairwise correlations were observed for CRP with fibrinogen (ρ=0.48), and interleukin-6 (ρ=0.39). Six genetic correlations were seen with highest correlation coefficients for fibrinogen and CRP (0.14), and for interleukin-6 and P-selectin (0.12).

Genetic association—To account for multiple testing we computed false discovery rates.[23] A total of 45 associations were significant at a cutoff q-value<0.25. Lowest p-values for *trans*-acting (not involving the protein-coding gene) SNPs were observed for *APCS* (rs1374486, $p=1.01*10^{-7}$, and rs6695377, 5' near gene, $p=1.85*10^{-7}$) with MCP-1 concentrations, *IL6R* (rs8192284, Ala/Asp missense, $p=3.36*10^{-5}$) with interleukin-6 concentrations, and *MPO* in relation to myeloperoxidase (rs28730837, Val/Ala missense , $p=1.9\times10^{-5}$). SNPs with a q-value<0.25 across phenotypes not previously reported in the Framingham Study (SNPs in the *CRP*, *CCL2 and SELP* genes) are tabulated (Table 2). The top *cis*-acting associations for SNPs not previously reported by our group (*SELP* SNPs- P-selectin concentrations were previously reported20) were observed in the *ICAM1* gene in relation to ICAM-1 concentrations (rs1799969, Arg/Gly missense, $p=1.32*10^{-8}$). Results for the top SNPs (q-value<0.25) presented in Table 2 were consistent with age- and sex-adjusted models (Supplement Table 10).

Secondary analyses

Interactions—Accounting for multiple testing, there was no evidence for strong interactions between the SNPs most highly associated with each phenotype and sex, age, smoking status, and body mass index (Supplement Table 4).

Replication from the literature—We were able to replicate two previously reported *ICAM1* SNPs in our database (Supplement Table 6); rs1799969 was congruent with our top ICAM1 finding. SNPs in *IL6*, *CD14* and *NOS3* genes in relation to interleukin-6 concentrations were not replicated. We could confirm rs8192284 in the *IL6R* gene in relation to interleukin-6 concentrations, as well as three SNPs in the *CCL2* gene in association with MCP-1.

External replication—In the Athero*Gene* cohort, predominantly consisting of coronary artery disease patients (n=895–1752), only rs3732764 in the *P2RY12* gene, reached borderline significance, p=0.05. None of the other top findings could be replicated (Supplement Table 7).

Discussion

Principal Findings

We report heritability and genetic associations for a broad panel of carefully selected inflammatory biomarkers and SNPs in a moderately-sized community-based sample. We observed significant heritability for 11 inflammatory biomarker traits, with heritability estimates ranging from 10.9% to 44.8%, including estimates for isoprostanes, myeloperoxidase and tumor necrosis factor receptor II that have not been published before. We detected substantial environmental correlations between many systemic biomarkers, and some pairwise genetic correlations between biomarker traits. Top findings of the broad candidate gene approach, comprising 1943 SNPs, confirmed recent results from the literature for *cis*-acting SNPs in *ICAM1* and *CRP* genes and *trans*-acting *IL6R* association with interleukin-6 concentrations. In addition, novel associations we report were significant *cis*-acting *MPO* SNPs with myeloperoxidase concentrations, and *trans*-acting *APCS* SNPs in relation to monocyte chemoattractant protein-1. We were not able to replicate our results in a cohort of patients with prevalent coronary artery disease. We present age- and sex-adjusted, as well as multivariable-

adjusted phenotype-genotype associations online, so that investigators can download the data and conduct their own analyses. Furthermore, to place our results into perspective, we include comprehensive reviews of the inflammatory biomarker heritability and candidate gene literature in the Supplement.

Environmental and genetic correlations

The examination of bivariate biomarker trait correlations, partitioned into shared genetic and environmental components,[24] clearly revealed that environmental factors contributed a larger extent to observed correlations of circulating biomarker concentrations compared to additive genetic effects. The strength of the genetic and environmental correlations we observed was lower than reported in recent twin studies.[25] Only CRP in relation to fibrinogen, and ICAM1 and interleukin-6 showed moderate genetic, as well as environmental correlations. Compared to the prior literature, we provide correlations for a large inflammatory marker panel.

Heritability

Heritability for inflammatory biomarkers has been reported by Framingham and other researchers for extensively investigated traits like CRP, interleukin-6, ICAM1 and monocyte chemoattractant protein-1 (Supplement Table 5).[10–12,26,27] The present cohort convincingly demonstrated a modest to moderate proportion of variability explained by descent in a large panel of distinct inflammatory biomarkers, even biomarkers with known higher intra-individual variability and measurement coefficients of variation like isoprostanes.[28]

Genetic Association

As coding genes have the highest likelihood of association with encoded proteins, the majority of biomarker candidate gene association analyses have been performed for *cis*-acting genes. Not surprisingly, our strongest association finding was in the *SELP* gene in relation to P-selectin concentrations, which has previously been reported in Framingham[20] and independent studies.[29,30] For several tagging SNPs we and others were able to show moderate associations between common genetic variation in the respective coding genes for CRP[14,21,31] and ICAM1[32,33] concentrations after accounting for known covariates (for additional replication please see Supplement Table 6).

We further hypothesized that inflammatory genes are related to circulating biomarkers not coded for by the gene (*trans*-acting genes). We extended current knowledge by examining a broad panel of 233 inflammatory candidate genes. The aim was to capture *trans*-acting genotypes that might contribute at the genetic level to the known strong interrelations of inflammatory pathways at the biomarker level. We confirmed the strong association of SNP rs8192284 in *IL6R* with interleukin-6 phenotype.[34] The observed relation has a functional explanation since the amino acid exchange leads to an alteration at the receptor cleavage site, which increases soluble interleukin-6 receptor concentrations and thus affects, circulating interleukin-6.[34,35] The independent evidence in ethnically different groups and a plausible pathophysiological explanation have turned this SNP into a very promising candidate polymorphism.

Less evidence is available that would help to explain the top finding of two SNPs in the *APCS* gene in relation to serum monocyte chemoattractant protein-1 concentrations. The *APCS* SNPs, all located in the 5' gene region without relevant LD to SNPs with known function, belong to a gene with multiple polyadenylation sites encoding a highly conserved glycoprotein of the pentraxin family, serum amyloid P component.[36] Serum amyloid component shares considerable sequence homology with CRP resulting from gene duplication during evolution. Serum amyloid P opsonizes apoptotic cells, an important step in their clearance.[37] Amyloid P is found in atherosclerotic plaques[38] and circulating concentrations have been related to clinical

cardiovascular disease in an elderly, multiethnic community-based cohort.[39] Genetic data in humans are scant. We reported a linkage peak for MCP-1 on chromosome 1 which extends over the *APCS* gene locus and provides additional evidence for a potential association.[10] Ongoing genome-wide association studies will help to identify the true variants. None of the polymorphisms in the *CCL2* gene reported in the Framingham Heart Study cohort reached experiment-wide significance, but showed nominally significant associations with the same directionality.[40]

Myeloperoxidase, has been recognized for its role in non-infectious inflammatory diseases, and as an important modulator of vasomotor function in vascular inflammation.[41] Two non-HapMap SNPs have been described in association with myeloperoxidase activity (rs28365049, rs34097845). The functional promotor polymorphism (−463G/A) containing an Alu element is related to myeloperoxidase expression.[42] It has been linked to inflammatory diseases like Alzheimer's disease,43 and atherosclerotic disease.44 In the current cohort we provide evidence on the significance of a new SNP, rs28730837, a Val/Ala missense variation, with regard to myeloperoxidase concentration.

Replication from the Literature

Compared to the large number of published studies, only few met our inclusion criteria of sample size ≥500 participants for an in-silico replication attempt. We were able to replicate mostly *cis*-acting SNPs from the literature for *ICAM1* and *CCL2* genes and one prominent *trans*-acting association of the recently reported SNP rs8192284 in *IL6R* gene in relation to interleukin-6. Associations for SNPs in *CD14* and *NOS3* genes with interleukin-6, previously seen in patients with coronary artery disease could not be confirmed and may indicate spurious or disease-specific findings.[45,46]

Strengths and Limitations—The Framingham Study constitutes a single center family-based community cohort with limited referral bias, stringent biomarker quality control, well-documented, and routinely ascertained environmental confounders, which facilitate multivariable models and heritability analyses. The choice of multiple biomarkers from scientifically sound candidate pathways based on basic and human studies further increases the current study's comprehensiveness. The broad range of SNPs chosen for association reduces bias observed in candidate gene approaches and may uncover both *cis and trans* regulators.[47] Whereas our study underscores the problems of multiple testing, in contrast to many recent publications, a q-value method was applied with conservative thresholds, to minimize false positive findings without instituting overly strict Bonferroni corrections. In addition, we provide a downloadable excel file at our website of all inflammatory marker-SNP associations tested that will facilitate replication by external investigators.[a] Furthermore, we provide a comprehensive review of most prior publications examining heritability and the associations between SNPs and circulating inflammatory biomarkers.

Some limitations meriting consideration are that the significant results are currently restricted to one study group. We were unable to replicate our findings in an independent cohort with coronary artery disease. Non-replication may be due to a relatively low number of genotyped individuals and their pre-existing coronary disease, which is known to elevate biomarker concentrations. As noted by Chanock and colleagues, phenotype and participant heterogeneity will compromise the likelihood of replication.[48] The selected nature of Athero*Gene*, is corroborated by the observation, that the repeatedly validated association of *IL6R* rs8192284[34] was replicated in the FHS cohort but not in the Athero*Gene* cohort. We used an older HapMap build (build 35) and thus may have missed important variants.

[a]Data file included as reviewers' electronic supplement will be posted upon publication.

Intermediate cardiovascular phenotypes (i.e. hypertension) are strongly correlated with inflammatory activity; multivariable-adjustment may limit our ability to detect pleiotropic environmental and genetic effects related to inflammation. To reduce the high multiple testing burden, we specified à priori that our primary analyses would be multivariable-adjusted models.

Generalizability of the results is limited by the ethnically homogenous cohort; biomarker concentrations vary by ethnicity.[49] For other ethnicities, a slightly different set of informative SNPs would have been chosen.[50] On the other hand, the potential for population stratification was reduced by racial homogeneity.[51] We acknowledge that our cohort had only moderate power to detect modest effects; a potential for false negative findings is evident. Inherent to single-cohort genetic association studies, our results should be viewed as hypothesis generating; replication in independent samples is necessary.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CRP	C-reactive protein
LD	linkage disequilibrium
ICAM1	intercellular adhesion molecule 1
SNP	single nucleotide polymorphism

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Reference List

1. Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12:1005–1015. [PubMed: 16960575]
2. Keller CR, Odden MC, Fried LF, Newman AB, Angleman S, Green CA, Cummings SR, Harris TB, Shlipak MG. Kidney function and markers of inflammation in elderly persons without chronic kidney disease: The health, aging, and body composition study. Kidney Int 2007;71:239–244. [PubMed: 17183246]
3. Barbieri M, Ferrucci L, Corsi AM, Macchi C, Lauretani F, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Paolisso G. Is chronic inflammation a determinant of blood pressure in the elderly? Am J Hypertens 2003;16:537–543. [PubMed: 12850386]
4. Gupta A, Watkins A, Thomas P, Majer R, Habubi N, Morris G, Pansari K. Coagulation and inflammatory markers in Alzheimer's and vascular dementia. Int J Clin Pract 2005;59:52–57. [PubMed: 15707465]
5. Snoeck-Stroband JB, Postma DS, Lapperre TS, Gosman MM, Thiadens HA, Kauffman HF, Sont JK, Jansen DF, Sterk PJ. Airway inflammation contributes to health status in COPD: a cross-sectional study. Respir Res 2006;7:140. [PubMed: 17137518]

6. Fogarty AW, Jones S, Britton JR, Lewis SA, McKeever TM. Systemic inflammation and decline in lung function in a general population: a prospective study. Thorax 2007;62:515–520. [PubMed: 17251312]
7. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. Exp Gerontol 2004;39:687–699. [PubMed: 15130663]
8. Pedersen M, Bruunsgaard H, Weis N, Hendel HW, Andreassen BU, Eldrup E, Dela F, Pedersen BK. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. Mech Ageing Dev 2003;124:495–502. [PubMed: 12714258]
9. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91. [PubMed: 7678183]
10. Dupuis J, Larson MG, Vasan RS, Massaro JM, Wilson PW, Lipinska I, Corey D, Vita JA, Keaney JF Jr, Benjamin EJ. Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q. Atherosclerosis 2005;182:307–314. [PubMed: 16159603]
11. Pankow JS, Folsom AR, Cushman M, Borecki IB, Hopkins PN, Eckfeldt JH, Tracy RP. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis 2001;154:681–689. [PubMed: 11257270]
12. de Maat MP, Bladbjerg EM, Hjelmborg JB, Bathum L, Jespersen J, Christensen K. Genetic influence on inflammation variables in the elderly. Arterioscler Thromb Vasc Biol 2004;24:2168–2173. [PubMed: 15345506]
13. Kathiresan S, Gona P, Larson MG, Vita JA, Mitchell GF, Tofler GH, Levy D, Newton-Cheh C, Wang TJ, Benjamin EJ, Vasan RS. Cross-sectional relations of multiple biomarkers from distinct biological pathways to brachial artery endothelial function. Circulation 2006;113:938–945. [PubMed: 16476848]
14. Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, Fornage M, Boerwinkle E, Gross M, Jaquish C, Nickerson DA, Myers RM, Siscovick DS, Reiner AP. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet 2005;77:64–77. [PubMed: 15897982]
15. Suk HJ, Ridker PM, Cook NR, Zee RY. Relation of polymorphism within the C-reactive protein gene and plasma CRP levels. Atherosclerosis 2005;178:139–145. [PubMed: 15585211]
16. Kannel WB, Feinleib M, McNamara PM, Garrison RJ. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol 1979;110:281–290. [PubMed: 474565]
17. Quan SF, Howard BV, Iber C, Kiley JP, Nieto FJ, O'Connor GT, Rapoport DM, Redline S, Robbins J, Samet JM, Wahl PW. The Sleep Heart Health Study: design, rationale, and methods. Sleep 1997;20:1077–1085. [PubMed: 9493915]
18. Schnabel R, Larson MG, Dupuis J, Lunetta KL, Lipinska I, Meigs JB, Yin X, Rong J, Vita JA, Newton-Cheh C, Levy D, Keaney JF Jr, Vasan RS, Mitchell GF, Benjamin EJ. Relations of inflammatory biomarkers and common genetic variants with arterial stiffness and wave reflection. Hypertension 2008;51:1651–1657. [PubMed: 18426996]
19. Storey JD. A direct approach to false discovery rates. Journal of the Royal Statistical Society 2002;64:479–498.
20. Lee DS, Larson MG, Lunetta KL, Dupuis J, Rong J, Keaney JF Jr, Lipinska I, Baldwin CT, Vasan RS, Benjamin EJ. Clinical and genetic correlates of soluble P-selectin in the community. J Thromb Haemost. 2007
21. Kathiresan S, Larson MG, Vasan RS, Guo CY, Gona P, Keaney JF Jr, Wilson PW, Newton-Cheh C, Musone SL, Camargo AL, Drake JA, Levy D, O'Donnell CJ, Hirschhorn JN, Benjamin EJ. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. Circulation 2006;113:1415–1423. [PubMed: 16534007]
22. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003;349:1605–1613. [PubMed: 14573732]
23. Storey JD. The positive false discovery rate: A Bayesian interpretation and the q-value. Annals of Statistics 2003;31:2013–2035.

24. Kent JW Jr, Comuzzie AG, Mahaney MC, Almasy L, Rainwater DL, VandeBerg JL, MacCluer JW, Blangero J. Intercellular adhesion molecule-1 concentration is genetically correlated with insulin resistance, obesity, and HDL concentration in Mexican Americans. Diabetes 2004;53:2691–2695. [PubMed: 15448102]
25. Su S, Snieder H, Miller AH, Ritchie J, Bremner JD, Goldberg J, Dai J, Jones L, Murrah NV, Zhao J, Vaccarino V. Genetic and environmental influences on systemic markers of inflammation in middle-aged male twins. Atherosclerosis. 2008
26. Keaney JF Jr, Massaro JM, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Sutherland P, Vita JA, Benjamin EJ. Heritability and correlates of intercellular adhesion molecule-1 in the Framingham Offspring Study. J Am Coll Cardiol 2004;44:168–173. [PubMed: 15234428]
27. Bielinski SJ, Pankow JS, Foster CL, Miller MB, Hopkins PN, Eckfeldt JH, Hixson J, Liu Y, Register T, Myers RH, Arnett DK. Circulating soluble ICAM-1 levels shows linkage to ICAM gene cluster region on chromosome 19: the NHLBI Family Heart Study follow-up examination. Atherosclerosis 2008;199:172–178. [PubMed: 18045607]
28. Helmersson J, Basu S. F(2)-isoprostane and prostaglandin F(2 alpha)metabolite excretion rate and day to day variation in healthy humans. Prostaglandins Leukot Essent Fatty Acids 2001;65:99–102. [PubMed: 11545626]
29. Barbaux SC, Blankenberg S, Rupprecht HJ, Francomme C, Bickel C, Hafner G, Nicaud V, Meyer J, Cambien F, Tiret L. Association between P-selectin gene polymorphisms and soluble P-selectin levels and their relation to coronary artery disease. Arterioscler Thromb Vasc Biol 2001;21:1668–1673. [PubMed: 11597943]
30. Volcik KA, Ballantyne CM, Coresh J, Folsom AR, Wu KK, Boerwinkle E. P-selectin Thr715Pro polymorphism predicts P-selectin levels but not risk of incident coronary heart disease or ischemic stroke in a cohort of 14595 participants: the Atherosclerosis Risk in Communities Study. Atherosclerosis 2006;186:74–79. [PubMed: 16125711]
31. Reiner AP, Barber MJ, Guan Y, Ridker PM, Lange LA, Chasman DI, Walston JD, Cooper GM, Jenny NS, Rieder MJ, Durda JP, Smith JD, Novembre J, Tracy RP, Rotter JI, Stephens M, Nickerson DA, Krauss RM. Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. Am J Hum Genet 2008;82:1193–1201. [PubMed: 18439552]
32. Ponthieux A, Lambert D, Herbeth B, Droesch S, Pfister M, Visvikis S. Association between Gly241Arg ICAM-1 gene polymorphism and serum sICAM-1 concentration in the Stanislas cohort. Eur J Hum Genet 2003;11:679–686. [PubMed: 12939654]
33. Li YF, Tsao YH, Gauderman WJ, Conti DV, Avol E, Dubeau L, Gilliland FD. Intercellular adhesion molecule-1 and childhood asthma. Hum Genet 2005;117:476–484. [PubMed: 16021473]
34. Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, Choy E, Hu D, Tamraz B, Pawlikowska L, Wassel-Fyr C, Huntsman S, Waliszewska A, Rossin E, Li R, Garcia M, Reiner A, Ferrell R, Cummings S, Kwok PY, Harris T, Zmuda JM, Ziv E. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. Am J Hum Genet 2007;80:716–726. [PubMed: 17357077]
35. Barille S, Collette M, Thabard W, Bleunven C, Bataille R, Amiot M. Soluble IL-6R alpha upregulated IL-6, MMP-1 and MMP-2 secretion in bone marrow stromal cells. Cytokine 2000;12:1426–1429. [PubMed: 10976008]
36. Walsh MT, Divane A, Whitehead AS. Fine mapping of the human pentraxin gene region on chromosome 1q23. Immunogenetics 1996;44:62–69. [PubMed: 8613143]
37. Mold C, Baca R, Du Clos TW. Serum amyloid P component and C-reactive protein opsonize apoptotic cells for phagocytosis through Fcgamma receptors. J Autoimmun 2002;19:147–154. [PubMed: 12419285]
38. Li XA, Hatanaka K, Ishibashi-Ueda H, Yutani C, Yamamoto A. Characterization of serum amyloid P component from human aortic atherosclerotic lesions. Arterioscler Thromb Vasc Biol 1995;15:252–257. [PubMed: 7749834]
39. Jenny NS, Arnold AM, Kuller LH, Tracy RP, Psaty BM. Serum amyloid P and cardiovascular disease in older men and women: results from the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol 2007;27:352–358. [PubMed: 17138933]

40. McDermott DH, Yang Q, Kathiresan S, Cupples LA, Massaro JM, Keaney JF Jr, Larson MG, Vasan RS, Hirschhorn JN, O'Donnell CJ, Murphy PM, Benjamin EJ. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. Circulation 2005;112:1113–1120. [PubMed: 16116069]
41. Baldus S, Heitzer T, Eiserich JP, Lau D, Mollnau H, Ortak M, Petri S, Goldmann B, Duchstein HJ, Berger J, Helmchen U, Freeman BA, Meinertz T, Munzel T. Myeloperoxidase enhances nitric oxide catabolism during myocardial ischemia and reperfusion. Free Radic Biol Med 2004;37:902–911. [PubMed: 15304260]
42. Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 1996;271:14412–14420. [PubMed: 8662930]
43. Reynolds WF, Rhees J, Maciejewski D, Paladino T, Sieburg H, Maki RA, Masliah E. Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. Exp Neurol 1999;155:31–41. [PubMed: 9918702]
44. Nikpoor B, Turecki G, Fournier C, Theroux P, Rouleau GA. A functional myeloperoxidase polymorphic variant is associated with coronary artery disease in French-Canadians. Am Heart J 2001;142:336–339. [PubMed: 11479475]
45. Antoniades C, Tousoulis D, Vasiliadou C, Pitsavos C, Chrysochoou C, Panagiotakos D, Tentolouris C, Marinou K, Koumallos N, Stefanadis C. Genetic polymorphism on endothelial nitric oxide synthase affects endothelial activation and inflammatory response during the acute phase of myocardial infarction. J Am Coll Cardiol 2005;46:1101–1109. [PubMed: 16168297]
46. Morange PE, Saut N, Alessi MC, Frere C, Hawe E, Yudkin JS, Tremoli E, Margaglione M, Di Minno G, Hamsten A, Humphries SE, Juhan-Vague I. Interaction between the C-260T polymorphism of the CD14 gene and the plasma IL-6 concentration on the risk of myocardial infarction: the HIFMECH study. Atherosclerosis 2005;179:317–323. [PubMed: 15777548]
47. Morley M, Molony CM, Weber TM, Devlin JL, Ewens KG, Spielman RS, Cheung VG. Genetic analysis of genome-wide variation in human gene expression. Nature 2004;430:743–747. [PubMed: 15269782]
48. Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JN, Abecasis G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni JF Jr, Freimer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn DM, Collins FS. Replicating genotype-phenotype associations. Nature 2007;447:655–660. [PubMed: 17554299]
49. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). Am J Cardiol 2004;93:1238–1242. [PubMed: 15135696]
50. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–120. [PubMed: 14681826]
51. Hinds DA, Stokowski RP, Patil N, Konvicka K, Kershenobich D, Cox DR, Ballinger DG. Matching strategies for genetic association studies in structured populations. Am J Hum Genet 2004;74:317–325. [PubMed: 14740319]
52. Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C, Hirschhorn JN, Berglund G, Hedblad B, Groop L, Altshuler DM, Newton-Cheh C, Orho-Melander M. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;358:1240–1249. [PubMed: 18354102]
53. Zee RY, Cheng S, Erlich HA, Lindpaintner K, Rifai N, Buring JE, Ridker PM. Intercellular adhesion molecule 1 (ICAM1) Lys56Met and Gly241Arg gene variants, plasma-soluble ICAM1 concentrations, and risk of incident cardiovascular events in 23,014 initially healthy white women. Stroke 2007;38:3152–3157. [PubMed: 17962597]
54. Ridker PM, Pare G, Parker A, Zee RY, Danik JS, Buring JE, Kwiatkowski D, Cook NR, Miletich JP, Chasman DI. Loci related to metabolic-syndrome pathways including LEPR,HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women's Genome Health Study. Am J Hum Genet 2008;82:1185–1192. [PubMed: 18439548]

Table 1

Inflammatory Biomarker Heritability, Pearson Correlation Coefficient and Genetic (Grey Shading) Component of Correlation

	Heritability												
	Age- & sex (%)*	**Multivariable (%)***	**CD40 Ligand**	**CRP**	**Fibrinogen**	**ICAM1¶**	**IL6**	**Isoprostanes**	**MCP-1‡**	**MPO**	**P-selectin**	**TNFRII**	**TNFA**
CD40 Ligand	**17.0**	**14.4**	--	0.02	0.03	**0.05**	0.01	−0.03	−0.01	0.02	0.01	**0.04**	**0.07**
CRP	**29.1**	**30.3**	**−0.09**	--	**0.48**	**0.16**	**0.39**	**0.09**	0.01	**0.10**	**0.09**	**0.17**	**0.11**
Fibrinogen	**37.6**	**36.3**	−0.05	**0.14**	--	**0.09**	**0.29**	−0.03	−0.01	**0.07**	**0.10**	**0.10**	**0.08**
ICAM1¶	**34.1**	**33.9**	**−0.09**	−0.04	0.00	--	**0.22**	**0.17**	**0.04**	0.02	**0.12**	**0.32**	**0.21**
Interleukin-6	**20.3**	**11.8**	−0.01	0.08	0.07	**0.10**	--	**0.13**	**0.10**	**0.11**	**0.10**	**0.23**	**0.22**
Isoprostanes	**16.8**	**10.9**	−0.05	0.06	0.04	0.01	0.06	--	**0.08**	0.01	**0.06**	**0.04**	**0.05**
MCP-1‡	**42.8**	**41.7**	−0.05	−0.01	0.01	−0.03	0.08	0.04	--	**0.12**	**0.07**	**0.07**	**0.06**
Myeloperoxidase	**23.8**	**25.1**	−0.01	0.04	−0.01	0.00	0.00	0.00	0.04	--	**0.11**	**0.06**	**0.07**
P-selectin	**44.7**	**44.8**	0.01	0.04	0.07	0.04	**0.12**	0.03	0.02	**0.09**	--	**0.10**	**0.09**
TNFRII#	**34.0**	**28.0**	−0.06	0.02	0.00	0.06	0.07	−0.03	−0.03	0.01	0.05	--	**0.32**
TNFA†	**15.6**	**14.2**	0.02	−0.03	−0.02	0.08	−0.01	−0.02	0.04	0.02	0.07	0.01	--

Estimates are bolded if p<0.05 (test for the hypothesis that heritability=0);

*SE (standard error) of all heritability estimates is about 6%.

§ ¶intercellular adhesion molecule-1;

‡monocyte chemoattractant protein-1;

#tumor necrosis factor receptor II;

†tumor necrosis factor-alpha N=3710 for heritability

Table 2

Association of SNPs from 233 Inflammatory Candidate Genes (n=1943 SNPs) with Multivariable-Adjusted Inflammatory Biomarker Residuals within Phenotype q-Value<0.25

Gene	Allelic Variant	Chr*	SNP Type	Major/ Minor Allele	Minor Allele Frequency	Heterozygote		Homozygote		Partial R^2	Multivar-iable P	Q-Value	Age- sex-adjusted *P*	Repli-cation *P*
						Beta	SE	Beta	SE					
C-reactive protein														
ADAMTS2	rs878933	5	intronic	G/A	16.7	−0.17	0.06	−0.49	0.14	0.011	1.4×10^{-4}	0.11	0.003	0.43
IL1RN	rs4251961	2	locus-region	T/C	38.9	−0.04	0.06	0.25	0.08	0.010	6.0×10^{-4}	0.13	0.002	--
ITGA4	rs16867464	2	intronic	C/T	12.6	−0.24	0.06	0.01	0.20	0.010	6.4×10^{-4}	0.13	0.002	0.28
P2RY12	rs1491974	3	intronic	A/G	48.5	0.18	0.06	0.30	0.07	0.011	2.0×10^{-4}	0.11	4.3×10^{-4}	0.10
P2RY12	rs17504	3	intronic	A/G	48.8	0.16	0.06	0.29	0.07	0.010	3.2×10^{-4}	0.11	6.4×10^{-4}	--
P2RY12	rs16863323	3	intronic	C/T	30.1	−0.07	0.05	0.30	0.09	0.010	4.3×10^{-4}	0.13	0.03	0.75
P2RY12	rs3732764	3	intronic	C/A	32.4	−0.17	0.05	−0.24	0.09	0.009	1.3×10^{-3}	0.18	0.003	0.05
P2RY12	rs3975403	3	intronic	C/G	31.2	−0.16	0.05	−0.26	0.09	0.009	1.4×10^{-3}	0.18	0.002	--
Fibrinogen														
TNFRSF11b	rs2875845	8	intronic	A/G	17.4	0.19	0.06	−0.30	0.15	0.011	2.7×10^{-4}	0.24	3.8×10^{-5}	--
TNFRSF11b	rs10955912	8	intronic	T/C	21.5	0.18	0.05	−0.23	0.13	0.011	2.8×10^{-4}	0.24	4.2×10^{-5}	--
ICAM1														
ICAM1	rs1799969	19	missense	G/A	10.3	−0.40	0.07	−0.57	0.22	0.023	1.3×10^{-8}	2.4×10^{-5}	1.5×10^{-7}	--
ICAM1	rs2075741	19	intronic	G/C	46.0	0.19	0.06	0.33	0.08	0.012	8.6×10^{-5}	0.08	4.5×10^{-4}	0.12
Isoprostanes														
CAT	rs3781710	11	intronic	T/G	35.9	0.05	0.06	−0.31	0.08	0.014	1.1×10^{-4}	0.21	1.9×10^{-4}	--
Interleukin-6														
IL6R	rs8192284	1	missense	A/C	38.6	0.16	0.05	0.34	0.08	0.01	3.4×10^{-5}	0.06	4.1×10^{-5}	0.21
Monocyte chemoattractant protein-1														
APCS	rs1374486	1	intergenic	G/A	19.9	−0.29	0.06	−0.38	0.12	0.02	1.0×10^{-7}	1.6×10^{-4}	3.5×10^{-8}	0.48

Gene	Allelic Variant	Chr*	SNP Type	Major/ Minor Allele	Minor Allele Frequency	Heterozygote		Homozygote		Partial R^2	Multivar-iable P	Q-Value	Age- sex-adjusted *P*	Repli-cation *P*
						Beta	SE	Beta	SE					
APCS	rs6695377	1	downstream	C/T	22.1	−0.27	0.05	−0.38	0.11	0.02	1.9×10^{-7}	1.6×10^{-4}	8.1×10^{-8}	--
APCS	rs1562388	1	unknown	G/C	42.0	−0.11	0.06	−0.32	0.07	0.01	7.2×10^{-5}	0.03	6.4×10^{-5}	--
APCS	rs1037143	1	upstream	T/C	42.0	−0.12	0.06	−0.32	0.07	0.01	7.4×10^{-5}	0.03	6.1×10^{-5}	0.88
APCS	rs10908734	1	intergenic	C/T	11.2	0.24	0.06	0.39	0.22	0.01	4.1×10^{-4}	0.12	6.1×10^{-4}	0.44
APCS	rs1156060	1	unknown	T/G	11.2	0.23	0.06	0.39	0.22	0.01	4.2×10^{-4}	0.12	6.2×10^{-4}	--
APCS	rs1446969	1	unknown	C/T	11.2	0.23	0.06	0.39	0.22	0.01	5.1×10^{-4}	0.13	7.7×10^{-4}	--
CRP	rs3093077	1	downstream	A/C	6.9	−0.26	0.07	−0.08	0.29	0.01	8.0×10^{-4}	0.15	3.6×10^{-4}	--
IL4R	rs3024622	16	intronic	C/G	34.4	−0.05	0.05	0.27	0.08	0.01	6.7×10^{-4}	0.15	4.1×10^{-4}	0.43
Myeloperoxidase														
MPO	rs28730837	17	missense	C/T	1.3	−0.65	0.15	NA	NA	0.01	1.9×10^{-5}	0.03	6.2×10^{-5}	--
P-selectin														
TNFSF10	rs1131532	3	synonymous	A/G	30.6	−0.17	0.05	−0.28	0.09	0.01	4.9×10^{-4}	0.15	8.5×10^{-4}	0.81
TNFSF10	rs1131542	3	3' untranslated	T/G	30.6	−0.17	0.05	−0.28	0.09	0.01	5.5×10^{-4}	0.15	9.4×10^{-4}	--
ITGB4	rs2838737	21	3' untranslated	T/C	15.7	−0.26	0.06	−0.38	0.21	0.02	6.4×10^{-5}	0.12	0.35	--
TNFRSF1b	rs235214	1	unknown	T/C	14.1	0.02	0.07	0.89	0.22	0.01	2.1×10^{-4}	0.19	0.91	--
Tumor necrosis factor receptor II														
TFPI	rs4666734	2	unknown	G/A	10.8	0.00	0.07	0.94	0.21	0.01	5.7×10^{-5}	0.10	1.6×10^{-5}	0.88

Response variables were multivariable-adjusted biomarker residuals (see methods). Comparison was made using the homozygotes of the major allele as the reference group. Partial R^2 is the proportion of residual variance explained by the SNP.

*Chr stands for chromosome.

The replication p value for Athero*Gene* cohort is provided for genotyped SNPs (details Supplement Table 7). Reported *P*-value for rs1799969 ranged from ≤0.05 to<0.0001;[32,53] for rs8192284 from $2*10^{-8}$ from to $2.0*10^{-9}$.[34,54]

R^2 linkage disequilibrium of nearby SNPs is provided in Supplement Table 8.
Means and standard deviation by genotype are provided in Supplement Table 9.
Betas are the regression coefficients.