Genetic Variants in Platelet Factor 4 Modulate Inflammatory and Platelet Activation Biomarkers

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Background—African Americans suffer from higher prevalence and severity of atherosclerosis compared with whites, highlighting racial and ethnic disparities in cardiovascular disease. Previous studies have pointed to the role of vascular inflammation and platelet activation in the formation of atherosclerotic lesions.

Methods and Results—We explored the role of genetic variation in 4 chemokine/chemokine receptor genes (*CX3CR1*, *CX3CL1*, *CXCR3*, and *PF4*) on systemic inflammation and platelet activation serum biomarkers (fractalkine, platelet P-selectin, platelet factor 4 [PF4], and tumor necrosis factor-α). In total, 110 single nucleotide polymorphisms were tested among 1042 African Americans and 763 whites. The strongest association with serum PF4 levels was observed for rs168449, which was significant in both racial groups (*P* value: African Americans=0.0017, whites=0.014, combined=1.2×10^{-4}), and remained significant after permutation-based multiple corrections (P_c value: combined=0.0013). After accounting for the effect of rs168449, we identified another significant single nucleotide polymorphism (rs1435520), suggesting a second independent signal regulating serum PF4 levels (conditional *P* value: African Americans=0.02, whites=0.02). Together, these single nucleotide polymorphisms explained 0.98% and 1.23% of serum PF4 variance in African Americans and whites, respectively. Additionally, in African Americans, we found an additional *PF4* variant (rs8180167), uncorrelated with rs168449 and rs1435520, associated with serum tumor necrosis factor-α levels (*P*=0.008, P_c=0.048).

Conclusions—Our study highlights the importance of *PF4* variants in the regulation of platelet activation (PF4) and systemic inflammation (tumor necrosis factor-α) serum biomarkers. **(*Circ Cardiovasc Genet*. 2012;5:412-421.)**

Key Words: association study ■ atherosclerosis ■ inflammation ■ platelets ■ chemokines ■ PF4 ■ TNF-α

Over the past decade, epidemiological studies comparing African Americans and other minority groups to whites have demonstrated persistent disparities in morbidity and mortality.[1,2] In particular, a disproportionately high prevalence and severity of atherosclerotic cardiovascular disease in African Americans is observed. The reasons for this health disparity are multiple and largely unresolved.[3] Atherosclerosis is a lifelong progressive disease, which leads to myocardial infarction, stroke, and higher mortality rates.[4] This disease was formerly characterized by the accumulation of lipids and fibrous elements in large arteries, but, in recent years, substantial advances in basic and experimental science have illuminated the importance of vascular inflammation[5,6] and platelet activation in disease etiology[7]; however, the underlying cellular and molecular mechanisms that contribute to atherogenesis are not fully known. It has been suggested that continuous immigration and infiltration of activated macrophages and T cells into and within atherosclerotic lesions are critical events during the initial phase of plaque formation,[8] progression, and even in acute complications such as plaque rupture and thrombosis.[9] The recruitment of these cells to lesions is largely guided by chemokines and contributes to the accumulation of inflammatory cells in the atherosclerotic plaque.[10]

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Chemokines constitute a family of structurally related chemotactic cytokines and classified into subgroups (CC, CXC, C, CX3C) based on the position of the conserved cysteine residues in the amino-terminal region of the molecule. In addition to the regulation of trafficking of macrophages and T cells, chemokines are also involved in platelet activation.[11,12] Fractalkine (*CX3CL1*) is not produced by

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endothelial cells under physiological conditions in vivo; however, its production is induced via inflammatory signals (ie, tumor necrosis factor [TNF-α]), vascular injury, or atherosclerosis. Monocytes and platelets are known to interact directly with membrane-bound *CX3CL1*, and fractalkine receptor (*CX3CR1*) and *CXCR3* are well-documented in the recruitment of inflammatory cells. Previous studies have demonstrated the presence of a functional *CX3CR1* on human platelets.[5] Blood platelets are mainly involved in hemostasis and acute thrombus formation but also have proinflammatory and growth-regulatory properties that contribute to the progression of atherosclerosis.[13,14] The activation of platelets leads to the release of a wide range of growth factors, inflammatory mediators, including chemokines (eg, PF4), and adhesion molecules (eg, P-selectin). Previous studies have suggested that platelet activation also induces the expression of proinflammatory TNF-α and chemokines in monocytes/macrophages.[15] Compelling evidence from functional[16–18] and genetic studies[19,20] also affirms a link between chemokines and the progression of atherosclerosis. Prior studies have shown that activated platelets can stimulate endothelial cells, leading to an increase in leukocyte-rolling along the vessel wall[7]; however, the mechanisms by which platelets activate endothelial cells and vascular inflammation is not well-understood. Platelet factor 4 (PF4 or CXCL4) is a chemokine stored in platelet α-granules and released during platelet activation.[8–11,] Our prior work demonstrated PF4's role in T-cell trafficking and development of experimental cerebral malaria.[21]

Given the importance of systemic inflammation and platelet activation to the development of atherosclerosis, we explored the role of genetic variants in regulating these processes. Indeed, these 2 processes yield predictive and prognostic information of considerable clinical use. Based on our current understanding, we assume a model in which inflammatory mediators can lead to platelet activation, which, in turn, intensifies the inflammatory response, thus forming an atherogenic amplification loop. The key inflammatory mediators on which we focused are chemokines and chemokine receptors; fractalkine receptor (*CX3CR1*), fractalkine (*CX3CL1*), chemokine (C-X-C motif) receptor 3 (*CXCR3*, which serves as a receptor for PF4), and *PF4*. The systemic inflammation and platelet activation serum biomarkers; fractalkine, P-selectin, PF4, and TNF-α were assessed as vascular risk factors for atherosclerosis. The overall goal of this study is to explore the influence of genetic variations in *CX3CR1* and *CXCR3* and determine if they are asymmetrically distributed in African Americans compared with whites or if they modulate in the chemokine/chemokine receptors on the systemic inflammation and platelet activation serum biomarkers in African Americans and whites.

Materials and Methods

Study Cohort

Study samples were drawn from participants of the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study of the National Institute on Aging Intramural Research Program.[22] HANDLS is a prospective longitudinal study of approximately 4000 black and white adults from Baltimore City, MD. The purpose of the HANDLS study is to unravel the effects of race and socioeconomic status (SES) on the development of age-associated health disparities over a 20-year period. Subjects were enrolled from 2004 to 2008 by household screenings from an area probability sample of 13 neighborhoods defined by contiguous US Census tracts in the city of Baltimore. These tracts were selected because they were likely to yield representative distributions of individuals who were 30 to 64 years old, African Americans or whites, males and females, and had a household income either <125% or ≥125% of the federal poverty level. Multi-ethnic individuals were included, provided they identified themselves as African Americans or whites but not both. To be included in the HANDLS study, participants must (1) be within age range of 30 to 64 years at baseline; (2) be able to give informed consent; (3) be able to perform at least 5 measures of the following evaluations: laboratory evaluation, medical history, physical examination, physical performance, cognitive testing, dietary recall, audio questionnaire, body composition, carotid Doppler, or pulse wave velocity assessment; (4) have valid picture identification; and (5) have a verifiable address at the time of entry. Exclusions included participants who were pregnant at the time of entry, had a diagnosis of AIDS, and were within 6 months of active treatment of cancer (chemotherapy, biological, or radiation). Details of the study design are given elsewhere.[22] Briefly, the data used herein consist of 1920 community-dwelling black and white Baltimore City residents who were enrolled after providing written informed consent and had available serum biomarker data. The MedStar Institutional Review Board approved this protocol.

Assessment of Serum Biomarkers

Measurements of the serum biomarkers were evaluated on cryopreserved (−80°C) serum aliquots. The serum samples were divided into 4 aliquots, and each aliquot was used for the assessment of 1 of the 4 biomarkers (fractalkine, P-selectin, PF4, and TNF-α). Both serum biomarker data and DNA genotypes were available for 1920 HANDLS participants (whites, n=828; African Americans, n=1092). Commercial Meso Scale Discovery kits (Meso Scale Discovery) were used for the measurement of serum fractalkine (Customized Fractalkine Single-Plex assay kit, Category No. N45ZA-1), PF4 (Customized PF4 Single-Plex assay kit, Category No. N45ZA-1), P-selectin (Cataloged P-Selectin Single-Plex assay kit, Category No. N451ENB-1) and TNF-α (Cataloged Human Proinflammatory 9-Plex Assay Ultra-Sensitive Kit, Category No. K15007C-4) levels. Dilution factors were determined using naive serum samples before the sample testing and measurements were made according to manufacturer's instructions. All the serum assessments were performed in duplicate.

Single Nucleotide Polymorphism Selection, Genotyping, and Quality Control

For each gene, we selected single nucleotide polymorphisms (SNPs) using 2 criteria. First, all the known missense and previously associated SNPs were selected for genotyping, and then additional tag SNPs[23] were selected from HapMap phase 1, 2, and 3, using Yoruba in Ibadan, Nigeria and Utah residents with Northern and Western European ancestry datasets. All the tag SNPs were captured from the 20-kb flanking 5′ and 3′ regions of the *CX3CL1*, *CX3CR1*, *CXCR3*, and *PF4* genes, with minor allele frequency >1% and $r^2 \geq 0.8$. Given the proximity of nearby genes to our genes of interest, some of the flanking SNPs lie in nearby genes. A total of 137 tag SNPs from the 4 genes (*CX3CL1*, n=30; *CX3CR1*, n=68; *CXCR3*, n=9; and *PF4*, n=30) were selected and grouped in 4 separate pools for genotyping using iPLEX Gold single base-pair extension with MALDI-TOF mass spectrometry (Sequenom MassArray). Quality control for both samples and SNPs was performed separately for each Sequenom genotyping pool, and the following inclusion/exclusion criteria were applied: SNPs with >5% missingness (23 SNPs) and monomorphic SNPs (4 SNPs) were excluded, and after removal of these SNPs, samples with >50% missingness (reflecting

poor quality DNA and/or poor genotyping) were removed. A sensitivity analysis using a stringent sample missingness exclusion threshold of >5% demonstrated no meaningful difference in results (data not shown). After quality control, 110/137 SNPs were analyzed in 1805 (94%) individuals (whites, n=763; African Americans, n=1042). The allele frequency comparison of the directly genotyped 110 SNPs with the HapMap (phase 1, 2, and 3) population of Utah residents with Northern and Western European ancestry and residents with African ancestry in southwest USA confirmed the quality of the genotyped data, and all the SNPs are in Hardy-Weinberg equilibrium.

Statistics

Before assessing genetic associations, serum biomarker levels in the studied samples were evaluated for normality, and appropriate transformation was applied on each biomarker (fractalkine: log transformed, P-selectin: square root transformed, PF4: log transformed, TNF-α: inverse log transformed). Association analysis and quality control measures were performed using PLINK software package[24] version 1.06 (http://pngu.mgh.harvard.edu/purcell/plink/). To assess the influence of genetic variation in the chemokine/chemokine receptors on the systemic inflammation and platelet activation serum biomarkers, all analyses were stratified by race and adjusted for age and sex in a multivariate linear regression, assuming an additive genetic model of inheritance. To create a more comprehensive fine map of the locus, imputation was performed using the Hidden Markov model, as implemented in the MACH software[25](version 1.0.16)(http://www.sph.umich.edu/csg/abecasis/MACH/). Utah residents with Northern and Western European ancestry and the combined panel of Utah residents with Northern and Western European ancestry and Yoruba in Ibadan, Nigeria, from the 1000 Genomes Project,[26] were used as reference populations for whites and African Americans, respectively. Imputation was performed in 2 steps, and quality control was performed both before and after imputation. In step 1, model parameters were estimated using 100 iterations and, thereafter, allele dosage, and maximum likelihood genotypes were imputed in step 2. To account for the uncertainty of the imputed data, the estimated allele dosage for each SNP was analyzed using ProABEL[27] under a linear regression framework. Standard quality metrics were applied, and only SNPs with high-quality scores (r^2 >0.8) were analyzed. Linkage disequilibrium (LD) patterns within the surrounding region of the significant SNPs were constructed using the solid spine method, as implemented in Haploview[28] (version 4.1) (http://www.broad.mit.edu/mpg/haploview/index.php). For each defined gene region, permutation-based multiple test correction was performed using 5000 permutations. To determine independent genetic effects, multivariate conditional regression analyses were performed using allele dosage data. To identify known functional regulatory variants within or in proximity to the region of interest, the Genotype-Tissue Expression expression quantitative trait loci database was queried (http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi).[29] Further, a long-range haplotype test was used to detect recent selection, and the inferences were made using integrated haplotype score, as described elsewhere.[30]

Results

Detailed demographic and clinical characteristics of the studied 1805 samples are shown in Table 1 and online-only Data Supplement Table I. The average age of the studied cohort was 48 years, with ≈45% males.

Table 1. Demographic and Clinical Characteristics

Characteristics	African Americans	Whites	*P* Value*
Samples, n (%)	1042 (57.7)	763 (42.3)	
Sex, n (%)			
Males	472 (45.3)	341 (44.7)	0.79
Age (y), mean±SD	48.18±8.9	48.39±9.3	0.51
Poverty status†, n (%)			
Below 125%	518 (49.7)	261 (34.2)	<0.001
Serum biomarkers, mean±SD			
Fractalkine (pg/mL)	895.7±438.8	835.9±334.7	< 0.001
P-selectin (ng/mL)	104.1±34.4	108.7±37.1	0.018
Platelet factor 4 (ng/mL)	18098±10796	20285±19480	< 0.001
TNF-α (pg/mL)	14.1±22.4	13.98±23.1	0.002
Diabetes mellitus, n (%)	138 (13.2)	98 (12.8)	0.58
Hypertension, n (%)	406 (38.9)	227 (29.7)	<0.001
Cigarette smoking, n (%)			0.54
Never smoked	195 (18.7)	165 (21.6)	
Smoked, never regularly	91 (8.7)	65 (8.5)	
Former smoker	186 (17.8)	143 (18.7)	
Current smoker	452 (43.4)	319 (41.8)	
Not known	118 (11.3)	71 (9.3)	
Total cholesterol	185.6±44.4	191.2±42.5	0.005
LDL (mg/dL)	108.1±38.6	112.9±36.7	0.004
HDL (mg/dL)	55.94±18.6	48.35±14.1	<0.001

y indicates years; SD, standard deviation; TNF-α, tumor necrosis factor-α; LDL, low-density lipoprotein; HDL, high-density lipoprotein

*P values for continuous and categorical variable comparisons were generated using Wilcoxon rank-sum and Pearson χ^2 test, respectively.

†Poverty status was determined whether a participant reported an annual household income <125% or ≥125% of the 2004 Department of Health and Human Services poverty guideline (http://aspe.hhs.gov/poverty/04poverty.shtml).

Association of *PF4* Genetic Variation With Serum PF4 Levels

Initially, 24 SNPs chosen to tag the *PF4* gene (see Methods) were tested for association with PF4 serum levels, using linear regression models, adjusting for age and sex. In African Americans, 3 SNPs were nominally associated with PF4 levels (P value <0.05), and, in whites, 8 SNPs were nominally associated, with 3 SNPs in common, all showing the same direction of effect in both populations, suggesting the presence of a shared genetic association in African Americans and whites (Table 2). In a joint analysis, adjusting for age, sex, and race, 6 of the SNPs were associated with PF4 levels, with the most significant effect observed for rs183028 (P value: 2.7×10^{-4}) (Table 2). The ancestral allele (A) of rs183028 is the minor allele and is significantly associated with higher serum PF4 levels in both racial groups (black: β=0.06, SE=0.02; whites: β=0.09, SE=0.04). The frequency of this allele (A) in whites was 9%, compared with 41% in African Americans. Given the large allele frequency difference, we explored the possibility of population-specific selection pressure at this locus. To detect signal for recent positive selection, we used integrated haplotype score statistics,[30] based on differential levels of LD surrounding a positively selected allele compared with the background allele at the same position, and no evidence of selection was observed (data not shown).

To fine-map the observed genetic effect and potentially identify additional independent effects, we imputed ungenotyped

Table 2. *PF4* Region SNPs and Genetic Association With Serum PF4 Levels

			African Americans				Whites				Combined		
SNP*	Position	Coded/ Noncoded Allele	Coded Allele Freq	β†	SE	*P* Value	Coded Allele Freq	β†	SE	P Value	β‡	SE	*P* Value
rs1429637	75 052 310	T/C	0.036	−0.11	0.05	0.02	0.060	−0.12	0.05	0.01	−0.12	0.03	6.3×10^{-4}
rs351997	75 052 923	T/C	0.087	0.004	0.03	0.91	0.090	0.09	0.04	0.02	0.04	0.02	0.15
rs6813952	75 063 296	C/A	0.073	−0.04	0.04	0.23	0.059	−0.12	0.05	0.01	−0.07	0.03	0.01
rs1435520	75 064 281	C/A	0.019	−0.16	0.07	0.02	0.053	−0.13	0.05	0.009	−0.14	0.04	3.9×10^{-4}
rs442155	75 070 096	G/A	0.059	0.07	0.04	0.08	0.084	0.10	0.04	0.02	0.08	0.03	0.0035
rs183028	75 070 782	A/G	0.411	0.06	0.02	0.003	0.092	0.09	0.04	0.03	0.06	0.02	2.7×10^{-4}
rs409336	75 076 522	C/A	0.537	0.02	0.02	0.18	0.104	0.09	0.04	0.01	0.04	0.02	0.02
rs2437285	75 079 102	T/C	0.410	0.01	0.02	0.54	0.098	0.09	0.04	0.01	0.03	0.02	0.09

SNP indicates single nucleotide polymorphism; Freq, frequency.
Positions are in reference to NCBI build 36.3.
*Only the SNPs which are nominally associated in at least 1 racial group are shown in the table.
†Effect size of the coded allele based on the multivariate linear regression adjusted for age and sex.
‡Effect size of the coded allele based on the multivariate linear regression adjusted for age, sex, and race.
§Most significant SNPs and the *P* values for their association.

SNPs over a 30-kb interval centered on rs183028, using the 1000 Genomes reference panels.[26] In total, 66 SNPs were imputed, and after removing poorly imputed SNPs (RSq <0.8), 44 SNPs were analyzed. The strongest evidence for association was observed in a region of high LD, encompassing the *PF4*, *PPBP* (proplatelet basic protein) and *CXCL5* genes (Figure 1A and 1B). In this region, 30 SNPs showed nominal association (*P* value <0.05) with PF4 serum levels (Table 3). In African Americans, the most significant association was observed for rs168449 (*P* value=0.0017), where the T allele (frequency 41%) was associated with higher PF4 serum levels (β=0.05; SE=0.02). This SNP is ≈1.3 kb upstream of rs183028, with which it is highly correlated in both racial groups (African Americans: r^2=0.97; whites: r^2=1.0) (Figure 1A, 1B). The association of rs168449 with serum PF4 levels remained significant (P_c value=0.02) even after gene-wide permutation-based multiple correction (Table 3). Similarly, in whites, this SNP showed nominal significance (*P* value=0.014) with the same direction effect (T allele, β=0.09; SE=0.04) (Table 3). The serum PF4 variance explained by rs168449 is 0.58% and 0.64% in African Americans and whites, respectively. In a joint analysis of both racial groups, rs168449 was the most significant SNP (*P* value=1.2×10^{-4}, P_c value=0.0013) from this region, (Table 3), and the distributions of the serum PF4 levels within each genotype group of rs168449 are shown in Figure 2. Individuals who were homozygous for the T allele have higher serum levels compared with those homozygous for the major allele (C) (Figure 2). Given the small number of individuals in the T-allele homozygous group, we also tested the association under a dominant genetic model. In both racial groups, rs168449 maintains significance assuming a dominant model of inheritance (*P* value: African Americans=0.02, whites=0.009, combined=0.001). To test for genetic effects independent of rs168449, we performed conditional multivariate regression analysis. After accounting for the effect of rs168449, rs1429637 and rs1435520 still showed significant association (Table 3). Notably, these SNPs are not in LD with rs168449 (r^2=0) in both racial groups but are in strong LD with each other (r^2=0.88 and 0.51 in whites and African Americans, respectively); thus likely representing the same genetic signal (Figure 1A and 1B). These 2 SNPs are directly genotyped, and rs1435520 showed greater significance in both African Americans (conditional *P* value: 0.022) and whites (conditional *P* value: 0.02). Given the small number of individuals with homozygous rare alleles observed for rs1435520, we also tested the genetic association of serum PF4 levels under a dominant genetic model, combining the rare homozygotes and heterozygous individuals (online-only Data Supplement Table II). As seen with the additive model, both SNPs, rs1429637 (*P* value: African Americans=0.06, whites=0.011, and combined=0.0016) and rs1435520 (*P* value: African Americans=0.045; whites=0.0084, and combined=0.0009), maintain a significant association. In both racial groups, rs1435520 explains more PF4 variability than rs1429637, explaining 0.46% and 0.77% of the variance in African Americans and whites, respectively. Since, rs168449 is an imputed SNP, we also performed conditional multivariate analysis with a directly genotyped SNP that is in complete or high correlation with rs168449, rs183028 (African Americans: r^2=0.97; whites: r^2=1.0). As expected, we only observed significant associations with rs1429637 and rs1435520, confirming the presence of 2 independent loci (rs168449 and rs1435520) regulating serum PF4 levels (online-only Data Supplement Table III). Combined, these 2 loci explain 0.98% (African Americans) and 1.23% (whites) of the variability in serum PF4 levels.

Association of *PF4* Genetic Variation and Serum TNF-α Levels

We also assessed the effect of *PF4* variants on 3 additional serum biomarkers (fractalkine, P-selectin, and TNF-α), and we observed significant association with TNF-α serum levels. The most significant association was seen in African Americans for rs8180167 (β=0.016, SE=0.006; *P* value=0.008), however, this SNP did not show an effect in whites (β=0.003; *P* value=0.66) (online-only Data Supplement Table IV).

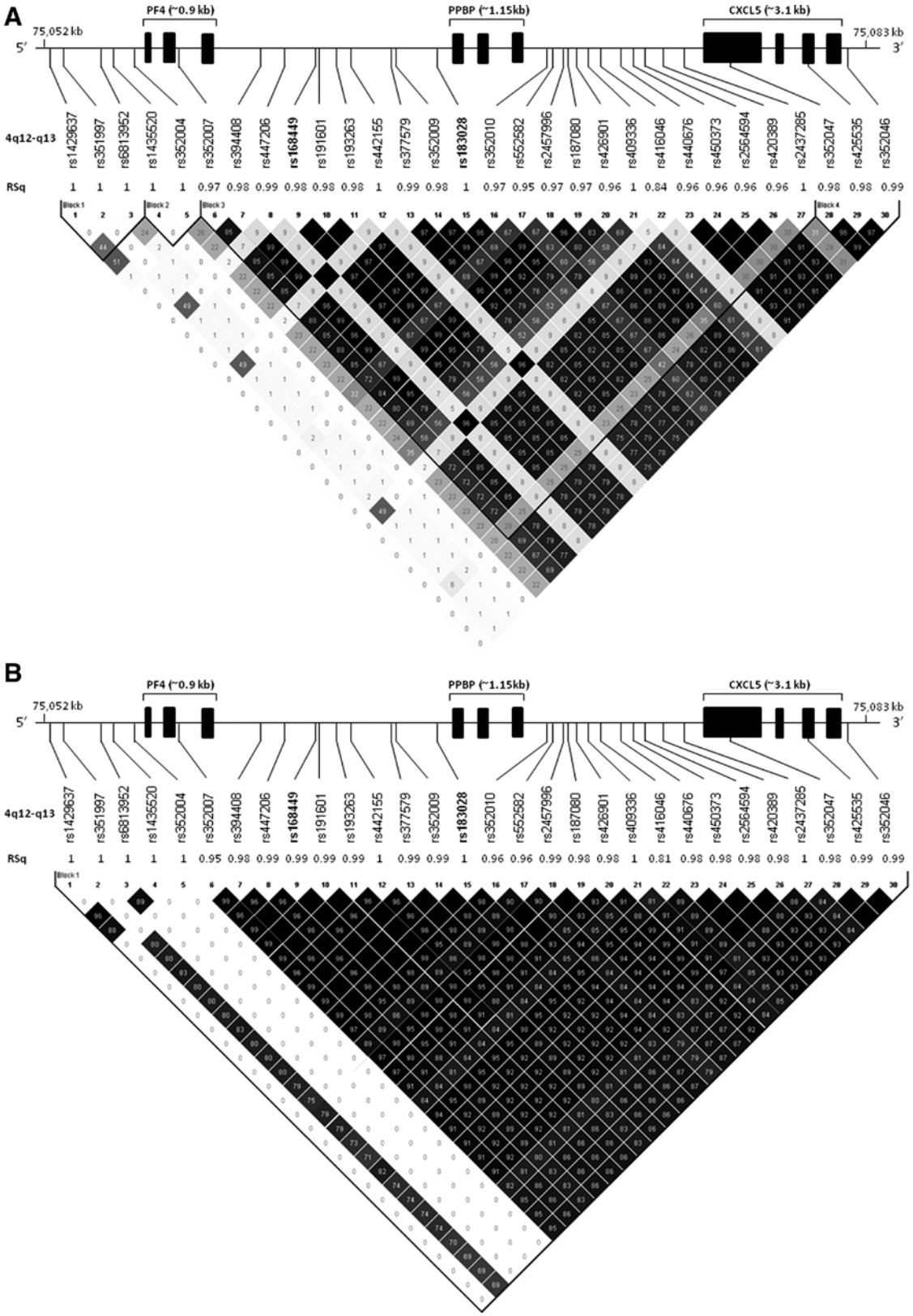

Figure 1. Schematic representation and linkage disequilibrium (LD) pattern of the 4q12-q31 region single nucleotide polymorphisms (SNPs). The upper panel shows the location of the *PF4*, *PPBP*, and *CXCL5* genes and neighboring significant SNPs (n=30). In the middle panel, imputation quality of these SNPs is shown as RSq (squared correlation between imputed and true genotypes), and directly genotyped SNPs are marked as 1. 1a, The bottom panel shows the LD pattern of these SNPs in African Americans, constructed using Haploview (version 4.1) software (http://www.broad.mit.edu/mpg/haploview/index.php). Numbers within diamonds and degree of shading represents the magnitude of the pairwise LD (measured as r^2, black to white gradient reflecting higher to lower LD values). The observed most significant SNP with serum PF4 levels is highlighted in bold. For whites, the pairwise correlation of these SNPs is shown in Figure 1B.

Table 3. Association and Conditional Analysis of the Genotyped and Imputed SNPs From the *PF4* Region

			African Americans							Whites							Combined				
SNPs	Position	Coded/ Noncoded Allele	Coded Allele Freq	Rsq*	β†	SE	*P* Value	P_c Value§	rs168449 Conditional *P* Value¶	Coded Allele Freq	Rsq*	β†	SE	*P* Value	P_c Value§	rs168449 Conditional *P*¶	β‡	SE	*P* Value	P_c Value§	rs168449 Conditional *P*¶
rs1429637	75052310	T/C	0.036	1	−0.11	0.05	0.022	0.18	0.030	0.060	1	−0.12	0.04	0.011	0.06	0.02	−0.12	0.03	0.0006	0.005	0.0014
rs351997	75052923	T/C	0.087	1	0.003	0.03	0.91	0.99	0.653	0.090	1	0.09	0.04	0.015	0.08	0.58	0.04	0.02	0.13	0.63	0.76
rs6813952	75063296	C/A	0.073	1	−0.04	0.03	0.23	0.86	0.112	0.059	1	−0.12	0.04	0.013	0.07	0.03	−0.07	0.03	0.01	0.10	0.06
rs1435520	75064281	C/A	0.019	1	−0.16	0.08	0.015	0.14	0.022	0.053	1	−0.13	0.05	0.009	0.06	0.02	−0.14	0.04	0.0004	0.002	0.0009
rs352004	75065434	T/C	0.253	1	−0.03	0.02	0.15	0.69	0.828	0.008	1	−0.32	0.13	0.077	0.86	0.10	−0.04	0.02	0.10	0.58	0.83
rs352007	75066156	G/C	0.440	0.97	0.05	0.02	0.0048	0.04	0.355	0.092	0.95	0.09	0.04	0.016	0.12	0.97	0.06	0.02	0.0003	0.003	0.33
rs394408	75068216	T/C	0.407	0.98	0.05	0.02	0.0079	0.05	0.364	0.091	0.98	0.09	0.04	0.015	0.09	0.64	0.06	0.02	0.0006	0.005	0.54
rs447206	75068787	A/G	0.060	0.99	0.07	0.04	0.07	0.41	0.277	0.088	0.99	0.10	0.04	0.014	0.08	0.68	0.08	0.03	0.002	0.02	0.13
rs168449	75069487	T/C	0.407	0.98	0.05	0.02	0.0017	0.02	...	0.091	0.99	0.09	0.04	0.014	0.09	...	0.06	0.02	1.2×10^{-4}	0.0013	...
rs191601	75069569	C/T	0.406	0.98	0.05	0.02	0.0077	0.05	0.493	0.091	0.99	0.09	0.04	0.015	0.09	0.99	0.06	0.02	0.0002	0.006	0.53
rs193263	75069687	T/A	0.406	0.98	0.05	0.02	0.0077	0.05	0.458	0.091	0.99	0.09	0.04	0.015	0.09	0.97	0.06	0.02	0.0002	0.006	0.51
rs442155	75070096	G/A	0.060	1	0.07	0.04	0.06	0.41	0.266	0.088	1	0.10	0.04	0.014	0.08	0.71	0.08	0.03	0.002	0.02	0.13
rs377579	75070524	T/C	0.412	0.99	0.06	0.02	0.0029	0.03	0.720	0.091	0.99	0.09	0.04	0.015	0.09	0.82	0.06	0.02	0.0002	0.002	0.31
rs352009	75070557	G/A	0.406	0.98	0.05	0.02	0.0074	0.05	0.780	0.091	0.99	0.09	0.04	0.015	0.09	0.84	0.06	0.02	0.0005	0.006	0.40
rs183028	75070782	A/G	0.412	1	0.06	0.02	0.0029	0.03	0.720	0.091	1	0.09	0.04	0.015	0.09	0.83	0.06	0.02	1.9×10^{-4}	0.0016	0.61
rs352010	75074095	A/G	0.409	0.97	0.05	0.02	0.0075	0.05	0.798	0.094	0.96	0.09	0.04	0.015	0.08	0.99	0.06	0.02	0.0005	0.005	0.83
rs552582	75074722	A/G	0.497	0.95	0.04	0.02	0.03	0.42	0.813	0.100	0.96	0.09	0.04	0.016	0.11	0.77	0.05	0.02	0.003	0.06	0.98
rs2457996	75075399	C/T	0.409	0.97	0.05	0.02	0.0078	0.05	0.951	0.091	0.99	0.09	0.04	0.015	0.08	0.98	0.06	0.02	0.0006	0.005	0.95
rs187080	75075527	T/C	0.416	0.97	0.05	0.02	0.01	0.09	0.780	0.093	0.98	0.10	0.04	0.011	0.08	0.39	0.06	0.02	0.0006	0.006	0.96
rs426901	75076379	G/A	0.455	0.96	0.03	0.02	0.10	0.64	0.100	0.098	0.98	0.10	0.04	0.008	0.06	0.28	0.04	0.02	0.01	0.09	0.23
rs409336	75076522	C/A	0.538	1	0.02	0.02	0.20	0.83	0.270	0.104	1	0.09	0.04	0.013	0.07	0.54	0.04	0.02	0.02	0.17	0.46
rs416046	75076546	A/G	0.053	0.84	0.09	0.04	0.06	0.49	0.254	0.072	0.81	0.12	0.05	0.013	0.07	0.59	0.10	0.03	0.002	0.02	0.12
rs440676	75076834	C/A	0.424	0.96	0.04	0.02	0.03	0.42	0.189	0.095	0.98	0.10	0.04	0.009	0.06	0.36	0.05	0.02	0.002	0.04	0.39
rs450373	75076915	G/A	0.424	0.96	0.04	0.02	0.03	0.42	0.181	0.095	0.98	0.10	0.04	0.009	0.06	0.35	0.05	0.02	0.002	0.04	0.38
rs2564594	75077164	T/A	0.424	0.96	0.04	0.02	0.03	0.42	0.170	0.095	0.98	0.10	0.04	0.009	0.06	0.35	0.05	0.02	0.002	0.04	0.37
rs420389	75077352	G/C	0.424	0.96	0.04	0.02	0.03	0.42	0.160	0.095	0.98	0.10	0.04	0.009	0.06	0.34	0.05	0.02	0.002	0.04	0.35
rs2437285	75079102	T/C	0.412	1	0.01	0.02	0.56	0.99	0.405	0.102	1	0.09	0.04	0.011	0.07	0.45	0.03	0.02	0.09	0.49	0.71
rs352047	75080954	C/G	0.427	0.98	0.02	0.02	0.22	0.90	0.070	0.098	0.98	0.11	0.04	0.009	0.06	0.11	0.04	0.02	0.02	0.15	0.07
rs425535	75082861	T/C	0.416	0.98	0.02	0.02	0.27	0.91	0.070	0.099	0.99	0.11	0.04	0.009	0.06	0.10	0.04	0.02	0.02	0.15	0.07
rs352046	75083414	G/C	0.425	0.99	0.02	0.02	0.27	0.89	0.070	0.100	0.99	0.11	0.04	0.009	0.06	0.10	0.04	0.02	0.02	0.15	0.07

SNPs indicate single nucleotide polymorphisms; Freq, frequency; RSq, squared correlation between imputed and true genotypes.

Positions are in reference to NCBI build 36.3.

*Directly genotyped SNPs are marked as 1.

†Effect size of the coded allele based on the multivariate linear regression adjusted for age and sex (using allele dosages).

‡Effect size of the coded allele based on the multivariate linear regression adjusted for age, sex, and race (using allele dosages).

§Corrected *P* values (P_c) were generated by gene-wide multiple test correction using 5000 permutations.

¶Uncorrected conditional *P* values.

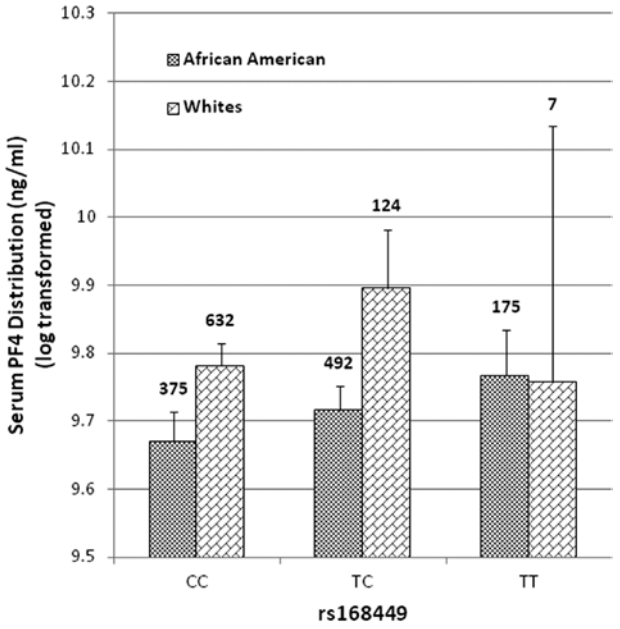

Figure 2. Distribution of serum PF4 levels. The histogram displays the mean values of transformed serum PF4 levels stratified by genotype at the rs168449 locus. The 95% confidence interval (CI) around the mean is represented as vertical bars, and the numbers above the bars indicate individual counts observed for the respective genotype group.

rs8180167 is ≈12.0 kb and ≈17.2 kb upstream of rs1435520 and rs168449, respectively, and is largely uncorrelated within African Americans (rs1435520, r^2=0 and rs168449, r^2=0.04) (online-only Data Supplement Figure I). The association of rs8180167 remained significant after multiple test correction (P_c value=0.048), and no other SNPs in the region show association after accounting for the effect of rs8180167 (online-only Data Supplement Table IV). rs8180167 explains ≈1.3% of serum TNF-α variance.

CX3CL1, *CX3CR1*, and *CXCR3* Genetic Variations and Serum Biomarker Levels

In total, 86 SNPs tagging the remaining 3 genes were tested for all 4 serum biomarkers. We did not detect any influence of these SNPs on biomarker levels in either of the racial groups or in the combined analysis after gene-wide permutation corrections. In addition, the *CX3CR1* gene, which encodes a chemokine receptor for fractalkine, contains 2 nonsynonymous SNPs, rs3732378 (Thr280Met) and rs3732379 (Val249Ile), that were previously associated with reduced prevalence of atherosclerosis and acute coronary events.[31,32] In whites, we observed a nominally significant association of serum fractalkine levels with Thr280Met (*P* value=0.05) (online-only Data Supplement Table V). Given a priori evidence of this variant, we reported the uncorrected *P* value, and results should therefore be interpreted with caution. Surprisingly, the observed effect estimates for rs3732378 (Thr280Met) is in the opposite direction as those previously reported for serum fractalkine. As opposed to the known atheroprotective effect of 280Met, the allele A (Met) was observed associated with higher serum fractalkine levels (β=0.05, SE=0.02) (online-only Data Supplement Table V). Neither SNP showed any significance with the other serum biomarkers.

Discussion

Atherosclerosis is a systemic disease that is responsible for most cardiovascular events and stroke.[4] Given the importance of the inflammatory and platelet activation cascade in the pathogenesis of disease,[5–7] clinical interest has focused on the identification of biomarkers for atherosclerotic risk prediction. Epidemiological studies have demonstrated that African Americans have higher prevalence and disease severity compared with whites.[1,2] The reason for this differential atherogenic susceptibility are multiple and largely unresolved. In this study, we investigated the influence of genetic variation in the chemokine/chemokine receptors on systemic inflammation and platelet activation serum biomarkers.

We performed a comprehensive genetic screen of 4 genes (*CX3CL1*, *CX3CR1*, *CXCR3*, and *PF4*), and report *PF4* locus variants associated with the modulation of serum PF4 and TNF-α levels. For serum PF4 levels, the most significant association was observed for rs168449, which showed consistent effect in both black and white populations. In African Americans, allele T (frequency 41%) was associated with higher levels (Table 3), whereas, in whites, this allele showed the same direction effect but had a ≈4.5-fold lower frequency (T allele frequency, 9%). In the combined dataset, rs168449 maintained a robust association and suggested the potential involvement in the modulation of serum PF4 levels in both racial groups. While accounting for the effect of rs168449, we also report another SNP (rs1435520) as a second independent signal regulating serum PF4 levels from this region. Together these SNPs explained 0.98% and 1.23% of serum PF4 variance in African Americans and whites, respectively. Our study also identified the association of another independent SNP (rs8180167) regulating proinflammatory serum TNF-α levels. In race-stratified analysis, this association was observed only in African Americans and revealed significant differences in allele frequencies between the 2 cohorts (online-only Data Supplement Table IV). Although the frequencies of the ancestral allele (A) in African Americans and whites were substantially different (75% and 18%, respectively), we did not observe any evidence for recent selection at this locus. In an attempt to identify potentially functional variants in this locus, we looked for known expression quantitative trait loci using the Genotype-Tissue Expression database, which queries lymphoblastoid, liver, brain cerebellum, frontal cortex, and temporal cortex tissues but found no known expression quantitative trait loci in this region.

As shown in Figure 1A and 1B, these 3 *PF4* region SNPs (rs168449, rs1435520, and rs8180167) are intergenic SNPs and lie close to the *PPBP* and *CXCL5* genes, which are known to encode inflammatory cytokines. The *PPBP* gene encodes platelet-derived growth factor and functions as a potent chemo-attractant and activator of neutrophils. Neutrophils are the most prominent leukocytes in acute inflammatory reactions and contribute in a number of inflammatory conditions. Recently, Rotzius et al examined the role of

neutrophils in a mouse model of atherosclerosis and noticed that neutrophils accumulate in atherosclerotic lesions.[31] Moreover, neutrophils are the predominant immune cells in the high inflammatory shoulder regions of plaques, suggesting that these cells may play an important role in the immunologic processes of atherogenesis. Similarly, *CXCL5* is also implicated in the chemotaxis of inflammatory cells and previously shown to be a recruiter of neutrophils and involved in their activation. In accordance to these reports, our results suggest the plausible involvement of *PF4*, *PPBP*, and/or *CXCL5* genes in the modulation of serum PF4 and pro-inflammatory TNF-α levels

Our study also confirms the role of a *CX3CR1* missense variant (Thr280Met) that has previously been reported associated with reduced prevalence of atherosclerosis and acute coronary events.[32,33] Previously, the *CX3CR1-280Met* mutant was demonstrated to have an atheroprotective effect[34] and showed a marked reduction in the kinetics of fractalkine binding.[34] The mutant form of *CX3CR1* was also associated with reduced fractalkine-induced chemotactic activity[35] and dysfunction of the receptor.[32,35–37] In this study, we observed a significant association of serum fractalkine levels with Thr280Met, but, surprisingly, the observed effect of 280Met allele is in the opposite direction as those reported previously. In whites, we observed higher fractalkine levels associated with this allele, whereas no significant association was observed in African Americans.

Previously, it has been shown that PF4 is localized to fatty streaks and atherosclerotic lesions.[38] To assess the role of PF4 in the formation of atherosclerotic lesions, Sachais et al investigated the effects of knocking out PF4 expression in mice and showed a significant decrease in lesion formation in the absence of PF4.[39] This finding provides in vivo evidence that establishes that elimination of PF4 is atheroprotective. Given the current knowledge available for the role of PF4 in atherosclerosis, our study demonstrates the importance of *PF4* gene variants in the modulation of serum PF4 and proinflammatory TNF-α levels. In our study, we assumed a model in which inflammatory responses lead to platelet activation, which can, in turn, intensify the inflammatory responses. This model contains regulatory elements that are known risk factors for atherosclerotic cardiovascular disease but has several important limitations that merit discussion. First, given the importance of the platelet activation and inflammatory mediators in the progression of atherosclerosis, we only tested 4 genes in our study, and possibly other genes have a greater biological impact on these alterations. Through this study, we observed that *PF4* SNPs explain a small proportion ($\approx$1%) of genetic variability for serum PF4 and TNF-α levels; hence, suggesting the involvement of other genes contributing alterations in serum levels. Second, our study is only limited to the association of the systemic inflammation and platelet activation serum biomarkers, whereas exploring subclinical measures of atherosclerosis (carotid intima media thickness, pulse wave velocity, heart rate variability, etc.) might have high biological relevance in context to atherosclerosis. Furthermore, although we have performed multiple test correction for each gene, we have not additionally corrected for the 4 traits studied. Knowing that the genetic puzzle of atherosclerotic cardiovascular disease will be complex and involve genes/loci from multiple biological pathways, the next step will be to understand the relationships between disease risk loci important in innate and adaptive immunity, inflammation, blood pressure control, and vascular reactivity and other factors; however, most critical will be an approach that keeps in mind that the relative risks of disease have different weights in the process of disease development and considerable influence especially when taken in the context of gene-environment interactions.[40–43] Examining those genes demonstrated to affect the risk of disease in African Americans might allow us to work toward a model that includes blood pressure control loci *SLC24A4* (rs11160059; rs17783630) and incident coronary heart disease loci *PFTK1* variant (rs1859023) identified to be important in African Americans.[44,45] Our finding in *PF4* highlights the possible relevance of markers of systemic inflammation in atherosclerotic heart disease among African Americans. These findings should be put in a model with other loci important in modulating levels of acute phase reactants such as C-reactive protein and other inflammatory molecules. The disparity in the incidence and severity of atherosclerotic cardiovascular disease is linked to unique inter-relationships between differentially expressed gene loci from different molecular pathways and, ultimately, with their interaction with the environment.

In conclusion, our results overall indicate that *PF4* genetic variants are likely involved in the alterations of serum PF4 levels in both black and white populations. We also demonstrate that another *PF4* variant (rs8180167) has a race-specific effect in the regulation of serum TNF-α levels in African Americans. Taken together, these results highlight the importance of *PF4* genetic variation in the regulation of systemic inflammation (TNF-α) and platelet activation (PF4) serum biomarkers.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Given the importance of systemic inflammation and platelet activation to the development of atherosclerosis, we explored the role of genetic variants in regulating these processes. We performed a comprehensive genetic screen of 4 genes (*CX3CL1*, *CX3CR1*, *CXCR3*, and *PF4*) and report *PF4* locus variants associated with the modulation of serum PF4 and TNF-α levels. For serum PF4 levels, the most significant association was observed for rs168449, which showed consistent effect in both black and white populations. In African Americans, allele T was associated with higher serum levels of PF4, whereas, in whites, this allele showed the same direction effect but had a several-fold lower frequency. Our study also identified the association of another independent SNP (rs8180167) regulating proinflammatory serum TNF-α levels. In conclusion, our results overall indicate that *PF4* genetic variants are likely involved in the alterations of serum PF4 levels in both black and white populations. We also demonstrate that another *PF4* variant (rs8180167) has a race-specific effect in the regulation of serum TNF-α levels in African Americans. Taken together, these results highlight the importance of *PF4* genetic variation in the regulation of systemic inflammation (TNF-α) and platelet activation (PF4) serum biomarkers. Knowing that the genetic puzzle of atherosclerotic cardiovascular disease will be complex and involve genes/loci from multiple biological pathways, the next step will be to understand the relationships between disease risk loci important in innate and adaptive immunity, inflammation, blood pressure control, and vascular reactivity and other factors.

SUPPLEMENTAL MATERIAL

Supplementary Table 1. Coefficient of variation of the studied 4 serum biomarkers

Serum Biomarkers, CV*	African Americans	Whites
Fractalkine (pg/mL)	0.36	0.39
P-selectin (ng/mL)	0.33	0.34
Platelet factor 4 (ng/mL)	0.45	0.44
TNFα (pg/mL)	1.13	1.08

* Coefficient of variation is defined as the ratio of the standard deviation to the mean

Supplementary Table 2. Association of rs1429637 and rs1435520 SNPs with serum PF4 levels under different genetic models

SNP	Position	Model	Genotypes	African Americans					Whites					Combined				
				PF4 levels		95% CI		P	PF4 levels		95% CI		P	PF4 levels		95% CI		P
				Mean	SE	Lower	Upper		Mean	SE	Lower	Upper		Mean	SE	Lower	Upper	
rs1429637	75,052,310	Dominant																
			CC	9.72	0.01	-	-	0.06	9.82	0.02	-	-	0.011	9.76	0.01	-	-	0.0016
			CT-TT	9.62	0.04	-0.2	0		9.68	0.04	-0.22	-0.03		9.66	0.03	-0.18	-0.04	
		Recessive																
			CC-CT	9.71	0.01	-	-	0.0039	9.8	0.02	-	-	0.613	9.75	0.01			0.02
			TT	8.83	0.41	-1.44	-0.28		9.67	0.26	-0.76	0.45		9.25	0.32	-0.91	-0.08	
rs1435520	75,064,281	Dominant																
			AA	9.71	0.01	-	-	0.045	9.82	0.02	-	-	0.0084	9.75	0.01	-	-	0.0009
			AC-CC	9.58	0.07	-0.28	0		9.67	0.05	-0.24	-0.04		9.64	0.04	-0.22	-0.06	
		Recessive																
			AA-AC	9.71	0.01	-	-	0.0023	9.8	0.02	-	-	0.613	9.75	0.01	-	-	0.03
			CC	8.41	0	-2.1	-0.46		9.67	0.26	-0.76	0.45		9.25	0.45	-1	-0.04	

Supplementary Table 3. Conditional analysis of rs183028 in the PF4 region

			African Americans			Whites			Combined
SNPs	Position	Coded/ Noncoded Allele	Coded Allele Freq	Rsq*	rs183028 Conditional P-value¶	Coded Allele Freq	Rsq*	rs183028 Conditional P-value¶	rs183028 Conditional P-value¶
rs1429637	**75,052,310**	**T/C**	**0.036**	**1**	**0.034**	**0.060**	**1**	**0.02**	**0.002**
rs351997	75,052,923	T/C	0.087	1	0.622	0.090	1	0.57	0.82
rs6813952	75,063,296	C/A	0.073	1	0.109	0.059	1	0.03	0.07
rs1435520	**75,064,281**	**C/A**	**0.019**	**1**	**0.025**	**0.053**	**1**	**0.02**	**0.001**
rs352004	75,065,434	T/C	0.253	1	0.918	0.008	1	0.10	0.93
rs352007	75,066,156	G/C	0.440	0.97	0.781	0.092	0.95	0.99	0.44
rs394408	75,068,216	T/C	0.407	0.98	0.220	0.091	0.98	0.72	0.32
rs447206	75,068,787	A/G	0.060	0.99	0.301	0.088	0.99	0.67	0.17
rs168449	75,069,487	T/C	0.407	0.98	0.690	0.091	0.99	0.81	0.72
rs191601	75,069,569	C/T	0.406	0.98	0.690	0.091	0.99	0.75	0.34
rs193263	75,069,687	T/A	0.406	0.98	0.690	0.091	0.99	0.76	0.34
rs442155	75,070,096	G/A	0.060	1	0.301	0.088	1	0.70	0.17
rs377579	75,070,524	T/C	0.412	0.99	0.800	0.091	0.99	0.99	0.69
rs352009	75,070,557	G/A	0.406	0.98	0.820	0.091	0.99	0.72	0.41
rs183028	**75,070,782**	**A/G**	**0.412**	**1**	**-**	**0.091**	**1**	**-**	**-**
rs352010	75,074,095	A/G	0.409	0.97	0.800	0.094	0.96	0.97	0.73
rs552582	75,074,722	A/G	0.497	0.95	0.254	0.100	0.96	0.76	0.58
rs2457996	75,075,399	C/T	0.409	0.97	0.119	0.091	0.99	0.96	0.78
rs187080	75,075,527	T/C	0.416	0.97	0.302	0.093	0.98	0.39	0.73
rs426901	75,076,379	G/A	0.455	0.96	0.260	0.098	0.98	0.28	0.11
rs409336	75,076,522	C/A	0.538	1	0.167	0.104	1	0.53	0.33
rs416046	75,076,546	A/G	0.053	0.84	0.357	0.072	0.81	0.59	0.16
rs440676	75,076,834	C/A	0.424	0.96	0.290	0.095	0.98	0.35	0.15
rs450373	75,076,915	G/A	0.424	0.96	0.290	0.095	0.98	0.35	0.14
rs2564594	75,077,164	T/A	0.424	0.96	0.290	0.095	0.98	0.34	0.14
rs420389	75,077,352	G/C	0.424	0.96	0.290	0.095	0.98	0.34	0.13
rs2437285	75,079,102	T/C	0.412	1	0.336	0.102	1	0.44	0.61
rs352047	75,080,954	C/G	0.427	0.98	0.060	0.098	0.98	0.10	0.10
rs425535	75,082,861	T/C	0.416	0.98	0.070	0.099	0.99	0.10	0.10
rs352046	75,083,414	G/C	0.425	0.99	0.070	0.100	0.99	0.10	0.10

Positions are in reference to NCBI build 36.3

*Squared correlation between imputed and true genotypes. Directly genotyped SNPs are marked as 1

¶ Uncorrected conditional P-values

Significant SNP and the P-values for their association are highlighted in bold

Supplementary Table 4. Association analysis of *PF4* variants with serum TNFα levels

SNP	Position	Coded/ Noncoded Allele	African Americans							Caucasians					Combined			
			Coded Allele Freq	Rsq*	β†	SE	P	Pc	rs8180167 Conditional P¶	Coded Allele Freq	Rsq*	β†	SE	P	β‡	SE	P	Pc
rs10017182	75,050,352	G/A	0.799	0.940	0.015	0.007	0.025	0.18	0.83	0.183	0.966	0.003	0.006	0.61	0.010	0.005	0.03	0.18
rs8180167	**75,052,237**	**A/T**	**0.752**	**0.987**	**0.016**	**0.006**	**0.008**	**0.048**	**-**	**0.180**	**0.982**	**0.003**	**0.006**	**0.66**	**0.011**	**0.004**	**0.01**	**0.07**
rs1429637	75,052,310	C/T	0.964	1	-0.010	0.017	0.531	0.99	0.64	0.940	1	-0.015	0.008	0.14	-0.013	0.008	0.15	0.55
rs1595008	75,053,322	C/A	0.798	0.949	0.015	0.007	0.024	0.19	0.87	0.179	0.962	0.003	0.006	0.61	0.010	0.005	0.03	0.19
rs1595009	75,053,520	C/T	0.812	0.878	0.016	0.007	0.025	0.19	0.86	0.230	0.717	0.003	0.006	0.64	0.010	0.005	0.04	0.19
rs2175498	75,054,231	A/G	0.800	0.954	0.015	0.007	0.025	0.19	0.86	0.180	0.975	0.003	0.006	0.63	0.010	0.005	0.03	0.19
rs4694659	75,056,016	C/A	0.800	0.954	0.015	0.007	0.025	0.19	0.86	0.179	0.973	0.003	0.006	0.65	0.010	0.005	0.04	0.19
rs4694180	75,056,266	T/C	0.800	0.954	0.015	0.007	0.025	0.19	0.86	0.179	0.973	0.003	0.006	0.65	0.010	0.005	0.04	0.19
rs6446973	75,057,336	A/T	0.800	0.954	0.015	0.007	0.026	0.19	0.87	0.179	0.973	0.003	0.006	0.65	0.009	0.005	0.04	0.19
rs6820253	75,057,935	C/T	0.582	0.974	-0.011	0.005	0.044	0.29	0.37	0.901	0.982	0.006	0.008	0.41	-0.007	0.004	0.13	0.57
rs4694660	75,059,309	T/A	0.800	0.955	0.015	0.007	0.026	0.19	0.87	0.179	0.973	0.003	0.006	0.66	0.009	0.005	0.04	0.20
rs4694661	75,059,477	A/T	0.800	0.955	0.015	0.007	0.026	0.19	0.87	0.179	0.973	0.003	0.006	0.66	0.009	0.005	0.04	0.20
rs1594230	75,060,249	C/T	0.582	0.976	-0.011	0.005	0.043	0.29	0.37	0.901	0.985	0.006	0.008	0.41	-0.007	0.004	0.13	0.57
rs1594229	75,060,428	C/G	0.582	0.977	-0.011	0.005	0.043	0.29	0.37	0.901	0.985	0.006	0.008	0.41	-0.007	0.004	0.13	0.57
rs6810940	75,061,053	C/T	0.801	0.957	0.015	0.007	0.026	0.19	0.88	0.178	0.974	0.003	0.006	0.67	0.009	0.005	0.04	0.20
rs1836663	75,061,436	T/C	0.582	0.979	-0.011	0.005	0.042	0.25	0.36	0.901	0.987	0.006	0.008	0.41	-0.007	0.004	0.13	0.51
rs2472655	75,061,882	T/C	0.582	0.979	-0.011	0.005	0.042	0.25	0.36	0.901	0.988	0.006	0.008	0.41	-0.007	0.004	0.13	0.51
rs1156554	75,063,996	C/T	0.584	0.970	-0.012	0.005	0.032	0.20	0.34	0.901	0.996	0.006	0.008	0.40	-0.007	0.005	0.10	0.44
rs1156553	75,064,018	T/G	0.591	0.995	-0.012	0.005	0.033	0.20	0.33	0.901	0.997	0.006	0.008	0.40	-0.007	0.005	0.11	0.44
rs1435520	75,064,281	A/C	0.981	1	-0.004	0.033	0.866	0.99	0.96	0.947	1	-0.017	0.008	0.10	-0.014	0.010	0.19	0.63
rs168449	75,069,487	T/C	0.407	0.980	0.001	0.006	0.816	0.99	0.55	0.091	0.988	-0.015	0.007	0.08	-0.003	0.005	0.51	0.99
rs183028	75,070,782	A/G	0.412	1	0.002	0.006	0.780	0.99	0.60	0.091	1	-0.015	0.007	0.08	-0.003	0.005	0.54	0.99
rs2472649	75,076,572	A/G	0.819	1	0.012	0.006	0.064	0.31	0.78	0.165	1	-0.008	0.006	0.21	0.004	0.005	0.40	0.92
rs12512838	75,078,267	C/T	0.817	0.928	0.012	0.007	0.078	0.36	0.70	0.201	0.772	-0.008	0.006	0.21	0.004	0.005	0.44	0.94

Positions are in reference to NCBI build 36.3

*Squared correlation between imputed and true genotypes. Directly genotyped SNPs are marked as 1

†Effect size of the coded allele based on the multivariate linear regression adjusted for age and sex (using allele dosages)

‡Effect size of the coded allele based on the multivariate linear regression adjusted for age, sex and race (using allele dosages)

Corrected P-values (Pc) were generated by gene-wide multiple correction (maximum likelihood genotypes) using 5000 permutations

¶ Uncorrected conditional P-values

Most significant SNP and the P-values for their association are highlighted in bold

Supplementary Table 5. Association of *CX3CR1* previously reported SNPs with serum fractalkine levels

Chr	SNP	Position	Coded/ Noncoded Allele	African Americans				Caucasians				Combined		
				Coded Allele Freq	β†	SE	P	Coded Allele Freq	β†	SE	P	β‡	SE	P
3	**rs3732378 (Thr280Met)**	**39,282,166**	**A/G**	**0.028**	**0.04**	**0.05**	**0.43**	**0.18**	**0.05**	**0.02**	**0.05**	**0.04**	**0.02**	**0.04**
3	rs3732379 (Val249Ile)	39,282,260	T/C	0.119	0.006	0.02	0.81	0.287	0.003	0.02	0.89	0.004	0.02	0.78

Positions are in reference to NCBI build 36.3
†Effect size of the coded allele based on the multivariate linear regression adjusted for age and sex
‡Effect size of the coded allele based on the multivariate linear regression adjusted for age, sex and race
Marginal significant SNP and the P-values for the association are highlighted in bold

Supplementary Figure 1. Linkage disequilibrium (LD) pattern of SNPs associated with serum TNFα level in African Americans