Relations of Inflammatory Biomarkers and Common Genetic Variants With Arterial Stiffness and Wave Reflection

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Abstract—Inflammation causes vascular dysfunction and perpetuates proatherosclerotic processes. We hypothesized that a broad panel of inflammatory biomarkers and single nucleotide polymorphisms in inflammatory genes is associated with vascular stiffness. We assessed 12 circulating inflammatory biomarkers (C-reactive protein, fibrinogen, interleukin-6, intercellular adhesion molecule-1, lipoprotein-associated phospholipase-A2 [mass and activity], monocyte chemoattractant protein-1, myeloperoxidase, CD40 ligand, osteoprotegerin, P-selectin, and tumor necrosis factor receptor-II) in relation to tonometry variables (central pulse pressure, mean arterial pressure, forward pressure wave, reflected pressure wave, carotid-femoral pulse wave velocity, and augmentation index) measured in 2409 Framingham Heart Study participants (mean age: 60 years; 55% women; 13% ethnic/racial minorities). Single nucleotide polymorphisms (n=2195) in 240 inflammatory candidate genes were related to tonometry measures in 1036 white individuals. In multivariable analyses, biomarkers explained <1% of any tonometry measure variance. Applying backward elimination, markers related to tonometry ($P<0.01$) were as follows: tumor necrosis factor receptor-II (inversely) with mean arterial pressure; C-reactive protein (positively) and lipoprotein-associated phospholipase-A2 (inversely) with reflected pressure wave; and interleukin-6 and osteoprotegerin (positively) with carotid-femoral pulse wave velocity. In genetic association analyses, lowest *P* values (false discovery rate <0.50) were observed for rs10509561 (*FAS*), $P=6.6\times10^{-5}$ for central pulse pressure and rs11559271 (*ITGB2*), $P=1.1\times10^{-4}$ for mean arterial pressure. These data demonstrate that, in a community-based sample, circulating inflammatory markers tumor necrosis factor receptor-II (mean arterial pressure), C-reactive protein, lipoprotein-associated phospholipase-A2 activity (reflected pressure wave), interleukin-6, and osteoprotegerin (carotid-femoral pulse wave velocity) were significantly but modestly associated with measures of arterial stiffness and wave reflection. Additional studies are needed to determine whether variation in inflammatory marker genes is associated with tonometry measures. **(*Hypertension*. 2008;51:1651-1657.)**

Key Words: tonometry ■ inflammation ■ epidemiology ■ polymorphism ■ single nucleotide ■ genetics

Arterial stiffness has been associated with increased cardiovascular morbidity and mortality.[1] The clinical correlates of increasing vascular stiffness are advancing age, sex, body mass index,[2] hypertension,[3] diabetes mellitus, and smoking.[4] The clinical applications and importance of arterial stiffness and wave reflection in cardiovascular disease assessment have been reviewed recently.[5] Experimental and human evidence suggest that inflammation may contribute to vascular stiffness. For instance, autoimmune diseases are accompanied by increased arterial stiffness and premature cardiovascular disease.[6] Current research suggests causal relations between acute inflammatory states induced by vaccination and impaired vascular function detected by reversible increases in pulse wave velocity (PWV).[7] In addition, cross-sectional human studies have reported that C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α concentrations are related to PWV[8–10] and arterial elasticity.[11] Even in apparently healthy individuals, large artery stiffness

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and central pulse pressure (CPP) are correlated with the extent of systemic inflammation.[12,13]

Recent studies suggest a potential role of genetic variation in tonometry response. Heritability for tonometry variables has been demonstrated, and linkage investigations identified several genomic regions for further research.[14] Single nucleotide polymorphisms (SNPs) in genes coding for proteins, such as the renin-angiotensin-aldosterone system,[15] endothelial NO synthase,[16] and inflammatory mediators (eg, CRP), have been reported to be related to arterial stiffness measures.[17]

We sought to examine associations between systemic inflammatory markers and SNPs with vascular stiffness measures in a community-based cohort. We hypothesized that ≥1 of a broad range of inflammatory biomarkers is related to noninvasive measures of arterial stiffness and wave reflection. Furthermore, we tested the hypothesis that SNPs in inflammatory candidate genes are associated with tonometry measures, in part because of the effect of SNPs on biomarker concentrations.

Materials and Methods

Study Sample

The Offspring cohort was recruited in the 1970s and has been examined routinely every 4 to 8 years.[18] Multiethnic Omni participants were recruited in the 1990s.[19] Participants attending examination 7 (n=3537) or Omni examination 2 (n=405) were eligible for analyses. Vascular tonometry data and inflammatory markers were available in 2095 Offspring participants and 314 Omni participants (for detailed exclusions, see supplemental data available online at http://hyper.ahajournals.org). Genotype data were available on 1036 Offspring participants. The Boston University Medical Center Institutional Review Board approved the study protocols.

Noninvasive Hemodynamic Data Acquisition

Noninvasive measures of arterial stiffness and wave reflection were assessed in supine participants as described previously, blinded to clinical and biomarker data (see supplemental data for further details).[20]

Biomarker Determination

Twelve circulating markers, representing different inflammatory pathways, were selected a priori, including the following: CD40 ligand, CRP, fibrinogen, intercellular adhesion molecule-1, IL-6, lipoprotein-associated phospholipase-A2 (Lp-PLA2) activity and mass, monocyte chemoattractant protein-1, myeloperoxidase, osteoprotegerin, P-selectin, and TNF receptor-II (TNFRII). Details of specimen type, measurement kit, and reproducibility are provided in Table S1.

Genotyping

Genotyping of common SNPs was conducted by Perlegen Sciences, Inc (232 genes and 2942 SNPs) and the Broad Institute of Harvard and Massachusetts Institute of Technology (9 genes and 125 SNPs) using methods described in the supplemental data. Inflammatory candidate genes were selected by Framingham investigators, guided by criteria outlined in Table S2. A total of 2195 SNPs in 240 genes passed quality control.

Statistical Analysis

Biomarker Analyses

We used log-normal transformed biomarkers. We conducted multivariable linear regression to relate tonometry measures (dependent variables) to circulating biomarkers, adjusting for cohort, sex, age, age^2, mean arterial pressure (MAP; except if MAP was the dependent measure), heart rate, height, weight, total/high-density lipoprotein cholesterol, glucose, diabetes, smoking, prevalent cardiovascular disease, hormone replacement therapy, hypertension treatment, aspirin (≥3 days per week), and lipid-lowering medication. Tonometry measures associated with the inflammatory marker panel with a global $P<0.01$ were studied further. Forcing in clinical covariates, we selected biomarkers associated with the respective tonometry measure with backward elimination models ($P<0.01$ for inclusion).

Genetic Analyses

Potential variation because of major confounders of tonometry variables was accounted for by the creation of multivariable-adjusted residuals (age, age^2, sex, height, and weight). We examined tonometry residuals in association with inflammatory SNPs using ANOVA with a general genetic model (2 degrees of freedom); if the lowest frequency genotype category had <10 individuals, nonparametric Kruskal-Wallis tests were performed. Mean residual tonometry measures and β-coefficients were computed for each genotype. In addition, we calculated within-phenotype q-values. We used q-value <0.50 as a threshold indicative of potentially important associations.[21] The q-value represents the expected proportion of false-positive associations among tests exhibiting the specified level of statistical significance and is less conservative than Bonferroni adjustments.

Secondary Analyses

Because of numerous SNP, tonometry, and biomarker measures, we were concerned about multiple testing but were aware that different research groups have assessed various tonometry and biomarker measures. To maximize results disclosure yet modestly reduce multiple testing penalties, we a priori specified secondary phenotypes. Table S3 displays biomarker and tonometry characteristics of primary (6 tonometry and 12 biomarker) and secondary (2 tonometry and 4 biomarker [available on a subset]) measures. We provide secondary analyses of Pearson partial correlation coefficients adjusted for age, age^2, sex, and cohort for individual biomarkers (12 primary and 4 secondary) and tonometry variables (dependent measures: 6 primary, in Table S4, and 2 secondary tonometry measures, in Table S5). We tested final models for interactions with hypertension (3 levels: hypertension treated, hypertension untreated, and no hypertension), lipid-lowering treatment, and hormone replacement therapy.

To explore mendelian randomization,[22] we examined biomarkers that were retained in backward elimination models relating biomarker concentrations to tonometry measures. The associations of SNPs significantly associated with each biomarker (ie, SNPs both cis [within] and trans [outside] the gene coding the marker) were assessed in relation to the same tonometry measures with which the biomarker was significantly related.

SAS version 8.1 (http://www.sas.com/presscenter/guidelines.html) was used for regression analyses and for creating phenotype residuals for genetic analyses. R was used for genetic analyses (www.r-project.org).

Results

Participant Characteristics

Clinical characteristics by study sample are presented in Table S6. Briefly, the phenotype sample included Omni ethnic minority participants and was slightly younger compared with the genotype sample, which consisted only of white Offspring cohort participants. Tonometry measures and inflammatory marker concentrations are provided in Table S3.

Inflammatory Biomarkers and Tonometry Variables

In multivariable-adjusted analyses, the inflammatory markers as a group were significantly related to 3 tonometry mea-

Table 1. Multiple Inflammatory Phenotypes in Relation to Tonometry Variables and Backward Elimination of Inflammatory Biomarkers

Response	Model R^2	Partial R^2	Global P*	Biomarker†	β‡	P
CPP, mm Hg	0.4633	0.0058	0.01			
MAP, mm Hg	0.1869	0.0094	0.007	TNFRII	−0.92±0.26	0.0005
Forward pressure wave (primary wave), mm Hg	0.3478	0.0041	0.25			
RPW (augmented pressure), mm Hg	0.3961	0.0069	0.008	CRP	0.40±0.15	0.009
				Lp-PLA2 activity	−0.45±0.16	0.005
CFPWV, m/s	0.5390	0.0080	<0.0001	IL-6	0.18±0.06	0.001
				Osteoprotegerin	0.19±0.06	0.001
Augmentation index, %	0.3809	0.0057	0.04			

Partial R^2 is provided for the tonometric variation explained by inflammatory biomarkers.

*Global P from testing whether the 12 inflammatory biomarker set is related to specified vascular function measure. Covariates (n=17) in the multivariable models are noted in the Statistical Analysis, Biomarker Analyses section.

†For tonometry measures with a global $P<0.01$, individual biomarkers were significantly ($P<0.01$) related to tonometry measures after backward elimination of 12 eligible inflammatory biomarkers.

‡β, the regression coefficient, shows estimated change in vascular function measure per 1-SD increment in a log-transformed inflammatory marker.

sures: MAP, reflected pressure wave (RPW), and carotid-femoral pulse wave velocity (CFPWV; global $P<0.01$). Therefore, we conducted backward elimination of the 12 inflammatory biomarkers in relation to the 3 tonometry measures, adjusting for 17 potential confounders (Table 1). TNFRII was inversely associated with MAP; CRP (positive) and Lp-PLA2 activity (inverse) were related to RPW; and IL-6 and osteoprotegerin were positively associated with CFPWV. After accounting for 17 potential clinical confounders, the inflammatory markers explained <1% of the tonometry measure variability (partial R^2 ranged from 0.69% for RPW to 0.94% for MAP).

Phenotype Genotype Association

We examined the relations of 2195 SNPs in 240 inflammatory candidate genes to tonometry variables. The top 5 SNPs associated with each tonometry variable are displayed (Table 2; full disclosure available at the Framingham Heart Study inflammation Web site [http://www.inflammation-framinghamheartstudy.org]). We observed that SNP rs10509561 in *FAS* (previously *TNFRSF6*; $P=6.6\times10^{-5}$) was associated with CPP. For SNP rs10509561, heterosis was seen with the lowest geometric mean for the heterozygote (standardized residual mean: −0.13±0.09 compared with means of 0.06±0.04 in the major allele and 0.27±0.11 in the minor allele homozygote). Rs10509561 in *FAS* was also among the top SNPs for forward pressure wave ($P=1.2\times10^{-3}$) and RPW (8.9×10^{-3}; see Figure S1). In addition, SNP rs11559271 in the *ITGB2* gene ($P=1.1\times10^{-4}$) was associated with MAP. Only 7 SNP biomarker associations showed q-values of <0.5.

Secondary Analyses

We observed modest correlations for pairwise comparisons of tonometry variables with inflammatory markers (partial correlation coefficients ranging from −0.11 to 0.17; Table S4). An unanticipated observation was that 2 of the markers selected with backward elimination in Table 1 in the 17 covariate-adjusted models were not significantly associated with the tonometry measure if examined individually (secondary analyses). As displayed in Table S4, with the model adjusting for 4 covariates (age, age^2, sex, and cohort), TNFRII concentrations were not associated with MAP ($r=0.00$; $P=0.82$), and CRP concentrations were not associated with RPW ($r=0.02$; $P=0.43$). We conducted posthoc exploratory analyses to understand which covariate(s) affected the TNFRII-MAP association; hypertension treatment and hormone replacement therapy were the factors most responsible for rendering the TNFRII-MAP relation significant in the 17 covariate-adjusted multivariable model.

Using $P<0.01$, we observed potential interactions between several inflammatory markers and hypertension categories. Specifically, the associations between Lp-PLA2 and RPW and between IL-6 and CFPW were stronger in those with compared with those without hypertension. Individuals taking lipid-lowering medication (versus those without) had a more positive slope between IL6 and CFPWV (Table S7).

For biomarkers significantly associated with tonometry measures (Table 1), we subsequently examined whether SNPs associated with specific biomarker concentrations were also associated with the respective tonometry measure (Table S8) at $P\leq0.05$ to test the "mendelian randomization" concept. Top associations were observed for *TNFSF15* SNP rs10817678 for osteoprotegerin concentrations ($P=0.003$) and CFPWV ($P=0.02$) and for rs6586166 in the *FAS* gene in relation to IL-6 ($P=0.009$) and CFPWV ($P=0.02$). The SNP rs1051931 in the *PLA2G7* gene was associated with Lp-PLA2 activity ($P=0.0001$), but the association with RPW was $P=0.054$.

Discussion

Principal Findings

In a community-based cohort, 12 circulating inflammatory biomarkers representing different pathophysiological pathways and 2195 SNPs in 240 inflammation-related candidate genes were related to tonometric measures of arterial stiffness and wave reflection. The circulating inflammatory biomarker panel revealed significant associations between IL-6 and

Table 2. Sex-Pooled Association of SNPs From 240 Inflammatory Candidate Genes With Multivariable-Adjusted Tonometry Measures

Gene	Allelic Variant	Chr	Location	LD	Major Minor Allele	MAF	Heterozygote β	Heterozygote SE	Homozygote β	Homozygote SE	Partial R^2	*P*	q Value
CPP													
GCLM	rs2301022	1	94084899		T C	33.7	−0.03	0.06	−0.34	0.10	0.010	2.1×10^{-3}	0.55
INDO	rs3808606	8	39888532		A G	48.5	0.12	0.07	−0.14	0.08	0.010	2.2×10^{-3}	0.55
FAS	rs10509561	10	90741892		T A	33.4	−0.18	0.06	0.21	0.10	0.016	6.6×10^{-5}	0.13
CCL7	rs3091324	17	29625029	0.99	G T	18.6	0.10	0.07	0.53	0.15	0.011	1.7×10^{-3}	0.55
CCL7	rs17735961	17	29636453		C A	18.4	0.09	0.07	0.54	0.16	0.011	1.8×10^{-3}	0.55
MAP													
VEGFC	rs3775203	4	177986180		G T	45.5	0.23	0.07	0.26	0.08	0.012	7.6×10^{-4}	0.50
ITGB2	rs84193	21	45094804	0.11	G C	35.5	−0.01	0.06	0.29	0.09	0.010	2.6×10^{-3}	0.76
ITGB2	rs11559271	21	45144741		C T	24.7	0.08	0.06	0.53	0.12	0.015	1.1×10^{-4}	0.22
PDGFB	rs5757573	22	37958122	0.99	C T	34.7	−0.21	0.06	−0.0004	0.09	0.011	1.3×10^{-3}	0.62
PDGFB	rs5757572	22	37957420		C G	34.5	−0.20	0.06	0.01	0.09	0.010	2.0×10^{-3}	0.76
Forward pressure wave													
DDAH1	rs2177461	1	85573997	0.88	C G	37.0	0.01	0.06	0.33	0.10	0.011	1.3×10^{-3}	0.46
DDAH1	rs233112	1	85497772		T C	38.6	0.04	0.07	0.32	0.09	0.010	2.0×10^{-3}	0.46
IL12B	rs13153734	5	158639291		C T	19.5	0.20	0.06	0.41	0.16	0.012	8.0×10^{-4}	0.46
FAS	rs10509561	10	90741892		T A	33.4	−0.14	0.06	0.22	0.10	0.012	8.8×10^{-4}	0.46
CCL7	rs3091324	17	29625029		G T	18.6	0.12	0.07	0.53	0.15	0.011	1.2×10^{-3}	0.46
RPW													
IL10	rs1554286	1	203332628		A G	17.1	0.05	0.06	0.55	0.17	0.009	4.7×10^{-3}	0.94
CD34	rs2556	1	204448754		G A	8.0	0.18	0.08	0.25	0.57	0.005	5.2×10^{-3}	0.94
ITGA4	rs17289831	2	182158467		A G	12.5	0.16	0.07	0.51	0.22	0.008	7.0×10^{-3}	0.94
CXCL12	rs2839695	10	44193855		A G	20.1	0.14	0.06	0.36	0.15	0.008	8.0×10^{-3}	0.94
FAS	rs10509561	10	90741892		T A	33.4	−0.16	0.06	0.08	0.09	0.008	8.9×10^{-3}	0.94
CFPWV													
CSF1	rs7540934	1	110195815		G A	43.8	0.24	0.07	0.08	0.09	0.011	2.4×10^{-3}	0.65
SELENBP1	rs2769264	1	148157814		T G	17.5	0.23	0.07	−0.13	0.21	0.011	2.4×10^{-3}	0.65
SELP	rs6028	1	166283340		T C	27.1	0.25	0.07	0.13	0.12	0.013	8.5×10^{-4}	0.65
NOTCH4	rs715299	6	32297819		T G	34.2	−0.15	0.07	0.22	0.10	0.012	1.1×10^{-3}	0.65
IL18	rs4937113	11	111534931		A T	42.0	0.24	0.07	0.02	0.09	0.012	1.5×10^{-3}	0.65
Augmentation index													
PTGER3	rs17482481	1	71081387		T G	29.0	0.06	0.06	0.37	0.11	0.010	3.1×10^{-3}	0.99
ADIPOR1	rs2185781	1	199638163		C T	20.8	0.01	0.06	−0.49	0.15	0.009	4.7×10^{-3}	0.99
CD34	rs2556	1	204448754		G A	8.0	0.23	0.08	0.29	0.58	0.007	3.2×10^{-3}	0.99
ITGA1	rs17211331	5	52163510		G C	10.7	0.08	0.07	0.87	0.26	0.010	2.4×10^{-3}	0.99
CXCL12	rs2839695	10	44193855		A G	20.1	0.15	0.06	0.35	0.15	0.008	6.2×10^{-3}	0.99

Response variables were tonometry residuals adjusted for age, age^2, sex, height, and weight analyzing each SNP under a general genetic model, using major allele homozygotes as the reference group. Partial R^2 is the proportion of residual variance explained by SNP. Chr denotes chromosome. LD indicates linkage disequilibrium r^2 for nearby significant SNPs. Location refers to the base pair from p-telomere.

osteoprotegerin with CFPWV; CRP and Lp-PLA2 activity (inverse) with RPW; and TNFRII with MAP (inverse). After accounting for potential clinical confounders, the inflammatory markers explained minor additional variability in the tonometry measures (<1%). Genetic findings interesting for follow-up were observed in both pulsatile (CPP) and steady-flow (MAP) measures of vascular function, which were associated with SNPs in the inflammatory genes *FAS* and *ITGB2*.

We prespecified a conservative strategy of analyzing the biomarkers as a group and conducting multivariable models adjusting for 17 potential confounders. An unanticipated finding was that 2 selected biomarkers, TNFRII and CRP, were not significantly associated with the tonometry measure in individual biomarker models adjusting for only 4 potential confounders (age, age^2, sex, and cohort). We acknowledge several alternative interpretations of the apparent inconsistency. One possibility is that TNFRII-CPP and CRP-RPW

associations represent false-positive findings because of multiple testing. Another explanation is that the 17-covariate model "overadjusted" by including potential confounders that pathophysiologically serve as intermediate mechanisms. For instance, based on previous longitudinal studies, one speculation is that inflammation contributes to the development of hypertension[23] and diabetes.[24] Hence, adjusting for diabetes or hypertension may be inappropriate if inflammation leads to diabetes and hypertension, which etiologically contribute to the development of arterial stiffness.

Comparison With Previous Literature

Recent investigations have demonstrated that arterial stiffness[3] and inflammation are both crucial factors in cardiovascular pathology and aging. Previous studies have related CRP and IL-6 to conduit vessel distensibility and arterial stiffness.[7,8,12,13,25] It has been shown that osteoprotegerin regulates vascular morphology and function in interaction with the immune system and may be responsible for vascular calcification.[26] In animal and in vitro models, osteoprotegerin has a favorable effect on arteries, yet higher osteoprotegerin was associated with high CFPWV in our data. In accordance with these findings, in human studies, osteoprotegerin concentrations have been associated positively with systolic blood pressure and brachial-ankle PWV[27] and are indicators of cardiovascular disease risk and mortality,[28] which might be due to compensatory overexpression of osteoprotegerin in an imbalanced system. TNFRII and TNF-α were associated with aortic and brachial-ankle PWV.[6,29] We found a modest relation to RPW (CRP) and CFPW (osteoprotegerin and IL-6) but not to CPP and MAP. After accounting for multiple testing, none of the markers was significantly related to augmentation index. A lack of association of augmentation index with CRP concentrations has been reported previously.[12]

SNPs and Tonometry Variables

Among the top associations of SNPs in inflammatory genes was *FAS* rs10509561 with CPP, which was also related to forward pressure wave and RPW. *FAS* codes for widely expressed membrane receptors, which belong to the TNF superfamily. FAS protein and its ligand induce apoptotic cell death and are integral to immune processes.[30] In addition, Fas signaling has been reported to regulate blood pressure and endothelial function through the modulation of endothelial NO synthase expression in mice[31] and, therefore, might have a pathophysiological relation to arterial stiffness.[2] We acknowledge that biological plausibility is only 1 criteria for establishing a causal relation; clearly our findings will need to be replicated in other studies and with other study designs.

Unreplicated reports have been published relating inflammatory candidate genes with tonometry measures. We were not able to replicate genetic findings reported for initial associations of *CRP* gene SNP rs1800947 with brachial-ankle PWV.[32] In the Rotterdam study, no relevant relations of CFPWV and pulse pressure to common polymorphisms of the *TGFB1* gene could be demonstrated,[33] similar to our data.

SNPs, Biomarkers, and Tonometry Variables

We hypothesized that inflammatory markers would be related to tonometry measures and sought to test whether SNPs significantly associated with the related inflammatory markers would also explain the variability in tonometry measures. According to the theory of mendelian randomization, an association of genetic variation with an inflammatory marker and a vascular phenotype would provide suggestive evidence of a causal biomarker-vascular phenotype relation. Genotype-vascular phenotype associations would be less likely to be secondary to reverse causation and residual confounding.[23,34] Most of the biomarker-SNP associations were in genes not coding for the respective biomarker and have, to our knowledge, not been investigated in association with biomarker concentrations. Of note, the 2 top SNPs for Lp-PLA2 activity and RPW are in the *PLA2G7* gene and were significantly associated with Lp-PLA2 activity in the Framingham Offspring cohort and in previous studies.[35] In our exploratory analyses, we were not able to show strong evidence for mendelian randomization for the inflammatory biomarkers examined (including Lp-PLA2). We acknowledge that many of the conditions required to infer causality under mendelian randomization were not met in the present project.[36,37] The lack of strong confirmation of an association among inflammatory marker SNPs, biomarkers, and tonometry phenotypes in our data may be explained by the fact that SNPs in the corresponding genes did not explain substantial variability in the biomarker or in tonometry variables.

Strengths and Limitations

The routine measurement of a broad inflammatory biomarker panel representing diverse pathways systematically ascertained tonometry data performed in accordance with strict quality control protocols, well-characterized cardiovascular risk factors enabling multivariable models, carefully prespecified analytic approaches with a priori designation of covariates and primary versus secondary models, and the large community-based cohort limiting referral bias constitute study strengths. It should be noted that, compared with previous studies, we did not observe very strong associations between tonometry measures and inflammatory biomarker concentrations or SNPs. Most previous studies were smaller (n=78 to 391[10,11,38–41]), were referral based (selected for vascular conditions[42,43]), included only small numbers of inflammatory biomarkers and vascular measures apart from blood pressure,[9,12,44–47] and tested only limited numbers of SNPs.[32,33]

Limitations of our study must be noted. A middle-aged to elderly study cohort may not be optimal to dissect genetic associations that might be found to be more prominent in younger samples. Clinical factors like age, sex, MAP, height, and heart rate account for ≤50% of the tonometry measures of variability.[2] It might be argued that the relative contribution of the biomarkers and SNPs toward explaining tonometric variability was low, because we accounted for variability related to potential confounders. In attempting to conservatively adjust for a broad range of potential confounders, as noted above, we acknowledge that we potentially overadjusted, if some of the clinical covariates included in the

multivariable models actually represent intermediate mechanisms. Nonrandom exclusions for nursing home and home visit participants could have introduced confounding, because these subjects suffer from illnesses that may affect inflammatory biomarkers and tonometry measures. We recognize that experts may disagree on the choice of inflammatory biomarkers and candidate genes selected for such a study. However, we sought to cover different inflammatory pathways that have been reported to be of central importance in vascular pathophysiology.

Although testing a broad panel of inflammatory biomarkers and SNPs is an asset of our study, we concede that the breadth of markers and SNPs studied introduces substantive concerns about multiple testing. We cannot exclude the possibility that the reported associations may be because of chance. To reduce the probability of false-positives, we restricted our analyses to 6 key measures of arterial stiffness and introduced more stringent thresholds for statistical significance than the typical $P<0.05$ as applied in other Framingham multi-inflammatory biomarker projects.[48,49] However, we emphasize that our clinical and genetic findings will need to be replicated. Conversely, because we accounted for measuring multiple biomarkers, SNPs, and tonometric measures, we cannot exclude the possibility of false-negatives; minor associations may have been missed. A strength of our study is that we did not selectively report only positive findings. We provide investigators with a Web-based resource of all of our primary, secondary, and exploratory results. We measured circulating inflammatory biomarkers as a surrogate for systemic vascular inflammation; we cannot exclude the possibility that local vascular inflammation may be causally related to local arterial stiffness without being manifest in systemic biomarker concentrations. Similarly, the clinical analyses were cross-sectional; we cannot infer the temporality of the observed associations. Although we modeled the association of inflammation to tonometry (dependent measure), it is equally plausible that arterial stiffness could lead to enhanced vascular shear and systemic inflammation.

Perspectives

Arterial stiffness and wave reflection are risk indicators of cardiovascular disease and mortality. Robust experimental and epidemiological evidence has shown that inflammatory pathways are implicated in vascular remodeling and disease. However, whether inflammatory processes are causal or are surrogate markers for vascular remodeling is still under investigation. In our study, although modest, we demonstrated associations in inflammatory markers carefully selected to represent different pathophysiological pathways and SNPs in inflammatory genes with tonometry variables. Candidate genes were chosen in a thorough review of known evidence using current literature and genetic databases. Yet, our findings must be replicated. Successful replication of the tonometry-inflammatory SNP association would provide additional motivation for further research into the mechanisms initiating and perpetuating the contribution of vascular inflammation to vascular remodeling and stiffness. Whereas inflammatory biomarkers and SNPs contribute only minor amounts to predicting tonometric indices, we submit that the associations after accounting for 17 covariates suggest that they may play a pathophysiological role in vascular stiffness. Although no direct clinical implications should be derived presently from the results, if our data are replicated, they might spur further investigations into the role of inflammatory markers and SNPs in risk stratification and potential targeted therapeutic interventions.

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Disclosures

G.F.M. is owner of Cardiovascular Engineering, Inc, which designs and manufactures vascular stiffness measurement devices. The remaining authors report no conflicts.

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