Interleukin-6 and atrial fibrillation in patients with coronary artery disease: Data from the Heart and Soul Study

Gregory M. Marcus, MD,[a] Mary A. Whooley, MD,[b,c,f] David V. Glidden, PhD,[c] Ludmila Pawlikowska, PhD,[d] Jonathan G. Zaroff, MD,[e] and Jeffrey E. Olgin, MD[a,d] *San Francisco, CA*

Background Previous studies suggest that markers of inflammation are elevated in patients with atrial fibrillation (AF). However, because inflammation has been implicated in contributing to risk of both AF and coronary artery disease (CAD), which are often present in the same populations, it is important to control for confounding by the presence of CAD. We therefore examined several biomarkers of inflammation and ultimately genotyped IL-6 polymorphisms in patients with AF in a cohort of subjects with known CAD.

Methods We performed a cross-sectional analysis of 971 participants in the Heart and Soul Study, 46 of whom had AF. Interleukin-6, C-reactive protein, tumor necrosis factor-α, CD-40 ligand, monocyte chemoattractant protein-1, and fibrinogen levels were measured.

Results In both unadjusted and adjusted analyses, IL-6 was the only biomarker significantly associated with AF (median IL-6 3.76 and 2.52 pg/mL in those with and without AF, respectively, $P = .0005$; adjusted odds ratio 1.77, $P = .032$). The *IL-6–174CC* genotype was significantly associated with the presence of AF in the adjusted analysis (odds ratio 2.34, $P = .04$) and with higher IL-6 levels ($P = .002$).

Conclusions In this cohort of subjects with CAD, AF was significantly associated with elevated IL-6 levels and the *IL-6–174CC* genotype. No associations were found with other biomarkers, including C-reactive protein. This suggests that IL-6 is a uniquely important mediator in the pathophysiology of AF. (Am Heart J 2008;155:303-9.)

Atrial fibrillation is the most common arrhythmia and is responsible for substantial morbidity and mortality.[1] Although the precise etiology remains unknown,

animal[2,3] and human[4,5] studies have suggested that inflammation and fibrosis play an important role in some types of AF. Earlier studies found C-reactive protein (CRP) to be elevated in patients with AF.[6] However, more recent investigation has suggested that the elevation in CRP may simply reflect underlying disease processes rather than something unique to AF itself.[7] In particular, coronary artery disease (CAD) is an important potential confounder because it is significantly associated with both elevated CRP levels[8] and a greater risk of AF.[9] However, to our knowledge, no previous study has determined whether the relationship between inflammatory markers and AF persists in a population restricted to subjects with known CAD.

In fact, the mechanism by which inflammation and stimulation of CRP secretion occur in AF remains unknown, and more recent studies have suggested that one of the most important stimuli of CRP release,[10] interleukin-6 (IL-6), may play an important role.[11,12] Interleukin-6 has been shown to significantly correlate with increased left atrial size,[13] an important risk factor for developing AF. In addition, polymorphisms in the promoter region of the *IL-6* gene have been associated with postoperative AF.[14,15] Importantly, IL-6 has not previously been shown to be independently associated

with spontaneous (non-postoperative) AF nor have polymorphisms of the IL-6 gene been studied in these patients.

We examined the relationship between several serologic markers of inflammation and the presence of AF in a well-characterized cohort of patients, all of whom had CAD.

Methods

Patients

We performed an analysis of baseline electrocardiograms (ECGs) and serum inflammatory markers collected for the Heart and Soul Study. The Heart and Soul Study is a prospective cohort study of health outcomes in patients with CAD. The details of eligibility and enrollment have been described previously.[16] Briefly, between September 2000 and December 2002, 1024 outpatients with stable CAD were recruited using administrative databases from 2 Department of Veterans Affairs Medical Centers (San Francisco and Palo Alto, CA), 1 university medical center (University of California, San Francisco), and 9 public health clinics in the Community Health Network of San Francisco. Patients were eligible to participate if they had a history of myocardial infarction (MI), angiographic evidence of ≥50% stenosis in one or more coronary vessels, evidence of exercise-induced ischemia by treadmill or nuclear testing, or prior coronary revascularization. Patients were not eligible if they had an acute coronary syndrome within the past 6 months, could not walk one block, or were planning to move out of the local area within 3 years.

In all participants, the cardiac rhythm was determined from a baseline 12-lead ECG at the time of enrollment. We used these data to perform a cross-sectional study. Patients with atrial flutter (n = 3), a multifocal atrial rhythm (n = 1), ventricular arrhythmia (n = 0), or a regular supraventricular tachycardia (n = 1) on their baseline ECG were excluded. An additional 32 patients were unable to provide blood samples (2 with AF), leaving a total of 971 participants for the analysis.

The institutional review board at each of the sites approved this protocol. All participants provided written informed consent.

Measurements

Atrial fibrillation. On the day of their visit, all patients underwent a standard 12-lead ECG with standard amplitudes obtained at a 25 mm/s paper speed. The diagnosis of AF was adjudicated by 2 physicians, and discrepancies resolved by a third physician reviewer. Only patients whose rhythm was AF, sinus rhythm, or an atrial paced rhythm were included in the analysis.

Inflammatory biomarkers. Participants were instructed to fast for 12 hours (except for medication, which they were able to take with water), not to take aspirin for 1 week, and not to smoke for 5 hours before their study appointment. Venous blood samples were obtained, and plasma and serum samples were stored at −70°C. Laboratory technicians who assayed the inflammatory markers were blinded to clinical characteristics.

Serum fibrinogen concentrations were determined by the Clauss assay. The Roche (Indianapolis, IN) Integra high-

sensitivity assay was used to measure CRP in the first 229 participants and (due to a change in the laboratory) the Beckman (Beckman Coulter, Inc, Galway, Ireland) Extended Range assay to measure CRP in the remaining samples. Results from these 2 assays were highly correlated (r = 0.99 in 185 participants). The R&D Systems (Minneapolis, MN) Quantikine HS IL-6 Immunoassay was used to determine the concentration of IL-6. The R&D Systems Quantikine HS CD40 Ligand Immunoassay was used to measure serum CD40 ligand using the quantitative sandwich enzyme immunoassay technique (lower limit of detection). The Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc, St Charles, MO) was used to measure tumor necrosis factor-α (TNF-α) and monocyte cheomoattractant protein-1 (MCP-1).

Single nucleotide polymorphisms. Whole blood was collected for genetic analysis from 986 participants, and buffy coat samples were stored in a freezer at −80°C. Genomic DNA was extracted from peripheral blood lymphocytes using a salt modification method (Gentra Systems). The *IL-6* −174G/C (rs1800795) and *IL-6* −572G/C (rs1800796) polymorphisms (single nucleotide polymorphisms [SNPs]) were genotyped by template-directed dye-terminator incorporation assay with fluorescence polarization detection,[17] using the AcycloPrime-FP kit (Perkin-Elmer) as previously described.[18] Genotyping was performed by investigators blinded to clinical status. Quality control included negative (blank) and positive controls on each 96-sample plate (44 duplicates, 100% concordance observed). Genotyping assays were completed in 871 of the 986 samples.

Other measurements. Age, medical history, and smoking status were determined by questionnaire. We measured height and weight and calculated body mass index (kg/m^2). Participants were instructed to bring their medication bottles to the study appointment and all medications were recorded. A complete resting echocardiogram was performed using an Acuson Sequoia Ultrasound System with a 3.5-MHz transducer with standard measurements as previously described.[16] Results from all echocardiograms were interpreted by an expert echocardiographer blinded to the results of the serologic markers and genetic analyses.

Statistical analysis

Normally distributed continuous variables are presented as means ± SD. Continuous variables that were not normally distributed are presented as medians and interquartile ranges. Consistent with previous studies, IL-6[13,19] and CRP[6] were right skewed. Tumor necrosis factor-α was noted to be significantly right skewed as well. Baseline characteristics were assessed using t tests and Spearman's rank correlation coefficient for continuous variables and χ^2 tests for categorical variables. The bivariate associations between each serologic marker and AF were performed using t tests and the Wilcoxon rank sum test as appropriate.

Interleukin-6, CRP, TNF-α were each log transformed for the multiple logistic regression model to improve linear fitting. Multiple logistic regression analysis was performed for each marker as a continuous variable and after categorizing each marker into quartiles. Covariates that were clinically known to be important to AF and the presence of inflammation (including age, sex, race, hypertension, ejection fraction)[1,20] as well as variables significantly associated with the predictors (inflammatory markers) and AF in the present cohort were included in

Table I. Baseline characteristics of subjects with and without AF on their baseline ECG

	AF (n = 46)	No AF (n = 925)	P value
Age (y)	74 ± 10	66 ± 11	<.0001
Male	43 (94%)	750 (81%)	.034
White	40 (87%)	548 (59%)	.003; pairwise
Black	0	155 (17%)	differences significant
Asian	4 (9%)	107 (12%)	for whites (P < .001)
Hispanic	1 (2%)	84 (9%)	and blacks (.002)
Hypertension	27 (60%)	654 (71%)	.12
Diabetes	8 (18%)	248 (27%)	.20
Prior MI	22 (50%)	496 (54%)	.61
Heart failure	12 (26%)	206 (22%)	.55
Ejection fraction (%)	60 ± 9	62 ± 10	.17
Left ventricular mass index (g/m^2)	100 ± 24	100 ± 34	.96
Left atrial volume (mL)	101 ± 36	62 ± 21	<.0001
Body mass index (kg/m^2)	30 ± 8	28 ± 5	.10
Statin use	25 (54%)	604 (65%)	.13
ACE inhibitor or angiotensin receptor blocker use	29 (63%)	467 (51%)	.096
Smoking	4 (9%)	189 (21%)	.057
Alcohol use	17 (38%)	268 (29%)	.22

Table II. Levels of biomarkers in those with and without AF on their baseline ECG

	AF (n = 46)	No AF (n = 925)	P value
IL-6 (pg/mL)	3.76 (2.61-5.21)	2.52 (1.58-4.06)	.0005
CRP (mg/dL)	2.46 (1.38-4.45)	2.15 (0.89-4.9)	.37
TNF-α (pg/mL)	4.48 (3.22-6.95)	3.76 (2.56-5.53)	.12
MCP-1 (pg/mL)	260 ± 129	243 ± 128	.38
CD-40 ligand (pg/mL)	5910 ± 3085	5078 ± 3069	.078
Fibrinogen (mg/dL)	392 ± 91	408 ± 72	.23

Interquartile ranges are shown in parentheses for the biomarkers that did not exhibit a normal distribution.

the model. Potentially important intermediaries were added to the model to evaluate mediation.

The SNPs were analyzed for dominant and recessive effects by recoding each polymorphism as a dichotomous variable (major allele homozygous vs other; minor allele homozygous vs other) and in an additive model (by allele frequency). χ^2 Tests were used to assess the relationships between each SNP and AF. Multiple logistic regression was performed using the dichotomous SNP variables and including the same covariates in the model included as above. Differences between IL-6 levels for each genotype across each SNP were assessed using the Kruskal-Wallis rank test.

Results

Of the 971 participants, 46 (5%) had AF and 925 (95%) had sinus rhythm (patients with an atrial paced rhythm [n = 11] were included in the sinus rhythm group). The

Figure 1
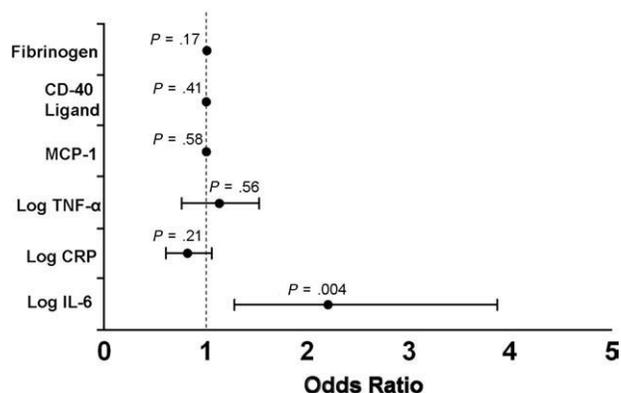
Odds ratios for the association between each biomarker and the presence of AF, adjusted for the independent effects of each biomarker (after entering each biomarker into a logistic regression model with AF as the binary outcome). Error bars denote 95% CIs. The dashed line denotes an OR of 1.

baseline demographics and clinical characteristics of these patients are shown in Table I.

Inflammatory biomarkers

Interleukin-6 was significantly higher in subjects with AF, whereas CRP was not (Table II). Although the higher CD-40 ligand levels in patients with AF approached statistical significance, neither this nor any of the other markers was significantly associated with AF. In a multiple logistic regression model in which each marker was included, IL-6 was the only one independently associated with AF (Figure 1).

Each progressive quartile of IL-6 compared to the first quartile of IL-6 was associated with progressively larger odds of AF (test for trend P = .0017) (Figure 2). This association was maintained after adjusting for age, sex, hypertension, diabetes, heart failure, ejection fraction, left ventricular mass index, and smoking (test for trend P = .033). No other markers demonstrated a significant association between progressive quartiles and larger odds of AF.

Table III lists continuous variables significantly correlated with IL-6. In assessing IL-6 versus categorical clinical characteristics, heart failure (P < .0001) and smoking (P = .0002) were significantly associated with elevated levels; no associations were found between IL-6 levels and sex, diabetes, hypertension, statin use, or ACE inhibitor/angiotensin receptor blocker use. Those with a previous MI had higher IL-6 levels (median 2.75 vs 2.42 pg/mL) that did not quite meet statistical significance (P = .05). Asians had lower IL-6 levels (median 1.88 pg/mL vs median 2.68 pg/mL in the remainder of the cohort, P < .0001), but IL-6 levels did not otherwise differ by race.

Figure 2
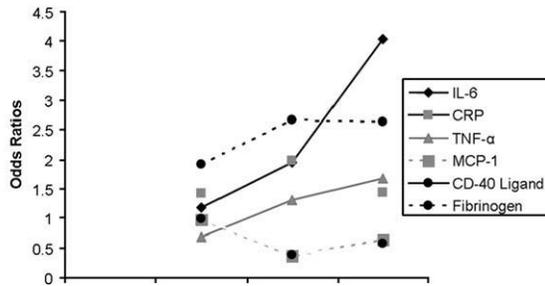
	Quartiles 1	2	3	4	p value
IL-6	6 (13%)	7 (15%)	11 (24%)	22 (48%)	0.002
CRP	8 (18%)	11 (24%)	33 (33%)	11 (24%)	0.48
TNF-α	10 (22%)	7 (15%)	13 (28%)	16 (34%)	0.23
MCP-1	15 (33%)	15 (33%)	6 (13%)	10 (22%)	0.15
CD-40 Ligand	15 (33%)	15 (33%)	6 (13%)	9 (20%)	0.12
Fibrinogen	6 (13%)	11 (23%)	15 (32%)	14 (30%)	0.17

Odds ratios of the second, third, and fourth quartiles compared to the first quartile for each biomarker as predictors of AF. The table exhibits the number of patients with AF in each quartile for each marker.

**Table III.** Correlations between continuous covariates and IL-6 levels

	Spearman's ρ (r)	P value
Age	0.15	<.0001
Atrial volume	0.14	<.0001
LV mass index	0.16	<.0001
Ejection fraction	−0.14	<.0001

The association between IL-6 and AF persisted in a logistic regression model controlling for age, sex, hypertension, diabetes, heart failure, ejection fraction, left ventricular mass index, and smoking (odds ratio [OR] 1.77, 95% CI 1.75-2.93, P = .025). Given the log transformation, this means that a doubling of IL-6 levels was associated with a 1.49 greater odds of AF (95% CI 1.05-2.12). Inclusion of left atrial volume attenuated the association between IL-6 and AF (OR 1.55, 95% CI 0.89-2.70, P = .12), consistent with an intermediary effect.

Genotypes of the IL-6 polymorphisms

Eight hundred and seventy-one subjects (40 with AF) were successfully genotyped for the *IL-6* −174G/C and the *IL-6* −572G/C SNPs. For the −174G/C SNP, 451 (52%), 302 (35%), and 123 (14%) carried the GG, GC, and CC genotypes, respectively. For the −572G/C SNP, 687 (76%), 153 (17%), and 63 (7%) carried the GG, GC, and CC genotypes, respectively. Restricting to whites only, the distributions of each SNP did not deviate

Figure 3
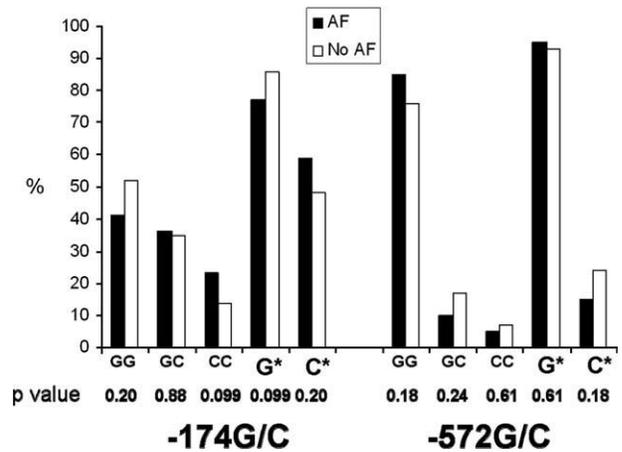
Proportions of patients with and without AF for each genotype and each allele (*denotes inclusion of both genotypes for a given allele). *P* values represent results of pairwise comparisons after collapsing for genotype or allele.

Figure 4
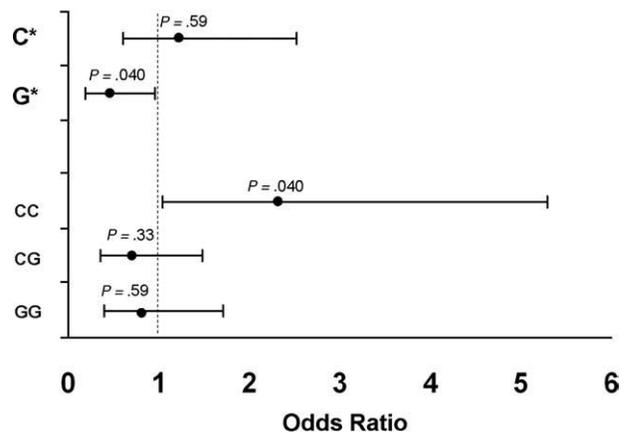
Odds ratios for the association between each −174G/C genotype and allele (*denotes inclusion of both genotypes for a given allele) and AF after adjustment for age, sex, hypertension, diabetes, heart failure, ejection fraction, left ventricular mass index, and smoking. Error bars denote 95% CIs. The dashed line denotes an OR of 1.

from Hardy-Weinberg expectations (*P* = .06 for −174G/C and *P* = .81 for −572G/C). However, the number of outcomes (n=34) was small in this restricted sample, and no significant associations were observed related to either of the SNPs and AF in this subgroup. Therefore, the genotype analysis includes the cohort as a whole; as expected given the multi-racial nature of the cohort,

Figure 5
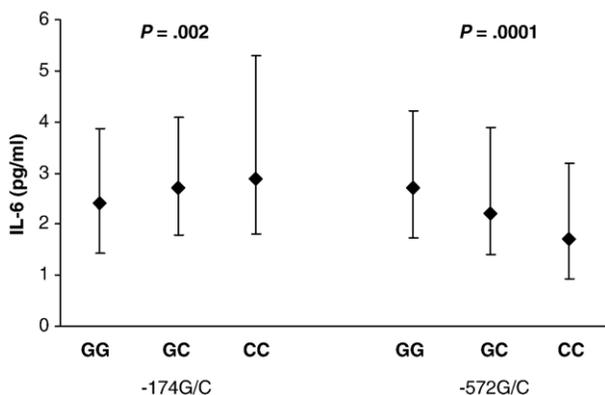
Median levels of IL-6 (pg/mL) for each genotype of the −174G/C and −572G/C polymorphisms. Error bars denote interquartile ranges.

the genotype distribution of each SNP in the entire cohort deviated from Hardy-Weinberg expectations ($P < .001$).

The genotype and allele frequencies for the 2 SNPs in patients with and without AF (with P values from χ^2 analyses) are shown in Figure 3. Although not statistically significant in the unadjusted analysis, there was a trend toward a progressively greater proportion with AF from the −174GG to −174GC to −174CC genotypes (test for trend $P = .08$). None of the unadjusted χ^2 analyses approached statistical significance in examining the relationship between AF and the −572G/C SNP (test for trend $P = .53$).

After applying the same multiple logistic regression model used in the biomarker analysis (adjusting for age, sex, hypertension, diabetes, heart failure, ejection fraction, left ventricular mass index, and smoking), the −174CC genotype was significantly more common in patients with AF ($P = .040$, Figure 4).

Interleukin-6 polymorphisms and IL-6 levels

Genotype of both *IL-6* SNPs was significantly associated with serum IL-6 levels. For the *IL-6* −174G/C SNP, serum IL-6 levels progressively increased with the number of C alleles present (highest in minor allele homozygotes, −174CC) (Figure 5). For the −572G/C polymorphism, IL-6 levels progressively decreased with the number of C alleles present (lowest in minor allele homozygotes, −572CC) (Figure 5).

Discussion

In a large cross-sectional cohort of patients with CAD, we found that, among 6 serologic markers of inflammation, IL-6 had a unique and independent association with AF that persisted after adjustment for multiple potential

confounders. In addition, the CC genotype of the *IL-6* −174G/C IL-6 polymorphism exhibited a 2.35 greater odds of being associated with AF than the GC and GG genotypes ($P = .040$) in multiple logistic regression, and the same genotype (CC) also demonstrated a significant association with elevated IL-6 levels.

Although AF is the most common arrhythmia and is responsible for substantial morbidity and mortality,[1] the cause remains unknown. Risk factors such as age, sex, heart failure, and CAD are well established.[9] Biopsy data suggest that AF may have an inflammatory etiology,[4] but the specific mechanism remains unknown. In particular, it is unclear whether inflammation is a cause or an effect of AF and, in either case, what mechanistic pathways may be important. Chung et al[6] demonstrated that CRP was significantly elevated in AF. However, subsequent study suggests that this association may have been confounded by concomitant conditions.[7] As both AF and CRP are associated with CAD, we chose to study biomarkers of inflammation in a population restricted to subjects with CAD. In this population, we were unable to find an association between AF and CRP.

We also studied several other markers previously reported to be associated with myocardial inflammation (eg, myocarditis) and/ or CAD: MCP-1,[21-23] TNF-α,[24-26] and CD-40 ligand.[27] Fibrinogen was included because of the suggestion that inflammation in AF may be related to the known aberrations in the coagulation cascade in AF.[12,28] None of these markers was independently associated with AF.

We did not find a significant association between AF and CRP, potentially reflecting confounding due to underlying coronary disease in previous studies,[7] bias due to choice of controls in previous case control (rather than cohort) studies, or insufficient power to detect a difference in CRP levels in this population.

To our knowledge, this is the first large cohort study in which the relationship between multiple biomarkers of inflammation and AF and the association between IL-6 and AF have been studied. IL-6 has been examined in case control studies of AF, but this study design is more likely to introduce bias. In those studies, elevated levels were consistently observed only in univariate analyses, with loss of a significant association after controlling for covariates.[11,28] Of importance, IL-6 has been associated with increased left atrial size.[13] Because increasing left atrial size is known to be a risk factor for AF,[21] one possibility is that elevated IL-6 results in AF via left atrial remodeling. In fact, we observed an association between IL-6 and increasing left atrial volume (albeit a weak one), and, as would be expected if left atrial volume were an important intermediary, the association between IL-6 and AF was attenuated when left atrial volume was added to the logistic regression model. The mechanism by which IL-6 might result in atrial

remodeling is unknown; however, animal data suggest that IL-6 may stimulate matrix metalloproteinase-2,[29] a molecule previously implicated in the atrial remodeling observed in AF.[30]

The −174G/C and the −572G/C SNPs both lie in the promoter region of IL-6 and have been reported to modulate IL-6 expression in vitro.[31,32] Although not statistically significant, there was a trend in the unadjusted analysis for IL-6 −174G/C genotypes associated with higher IL-6 levels to be found at greater frequency in the AF group. After controlling for important covariates, the −174CC genotype was significantly associated with AF. Importantly, this same genotype was associated with higher IL-6 levels.

The only other studies to examine the −174G/C SNP in patients with AF involved postoperative AF after coronary artery bypass grafting, and these 2 studies, involving only 110[14] and 96[15] patients, reported contradictory results: although both reported that increased IL-6 levels were associated with postoperative AF, one found that the area under the curve of IL-6 over several days was higher for the GG allele[14] and the other found higher acute IL-6 levels associated with the CC allele.[15] The study that had results contradictory to ours was based on 24 outcomes.[14] Regardless, postoperative AF is felt to be a different entity than spontaneously occurring AF,[20] and ours is the first study to examine this SNP in subjects with the more common spontaneous form.

This study has several limitations. Most importantly, we erred on the side of specificity in identifying our outcome (AF). Because we did not have reliable and uniform data regarding a history of AF and because historical reports can be inaccurate, we limited our outcome to the presence of AF documented on the baseline ECG performed at the time of enrollment in this cross-sectional analysis and required that 2 blinded physicians confirm the diagnosis. As any misclassification would therefore affect only sensitivity and is almost certainly nondifferential with regard to the predictors, this lack of sensitivity will bias our findings towards the *null* hypothesis. In other words, if anything, a reduced sensitivity in this case would increase the probability of a type II error, that we would miss a difference when one actually existed; because of the high specificity (which should be at or near 100%), it is crucial to emphasize that significant differences in outcome groups that were detected (such as those seen with IL-6 levels and the polymorphisms) are almost certainly real (the design works to minimize the chance of a type I error). In addition, our findings are best extrapolated to those with chronic or more frequent AF, as, by definition, such subjects would more likely have AF on their baseline ECG. Given our racially heterogeneous population, we cannot exclude population stratification as a possible explanation for the associations found between the −174G/C SNP and AF. We had insufficient power (too few outcomes per racial group) to examine these associations for each race and larger studies are required to validate our findings.

In summary, serum IL-6 levels are significantly associated with AF in patients with CAD. This association is independent of CRP, TNF-α, MCP-1, CD-40 ligand, or fibrinogen, and persists after adjusting for multiple potential confounders. The CC genotype of the −174G/C SNP was associated with elevated IL-6 levels in this population, and the same genotype was associated with AF. These findings suggest that IL-6 may play an active role in the pathophysiology of AF rather than simply representing an epiphenomenon related to inflammation. Future studies aimed at investigating the effects of IL-6 on atrial myocardium and AF inducibility will be necessary to determine whether this cytokine is indeed active and sufficient to increase the propensity to develop AF.

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