INFLAMMATORY AND HEMOSTATIC BIOMARKERS AND CORONARY ARTERY CALCIFICATION IN WOMEN UNDERGOING THE MENOPAUSAL TRANSITION

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ABSTRACT

Aims: Coronary heart disease (CHD) is a significant public health issue in the United States. The relationships of inflammatory and hemostatic biomarkers to subclinical CHD in women are uncertain. The aims of this study were to test the associations of baseline levels of four novel biomarkers with baseline coronary artery calcification (CAC) and CAC progression; and to evaluate if changes in these biomarkers were associated with CAC progression.

Methods: Subjects were obtained from the Study of Women's Health Across the Nation Heart Study. C-reactive protein (CRP), fibrinogen, plasminogen-activator inhibitor type 1 (PAI-1), and tissue plasminogen activator antigen (tPA-ag) were log-transformed. Logistic regression was used for the categorical outcomes (presence of baseline CAC and CAC progression), and tobit and linear regression were used for the continuous outcomes (extent of baseline CAC and CAC progression, respectively). Univariable and multivariable analyses were performed.

Results: A total of 372 women with a mean age of 51.3 years (SD, 2.8) were included for the baseline CAC analyses of which 131 (35.2%) were black. In the univariable analyses, all novel biomarkers were positively associated with baseline CAC presence and extent ($p<0.001$). These were significant after adjusting for traditional risk factors, but not after adjusting for body mass index. A significant interaction of race on log(CRP) and CAC was present. In race-stratified multivariable analyses, log(CRP) was significantly associated with CAC presence and CAC

extent in blacks, but not whites. There were 252 women for the CAC progression analyses. In the univariable analyses, log(PAI-1) and log(tPA-ag) were associated with presence of CAC progression and log(PAI-1) was associated with extent of CAC progression. After adjustment, only log(PAI-1) was associated with presence of CAC progression (OR, 1.91; 95% CI, 1.24-2.93; p=0.003). There was a trend towards an association between log(PAI-1) and extent of CAC progression (p=0.06). Changes in biomarkers were not associated with CAC progression.

Conclusions: Inflammatory and hemostatic biomarkers are associated with baseline CAC through obesity, except for CRP and CAC in blacks. Log(PAI-1) is associated with presence of CAC progression. These findings may improve CHD prevention in midlife women.

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PREFACE

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1.0 INTRODUCTION

Coronary heart disease (CHD) is the cause of approximately 1 of every 6 deaths in the United States (1). Risk prediction has traditionally been based on sex, age, blood pressure, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), smoking, and diabetes status (2). Current risk assessment based on the estimated risk for a cardiovascular (CV) event within 10-years, remains insufficient (3).Those with low short-term, or 10-year, risk estimates are still at risk for CV disease (CVD) during their lifetimes (4). Studying relationships between novel biomarkers and CHD development is therefore important.

1.1 CORONARY HEART DISEASE RISK PREDICTION IN WOMEN

The Global Registry of Acute Coronary Events demonstrated that women with suspected acute coronary syndromes (ACS) are more likely to present with atypical symptoms, to be older, and to have more CV risk factors when compared to men (5). Despite similar rates of positive cardiac enzymes, women were twice as likely as men to have normal or mild CHD on cardiac catheterization (12% vs. 6%, $p<0.001$). From the Get With the Guidelines-Coronary Artery Disease database, women with a diagnosis of ST-elevation myocardial infarction were less likely to receive reperfusion therapy and more likely to have in-hospital death (6). There is continued debate as to whether the mortality differences are related to higher baseline risk in women versus

treatment bias. There is no debate, however, that differences exist in CHD development between women and men.

Women at middle-age are of particular interest. Sex-related differences in CHD risk profile are particularly notable around the menopausal transition (7). The ratio of male-to-female CHD deaths begins to decrease at this point and continues until the eighth decade at which point the ratio approaches equivalency. In a study of individuals with CHD events, over 10% of women aged 46-55 years did not have any traditional risk factors and over 35% had only one risk factor (3). Understanding the mechanisms underlying CHD in women at midlife may optimize prevention strategies.

1.2 CORONARY ARTERY CALCIFICATION

Coronary artery calcification (CAC), as detected via computed tomography (CT) scanning, is absent in normal arteries and present in late stage atherosclerotic lesions (8). The amount detected on CT has been demonstrated to correlate with histomorphometric calcium and degree of coronary artery stenosis (9).

1.2.1 CAC clinical implications and epidemiology

In asymptomatic individuals, positive results in both a single quantification of CAC and CAC progression over time predict CHD events and all-cause mortality (10-13). Adjusted 10-year survival is 99.4% with a CAC score of 0 and 87.8% when >1,000 (11). CAC has emerged as a potential means to improve risk stratification (14-15). In an observational study, incorporation of

CAC into a traditional risk model improved accuracy by increasing the number of individuals with events into the high-risk category, and increasing the number without events into the low-risk category (15).

Men and women in the Multi-Ethnic Study of Atherosclerosis (MESA) who were considered to be low-risk for CHD event, or a predicted 10-year CHD risk <10% based on the Framingham risk score (FRS), had a CAC prevalence of 30% (16). The sample population included those without known CV disease with an average age of 55-60 years and included a broad ethnic base. CAC presence was defined as an Agatston score >0. The prevalence of advanced CAC, defined as a score >300, was 3.5%.

1.2.2 CAC pathophysiology and CHD events

The process of calcification in coronary atheromas is related to bone formation via an active process involving factors promoting and inhibiting calcification (17). Historically, the two major forms of vascular calcification are intimal atherosclerotic calcification and arterial medial calcification (18). Intimal atherosclerotic calcification is the more common, with dyslipidemia implicated in most studies. Arterial medial calcification, also known as Mönckeberg's sclerosis, is prominent in type 2 diabetes mellitus.

Despite the association between CAC and coronary events, calcified plaque within luminal calcified nodules do not contribute to the vast majority of ACS (19). ACS events are believed to be the result of sudden luminal thrombosis. The chief pathological causes consist of plaque rupture of a thin fibrous cap overlying a lesion with a necrotic core, plaque erosion in the absence of a connection with a necrotic core, and calcified nodules with discontinuity of the fibrous cap as a result of bony nodules overlying a calcified plate. Coronary thrombi are found in

60% of patients with sudden death. Of these, calcified nodules account for only 2% to 7%. Calcification has not been shown to disrupt mechanical stability of coronary atheroma (20). CAC, however, is strongly correlated with atherosclerotic plaque burden (21).

As CAC is a part of late-stage atheromas, the use of statin medication was hoped to retard CAC progression. In a recent meta-analysis, statins lowered LDL levels and decreased the size of coronary atheromas, but had no effect on CAC (22). It has been speculated that CAC progression while individuals are on statins is a benign process that signifies conversion of non-calcified plaque to calcified plaque in the absence of worsening atherosclerosis. In those sustaining a CHD event, greater CAC progression in statin users compared with non-statin users suggests that this is unlikely to be true (13). While CAC is a marker for increased CHD risk, further pathophysiologic underpinnings must be elucidated before it can be effectively used as a therapeutic target.

1.2.3 CAC methods of measurement

The most commonly used technique to quantify CAC is the Agatston scoring system (23). A CT density of 130 Hounsfield units with an area of 1 mm^2 is set as the threshold for a calcified lesion. A density factor is assigned based on the peak x-ray density in Hounsfield units: 1 for 130 to 199, 2 for 200 to 299, 3 for 300 to 399, and 4 for ≥400. The Agatston unit score (U) is calculated by multiplying the area (mm^2) by the density factor. The score corresponds with the degree of atherosclerotic plaque burden: 0 is none, 1 to 10 is minimal, 11 to 100 is mild, 101 to 400 is moderate, and >400 is extensive or severe (24). Individuals with even mild atherosclerotic disease, or a CAC score between 11 and 100, have an adjusted relative risk for all-cause death of 2.2, compared with those with no CAC (10).

An alternative method is the calcium volume score (CVS). It represents the actual volume of CAC as automatically calculated by software. The volume measured with units of mm^3 with Hounsfield unit greater than 130 is multiplied by a factor of 1,000 in order to express the result as a whole number (25).

1.2.4 CAC methods of progression measurement

More recently, CAC progression has gained attention as a potential means to identify high risk individuals. In a study of subjects without known CHD who underwent annual CAC scoring, individuals with a baseline CAC score of 0 had a non-linear development of any CAC on subsequent scans (26). By year 5 of follow-up, 11.6% had converted to any CAC. In those who converted to any CAC, the mean average ± standard deviation (SD) time to conversion was 4.1 ± 0.9 years with a mean average ± SD CAC score of 19 ± 19 at the time of conversion. No traditional risk factor was associated with conversion. The strongest predictor of CAC progression was the presence of any baseline CAC.

There is no universally accepted method to define significant CAC progression (8). The Hokanson, or square root transformed difference, method defines significant change as a square root-transformed CAC score of ≥ 2.5 mm^3. It was found to be the best predictor of mortality among several methods (12). Other accepted methods include the absolute difference between the second and first CAC measurements, the natural logarithm plus 25, and the percent change. The time between scans may be accounted for by dividing by the number of years (12). In those without CAC at baseline, an annual increase of 5-units in the Agatston score has been associated with an adjusted hazard ratio of 1.5 (95% confidence interval (CI), 1.1-2.1) for hard CHD

endpoints (13). For those with CAC at baseline, an annual increase of 100-units has been associated with an adjusted hazard ratio of 1.3 (95% CI, 1.1-1.5) for hard CHD endpoints.

1.2.5 Sex differences in CAC

CAC appears to be delayed in women by approximately one decade (27). This delay is similar to that in CHD deaths between women and men, which appears around the time of the menopausal transition (7). Significant increases in total cholesterol, LDL-C, and apolipoprotein B occur around the time of the final menstrual period (28).

In a study of 70 men and 70 women, women were less likely to have calcification of atherosclerotic lesions compared to men despite similar degrees of luminal narrowing on coronary angiography (29). Women were significantly older than men (mean age 60 ± 12 years vs. 56 ± 12 years, $p<0.05$). They were also more likely to have hypertension and diabetes mellitus. Screening for CHD using CT may therefore be limited in younger women.

A large cohort study of 10,746 white men and women without known CHD who underwent CAC evaluation highlight some of these differences (30). Among those who had a CHD event, women were much more likely to have had a CAC score of 0 compared with men (20.4% vs. 2.1%, $p<0.01$). The median CAC score was also markedly less in women (111 vs. 634.5). This was despite the fact that women with CHD events were more likely to be older, to have hyperlipidemia, to have hypertension, and to have diabetes. The type of CHD events were also more significant as 38.8% were CHD deaths or nonfatal myocardial infarctions in women, compared with 26.1% in men. These differences highlight the importance of gender in the process of studying the atherosclerosis. The presence of even small amounts of CAC appears to

portend a more ominous prognosis in females compared to their male counterparts. Ethnicity does not appear to alter the predictive ability of CAC score for incident CHD (31).

1.2.6 Diabetes and CAC

The mechanism of vascular calcification in diabetes involves the process of arterial medial calcification. When present in the vascular extremities of diabetics, it predicts CV mortality and complications (32,33). In contrast to discrete plaques seen in intimal type calcification, a uniform linear "railroad track" appearance is seen in medial type calcification on radiography.

Hoff et al. demonstrated that CAC had a greater prevalence and extent in diabetic individuals without known CHD, compared with non-diabetics (34). Male diabetics also exhibited a greater CAC burden compared with women. Raggi et al. reported that the absence of CAC in diabetics is associated with a similar 5-year mortality rate when compared to non-diabetics (98.8 vs. 99.4%, $p = 0.5$) (35). A limitation of these two studies was that the diagnosis of diabetes was self-reported. No distinction was made between type 1 and type 2 diabetes mellitus, and current diabetic treatment was not reported.

The Coronary Artery Calcification in Type 1 Diabetes (CACT1) study evaluated the association between CAC and type 1 diabetes mellitus in 1,420 subjects aged 20-55 years with no known CHD (36). The age-adjusted odds ratios (OR) for CAC prevalence for men versus women were 4.6 (95% CI, 3.2-6.9) in non-diabetics, and 2.5 (95% CI, 1.7-3.6) in type 1 diabetics. The difference was diminished on adjustment for traditional risk factors, and then eliminated on adjustment for components used to measure insulin resistance.

From the MESA study, CAC progression was found to be significantly greater in those with diabetes compared to those without (37). In those with diabetes and metabolic syndrome,

CAC progression was associated with CHD events that included myocardial infarction, angina, resuscitated cardiac arrest, or CHD death.

It is therefore evident that CAC has a higher prevalence in diabetics, compared to non-diabetes. Diabetics also demonstrate a greater rate of progression. Different pathophysiologic mechanisms are known to play a role in CAC development, as evident by different patterns on non-invasive imaging. Further insights into these mechanisms may improve preventative care.

1.3 NOVEL CV RISK FACTORS

1.3.1 C-reactive protein

Detection of immune cells and mediators within atheromas led to the current understanding that inflammation has a vital role in the atherosclerotic process (38). C-reactive protein (CRP) is a protein synthesized mainly in the liver as an acute phase reactant (39). It is a nonspecific marker for conditions such as inflammation, infection, tissue damage and malignancy. CRP as a marker for atherosclerotic activity has been the subject of considerable investigation in part due to its stability, long plasma half-life, and availability of testing. Plasma-derived CRP proteins have been found concentrated on the surface of macrophages of ruptured plaques in human coronary arteries (40). Pro-inflammatory cytokines resulting from macrophages promote calcification of vascular smooth muscle cells (41). The role CRP in atherogenesis and calcification is still not well understood, but may involve a feedback amplification loop between persistent inflammatory cytokines and osteogenesis.

The first reported association between elevated CRP and CAC was from the Framingham Offspring Study (42). The 321 subjects were free of known CHD and had a low rate of diabetes. The Spearman correlation coefficient remained significant for men (0.19, $P<0.05$) but not women (0.14, $P=0.09$) after adjusting for age, FRS, and body mass index. A subsequent analysis of 3373 subjects enrolled in the Dallas Heart Study did not find a significant association between CRP and CAC when adjusted for traditional risk factors, BMI, estrogen, and statin use (43). Subsequent analysis from the Dallas Heart Study, however, found that increasing levels of CRP were associated with increased CAC prevalence in normal and overweight (but not obese) men, and in normal weight women (44).

A study that combined 1299 subjects with type 2 diabetes mellitus from the Penn Diabetes Heart Study and 860 subjects without diabetes from the Study of Inherited Risk of Coronary Atherosclerosis assessed the relationships between CRP and CAC based on gender and diabetes (45). The associations were significant in diabetic and non-diabetic women, but not in men, when adjusted for age and race. This association was only significant in diabetic women after including medications, FRS, metabolic syndrome, and BMI into the model.

Ultimately, the relationship of CRP to CAC among healthy middle-aged women is yet to be conclusively established. More study, particularly in those undergoing the menopausal transition, is warranted.

1.3.2 Fibrinogen

Fibrinogen is a glycoprotein that is mainly synthesized in hepatocytes (46). Synthesis is increased in the setting of inflammation. In the setting of vascular injury, fibrinogen plays an important role in hemostasis. First, it is an adhesive protein essential for platelet aggregation.

Second, it forms an insoluble fibrin clot as part of blood coagulation. In a large meta-analysis, fibrinogen has been associated with increased risk for CHD, stroke, vascular mortality, and nonvascular mortality (47).

Histological studies of carotid artery plaques in subjects with prior transient ischemic attacks have noted elevated levels of plasma fibrinogen associated with a decrease in cap thickness, macrophage infiltration of the cap, and thrombosis (48). In diabetics, elevated fibrinogen is associated with a lower rate of clopidogrel-induced adenosine diphosphate receptor inhibition on platelet membranes (49). Increasing evidence suggests that activated platelets have a role in promoting atherosclerosis (50).

Fibrinogen degradation products are not seen in normal and early stage atherosclerotic lesions, but are present in late stage atherosclerotic lesions using monoclonal antibody techniques (51). A positive-feedback loop between calcification and inflammation has been noted between microcalcifications and proinflammatory activity from macrophages (52). In a cross-sectional study of 354 Caucasian subjects with hypertension, a significant correlation of CAC with increasing quintiles of fibrinogen was present in women, but not men (53). Additional studies in apparently healthy women that take into account menopausal status may uncover important insight into the relationship.

1.3.3 Plasminogen-activator inhibitor type 1

Plasminogen-activator inhibitor type 1 (PAI-1) is a glycoprotein synthesized in platelets and endothelial cells that serves as a major inhibitor of fibrinolysis (54). Theoretically, inhibition of fibrinolysis may lead to an increase in arterial fibrin deposition and thrombosis. The presence of coronary artery disease has been associated with decreased fibrinolysis, and believed to be

mediated through increased PAI-1 activity (55). Higher concentrations of PAI-1 mRNA have been noted in atherosclerotic human arteries compared with normal human arteries, thereby suggesting a potential role in atherosclerotic progression (56).

In a study of 560 subjects with type 1 diabetes and 693 subjects without diabetes, PAI-1 was independently associated with CAC in those with type 1 diabetes who were 35 years and younger (57). PAI-1 expression and activity is enhanced by CRP in human aortic endothelial cells in the setting of hyperglycemia (58). Other inflammatory mediators, such as interleukin-6, have been demonstrated to increase PAI-1 activity in human adipocytes (59).

Elevated levels of PAI-1 have been noted in healthy women with insulin resistance compared with normal healthy women (60). PAI-1 concentrations are higher in postmenopausal women when compared to premenopausal women (61). There are no studies, however, that have focused on healthy, midlife women and the relationship of PAI-1 and CAC.

1.3.4 Tissue plasminogen activator antigen

Tissue plasminogen activator (tPA) is responsible for endogenous fibrinolysis through conversion of plasminogen to plasmin. Measurement of tissue plasminogen activator antigen (tPA-ag) levels consists of active free tPA and inert tPA that is bound in activator/inhibitor complexes. Elevated levels of tPA-ag, therefore may reflect increased or decreased fibrinolytic activity. The British Regional Heart Study demonstrated a significant association between serum tPA-ag levels and CHD (62).

Elevated levels of tPA-ag have been associated with CHD in post-menopausal women (63). In a group of women aged 45 years or less with acute myocardial infarction, there was significant association between non-fatal myocardial infarction and plasma tPA-ag that was felt

to result from reduced fibrinolytic activity (64). To date, there have been no studies to evaluate potential associations between tPA-ag and CAC in women.

1.4 HYPOTHESES

We conducted a literature review of the associations of CRP, fibrinogen, PAI-1, and tPA-ag with baseline CAC and CAC progression (**APPENDIX A**). Ultimately, questions still remain on the role of novel CV risk factors in atherogenesis in women around the menopausal transition.

We sought to investigate the relationship between the novel CV risk factors CRP, fibrinogen, PAI-1, and tPA-ag with baseline CAC and CAC progression in a multicenter prospective cohort of middle-age women designed to study the menopausal transition. We also sought to examine the association between change in the four novel risk factors and progression of CAC. Our hypotheses were that baseline level and change in these biomarkers were associated with baseline level and progression of CAC beyond traditional risk factors for CHD.

2.0 METHODS

2.1 STUDY POPULATION

The Study of Women's Health Across the Nation (SWAN) is a multicenter, multiethnic prospective cohort study in the United States that was designed to examine the menopausal transition (65). Seven clinical sites recruited women who were Caucasian and one additional pre-determined ethnicity (African-Americans in Boston, Chicago, Detroit, Pittsburgh; Chinese in Oakland; Hispanic in New Jersey; Japanese in Los Angeles). Eligibility criteria for the SWAN parent study included age 42-52 years, an intact uterus, menstruating within the prior 3 months, and not using reproductive hormones. Those with a prior hysterectomy or bilateral oophorectomy were excluded.

Two sites, Pittsburgh and Chicago, participated in the SWAN Heart ancillary study. It was designed to evaluate the changes in subclinical measures of atherosclerosis during the menopausal transition. These two sites, by design, enrolled only self-identified Caucasian and African-American women. This present study utilized data from both the baseline and follow-up visits of the SWAN Heart study. The baseline SWAN Heart visit occurred during SWAN parent study annual visits 4 through 7 (2001-2005) within 3 months after the corresponding annual core SWAN assessment (66). The baseline CAC measurement was obtained between years 2001 to 2003, and the follow-up CAC measurement was obtained between years 2002 to 2005. For the

current project, subjects were excluded if they reported myocardial infarction, angina or stroke at any of the two evaluated visits (baseline and follow-up SWAN Heart visits); did not have baseline levels of CRP, fibrinogen, PAI-1, and tPA-ag; or did not have baseline and follow-up CAC measurements.

The SWAN Heart cohort originally consisted of 608 women from 2 sites in Chicago and 1 site in Pittsburgh (**Figure 1**). Baseline CAC measurement were obtained in 561 subjects. Subjects with a history of myocardial infarction, angina, or stroke at the baseline SWAN Heart visit were excluded from the present study. One Chicago site did not collect the biomarkers of interest for the present analysis. The final sample size for those with both CAC determination and novel CV risk factors of interest was therefore 372 subjects. There were 258 subjects with baseline and follow-up CAC measurements, and baseline novel CV risk factors. For the change in novel risk factors and CAC progression analyses, 223 subjects had CAC measurements and biomarkers at baseline and follow-up.

2.2 CV RISK FACTOR MEASUREMENTS

Blood pressure was measured twice with a minimum 2-minute rest period between measures, with readings taken on the right arm, with the respondent seated and feet flat on the floor for at least 5 minutes before the measurement. Respondents had not smoked or consumed any caffeinated beverage within 30 minutes of blood pressure measurement. Appropriate cuff size was determined based on arm circumference. Two sequential blood pressure values were averaged. Smoking was coded into current versus past or never smoker. BMI was derived from in-clinic measures of weight and height.

Figure 1. Sample size flow chart.

Total serum cholesterol, HDL-C, LDL-C, triglycerides, and glucose were determined from a fasting blood sample with standard methods described previously (67). The Homeostasis Model Assessment (HOMA) insulin resistance index was calculated using the following equation:

[insulin x glucose] / 22.5. Glucose in mg/dL was converted to millimoles per liter by multiplying by 0.0555. Insulin was in mg/dL. HOMA represents a computer model of the glucose-insulin feedback system during a fasting state, specifically regarding the functions of the tissues and organs related to glucose regulation. FRS assessment was the summation of all Framingham risk-related points: age, LDL-C or total cholesterol, HDL-C, blood pressure, diabetes, and smoking (2).

CRP, fibrinogen, PAI-1, and t-PA-ag were all measured in plasma. CRP was measured using ultrasensitive rate immunonephelometry (Dade-Behring, Marburg, Germany). Fibrinogen was measured in frozen citrated plasma using a clot-based turbidometric detection system (MLA ELECTRA 1400C; Medical Laboratory Automation Inc., Mt. Vernon, NY). PAI-1 was measured using a solid phased monoclonal antibody and a second enzyme-labeled goat antiserum for detection (IMUBIND plasma PAI-1 enzyme-linked immunosorbent assay; American Diagnostica, Greenwich, Connecticut). A double antibody in an enzyme-linked immunosorbent assay (American Diagnostica) measured t-PA-ag, with a human single chain t-PA-ag as a standard calibrated against an international standard (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom).

2.3 CAC MEASUREMENTS

A CT scanner (C-150 Ultrafast CT Scanner, GE Imatron, San Francisco, California) was used to quantify levels of calcification in the coronary arteries. The first pass allowed an evaluation of the participant's anatomy so that the landmarks for the coronary scans were identified. The second pass was for the coronary arteries in which 30 to 40 contiguous 3-mm thick transverse

images were obtained from the level of the aortic root to the apex of the heart. Images were obtained during a maximal breath hold, using electrocardiographic triggering so each 100-millisecond exposure was obtained during the same phase of the cardiac cycle (60%) of the R-R interval. All scan data were saved to an optical disk for central scoring, using a DICOM workstation and software by AcuImage, Inc (South San Francisco, California). This software program implements the Agatston scoring method (23). CAC is defined as a hyper-attenuating lesion >130 Hounsfield units with an area of ≥3 pixels. The Agatston unit (U) score is calculated by multiplying the lesion area (mm^2) by a density factor (between 1 and 4). The total calcium score was the sum of the individual scores for the four major epicardial coronary arteries. Under the supervision of a cardiologist, a technologist scored the scans.

2.4 COVARIATES

Menopausal status, obtained annually from reported bleeding patterns in the year preceding the visit, was categorized as premenopausal (bleeding in previous 3 months with no past year change in cycle predictability), early perimenopausal (bleeding the previous 3 months with decrease in cycle predictability in the past year), late perimenopausal (<12 to >3 months of amenorrhea), or postmenopausal (≥12 months of amenorrhea). Pre-menopausal and early perimenopausal women were combined into one group, as were late perimenopausal and post-menopausal women (68). For the baseline CAC analyses, surgical menopause was assigned for those who had undergone hysterectomy without bilateral oophorectomy. Women last classified as premenopausal or perimenopausal who reported taking hormones (oral contraceptives, oral estrogens and/or progestins, estrogen injections or patch) in the past year were considered indeterminate status

because of the impact of hormone use, even if discontinued, on bleeding patterns. For the CAC progression analysis, subjects with surgical menopause were excluded due to small numbers, and subjects who were indeterminate status and hormone therapy users were collapsed into a single category as hormone therapy users.

Income was initially reported in eight categories. These were compressed into three categories: <$50,000; $50,000-$150,000, and >$150,000. A three level measurement, "how hard to make ends meet", was measured as very hard, somewhat hard, and not very hard. This produced similar results to income, so income was used in the final models. Education was reported in 5 categories. These were compressed into three categories: high school graduate or less, some college or college graduate, and graduate degree.

The HOMA insulin resistance index and FRS were derived from the SWAN longitudinal cardiovascular created variables dataset. All other covariates were derived from the annual SWAN interview or examination most closely corresponding to the SWAN HEART baseline. Race/ethnicity was determined in response to "How would you describe your primary racial or ethnic group?" Other covariates included age, SWAN Heart clinical site, CV medication use (blood pressure medication use and cholesterol medication use), diabetes, family history of CVD, and current smoking. Diabetes was defined as the use of any medication for diabetes or a fasting blood glucose of >125 mg/dl.

2.5 STATISTICAL ANALYSIS

Descriptive data was presented as frequencies of subjects for categorical variables and mean with standard deviation (SD) for continuous variables, except when skewed. Continuous variables

with skewed distributions were presented as median with first quartile (Q1) and third quartile (Q3) where indicated. Differences in descriptive data between groups were assessed by the Wilcoxon-Mann-Whitney test for continuous variables and the chi-square test for categorical variables. Preliminary analyses included examination of the distributions of the continuous variables for outliers and violations of the assumptions of normality and the frequencies of the categorical variables for small cell size. Test for transformations of data was used as needed. The change in risk factor variables was measured as the difference between the follow-up value and the baseline value, divided by the time in years between risk factor measurements. A 2-tailed value of $p<0.05$ was considered significant for all analyses.

Of the four novel CV risk factors, fibrinogen levels were not obtained during SWAN annual visits 4 and 6. This missing data was adjusted for with imputation using two methods. In the first method, novel CV risk factor values from the prior annual visits were entered for the missing values. Values from visit 3 were used for missing visit 4 values, and values from visit 5 were used for missing visit 6 values. In the second method, novel CV risk factor values from the prior annual visit and the subsequent annual visit were averaged and entered for the missing baseline values. Averaged values from visits 3 and 5 were used for missing visit 4 values, and averaged values from visits 5 and 7 were used for missing visit 6 values. Results from these two methods were used for the univariable analysis between novel CV risk factors and CAC presence. Both yielded similar results. Results from the first method resulted in greater statistical power and was selected for the final analysis.

Spearman rank order correlations were utilized to examine the associations of the four novel CV risk factors with each other. Correlations of BMI and HOMA to the novel biomarkers were also examined. CRP, fibrinogen, PAI-1, and tPA-ag had skewed distributions and were

therefore log transformed using a base of *e*. For interpretation, one log unit was equivalent to 2.7 units of the non-log transformed variable (i.e. 1 log(CRP) = 2.7 mg/L CRP).

2.5.1 Baseline novel risk factors and presence of baseline CAC analyses

Baseline CAC was evaluated as both a categorical outcome (presence of calcification), and a continuous outcome (extent of calcification). For the baseline CAC presence analyses, presence of calcification was analyzed as a two-level variable as those without CAC (CAC score = 0) and those with CAC (CAC score >0). Univariable logistic regression analysis was be performed to assess the associations between the novel CV risk factors and covariates with the presence of CAC. Covariates that were known to be associated with CAC were forced into the adjusted models as well as those with $p<0.25$ in the univariable analysis. Given the design of the study, study site and menopausal status were also forced into the models. Due to small numbers, diabetes was not used in the adjusted models.

Multivariable logistic regression was performed using two different strategies. First, significant variables from the univariable analysis were introduced in a step-wise fashion. This was designated the traditional CV risk factor based models. Second, adjustment was made for the aggregate FRS and then other significant variables that were not part of the FRS, and designated the FRS based models. Interactions were assessed for race, menopausal status, and BMI on the relationships between the CV risk factors and CAC presence after introduction of BMI into the adjusted models. Interactions with menopausal status or BMI were not significant.

2.5.2 Baseline novel risk factors and extent of baseline CAC analyses

For the baseline CAC extent analyses, log transformation of the CAC score plus a constant of 1 was performed and analyzed as a continuous outcome measure. Univariable tobit regression models were used to assess the associations between baseline variables and log(CAC+1). This method allowed for retention of subjects with CAC=0 and has been previously described (69,70). Multivariable tobit regression was performed using strategies described above. Interactions were assessed for race, menopausal status, and BMI on the relationships between the CV risk factors and CAC extent after introduction of BMI into the adjusted models.

2.5.3 Baseline novel risk factors and presence of CAC progression analyses

There is no agreed upon definition of CAC progression in the literature and we observed a skewed distribution for CAC progression data with large number of zeros. CAC progression was evaluated as both a categorical and continuous variable.

For the presence of CAC progression analyses, a dichotomous variable of significant progression was created. Significant progression of CAC was defined as CAC >0 at follow-up in subjects with CAC = 0 at baseline, an annualized change of ≥10 U in subjects with 0<CAC<100 at baseline, and annualized percent change ≥10% at follow-up in subjects with CAC ≥100 at baseline. This definition has been previously used in a study of low-risk subjects from the Coronary Artery Risk Development in Young Adults (CARDIA) study and MESA (71). Logistic regression was used to determine OR and 95% CI between novel CV risk factors and CAC

progression. Multivariable analyses were performed using strategies previously described in the baseline CAC analyses, with the addition of baseline CAC.

2.5.4 Baseline novel risk factors and extent of CAC progression analyses

Extent of CAC progression as a continuous outcome was assessed as the log transformed difference between the second and first CAC measurement plus a constant of 25 (72):

$$[\log(CAC_{(follow\text{-}up)}+25) - \log(CAC_{(baseline)}+25)] \,/\, \text{time (years) between first and second scans}$$

This method, known as the "MESA method", has been shown to predict all-cause mortality (12). Univariable linear regression models were used to assess the significance between novel CV risk factors and change in the log-transformed CAC. Multivariable analyses were performed using strategies previously described in the baseline CAC analyses.

2.5.5 Change in novel risk factors and CAC progression analyses

The relationships between the change in CRP, fibrinogen, PAI-1, and tPA-ag with the presence and extent of CAC progression were evaluated. Change in the novel CV risk factors was calculated by the difference between the follow-up and baseline values, divided by the time between the visits corresponding to when the CAC measurements were performed.

Logistic regression analyses were performed for the outcome variable of presence of CAC progression based on the definition described above. Linear regression analyses were

performed for the continuous outcome of extent of CAC progression based on the MESA method described above (72). Univariable and multivariable analyses were performed for both.

3.0 RESULTS

3.1 BASELINE CHARACTERISTICS OF THE BASELINE CAC POPULATION

The characteristics of the population by presence of CAC at baseline are listed in **Table 1**. There were 372 subjects with baseline CAC and novel CV risk factor measurements available. The mean age of the population was 51.3 ± 2.8 years. The racial distribution was 35.2% black and 64.8% white. CAC was present in 184 (49.5%) subjects of the study population. For those with CAC, the median Agatston score was 8.6 U with a range from 1.0 U to 598.6 U.

Subjects with CAC present had a worse CV risk profile when compared to subjects without CAC. They were older and had a higher systolic blood pressure (SBP), BMI, LDL-C, triglycerides, and FRS. They had lower HDL-C. As a result, they were more likely to have used medications to control hypertension and hyperlipidemia. All four novel CV risk factors were also significantly higher in those with baseline CAC present. There was no significant difference between the two groups in relation to menopausal status or hormone therapy.

There were no significant differences in socio-economic status covariates of income and education between groups with and without the presence of CAC. Approximately one-fourth of women had an income of >$100,000 per year and nearly one-third had a graduate degree.

Table 1. Baseline characteristics of the baseline CAC study population by the presence and absence of baseline CAC.

Characteristics	Total n = 372	CAC = 0 n = 188	CAC >0 n = 184	P value*
Age at baseline CAC, mean (SD), y	51.3 (2.8)	50.9 (2.8)	51.6 (2.7)	0.005
Site, n (%)				0.5
Chicago, site 13	160 (43.0)	78 (41.5)	82 (44.6)	
Pittsburgh, site 17	212 (57.0)	110 (58.5)	102 (55.4)	
Race, n (%)				0.004
Black	131 (35.2)	53 (28.2)	78 (42.3)	
White	241 (64.8)	135 (71.8)	106 (57.6)	
Menopausal status, n (%)				0.7
Pre- and early perimenopausal	200 (53.8)	106 (56.4)	94 (51.1)	
Late peri- and postmenopausal	141 (37.9)	66 (35.1)	75 (40.8)	
Surgical menopause	12 (3.2)	7 (3.7)	5 (2.7)	
Indeterminate	19 (5.1)	9 (4.8)	10 (5.4)	
Income, n (%)				0.1
<$50,000	121 (32.7)	55 (29.3)	66 (36.3)	
$50,000-$100,000	154 (41.62)	77 (41.0)	77 (42.3)	
>$100,000	95 (25.7)	56 (29.8)	39 (21.4)	
Education, n (%)				0.4
High school or less	58 (15.6)	25 (13.3)	33 (17.9)	
Some college or college	192 (51.6)	102 (54.3)	90 (48.9)	
Graduate	122 (32.8)	61 (32.5)	61 (33.2)	
SBP, mean (SD), mmHg	118.4 (17.1)	113.3 (14.7)	123.5 (18.0)	<0.0001
BMI, mean (SD), kg/m^2	29.4 (6.4)	25.7 (3.6)	33.0 (6.5)	<0.0001
HDL-C, mean (SD), mg/dL	57.4 (14.3)	60.4 (14.6)	54.5 (13.4)	<0.0001
LDL-C, mean (SD), mg/dL	119.9 (33.2)	116.0 (29.8)	123.9 (36.0)	0.03
Triglycerides, median (Q1, Q3), mg/dL	99.0 (75.5, 137.5)	91.0 (70.0, 119.5)	116.5 (84.0, 170.0)	<0.0001
Hormone therapy, n (%)	55 (14.8)	31 (16.5)	24 (13.0)	0.3
CV medication use, n (%)	81 (21.8)	24 (12.8)	57 (31.0)	<0.0001
Diabetes, n (%)	18 (4.8)	6 (3.2)	12 (6.5)	0.1
Family history of CV disease, n (%)	251 (67.5)	118 (62.8)	133 (72.3)	0.05
Current smoking, n (%)	50 (13.4)	29 (15.4)	21 (11.4)	0.3
HOMA, median (Q1, Q3)	2.0 (1.5, 3.1)	1.6 (1.3, 2.2)	2.8 (1.8, 4.7)	<0.0001
FRS, mean (SD)	10.3 (3.5)	9.5 (3.3)	11.1 (3.5)	<0.0001
CRP, median (Q1, Q3), mg/L	2.1 (0.8, 5.6)	1.3 (0.6, 2.9)	3.9 (1.3, 8.0)	<0.0001
Fibrinogen, median (Q1, Q3), mg/dL	279 (242, 316)	262 (230, 300)	279 (256, 316)	0.0005
PAI-1, median (Q1, Q3), ng/mL	13.2 (7.8, 24.7)	10.6 (6.8, 19.2)	18.2 (9.2, 32.4)	<0.0001
tPA-ag, median (Q1, Q3), ng/mL	6.8 (5.2, 9.3)	6.3 (4.7, 7.6)	8.1 (6.0, 10.4)	<0.0001

* P-value for CAC groups - Wilcoxon rank-sum test for continuous variables; X^2 test for categorical variables

Spearman correlation coefficients demonstrated significant correlations between all four novel biomarkers, as well as BMI and HOMA, with the exception of fibrinogen and PAI-1 (**Table 2**). There was a moderate correlation between CRP and fibrinogen and a moderate-to-strong correlation between PAI-1 and tPA-ag.

Table 2. Spearman correlation coefficients between baseline novel CV risk factors, BMI, and HOMA to baseline novel CV risk factors.

	CRP (P value)	Fibrinogen (P value)	PAI-1 (P value)	tPA-ag (P value)
CRP, mg/dL	1.00	0.46 (<0.0001)	0.33 (<0.0001)	0.33 (<0.0001)
Fibrinogen, mg/dL		1.00	0.1 (0.07)	0.22 (<0.0001)
PAI-1, ng/mL			1.00	0.6 (<0.0001)
tPA-ag, ng/mL				1.00
BMI, kg/m^2	0.53 (<0.0001)	0.31 (<0.0001)	0.43 (<0.0001)	0.44 (<0.0001)
HOMA	0.43 (<0.0001)	0.17 (0.004)	0.43 (<0.0001)	0.49 (<0.0001)

3.2 BASELINE NOVEL BIOMARKERS AND THE PRESENCE OF BASELINE CAC

Results of the univariable logistic regression analyses for the presence of baseline CAC are listed in **Table 3**. Older age was associated with increased odds of CAC presence. The odds of white race being positive for CAC was significantly lower compared to black race (OR, 0.53; 95% CI, 0.35-0.82, p=0.004). Higher SBP, BMI, LDL-C levels, triglyceride levels, and CV medication use, and lower HDL-C levels were all associated with increased odds of CAC presence ($p \leq 0.0003$). The log-transformed values of all four novel CV risk factors had significant positive associations with CAC presence.

Multivariable logistic regression with individual traditional CV risk factors demonstrated significant associations between novel CV risk factors and the presence of CAC after adjusting for age, study site, race, menopausal status, income, education, and SBP (**Table 4**). None of

these associations were significant after adjusting for BMI. Using the FRS based strategy, the associations between novel CV risk factors and the presence of CAC remained significant after adjusting for FRS, study site, race, menopausal status, income, and education (**Table 5**). Similarly, none of the associations were significant after introduction of BMI into the models.

Effect modification for race, menopausal status, and BMI on novel CV risk factors and CAC presence was tested. There was a significant interaction with race on the relationship between log(CRP) and presence of CAC. Stratified analyses were performed on the relationship between log(CRP) and presence of CAC by race using the traditional CV risk factor based models (**Table 6**) and the FRS based models (**Table 7**). The models were similar.

In black women, the significant association of log(CRP) to CAC presence remained even after introduction of BMI, log(HOMA), family history of CV disease, lipids, CV medication use and current smoking in the traditional model (OR, 3.38; 95% CI, 1.51-7.58; p=0.003); and BMI, log(HOMA), family history of CV disease, and CV medication use in the FRS based model (OR, 3.25; 95% CI, 1.53-6.90; p=0.002). In white women, the relationship between log(CRP) and presence of CAC was similar to that seen in the total population in both the final multivariable traditional model (OR, 0.91; 95% CI 0.63-1.30; p=0.6) and the FRS based model (OR, 0.87; 95% CI, 0.62-1.23; p=0.4).

In the final multivariable models for the four novel CV risk factors, the strongest relationship with presence of CAC was related to BMI ($p<0.0001$ for all). This was present in both the traditional CV risk factor based models and the FRS based models. Log(HOMA) was significant in all traditional models ($p\leq0.02$ for all) and for 3 of the risk factors in the FRS based models ($p\leq0.04$ for all, except $p=0.1$ for log(PAI-1)). Family history of CV disease was significant in all models ($p\leq0.02$ for all).

Table 3. Univariable logistic regression analyses between baseline characteristics and the presence and absence of baseline CAC.

	n	Odds Ratio	95% CI	P Value	C
Age, y	372	1.10	1.03-1.19	0.009	0.583
Site[a]	372	0.88	0.59-1.33	0.5	0.515
Race[b]	372	0.53	0.35-0.82	0.004	0.571
Menopausal status[c]	372			0.1	0.534
Late peri- and postmenopausal		1.28	0.83-1.97	0.3	
Surgical menopause		0.81	0.25-2.62	0.7	
Indeterminate		1.25	0.49-3.22	0.6	
Income[d]	370			0.1	0.554
$50,000-$100,000		0.83	0.52-1.34	0.5	
>$100,000		0.58	0.34-1.00	0.05	
Education[e]	372			0.4	0.534
Some college or college		0.67	0.37-1.21	0.2	
Graduate		0.76	0.40-1.42	0.4	
SBP, mmHg	372	1.04	1.03-1.06	<0.0001	0.672
BMI, kg/m^2	372	1.32	1.24-1.34	<0.0001	0.833
HDL-C, mg/dL	372	0.97	0.95-0.99	0.0001	0.636
LDL-C, mg/dL	372	1.01	1.00-1.01	0.02	0.564
Log(Triglycerides), log mg/dL	372	2.88	1.80-4.61	<0.0001	0.639
Hormone therapy	372	0.76	0.43-1.35	0.4	0.517
CV medication use	372	3.07	1.81-5.21	<0.0001	0.591
Diabetes	372	2.12	0.78-5.76	0.1	0.517
Family history of CV disease	372	1.55	1.00-2.40	0.05	0.548
Current smoking	372	0.71	0.39-1.29	0.3	0.520
Log(HOMA)	336	5.52	3.41-8.94	<0.0001	0.750
FRS	341	1.15	1.08-1.23	<0.0001	0.645
Log(CRP), log mg/L	367	1.91	1.58-2.32	<0.0001	0.712
Log(Fibrinogen), log mg/dL	317	8.19	2.61-25.72	0.0003	0.613
Log(PAI-1), log ng/mL	356	1.67	1.31-2.12	<0.0001	0.640
Log(tPA-ag), log ng/mL	357	3.67	2.16-6.25	<0.0001	0.668

[a]site 17 versus site 13; [b]White versus Black; [c] Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less
1 Log(biomarker) = 2.7 non-log transformed value

Table 4. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence and absence of baseline CAC using traditional CV risk factor based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: Age, Site, Race					
Log(CRP), log mg/L	367	1.84	1.51-2.24	<0.0001	0.723
Log(Fibrinogen), log mg/dL	317	6.16	1.91-19.89	0.002	0.640
Log(PAI-1), log ng/mL	356	1.71	1.33-2.19	<0.0001	0.683
Log(tPA-ag), log ng/mL	357	3.54	2.04-6.14	<0.0001	0.691
Model 2: Age, Site, Race, Menopausal status					
Log(CRP), log mg/L	367	1.85	1.52-2.26	<0.0001	0.724
Log(Fibrinogen), log mg/dL	317	6.32	1.94-20.57	0.002	0.649
Log(PAI-1), log ng/mL	356	1.74	1.35-2.24	<0.0001	0.691
Log(tPA-ag), log ng/mL	357	3.81	2.16-6.70	<0.0001	0.700
Model 3: Age, Site, Race, Menopausal status, Income, Education					
Log(CRP), log mg/L	365	1.88	1.53-2.30	<0.0001	0.733
Log(Fibrinogen), log mg/dL	315	6.32	1.91-20.94	0.003	0.670
Log(PAI-1), log ng/mL	354	1.73	1.34-2.24	<0.0001	0.695
Log(tPA-ag), log ng/mL	355	3.77	2.13-6.70	<0.0001	0.710
Model 4: Age, Site, Race, Menopausal status, Income, Education, SBP					
Log(CRP), log mg/L	365	1.80	1.46-2.21	<0.0001	0.762
Log(Fibrinogen), log mg/dL	315	5.88	1.70-20.39	0.005	0.724
Log(PAI-1), log ng/mL	354	1.56	1.20-2.03	0.001	0.730
Log(tPA-ag), log ng/mL	355	3.17	1.76-5.72	0.0001	0.745
Model 5: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI					
Log(CRP), log mg/L	365	1.15	0.89-1.48	0.3	0.851
Log(Fibrinogen), log mg/dL	315	1.49	0.33-6.62	0.6	0.850
Log(PAI-1), log ng/mL	354	0.97	0.71-1.32	0.8	0.849
Log(tPA-ag), log ng/mL	355	1.15	0.60-2.21	0.7	0.848
Model 6: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)					
Log(CRP), log mg/L	331	1.17	0.89-1.53	0.3	0.864
Log(Fibrinogen), log mg/dL	282	2.64	0.52-13.47	0.2	0.866
Log(PAI-1), log ng/mL	319	0.87	0.61-1.25	0.5	0.865
Log(tPA-ag), log ng/mL	320	0.94	0.48-1.84	0.9	0.863
Model 7: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history					
Log(CRP), log mg/L	331	1.16	0.88-1.52	0.3	0.870
Log(Fibrinogen), log mg/dL	282	2.36	0.46-12.25	0.3	0.872
Log(PAI-1), log ng/mL	319	0.81	0.56-1.17	0.3	0.872
Log(tPA-ag), log ng/mL	320	0.78	0.39-1.57	0.5	0.869
Model 8: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)					
Log(CRP), log mg/L	331	1.22	0.92-1.61	0.2	0.874
Log(Fibrinogen), log mg/dL	282	2.18	0.41-11.57	0.4	0.878
Log(PAI-1), log ng/mL	319	0.84	0.58-1.23	0.4	0.874
Log(tPA-ag), log ng/mL	320	0.87	0.42-1.79	0.7	0.874
Model 9: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking					
Log(CRP), log mg/L	331	1.23	0.92-1.64	0.2	0.873
Log(Fibrinogen), log mg/dL	282	2.26	0.42-12.12	0.3	0.878
Log(PAI-1), log ng/mL	319	0.84	0.57-1.23	0.4	0.874
Log(tPA-ag), log ng/mL	320	0.86	0.42-1.78	0.7	0.874

1 Log(biomarker) = 2.7 non-log transformed value

Table 5. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence or absence of baseline CAC using FRS based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: FRS, Site, Race					
Log(CRP), log mg/L	339	1.84	1.50-2.25	<0.0001	0.739
Log(Fibrinogen), log mg/dL	292	6.45	1.91-21.77	0.003	0.660
Log(PAI-1), log ng/mL	327	1.67	1.28-2.16	0.0001	0.689
Log(tPA-ag), log ng/mL	328	3.37	1.93-5.89	<0.0001	0.707
Model 2: FRS, Site, Race, Menopausal status					
Log(CRP), log mg/L	339	1.86	1.51-2.28	<0.0001	0.740
Log(Fibrinogen), log mg/dL	292	6.97	2.04-23.89	0.002	0.676
Log(PAI-1), log ng/mL	327	1.70	1.31-2.21	<0.0001	0.700
Log(tPA-ag), log ng/mL	328	3.64	2.06-6.46	<0.0001	0.720
Model 3: FRS, Site, Race, Menopausal status, Income, Education					
Log(CRP), log mg/L	337	1.86	1.52-2.31	<0.0001	0.753
Log(Fibrinogen), log mg/dL	290	7.07	2.01-24.94	0.002	0.695
Log(PAI-1), log ng/mL	325	1.72	1.32-2.25	<0.0001	0.712
Log(tPA-ag), log ng/mL	326	3.74	2.08-6.74	<0.0001	0.731
Model 4: FRS, Site, Race, Menopausal status, Income, Education, BMI					
Log(CRP), log mg/L	337	1.19	0.92-1.54	0.2	0.849
Log(Fibrinogen), log mg/dL	290	1.60	0.34-7.52	0.6	0.845
Log(PAI-1), log ng/mL	325	1.04	0.75-1.44	0.8	0.843
Log(tPA-ag), log ng/mL	326	1.36	0.70-2.61	0.4	0.844
Model 5: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)					
Log(CRP), log mg/L	305	1.16	0.88-1.52	0.3	0.855
Log(Fibrinogen), log mg/dL	259	2.37	0.46-13.33	0.3	0.857
Log(PAI-1), log ng/mL	292	0.91	0.63-1.32	0.6	0.856
Log(tPA-ag), log ng/mL	293	1.07	0.54-2.13	0.9	0.856
Model 6: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history,					
Log(CRP), log mg/L	305	1.15	0.88-1.52	0.3	0.863
Log(Fibrinogen), log mg/dL	259	2.23	0.40-12.29	0.4	0.865
Log(PAI-1), log ng/mL	292	0.83	0.57-1.22	0.3	0.863
Log(tPA-ag), log ng/mL	293	0.88	0.43-1.79	0.7	0.863
Model 7: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use					
Log(CRP), log mg/L	305	1.16	0.88-1.53	0.3	0.863
Log(Fibrinogen), log mg/dL	259	2.22	0.40-12.28	0.4	0.865
Log(PAI-1), log ng/mL	292	0.83	0.57-1.22	0.3	0.863
Log(tPA-ag), log ng/mL	293	0.88	0.43-1.79	0.7	0.865

1 Log(biomarker) = 2.7 non-log transformed value

Table 6. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence and absence of baseline CAC using traditional CV risk factor based models by race.

	n	Odds Ratio	95% CI	P Value	C
Unadjusted					
Log(CRP), log mg/L - Black	131	2.41	1.68-3.47	<0.0001	0.761
Log(CRP), log mg/L - White	236	1.65	1.31-2.08	<0.0001	0.670
Model 1: Age, Site					
Log(CRP), log mg/L - Black	131	2.41	1.67-3.47	<0.0001	0.768
Log(CRP), log mg/L - White	236	1.60	1.26-2.02	0.0001	0.674
Model 2: Age, Site, Menopausal status					
Log(CRP), log mg/L - Black	131	2.41	1.66-3.49	<0.0001	0.767
Log(CRP), log mg/L - White	236	1.62	1.27-2.05	<0.0001	0.682
Model 3: Age, Site, Menopausal status, Income, Education					
Log(CRP), log mg/L - Black	130	2.43	1.66-3.55	<0.0001	0.778
Log(CRP), log mg/L - White	235	1.65	1.29-2.11	<0.0001	0.692
Model 4: age, site, menopausal status, income, education, SBP					
Log(CRP), log mg/L - Black	130	2.41	1.62-3.59	<0.0001	0.802
Log(CRP), log mg/L - White	235	1.54	1.20-1.98	0.0008	0.749
Model 5: Age, Site, Menopausal status, Income, Education, SBP, BMI					
Log(CRP), log mg/L - Black	130	1.66	1.01-2.71	0.05	0.896
Log(CRP), log mg/L - White	235	0.93	0.67-1.28	0.6	0.835
Model 6: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)					
Log(CRP), log mg/L - Black	119	2.58	1.34-4.96	0.005	0.932
Log(CRP), log mg/L - White	212	0.87	0.62-1.22	0.4	0.831
Model 7: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history					
Log(CRP), log mg/L - Black	119	2.56	1.33-4.94	0.005	0.932
Log(CRP), log mg/L - White	212	0.86	0.61-1.22	0.4	0.841
Model 8: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)					
Log(CRP), log mg/L - Black	119	3.00	1.42-6.31	0.004	0.941
Log(CRP), log mg/L - White	212	0.90	0.63-1.29	0.6	0.847
Model 9: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking					
Log(CRP), log mg/L - Black	119	3.38	1.51-7.58	0.003	0.946
Log(CRP), log mg/L - White	212	0.91	0.63-1.30	0.6	0.849

1 Log(CRP) = 2.7 non-log transformed value

Table 7. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence and absence of baseline CAC using FRS based models by race.

	n	Odds Ratio	95% CI	P Value	C
Unadjusted					
Log(CRP), log mg/L - Black	131	2.41	1.68-3.47	<0.0001	0.761
Log(CRP), log mg/L - White	236	1.65	1.31-2.08	<0.0001	0.670
Model 1: FRS, Site					
Log(CRP), log mg/L - Black	115	2.76	1.81-4.22	<0.0001	0.808
Log(CRP), log mg/L - White	224	1.56	1.23-1.97	0.0002	0.696
Model 2: FRS, Site, Menopausal status					
Log(CRP), log mg/L - Black	115	2.70	1.77-4.14	<0.0001	0.810
Log(CRP), log mg/L - White	224	1.60	1.26-2.03	0.0001	0.705
Model 3: FRS, Site, Menopausal status, Income, Education					
Log(CRP), log mg/L - Black	114	2.79	1.79-4.35	<0.0001	0.835
Log(CRP), log mg/L - White	223	1.61	1.26-2.06	0.0002	0.717
Model 4: FRS, Site, Menopausal status, Income, Education, BMI					
Log(CRP), log mg/L - Black	114	2.33	1.32-4.14	0.004	0.916
Log(CRP), log mg/L - White	223	0.94	0.68-1.30	0.7	0.825
Model 5: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA)					
Log(CRP), log mg/L - Black	105	2.86	1.44-5.68	0.003	0.934
Log(CRP), log mg/L - White	200	0.88	0.63-1.23	0.5	0.825
Model 6: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA), Family history					
Log(CRP), log mg/L - Black	105	3.18	1.53-6.63	0.002	0.941
Log(CRP), log mg/L - White	200	0.87	0.62-1.22	0.4	0.834
Model 7: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use					
Log(CRP), log mg/L - Black	105	3.25	1.53-6.90	0.002	0.942
Log(CRP), log mg/L - White	200	0.87	0.62-1.23	0.4	0.837

1 Log(CRP) = 2.7 non-log transformed value

3.3 BASELINE NOVEL BIOMARKERS AND THE EXTENT OF BASELINE CAC

Univariable tobit regression analyses for the relationships between baseline variables and baseline log(CAC+1) as a continuous variable demonstrated significant positive correlations for age, race, SBP, BMI, HDL-C level, LDL-C level, log(triglyceride) level, CV medication use, diabetes, log(HOMA), FRS, and the four novel CV risk factors (**Table 8**). In the adjusted traditional CV risk factor based models, all novel biomarkers were significantly associated with extent of CAC after adjusting for age, study site, race, menopausal status, income, education, and SBP (**Table 9**). None had significant associations after the introduction of BMI. Similar results were demonstrated in the FRS based models (**Table 10**).

Tests for interactions for race, menopausal status, and BMI on the relationship between the four novel CV risk factors and CAC extent were significant for race on the relationship between log(CRP) and CAC. In the univariable analyses, log(CRP) was significantly associated with baseline CAC extent in both black and white women (<0.0001) (**Table 11**). These relationships held in multivariable traditional models when adjusted for age, study site, menopausal status, income, education, and SBP in both black and white women. Upon introduction of BMI, the association was significant in blacks but not whites. The introduction of log(HOMA), family history of CV disease, HDL-C, LDL-C, log(triglycerides), CV medication use, and current smoking yielded similar results. Black women had a significant association of log(CRP) with log(CAC+1) (parameter estimate (β), 0.464; standard error (SE), 0.134; p=0.0006) whereas white women did not (β, -0.103; SE, 0.201; p=0.6).

In the multivariable FRS based models, log(CRP) was significantly associated with baseline log(CAC+1) after adjusting for FRS, site, menopausal status, income, and education in both black (p<0.0001) and white women (p=0.0005) (**Table 12**). Introducing BMI into the

models attenuated the relationship in white women (p=0.9), but the relationship was still significant in black women (p=0.0005). After adding log(HOMA), family history, and CV medication use, the relationship of log(CRP) and baseline(CAC+1) was still significant in blacks (β, 0.488; SE, 0.141; p=0.0005), but not whites (β, -0.088; SE, 0.198; p=0.7).

In the final adjusted models, BMI was the covariate that was most strongly associated with extent of CAC at baseline (p<0.0001). Age was significant in the traditional CV risk factor based models for all novel biomarkers (p≤0.02), except for log(CRP). The FRS also had a strong association (p≤0.002). Log(HOMA) had a borderline association with log(CRP) in the traditional model and FRS based model with p=0.06 and p=0.07, respectively. The associations of log(HOMA) to log(fibrinogen), log(PAI-1), and log(tPA-ag) were significant.

Table 8. Univariable tobit regression analyses between baseline characteristics and the extent of baseline CAC.

	n	Parameter Estimate	Standard Error	P value
Age, y	372	0.191	0.055	0.0005
Site[a]	372	-0.091	0.312	0.8
Race[b]	372	-0.696	0.321	0.03
Menopausal status[c]	372			
Late peri- and postmenopausal		0.525	0.326	0.1
Surgical menopause		0.370	0.894	0.7
Indeterminate		0.315	0.708	0.7
Income[d]	370			
$50,000-$100,000		-0.080	0.360	0.8
>$100,000		-0.583	0.416	0.2
Education[e]	372			
Some college or college		-0.746	0.437	0.09
Graduate		-0.634	0.465	0.2
SBP, mmHg	372	0.051	0.009	<0.0001
BMI, kg/m^2	372	0.232	0.021	<0.0001
HDL-C, mg/dL	372	-0.510	0.011	<0.0001
LDL-C, mg/dL	372	0.011	0.004	0.01
Log(Triglycerides), log mg/dL	372	1.710	0.306	<0.0001
Hormone therapy	372	-0.405	0.444	0.4
CV medication use	372	1.317	0.358	0.0002
Diabetes	372	1.430	0.681	0.04
Family history of CV disease	372	0.439	0.335	0.2
Current smoking	372	-0.166	0.462	0.7
Log(HOMA)	336	1.721	0.232	<0.0001
FRS	341	0.235	0.044	<0.0001
Log(CRP), log mg/L	367	0.804	0.123	<0.0001
Log(Fibrinogen), log mg/dL	317	2.905	0.797	0.0003
Log(PAI-1), log ng/mL	356	0.764	0.165	<0.0001
Log(tPA-ag), log ng/mL	357	1.461	0.301	<0.0001

[a]site 17 versus site 13; [b]White versus Black; [c]Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less
1 Log(biomarker) = 2.7 non-log transformed value

Table 9. Multivariable tobit regression analyses between baseline novel CV risk factors and the extent of baseline CAC using traditional CV risk factor based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: Age, Site, Race				
Log(CRP), log mg/L	367	0.725	0.125	<0.0001
Log(Fibrinogen), log mg/dL	317	2.447	0.806	0.002
Log(PAI-1), log ng/mL	356	0.777	0.164	<0.0001
Log(tPA-ag), log ng/mL	357	1.343	0.297	<0.0001
Model 2: Age, Site, Race, Menopausal status				
Log(CRP), log mg/L	367	0.737	0.125	<0.0001
Log(Fibrinogen), log mg/dL	317	2.414	0.806	0.003
Log(PAI-1), log ng/mL	356	0.805	0.165	<0.0001
Log(tPA-ag), log ng/mL	357	1.422	0.301	<0.0001
Model 3: Age, Site, Race, Menopausal status, Income, Education				
Log(CRP), log mg/L	365	0.759	0.128	<0.0001
Log(Fibrinogen), log mg/dL	315	2.296	0.808	0.005
Log(PAI-1), log ng/mL	354	0.826	0.167	<0.0001
Log(tPA-ag), log ng/mL	355	1.407	0.305	<0.0001
Model 4: Age, Site, Race, Menopausal status, Income, Education, SBP				
Log(CRP), log mg/L	365	0.645	0.125	<0.0001
Log(Fibrinogen), log mg/dL	315	2.085	0.774	0.007
Log(PAI-1), log ng/mL	354	0.670	0.162	<0.0001
Log(tPA-ag), log ng/mL	355	1.131	0.296	0.0001
Model 5: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI				
Log(CRP), log mg/L	365	0.157	0.121	0.2
Log(Fibrinogen), log mg/dL	315	0.609	0.695	0.4
Log(PAI-1), log ng/mL	354	0.204	0.151	0.2
Log(tPA-ag), log ng/mL	355	0.270	0.272	0.3
Model 6: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)				
Log(CRP), log mg/L	331	0.135	0.125	0.3
Log(Fibrinogen), log mg/dL	282	1.046	0.689	0.1
Log(PAI-1), log ng/mL	319	0.097	0.161	0.5
Log(tPA-ag), log ng/mL	320	0.124	0.277	0.7
Model 7: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history				
Log(CRP), log mg/L	331	0.132	0.125	0.3
Log(Fibrinogen), log mg/dL	282	1.032	0.687	0.1
Log(PAI-1), log ng/mL	319	0.070	0.162	0.7
Log(tPA-ag), log ng/mL	320	0.048	0.278	0.9
Model 8: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)				
Log(CRP), log mg/L	331	0.159	0.126	0.2
Log(Fibrinogen), log mg/dL	282	0.997	0.688	0.1
Log(PAI-1), log ng/mL	319	0.054	0.162	0.7
Log(tPA-ag), log ng/mL	320	-0.009	0.283	1.0
Model 9: Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking				
Log(CRP), log mg/L	331	0.159	0.125	0.2
Log(Fibrinogen), log mg/dL	282	0.942	0.693	0.2
Log(PAI-1), log ng/mL	319	0.053	0.162	0.7
Log(tPA-ag), log ng/mL	320	-0.032	0.283	0.9

1 Log(biomarker) = 2.7 non-log transformed value

Table 10. Multivariable tobit regression analyses between baseline novel CV risk factors and the extent of baseline CAC using FRS based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: FRS, Site, Race				
Log(CRP), log mg/L	339	0.699	0.123	<0.0001
Log(Fibrinogen), log mg/dL	292	2.471	0.808	0.002
Log(PAI-1), log ng/mL	327	0.702	0.164	<0.0001
Log(tPA-ag), log ng/mL	328	1.189	0.297	<0.0001
Model 2: FRS, Site, Race, Menopausal status				
Log(CRP), log mg/L	339	0.709	0.124	<0.0001
Log(Fibrinogen), log mg/dL	292	2.496	0.808	0.002
Log(PAI-1), log ng/mL	327	0.198	0.045	<0.0001
Log(tPA-ag), log ng/mL	328	1.257	0.300	<0.0001
Model 3: FRS, Site, Race, Menopausal status, Income, Education				
Log(CRP), log mg/L	337	0.714	0.127	<0.0001
Log(Fibrinogen), log mg/dL	290	2.346	0.809	0.004
Log(PAI-1), log ng/mL	325	0.768	0.166	<0.0001
Log(tPA-ag), log ng/mL	326	1.248	0.300	<0.0001
Model 4: FRS, Site, Race, Menopausal status, Income, Education, BMI				
Log(CRP), log mg/L	337	0.193	0.123	0.1
Log(Fibrinogen), log mg/dL	290	0.782	0.713	0.3
Log(PAI-1), log ng/mL	325	0.228	0.155	0.1
Log(tPA-ag), log ng/mL	326	0.282	0.276	0.3
Model 5: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)				
Log(CRP), log mg/L	305	0.148	0.129	0.3
Log(Fibrinogen), log mg/dL	259	1.064	0.715	0.1
Log(PAI-1), log ng/mL	292	0.125	0.169	0.5
Log(tPA-ag), log ng/mL	293	0.133	0.285	0.6
Model 6: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history				
Log(CRP), log mg/L	305	0.145	0.129	0.3
Log(Fibrinogen), log mg/dL	259	1.052	0.715	0.1
Log(PAI-1), log ng/mL	292	0.098	0.170	0.6
Log(tPA-ag), log ng/mL	293	0.061	0.286	0.8
Model 7: FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use				
Log(CRP), log mg/L	305	0.153	0.129	0.2
Log(Fibrinogen), log mg/dL	259	1.013	0.720	0.2
Log(PAI-1), log ng/mL	292	0.096	0.170	0.6
Log(tPA-ag), log ng/mL	293	0.044	0.287	0.9

1 Log(biomarker) = 2.7 non-log transformed value

Table 11. Multivariable tobit regression analyses between baseline novel CV risk factors and the extent of baseline CAC using traditional CV risk factor based models by race.

	n	Parameter Estimate	Standard Error	P value
Unadjusted				
Log(CRP), log mg/L - Black	131	0.817	0.161	<0.0001
Log(CRP), log mg/L - White	236	0.755	0.181	<0.0001
Model 1: Age, Site				
Log(CRP), log mg/L - Black	131	0.797	0.160	<0.0001
Log(CRP), log mg/L - White	236	0.640	0.182	0.0004
Model 2: Age, Site, Menopausal status				
Log(CRP), log mg/L - Black	131	0.806	0.162	<0.0001
Log(CRP), log mg/L - White	236	0.662	0.182	0.0003
Model 3: Age, Site, Menopausal status, Income, Education				
Log(CRP), log mg/L - Black	130	0.822	0.165	<0.0001
Log(CRP), log mg/L - White	235	0.684	0.186	0.0002
Model 4: Age, Site, Menopausal status, Income, Education, SBP				
Log(CRP), log mg/L - Black	130	0.729	0.159	<0.0001
Log(CRP), log mg/L - White	235	0.546	0.182	0.003
Model 5: Age, Site, Menopausal status, Income, Education, SBP, BMI				
Log(CRP), log mg/L - Black	130	0.357	0.133	0.007
Log(CRP), log mg/L - White	235	-0.079	0.192	0.7
Model 6: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)				
Log(CRP), log mg/L - Black	119	0.443	0.135	0.001
Log(CRP), log mg/L - White	212	-0.148	0.199	0.5
Model 7: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history				
Log(CRP), log mg/L - Black	119	0.443	0.135	0.001
Log(CRP), log mg/L - White	212	-0.157	0.200	0.4
Model 8: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)				
Log(CRP), log mg/L - Black	119	0.452	0.135	0.0009
Log(CRP), log mg/L - White	212	-0.103	0.204	0.6
Model 9: Age, Site, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking				
Log(CRP), log mg/L - Black	119	0.464	0.134	0.0006
Log(CRP), log mg/L - White	212	-0.103	0.201	0.6

1 Log(CRP) = 2.7 non-log transformed value

Table 12. Multivariable tobit regression analyses between baseline novel CV risk factors and the extent of baseline CAC using FRS based models by race.

	n	Parameter Estimate	Standard Error	P value
Unadjusted				
Log(CRP), log mg/L - Black	131	0.817	0.161	<0.0001
Log(CRP), log mg/L - White	236	0.755	0.181	<0.0001
Model 1: FRS, Site				
Log(CRP), log mg/L - Black	115	0.841	0.164	<0.0001
Log(CRP), log mg/L - White	224	0.581	0.172	0.0007
Model 2: FRS, Site, Menopausal status				
Log(CRP), log mg/L - Black	115	0.817	0.164	<0.0001
Log(CRP), log mg/L - White	224	0.618	0.173	0.0004
Model 3: FRS, Site, Menopausal status, Income, Education				
Log(CRP), log mg/L - Black	114	0.837	0.166	<0.0001
Log(CRP), log mg/L - White	223	0.622	0.178	0.0005
Model 4: FRS, Site, Menopausal status, Income, Education, BMI				
Log(CRP), log mg/L - Black	114	0.483	0.138	0.0005
Log(CRP), log mg/L - White	223	-0.024	0.189	0.9
Model 5: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA)				
Log(CRP), log mg/L - Black	105	0.488	0.142	0.0006
Log(CRP), log mg/L - White	200	-0.090	0.196	0.6
Model 6: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA), Family history				
Log(CRP), log mg/L - Black	105	0.490	0.141	0.0005
Log(CRP), log mg/L - White	200	-0.104	0.197	0.6
Model 7: FRS, Site, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use				
Log(CRP), log mg/L - Black	105	0.488	0.141	0.0005
Log(CRP), log mg/L - White	200	-0.088	0.198	0.7

1 Log(CRP) = 2.7 non-log transformed value

3.4 BASELINE NOVEL BIOMARKERS AND THE PRESENCE OF CAC PROGRESSION

There were 258 subjects with baseline CAC, follow-up CAC, and novel CV risk factor measurements. The final study population for these analyses was 252 subjects after excluding those with surgical menopause. The mean average time between scans was 2.3 ± 0.5 years. For the dichotomous outcome variable of presence of CAC progression, there were 199 subjects who did not progress and 53 subjects who demonstrated progression. Of the 132 subjects with CAC=0 at baseline, 29 (22.0%) had any CAC in follow-up. Of the 113 subjects with 0<baseline CAC<100, there were 18 subjects (15.9%) who demonstrated an annualized increase of ≥10 U. Of the 7 subjects who demonstrated an annualized increase of ≥10% compared to baseline, there were 6 (85.7%) that demonstrated progression.

Subjects who had CAC progression by the categorical outcome variable were similar in age, study site, race, menopausal status, income, education, triglycerides, CV medication use, family history of CV disease, current smoking, and baseline CAC compared with subjects who did not exhibit progression (**Table 13**). Subjects who had CAC progression had a worse CV profile with higher SBP, higher BMI, lower HDL-C, higher LDL-C, higher HOMA, and higher FRS. Among the 4 novel CV biomarkers, PAI-1 and tPA-ag were significantly higher in CAC progressors compared with non-progressors. There was no significant difference in CRP and fibrinogen levels.

Univariable logistic regression demonstrated significant associations for SBP, BMI, HDL-C, LDL-C, log(triglycerides), FRS, and baseline log(CAC+1) (**Table 14**). For the novel CV risk factors, significant positive correlations were present for the presence of CAC

progression for log(PAI-1) (OR, 1.94; 95% CI, 1.39-2.72; p=0.0001) and log(tPA-ag) (OR, 2.21; 95% CI, 1.06-4.61; p=0.03), but not log(CRP) or log(fibrinogen).

In the multivariable model based on traditional CV risk factors, log(tPA-ag) was no longer significantly associated with CAC progression after adjusting for baseline CAC, age, site, and race (**Table 15**). Of these covariates, log(CAC+1) at baseline attenuated the relationship between log(tPA-ag) and CAC progression. In contrast, log(PAI-1) maintained a significantly positive relationship after adjusting for baseline CAC, age, site, race, menopausal status, income, education, SBP, BMI, log(HOMA), family history of CV disease, HDL-C, LDL-C, log(triglycerides), CV medication use, and current smoking (OR, 1.91; 95% CI, 1.24-2.93; p=0.003).

Similarly, log(tPA-ag) was no longer significantly associated with presence of CAC progression in the FRS based multivariable model after adjusting for baseline CAC, FRS, study site, and race (**Table 16**). Log(PAI-1) was significantly associated with presence of CAC progression after adjusting for multiple variables that included baseline CAC, FRS, study site, race, menopausal status, income, education, BMI, log(HOMA), family history of CV disease, and CV medication use (OR, 1.82; 95% CI, 1.18-2.79, p=0.006).

In the final multivariable traditional CV risk factor based models for the four novel CV biomarkers, there was no covariate that was significantly associated with CAC progression using a dichotomous variable. Similar findings were present in the final multivariable FRS based models.

Table 13. Baseline characteristics of the CAC progression study population and by the presence and absence of CAC progression.

Characteristics	Total n = 252	CAC Stable n = 199	CAC Progressed† n = 53	P value*
Age at baseline CAC, mean (SD), y	51.2 (2.6)	51.1 (2.6)	51.7 (2.9)	0.2
Site, n (%)				0.6
Chicago, site 13	102 (40.5)	79 (39.7)	23 (43.4)	
Pittsburgh, site 17	150 (59.5)	120 (60.3)	30 (56.6)	
Race, n (%)				0.6
Black	82 (32.5)	63 (31.7)	19 (35.9)	
White	170 (67.5)	136 (68.3)	34 (64.2)	
Menopausal status, n (%)				0.1
Pre- and early perimenopausal	142 (56.4)	113 (56.8)	29 (54.7)	
Late peri- and postmenopausal	77 (30.6)	56 (28.1)	21 (39.6)	
Hormone therapy user	33 (13.1)	30 (15.1)	3 (5.7)	
Income, n (%)				0.7
<$50,000	82 (32.7)	65 (32.7)	17 (32.7)	
$50,000-$100,000	96 (38.3)	74 (37.2)	22 (42.3)	
>$100,000	73 (29.1)	60 (30.2)	13 (25.0)	
Education, n (%)				0.5
High school or less	41 (16.3)	32 (16.1)	9 (17.0)	
Some college or college	133 (52.8)	102 (51.3)	31 (58.5)	
Graduate	78 (31.0)	65 (32.7)	13 (24.5)	
SBP, mean (SD), mmHg	117.1 (15.8)	116.0 (15.8)	121.4 (14.8)	0.009
BMI, mean (SD), kg/m^2	29.4 (6.5)	28.8 (6.4)	31.4 (6.7)	0.008
HDL-C, mean (SD), mg/dL	57.2 (13.4)	58.3 (13.6)	53.4 (11.7)	0.02
LDL-C, mean (SD), mg/dL	119.3 (34.4)	116.8 (33.2)	128.5 (37.5)	0.08
Triglycerides, median (Q1, Q3), mg/dL	98.0 (74.0, 136.5)	97.0 (73.0, 133.0)	105.0 (76.0, 170.0)	0.1
CV medication use, n (%)	56 (22.2)	40 (20.1)	16 (30.2)	0.1
Diabetes, n (%)	9 (3.6)	4 (2.0)	5 (9.4)	0.01
Family history of CV disease, n (%)	166 (65.9)	132 (66.3)	34 (64.2)	0.8
Current smoking, n (%)	30 (11.9)	22 (11.1)	8 (15.1)	0.4
HOMA, median (Q1, Q3)	1.9 (1.5, 3.1)	1.8 (1.4, 2.9)	2.4 (1.5, 3.9)	0.05
FRS, mean (SD)	10.1 (3.3)	9.7 (3.1)	11.3 (3.7)	0.006
CAC - baseline, median (Q1, Q3), U	0 (0, 7.2)	0 (0, 5.8)	0 (0, 31.2)	0.2
CRP, median (Q1, Q3), mg/L	2.1 (1.0, 5.9)	2.1 (1.0, 5.9)	2.5 (1.3, 5.6)	0.5
Fibrinogen, median (Q1, Q3), mg/dL	273 (242, 312)	273 (238, 316)	279 (247, 300)	0.7
PAI-1, median (Q1, Q3), ng/mL	13.0 (7.2, 23.5)	12.0 (6.6, 21.8)	20.2 (10.0, 52.0)	0.0004
tPA-ag, median (Q1, Q3), ng/mL	6.6 (5.0, 9.4)	6.6 (4.7, 9.2)	6.8 (5.8, 10.1)	0.06

* P-value for CAC groups - Wilcoxon rank-sum test for continuous variables; X^2 test for categorical variables

†CAC progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100, ≥10% for baseline CAC≥100

Table 14. Univariable logistic regression analyses between baseline characteristics and the presence and absence of CAC progression.

	n	Odds Ratio	95% CI	P Value	C
Age, y	252	1.09	0.97-1.22	0.1	0.556
Site[a]	252	0.86	0.47-1.59	0.6	0.518
Race[b]	252	0.83	0.44-1.57	0.6	0.521
Menopausal status[c]	252				0.583
Late peri- and postmenopausal		1.46	0.77-2.79	0.3	
Hormone therapy user		0.39	0.11-1.37	0.1	
Income[d]	251				0.534
$50,000-$100,000		1.14	0.56-2.32	0.7	
>$100,000		0.83	0.37-1.85	0.6	
Education[e]	252				0.544
Some college or college		1.08	0.47-2.51	0.9	
Graduate		0.71	0.28-1.84	0.5	
SBP, mmHg	252	1.02	1.00-1.04	0.03	0.618
BMI, kg/m^2	252	1.06	1.01-1.11	0.01	0.618
HDL-C, mg/dL	252	0.97	0.94-1.00	0.02	0.605
LDL-C, mg/dL	252	1.01	1.00-1.02	0.03	0.579
Log(Triglycerides), log mg/dL	252	2.08	1.13-3.82	0.02	0.571
CV medication use	252	1.72	0.87-2.40	0.1	0.550
Diabetes	252	5.08	1.31-19.63	0.02	0.537
Family history of CV disease	252	0.91	0.48-1.71	0.8	0.511
Current smoking	252	1.43	0.60-3.42	0.4	0.520
Log(HOMA)	229	1.49	0.91-2.43	0.1	0.592
FRS	232	1.15	1.05-1.27	0.003	0.628
Log(CAC+1), baseline	252	1.29	1.06-1.56	0.01	0.554
Log(CRP), log mg/L	247	1.06	0.83-1.36	0.6	0.522
Log(Fibrinogen), log mg/dL	212	1.29	0.28-5.95	0.7	0.527
Log(PAI-1), log ng/mL	239	1.94	1.39-2.72	0.0001	0.667
Log(tPA-ag), log ng/mL	240	2.21	1.06-4.61	0.03	0.589

[a]site 17 versus site 13; [b]White versus Black; [c] Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less
CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100
1 Log(biomarker) = 2.7 non-log transformed value

Table 15. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence and absence of CAC progression using traditional CV risk factor based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: Baseline CAC, Age, Site, Race					
Log(CRP), log mg/L	247	0.95	0.73-1.24	0.7	0.574
Log(Fibrinogen), log mg/dL	212	0.90	0.18-4.61	0.9	0.581
Log(PAI-1), log ng/mL	239	1.85	1.30-2.64	0.0006	0.681
Log(tPA-ag), log ng/mL	240	1.84	0.85-3.98	0.1	0.614
Model 2: Baseline CAC, Age, Site, Race, Menopausal status					
Log(CRP), log mg/L	247	0.97	0.74-1.27	0.8	0.614
Log(Fibrinogen), log mg/dL	212	0.77	0.14-4.18	0.8	0.647
Log(PAI-1), log ng/mL	239	1.79	1.25-2.56	0.002	0.691
Log(tPA-ag), log ng/mL	240	1.70	0.77-3.76	0.2	0.622
Model 3: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education					
Log(CRP), log mg/L	246	0.98	0.74-1.29	0.9	0.634
Log(Fibrinogen), log mg/dL	211	0.80	0.14-4.42	0.8	0.671
Log(PAI-1), log ng/mL	238	1.95	1.33-2.84	0.0005	0.705
Log(tPA-ag), log ng/mL	239	1.94	0.86-4.38	0.1	0.639
Model 4: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP					
Log(CRP), log mg/L	246	0.96	0.72-1.27	0.8	0.658
Log(Fibrinogen), log mg/dL	211	0.82	0.15-4.56	0.8	0.686
Log(PAI-1), log ng/mL	238	1.93	1.32-2.83	0.0007	0.711
Log(tPA-ag), log ng/mL	239	1.90	0.83-4.31	0.1	0.668
Model 5: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI					
Log(CRP), log mg/L	246	0.89	0.65-1.21	0.5	0.668
Log(Fibrinogen), log mg/dL	211	0.81	0.14-4.65	0.8	0.687
Log(PAI-1), log ng/mL	238	1.93	1.31-2.85	0.0009	0.711
Log(tPA-ag), log ng/mL	239	1.81	0.76-4.30	0.2	0.670
Model 6: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)					
Log(CRP), log mg/L	225	0.87	0.63-1.22	0.4	0.654
Log(Fibrinogen), log mg/dL	191	0.70	0.11-4.37	0.7	0.693
Log(PAI-1), log ng/mL	216	1.92	1.26-2.92	0.002	0.695
Log(tPA-ag), log ng/mL	217	1.78	0.70-4.50	0.2	0.652
Model 7: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history					
Log(CRP), log mg/L	225	0.87	0.63-1.21	0.4	0.653
Log(Fibrinogen), log mg/dL	191	0.71	0.11-4.41	0.7	0.702
Log(PAI-1), log ng/mL	216	1.91	1.25-2.91	0.003	0.695
Log(tPA-ag), log ng/mL	217	1.73	0.68-4.43	0.3	0.654
Model 8: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)					
Log(CRP), log mg/L	225	0.87	0.62-1.23	0.4	0.686
Log(Fibrinogen), log mg/dL	191	0.79	0.12-5.19	0.8	0.714
Log(PAI-1), log ng/mL	216	1.88	1.23-2.89	0.004	0.713
Log(tPA-ag), log ng/mL	217	1.42	0.53-3.80	0.5	0.681
Model 9: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking					
Log(CRP), log mg/L	225	0.86	0.61-1.22	0.4	0.688
Log(Fibrinogen), log mg/dL	191	0.77	0.11-5.20	0.8	0.721
Log(PAI-1), log ng/mL	216	1.91	1.24-2.93	0.003	0.712
Log(tPA-ag), log ng/mL	217	1.42	0.53-3.84	0.5	0.680

CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100
1 Log(biomarker) = 2.7 non-log transformed value

Table 16. Multivariable logistic regression analyses between baseline novel CV risk factors and the presence and absence of CAC progression using FRS based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: Baseline CAC, FRS, Site, Race					
Log(CRP), log mg/L	230	0.95	0.72-1.25	0.7	0.643
Log(Fibrinogen), log mg/dL	197	1.11	0.21-5.96	0.9	0.653
Log(PAI-1), log ng/mL	221	1.71	1.19-2.44	0.003	0.691
Log(tPA-ag), log ng/mL	222	1.59	0.71-3.52	0.3	0.650
Model 2: Baseline CAC, FRS, Site, Race, Menopausal status					
Log(CRP), log mg/L	230	0.98	0.74-1.29	0.9	0.658
Log(Fibrinogen), log mg/dL	197	1.04	0.19-5.77	1.0	0.687
Log(PAI-1), log ng/mL	221	1.66	1.15-2.39	0.006	0.703
Log(tPA-ag), log ng/mL	222	1.47	0.65-3.33	0.4	0.657
Model 3: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education					
Log(CRP), log mg/L	229	1.00	0.75-1.33	1.0	0.654
Log(Fibrinogen), log mg/dL	196	1.06	0.18-6.07	1.0	0.702
Log(PAI-1), log ng/mL	220	1.81	1.24-2.65	0.002	0.711
Log(tPA-ag), log ng/mL	221	1.69	0.73-3.91	0.2	0.665
Model 4: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI					
Log(CRP), log mg/L	229	0.90	0.65-1.25	0.5	0.684
Log(Fibrinogen), log mg/dL	196	0.99	0.17-5.82	1.0	0.706
Log(PAI-1), log ng/mL	220	1.79	1.21-2.64	0.004	0.715
Log(tPA-ag), log ng/mL	221	1.50	0.61-3.66	0.4	0.681
Model 5: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)					
Log(CRP), log mg/L	209	0.91	0.65-1.27	0.6	0.665
Log(Fibrinogen), log mg/dL	177	0.91	0.14-5.78	0.9	0.694
Log(PAI-1), log ng/mL	199	1.78	0.16-2.73	0.008	0.688
Log(tPA-ag), log ng/mL	200	1.46	0.56-3.81	0.4	0.658
Model 6: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history					
Log(CRP), log mg/L	209	0.91	0.65-1.27	0.6	0.665
Log(Fibrinogen), log mg/dL	177	0.91	0.14-5.78	0.9	0.694
Log(PAI-1), log ng/mL	199	1.80	1.17-2.76	0.007	0.687
Log(tPA-ag), log ng/mL	200	1.49	0.57-3.94	0.4	0.657
Model 7: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use					
Log(CRP), log mg/L	209	0.90	0.64-1.26	0.5	0.678
Log(Fibrinogen), log mg/dL	177	1.00	0.16-6.45	1.0	0.714
Log(PAI-1), log ng/mL	199	1.82	1.18-2.79	0.006	0.691
Log(tPA-ag), log ng/mL	200	1.53	0.58-4.05	0.4	0.665

CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100
1 Log(biomarker) = 2.7 non-log transformed value

3.5 BASELINE NOVEL BIOMARKERS AND THE EXTENT OF CAC PROGRESSION

In the univariable linear regression analyses for extent of CAC, there were not significant relationships for study site, race, menopausal status, income, education, SBP, HDL-C, LDL-C, CV medication use, family history of CV disease, current smoking, and log(HOMA) (**Table 17**). There were positive associations for age, BMI, log(triglycerides), FRS, and baseline log(CAC+1). Of the four novel CV risk factors, only log(PAI-1) was significantly associated with extent of CAC progression (β, 0.020; SE, 0.007; p=0.006).

Multivariable linear regression analysis using the traditional CV risk factor based model demonstrated that log(PAI-1) was positively and significantly associated with extent of CAC progression after adjusting for baseline CAC, age, study site, race, menopausal status, income, and education (**Table 18**). When BMI was added to the model, the association lost statistical significance (p=0.06). When log(HOMA) was added in the next model, the association regained borderline statistical significance (p=0.05). The addition for family history of CV disease, HDL-C, LDL-C, log(triglycerides), CV medication use, and current smoking to the model no longer resulted in a significant positive association of log(PAI-1) to extent of CAC progression (β,0.017; SE, 0.009; p=0.06). Log(CRP), log(fibrinogen), and log(tPA-ag) did not have significant associations in any of the models.

In the FRS based multivariable linear regression analyses of baseline novel CV risk factors, log(PAI-1) was not significantly associated to extent of CAC progression after adjusting for FRS, study site, race, and menopausal status (**Table 19**). This relationship was not significant through the rest of the adjusted models. Due to missing data, the final multivariable models for log(PAI-1) in the traditional CV risk factor based models had 17 more subjects than that in FRS

based models. As with the traditional CV risk factor based models, there was no significant associations between extent of CAC progression and log(CRP), log(fibrinogen), and log(tPA-ag).

In contrast to the final multivariable models for presence of CAC progression, the final models for extent of CAC progression were notable for particularly strong associations with BMI. This was present in models for all four novel biomarkers in the traditional CV risk factor based models ($p \leq 0.04$ for all), as well as the FRS based models ($p \leq 0.02$).

Table 17. Univariable linear regression analyses between baseline characteristics and the extent of CAC progression.

	n	Parameter Estimate	Standard Error	P value
Age, y	252	0.005	0.003	0.04
Site[a]	252	0.003	0.014	0.8
Race[b]	252	0.011	0.015	0.4
Menopausal status	252			0.2
Late peri- and postmenopausal		0.028	0.015	0.07
Hormone therapy user		0.0004	0.021	1.0
Income[d]	251			1.0
$50,000-$100,000		-0.003	0.016	0.9
>$100,000		0.002	0.017	0.9
Education[e]	252			0.3
Some college or college		-0.012	0.019	0.5
Graduate		-0.030	0.021	0.2
SBP, mmHg	252	0.0005	0.0004	0.3
BMI, kg/m^2	252	0.003	0.001	0.01
HDL-C, mg/dL	252	-0.0004	0.0005	0.4
LDL-C, mg/dL	252	0.0002	0.0002	0.3
Log(Triglycerides), log mg/dL	252	0.037	0.014	0.009
CV medication use	252	0.015	0.016	0.4
Diabetes	252	0.056	0.037	0.1
Family history of CV disease	252	0.0004	0.014	1.0
Current smoking	252	0.003	0.021	0.9
Log(HOMA)	229	0.009	0.011	0.5
FRS	232	0.005	0.002	0.03
Log(CAC+1), baseline	252	0.011	0.005	0.01
Log(CRP), log mg/L	247	0.003	0.006	0.6
Log(Fibrinogen), log mg/dL	212	-0.010	0.036	0.8
Log(PAI-1), log ng/mL	239	0.020	0.007	0.006
Log(tPA-ag), log ng/mL	240	0.012	0.016	0.4

[a]site 17 versus site 13; [b]White versus Black; [c]Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less
1 Log(biomarker) = 2.7 non-log transformed value

Table 18. Multivariable linear regression analyses between baseline novel CV risk factors and the extent of CAC progression using traditional CV risk factor based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: Baseline CAC, Age, Site, Race				
Log(CRP), log mg/L	247	-0.002	0.006	0.8
Log(Fibrinogen), log mg/dL	212	-0.021	0.037	0.6
Log(PAI-1), log ng/mL	239	0.017	0.008	0.03
Log(tPA-ag), log ng/mL	240	0.005	0.016	0.8
Model 2: Baseline CAC, Age, Site, Race, Menopausal status				
Log(CRP), log mg/L	247	-0.001	0.006	0.8
Log(Fibrinogen), log mg/dL	212	-0.022	0.037	0.6
Log(PAI-1), log ng/mL	239	0.016	0.008	0.04
Log(tPA-ag), log ng/mL	240	0.002	0.017	0.9
Model 3: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education				
Log(CRP), log mg/L	246	0.0004	0.006	0.9
Log(Fibrinogen), log mg/dL	211	-0.017	0.037	0.6
Log(PAI-1), log ng/mL	238	0.018	0.008	0.02
Log(tPA-ag), log ng/mL	239	0.005	0.017	0.7
Model 4: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP				
Log(CRP), log mg/L	246	0.0001	0.006	1.0
Log(Fibrinogen), log mg/dL	211	-0.017	0.037	0.6
Log(PAI-1), log ng/mL	238	0.018	0.008	0.02
Log(tPA-ag), log ng/mL	239	0.005	0.017	0.8
Model 5: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI				
Log(CRP), log mg/L	246	-0.006	0.007	0.4
Log(Fibrinogen), log mg/dL	211	-0.035	0.038	0.4
Log(PAI-1), log ng/mL	238	0.016	0.008	0.06
Log(tPA-ag), log ng/mL	239	-0.005	0.018	0.8
Model 6: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)				
Log(CRP), log mg/L	225	-0.004	0.007	0.6
Log(Fibrinogen), log mg/dL	191	-0.041	0.040	0.3
Log(PAI-1), log ng/mL	216	0.017	0.009	0.05
Log(tPA-ag), log ng/mL	217	-0.002	0.019	0.9
Model 7: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), family history				
Log(CRP), log mg/L	225	-0.004	0.007	0.6
Log(Fibrinogen), log mg/dL	191	-0.042	0.040	0.3
Log(PAI-1), log ng/mL	216	0.017	0.009	0.06
Log(tPA-ag), log ng/mL	217	-0.003	0.019	0.9
Model 8: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)				
Log(CRP), log mg/L	225	-0.004	0.007	0.6
Log(Fibrinogen), log mg/dL	191	-0.040	0.040	0.3
Log(PAI-1), log ng/mL	216	0.017	0.009	0.06
Log(tPA-ag), log ng/mL	217	-0.007	0.020	0.7
Model 9: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking				
Log(CRP), log mg/L	225	-0.0004	0.007	0.6
Log(Fibrinogen), log mg/dL	191	-0.040	0.040	0.3
Log(PAI-1), log ng/mL	216	0.017	0.009	0.06
Log(tPA-ag), log ng/mL	217	-0.007	0.020	0.7

1 Log(biomarker) = 2.7 non-log transformed value

Table 19. Multivariable linear regression analyses between baseline novel CV risk factors and the extent of CAC progression using FRS based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: Baseline CAC, FRS, Site, Race				
Log(CRP), log mg/L	230	-0.0005	0.006	0.9
Log(Fibrinogen), log mg/dL	197	0.004	0.036	0.9
Log(PAI-1), log ng/mL	221	0.015	0.008	0.05
Log(tPA-ag), log ng/mL	222	0.004	0.016	0.8
Model 2: Baseline CAC, FRS, Site, Race, Menopausal status				
Log(CRP), log mg/L	230	-0.0007	0.006	0.9
Log(Fibrinogen), log mg/dL	197	0.002	0.036	1.0
Log(PAI-1), log ng/mL	221	0.014	0.008	0.07
Log(tPA-ag), log ng/mL	222	0.0008	0.017	1
Model 3: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education				
Log(CRP), log mg/L	229	0.001	0.006	0.8
Log(Fibrinogen), log mg/dL	196	0.005	0.036	0.9
Log(PAI-1), log ng/mL	220	0.017	0.008	0.04
Log(tPA-ag), log ng/mL	221	0.005	0.017	0.8
Model 4: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI				
Log(CRP), log mg/L	229	-0.006	0.007	0.4
Log(Fibrinogen), log mg/dL	196	-0.014	0.036	0.7
Log(PAI-1), log ng/mL	220	0.012	0.008	0.1
Log(tPA-ag), log ng/mL	221	-0.009	0.017	0.6
Model 5: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)				
Log(CRP), log mg/L	209	-0.003	0.007	0.6
Log(Fibrinogen), log mg/dL	177	-0.017	0.037	0.7
Log(PAI-1), log ng/mL	199	0.014	0.009	0.1
Log(tPA-ag), log ng/mL	200	-0.006	0.019	0.7
Model 6: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history				
Log(CRP), log mg/L	209	-0.004	0.007	0.6
Log(Fibrinogen), log mg/dL	177	-0.018	0.037	0.6
Log(PAI-1), log ng/mL	199	0.013	0.009	0.1
Log(tPA-ag), log ng/mL	200	-0.008	0.019	0.7
Model 7: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use				
Log(CRP), log mg/L	209	-0.004	0.007	0.6
Log(Fibrinogen), log mg/dL	177	-0.016	0.037	0.7
Log(PAI-1), log ng/mL	199	0.013	0.009	0.1
Log(tPA-ag), log ng/mL	200	-0.008	0.019	0.7

1 Log(biomarker) = 2.7 non-log transformed value

3.6 CHANGE IN NOVEL BIOMARKERS AND THE PRESENCE AND EXTENT OF CAC PROGRESSION

There were 223 subjects who had baseline and follow-up CAC measurements, and novel biomarker measurements (**Table 20**). The mean average time between scans was 2.3 ± 0.4 years. For the dichotomous outcome variable of presence of CAC progression, there were 174 subjects who did not progress and 49 subjects who demonstrated progression. Of the 119 subjects with CAC=0 at baseline, 28 (23.5%) had any CAC in follow-up. Of the 97 subjects with 0<baseline CAC<100, there were 15 subjects (15.5%) who demonstrated an annualized increase of ≥10 U. Of the 7 subjects who demonstrated an annualized increase of ≥10% compared to baseline, there were 6 (85.7%) that demonstrated progression.

There were no significant differences in the change of novel CV risk factors between subjects with the presence of CAC progression and subjects without (**Table 20** and **Table 21**). The magnitude of change was not large for any of the biomarkers: -0.5 ± 1.2 mg/L for CRP, 5.6 ± 30.4 mg/dL for fibrinogen, -1.9 ± 14.9 ng/mL for PAI-1, and 0.06 ± 2.1 ng/mL for tPA-ag. Multivariable adjusted models did not demonstrate any significant association between the change in novel biomarkers and presence of CAC progression using traditional CV risk factor based models (**Table 22**) or FRS based models (**Table 23**).

Similar to the presence of CAC progression analyses, there were no statistically significant associations between any of the novel CV risk factors and extent of CAC progression in the univariable linear regression models (**Table 24**). The non-significant relationships were maintained in all traditional risk factor based models (**Table 25**) and the FRS based models (**Table 26**).

Table 20. Characteristics of the change in novel CV risk factors study population by the presence and absence of CAC progression.

Characteristics	Total n = 223	CAC Stable n = 174	CAC Progressed† n = 49	P value*
Age at baseline CAC, mean (SD), y	51.1 (2.6)	51.0 (2.5)	51.5 (2.8)	0.4
Site, n (%)				0.8
Chicago, site 13	92 (41.3)	71 (40.8)	21 (42.9)	
Pittsburgh, site 17	131 (58.7)	103 (59.2)	28 (57.1)	
Race, n (%)				0.4
Black	70 (31.4)	52 (29.9)	18 (36.7)	
White	153 (68.6)	122 (70.1)	31 (63.3)	
Menopausal status, n (%)				0.1
Pre- and early perimenopausal	128 (57.4)	101 (58.1)	27 (55.1)	
Late peri- and postmenopausal	66 (29.6)	47 (27.0)	19 (38.8)	
Hormone therapy user	29 (13.0)	26 (14.9)	3 (6.1)	
Income, n (%)				0.6
<$50,000	74 (33.3)	58 (33.3)	16 (33.3)	
$50,000-$100,000	85 (38.3)	64 (36.8)	21 (43.8)	
>$100,000	63 (28.4)	52 (29.9)	11 (22.9)	
Education, n (%)				0.3
High school or less	38 (17.0)	30 (17.2)	8 (16.3)	
Some college or college	115 (51.6)	85 (48.9)	30 (61.2)	
Graduate	70 (31.4)	59 (33.9)	11 (22.5)	
SBP, mean (SD), mmHg	117.4 (15.9)	116.1 (16.0)	122.0 (14.9)	0.007
BMI, mean (SD), kg/m^2	29.4 (6.6)	28.7 (6.4)	31.8 (6.6)	0.001
HDL-C, mean (SD), mg/dL	56.8 (13.2)	57.9 (13.6)	52.9 (10.8)	0.01
LDL-C, mean (SD), mg/dL	118.3 (34.1)	115.7 (32.9)	127.5 (37.2)	0.09
Triglycerides, median (Q1, Q3), mg/dL	99.0 (75.0, 136.0)	97.5 (75.0, 133.0)	102.0 (76.0, 169.0)	0.2
CV medication use, n (%)	50 (22.4)	36 (20.7)	14 (28.6)	0.2
Diabetes, n (%)	8 (3.6)	3 (1.7)	5 (10.2)	0.005
Family history of CV disease, n (%)	143 (64.1)	113 (64.9)	30 (61.2)	0.6
Current smoking, n (%)	26 (11.7)	19 (10.9)	7 (14.3)	0.5
HOMA, median (Q1, Q3)	2.0 (1.5, 3.1)	1.8 (1.4, 2.9)	2.5 (1.6, 3.9)	0.02
FRS, mean (SD)	9.9 (3.2)	9.6 (3.0)	11.1 (3.7)	0.02
CAC - baseline, median (Q1, Q3), U	0 (0, 6.52)	0 (0, 5.5)	0 (0, 32.6)	0.3
ΔCRP/year, mean (SD), (mg/L)/y	-0.06 (2.3)	-0.07 (2.5)	-0.05 (1.2)	0.3
ΔFibrinogen/year, mean (SD), (mg/dL)/y	2.1 (28.3)	1.2 (27.8)	5.6 (30.4)	0.8
ΔPAI-1/year, mean (SD), (ng/mL)/y	-0.5 (12.1)	-0.1 (11.3)	-1.9 (14.9)	0.3
ΔtPA-ag/year, mean (SD), (ng/mL)/y	0.4 (6.7)	0.5 (7.5)	0.06 (2.1)	0.9

* P-value for CAC groups - Wilcoxon rank-sum test for continuous variables; X^2 test for categorical variables

†CAC progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100, ≥10% for baseline CAC≥100

Table 21. Univariable logistic regression analyses between characteristics of the change in novel CV risk factors study population and the presence and absence of CAC progression.

	n	Odds Ratio	95% CI	P Value	C
Age, y	223	1.07	0.95-1.21	0.3	0.543
Site[a]	223	0.92	0.48-1.75	0.8	0.510
Race[b]	223	0.73	0.38-1.43	0.4	0.534
Menopausal status[c]	223				0.582
Late peri- and postmenopausal		1.51	0.77-2.99	0.2	
Hormone therapy user		0.43	0.12-1.53	0.2	
Income[d]	222				0.546
$50,000-$100,000		1.19	0.57-2.50	0.6	
>$100,000		0.77	0.33-1.80	0.5	
Education[e]	223				0.570
Some college or college		1.32	0.55-3.20	0.5	
Graduate		0.70	0.25-1.92	0.5	
SBP, mmHg	223	1.02	1.00-1.04	0.02	0.627
BMI, kg/m^2	223	1.07	1.02-1.12	0.004	0.649
HDL-C, mg/dL	223	0.97	0.94-1.00	0.02	0.604
LDL-C, mg/dL	223	1.01	1.00-1.02	0.03	0.580
Log(Triglycerides), log mg/dL	223	1.99	1.04-3.81	0.04	0.559
CV medication use	223	1.53	0.75-3.15	0.2	0.539
Diabetes	223	6.47	1.49-28.13	0.01	0.542
Family history of CV disease	223	0.85	0.44-1.64	0.6	0.519
Current smoking	223	1.36	0.54-3.45	0.5	0.517
Log(HOMA)	201	1.53	0.91-2.54	0.1	0.612
FRS	205	1.16	1.04-1.28	0.006	0.618
Log(CAC+1), baseline	223	1.27	1.05-1.55	0.02	0.545
ΔCRP/year, (mg/L)/y	218	1.00	0.87-1.15	1.0	0.514
ΔFibrinogen/year, (mg/dL)/y	138	1.01	0.99-1.02	0.5	0.513
ΔPAI-1/year, (ng/mL)/y	199	0.99	0.96-1.02	0.4	0.556
ΔtPA-ag/year, (ng/mL)/y	201	0.99	0.91-1.07	0.7	0.510

[a]site 17 versus site 13; [b]White versus Black; [c] Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less
CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100

Table 22. Multivariable logistic regression analyses between the change in novel CV risk factors and the presence and absence of CAC progression using traditional CV risk factor based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: Baseline CAC, Age, Site, Race					
ΔCRP/year, (mg/L)/y	218	1.02	0.88-1.17	0.8	0.573
ΔFibrinogen/year, (mg/dL)/y	138	1.01	0.99-1.02	0.4	0.568
ΔPAI-1/year, (ng/mL)/y	199	0.99	0.96-1.02	0.4	0.585
ΔtPA-ag/year, (ng/mL)/y	201	0.99	0.92-1.06	0.7	0.567
Model 2: Baseline CAC, Age, Site, Race, Menopausal status					
ΔCRP/year, (mg/L)/y	218	1.01	0.87-1.17	0.9	0.609
ΔFibrinogen/year, (mg/dL)/y	138	1.01	0.99-1.02	0.3	0.654
ΔPAI-1/year, (ng/mL)/y	199	0.99	0.96-1.02	0.6	0.608
ΔtPA-ag/year, (ng/mL)/y	201	0.99	0.92-1.06	0.7	0.594
Model 3: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education					
ΔCRP/year, (mg/L)/y	217	1.00	0.86-1.15	1.0	0.644
ΔFibrinogen/year, (mg/dL)/y	138	1.01	1.00-1.03	0.2	0.689
ΔPAI-1/year, (ng/mL)/y	198	0.99	0.96-1.02	0.5	0.649
ΔtPA-ag/year, (ng/mL)/y	200	0.98	0.91-1.06	0.6	0.627
Model 4: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP					
ΔCRP/year, (mg/L)/y	217	1.00	0.86-1.15	0.9	0.664
ΔFibrinogen/year, (mg/dL)/y	138	1.01	0.99-1.03	0.3	0.697
ΔPAI-1/year, (ng/mL)/y	198	0.99	0.96-1.02	0.5	0.668
ΔtPA-ag/year, (ng/mL)/y	200	0.98	0.91-1.06	0.7	0.662
Model 5: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI					
ΔCRP/year, (mg/L)/y	217	1.01	0.87-1.17	0.9	0.683
ΔFibrinogen/year, (mg/dL)/y	138	1.01	0.99-1.03	0.2	0.712
ΔPAI-1/year, (ng/mL)/y	198	0.99	0.96-1.02	0.5	0.698
ΔtPA-ag/year, (ng/mL)/y	200	0.99	0.92-1.06	0.7	0.688
Model 6: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA)					
ΔCRP/year, (mg/L)/y	197	1.01	0.87-1.18	0.9	0.664
ΔFibrinogen/year, (mg/dL)/y	123	1.01	0.99-1.03	0.2	0.696
ΔPAI-1/year, (ng/mL)/y	177	1.00	0.97-1.03	1.0	0.690
ΔtPA-ag/year, (ng/mL)/y	179	0.97	0.88-1.08	0.6	0.682
Model 7: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history					
ΔCRP/year, (mg/L)/y	197	1.01	0.86-1.18	0.9	0.665
ΔFibrinogen/year, (mg/dL)/y	123	1.01	0.99-1.03	0.2	0.694
ΔPAI-1/year, (ng/mL)/y	177	1.00	0.97-1.03	1.0	0.688
ΔtPA-ag/year, (ng/mL)/y	179	0.97	0.88-1.08	0.6	0.682
Model 8: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)					
ΔCRP/year, (mg/L)/y	197	1.02	0.86-1.21	0.8	0.697
ΔFibrinogen/year, (mg/dL)/y	123	1.01	0.99-1.03	0.3	0.692
ΔPAI-1/year, (ng/mL)/y	177	1.00	0.97-1.04	0.8	0.708
ΔtPA-ag/year, (ng/mL)/y	179	0.97	0.88-1.08	0.6	0.703
Model 9: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking					
ΔCRP/year, (mg/L)/y	197	1.02	0.86-1.21	0.8	0.699
ΔFibrinogen/year, (mg/dL)/y	123	1.01	0.99-1.03	0.3	0.711
ΔPAI-1/year, (ng/mL)/y	177	1.00	0.97-1.04	0.9	0.708
ΔtPA-ag/year, (ng/mL)/y	179	0.97	0.87-1.08	0.6	0.704

CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100

Table 23. Multivariable logistic regression analyses between change in novel CV risk factors and the presence and absence of CAC progression using FRS based models.

	n	Odds Ratio	95% CI	P Value	C
Model 1: Baseline CAC, FRS, Site, Race					
ΔCRP/year, (mg/L)/y	203	1.01	0.87-1.17	0.9	0.634
ΔFibrinogen/year, (mg/dL)/y	130	1.01	0.99-1.02	0.5	0.590
ΔPAI-1/year, (ng/mL)/y	184	0.99	0.97-1.02	0.6	0.612
ΔtPA-ag/year, (ng/mL)/y	186	0.99	0.91-1.07	0.7	0.612
Model 2: Baseline CAC, FRS, Site, Race, Menopausal status					
ΔCRP/year, (mg/L)/y	203	1.00	0.86-1.17	1.0	0.648
ΔFibrinogen/year, (mg/dL)/y	130	1.01	0.99-1.02	0.4	0.637
ΔPAI-1/year, (ng/mL)/y	184	1.00	0.97-1.02	0.8	0.629
ΔtPA-ag/year, (ng/mL)/y	186	0.99	0.91-1.07	0.7	0.624
Model 3: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education					
ΔCRP/year, (mg/L)/y	202	1.00	0.85-1.18	1.0	0.653
ΔFibrinogen/year, (mg/dL)/y	130	1.01	0.99-1.03	0.3	0.679
ΔPAI-1/year, (ng/mL)/y	183	0.99	0.97-1.02	0.7	0.639
ΔtPA-ag/year, (ng/mL)/y	185	0.98	0.91-1.07	0.7	0.634
Model 4: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI					
ΔCRP/year, (mg/L)/y	202	1.02	0.86-1.20	0.9	0.691
ΔFibrinogen/year, (mg/dL)/y	130	1.01	0.99-1.03	0.2	0.698
ΔPAI-1/year, (ng/mL)/y	183	0.99	0.97-1.02	0.6	0.703
ΔtPA-ag/year, (ng/mL)/y	185	0.99	0.91-1.06	0.7	0.696
Model 5: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)					
ΔCRP/year, (mg/L)/y	183	1.01	0.86-1.19	0.9	0.645
ΔFibrinogen/year, (mg/dL)/y	115	1.01	0.99-1.03	0.4	0.671
ΔPAI-1/year, (ng/mL)/y	163	1.01	0.98-1.04	0.7	0.653
ΔtPA-ag/year, (ng/mL)/y	165	0.97	0.86-1.10	0.6	0.653
Model 6: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history					
ΔCRP/year, (mg/L)/y	183	1.02	0.86-1.21	0.8	0.671
ΔFibrinogen/year, (mg/dL)/y	115	1.01	0.99-1.03	0.2	0.674
ΔPAI-1/year, (ng/mL)/y	163	1.00	0.97-1.03	0.9	0.702
ΔtPA-ag/year, (ng/mL)/y	165	0.97	0.86-1.10	0.6	0.694
Model 7: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use					
ΔCRP/year, (mg/L)/y	183	1.02	0.87-1.21	0.8	0.676
ΔFibrinogen/year, (mg/dL)/y	115	1.01	0.99-1.03	0.3	0.689
ΔPAI-1/year, (ng/mL)/y	163	1.00	0.97-1.04	0.9	0.705
ΔtPA-ag/year, (ng/mL)/y	165	0.97	0.86-1.10	0.6	0.694

CAC Progression: any CAC for baseline CAC=0, ≥10 for 0<baseline CAC<100; ≥10% for baseline CAC ≥100

Table 24. Univariable linear regression analyses between characteristics of the change in novel CV risk factors study population and the extent of CAC progression.

	n	Parameter Estimate	Standard Error	P value
Age, y	223	0.004	0.003	0.1
Site[a]	223	0.003	0.015	0.8
Race[b]	223	0.011	0.016	0.5
Menopausal status	223			0.1
Late peri- and postmenopausal		0.032	0.017	0.05
Hormone therapy user		-0.009	0.023	0.7
Income[d]	222			1.0
$50,000-$100,000		0.002	0.018	0.9
>$100,000		0.003	0.019	0.9
Education[e]	223			0.3
Some college or college		-0.006	0.021	0.8
Graduate		-0.030	0.022	0.2
SBP, mmHg	223	0.0005	0.0005	0.3
BMI, kg/m^2	223	0.003	0.001	0.004
HDL-C, mg/dL	223	-0.0007	0.0006	0.2
LDL-C, mg/dL	223	0.0003	0.0002	0.2
Log(Triglycerides), log mg/dL	223	0.036	0.015	0.02
CV medication use	223	0.014	0.018	0.4
Diabetes	223	0.063	0.040	0.1
Family history of CV disease	223	-0.005	0.015	0.7
Current smoking	223	0.0009	0.023	1.0
Log(HOMA)	201	0.007	0.012	0.6
FRS	205	0.005	0.002	0.05
Log(CAC+1), baseline	223	0.010	0.005	0.04
ΔCRP/year, (mg/L)/y	218	0.002	0.003	0.6
ΔFibrinogen/year, (mg/dL)/y	138	0.0001	0.0003	0.6
ΔPAI-1/year, (ng/mL)/y	199	-0.0004	0.0007	0.5
ΔtPA-ag/year, (ng/mL)/y	201	-0.0007	0.001	0.5

[a]site 17 versus site 13; [b]White versus Black; [c]Referenced to pre- and early perimenopausal;
[d]Referenced to <$50,000; [e]Referenced to high school or less

Table 25. Multivariable linear regression analyses between change in novel CV risk factors and the extent of CAC progression using traditional risk factor based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: Baseline CAC, Age, Site, Race				
ΔCRP/year, mg/L*y	218	0.002	0.003	0.5
ΔFibrinogen/year, mg/dL*y	138	0.0001	0.0003	0.7
ΔPAI-1/year, ng/mL*y	199	-0.0004	0.0007	0.6
ΔtPA-ag/year, ng/mL*y	201	-0.0008	0.001	0.5
Model 2: Baseline CAC, Age, Site, Race, Menopausal status				
ΔCRP/year, mg/L*y	218	0.002	0.003	0.6
ΔFibrinogen/year, mg/dL*y	138	0.0002	0.0003	0.6
ΔPAI-1/year, ng/mL*y	199	-0.0002	0.0007	0.8
ΔtPA-ag/year, ng/mL*y	201	-0.0007	0.001	0.6
Model 3: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education				
ΔCRP/year, mg/L*y	217	0.001	0.003	0.7
ΔFibrinogen/year, mg/dL*y	138	0.0003	0.0003	0.4
ΔPAI-1/year, ng/mL*y	198	-0.0003	0.0007	0.7
ΔtPA-ag/year, ng/mL*y	200	-0.0007	0.001	0.5
Model 4: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP				
ΔCRP/year, (mg/L)/y	217	0.001	0.003	0.7
ΔFibrinogen/year, (mg/dL)/y	138	0.0003	0.0004	0.4
ΔPAI-1/year, (ng/mL)/y	198	-0.0003	0.0007	0.7
ΔtPA-ag/year, (ng/mL)/y	200	-0.0007	0.001	0.6
Model 5: Baseline CAC, Age, Site, Race, Menopausal status, Income, Education, SBP, BMI				
ΔCRP/year, (mg/L)/y	217	0.002	0.003	0.5
ΔFibrinogen/year, (mg/dL)/y	138	0.0004	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	198	-0.0003	0.0007	0.7
ΔtPA-ag/year, (ng/mL)/y	200	-0.0006	0.001	0.6
Model 6: Baseline CAC, Age, Site, Race, Menopausal Status, Income, Education, SBP, BMI, Log(HOMA)				
ΔCRP/year, (mg/L)/y	197	0.002	0.003	0.5
ΔFibrinogen/year, (mg/dL)/y	123	0.0004	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	177	-0.0001	0.0007	0.9
ΔtPA-ag/year, (ng/mL)/y	179	-0.001	0.001	0.4
Model 7: Baseline CAC, Age, Site, Race, Menopausal Status, Income, Education, SBP, BMI, Log(HOMA), Family history				
ΔCRP/year, (mg/L)/y	197	0.002	0.003	0.5
ΔFibrinogen/year, (mg/dL)/y	123	0.0004	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	177	-0.0001	0.0007	0.9
ΔtPA-ag/year, (ng/mL)/y	179	-0.001	0.001	0.4
Model 8: Baseline CAC, Age, Site, Race, Menopausal Status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides)				
ΔCRP/year, (mg/L)/y	197	0.003	0.003	0.4
ΔFibrinogen/year, (mg/dL)/y	123	0.0004	0.0004	0.3
ΔPAI-1/year, (ng/mL)/y	177	-0.00005	0.0007	1.0
ΔtPA-ag/year, (ng/mL)/y	179	-0.0009	0.001	0.4
Model 9: Baseline CAC, Age, Site, Race, Menopausal Status, Income, Education, SBP, BMI, Log(HOMA), Family history, HDL-C, LDL-C, Log(Triglycerides), CV medication use, Current smoking				
ΔCRP/year, (mg/L)/y	197	0.003	0.003	0.4
ΔFibrinogen/year, (mg/dL)/y	123	0.0004	0.0004	0.3
ΔPAI-1/year, (ng/mL)/y	177	-0.00007	0.0008	0.9
ΔtPA-ag/year, (ng/mL)/y	179	-0.0009	0.001	0.4

Table 26. Multivariable linear regression analyses between change in novel CV risk factors and the extent of CAC progression using FRS based models.

	n	Parameter Estimate	Standard Error	P value
Model 1: Baseline CAC, FRS, Site, Race				
ΔCRP/year, (mg/L)/y	203	0.002	0.003	0.6
ΔFibrinogen/year, (mg/dL)/y	130	0.0001	0.0004	0.7
ΔPAI-1/year, (ng/mL)/y	184	-0.0003	0.0006	0.7
ΔtPA-ag/year, (ng/mL)/y	186	-0.0008	0.001	0.5
Model 2: Baseline CAC, FRS, Site, Race, Menopausal status				
ΔCRP/year, (mg/L)/y	203	0.002	0.003	0.6
ΔFibrinogen/year, (mg/dL)/y	130	0.0002	0.0004	0.5
ΔPAI-1/year, (ng/mL)/y	184	-0.0001	0.0006	0.8
ΔtPA-ag/year, (ng/mL)/y	186	-0.0007	0.001	0.5
Model 3: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education				
ΔCRP/year, (mg/L)/y	202	0.002	0.003	0.6
ΔFibrinogen/year, (mg/dL)/y	130	0.0003	0.0004	0.4
ΔPAI-1/year, (ng/mL)/y	183	-0.0003	0.0007	0.7
ΔtPA-ag/year, (ng/mL)/y	185	-0.0008	0.001	0.5
Model 4: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI				
ΔCRP/year, (mg/L)/y	202	0.002	0.003	0.5
ΔFibrinogen/year, (mg/dL)/y	130	0.0005	0.0003	0.2
ΔPAI-1/year, (ng/mL)/y	183	-0.0003	0.0006	0.7
ΔtPA-ag/year, (ng/mL)/y	185	-0.0007	0.001	0.5
Model 5: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA)				
ΔCRP/year, (mg/L)/y	183	0.002	0.003	0.5
ΔFibrinogen/year, (mg/dL)/y	115	0.0005	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	163	-0.0001	0.0007	0.9
ΔtPA-ag/year, (ng/mL)/y	165	-0.001	0.001	0.3
Model 6: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history				
ΔCRP/year, (mg/L)/y	183	0.003	0.003	0.4
ΔFibrinogen/year, (mg/dL)/y	115	0.0005	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	163	-0.0001	0.0007	0.9
ΔtPA-ag/year, (ng/mL)/y	165	-0.001	0.001	0.3
Model 7: Baseline CAC, FRS, Site, Race, Menopausal status, Income, Education, BMI, Log(HOMA), Family history, CV medication use				
ΔCRP/year, (mg/L)/y	183	0.003	0.003	0.4
ΔFibrinogen/year, (mg/dL)/y	115	0.0004	0.0004	0.2
ΔPAI-1/year, (ng/mL)/y	163	-0.0001	0.0007	0.9
ΔtPA-ag/year, (ng/mL)/y	165	-0.001	0.001	0.3

4.0 DISCUSSION

Our investigation yielded several important observations. First, CRP, fibrinogen, PAI-1, and tPA-ag were all associated with the presence and extent of baseline CAC. These relationships were all attenuated by BMI in the multivariable models. Second, a significant interaction was noted for race on the relationship between CRP and baseline CAC. CRP was associated with CAC in black women, but not white women. Third, PAI-1 levels obtained at the baseline visits were associated with CAC progression after adjustment for traditional CV risk factors and socio-economic status. Fourth, changes in measurements of the four novel CV risk factors of interest were not associated with CAC progression. The magnitudes of change were small when compared to baseline values.

4.1 BASELINE NOVEL RISK FACTORS AND BMI IN WOMEN

Our study demonstrated that four novel CV risk factors associated with abnormalities in the inflammation and hemostasis were associated with the presence and extent of baseline CAC. These relationships were significant even after adjusting for traditional CV risk factors, or FRS. A notable finding was that all relationships were attenuated when BMI was added into the multivariable models. Therefore, the relationships between biomarkers and CAC appear to be largely mediated through the metabolic changes that are linked with increasing BMI. Higher

BMI levels were found to be independently associated with higher levels of CRP, fibrinogen, and PAI-1 in women, but not men, in a cross-sectional, community-based study of 166 healthy men and women with a mean age of 52.1 years (26-80 years) (73).

Prior investigators have suggested that the association between adipose tissue and CRP, mediated through interleukin-6, may diminish the predictive value of CRP for CHD in obese individuals. The CRP signal from adipose tissue may overwhelm that from subclinical atherosclerosis (44). A similar mechanism may explain the correlation between PAI-1 levels and BMI (59). Thus, increasing adipose tissue may be the primary reason for increasing levels of CRP, fibrinogen, PAI-1, and tPA-ag in midlife women.

High BMI was found to be a contributor to CHD deaths, independent of the FRS, in a sibling study of individuals with CHD who were younger than 60 years of age (74). In the United States, recent data estimated the prevalence rates for overweight and obese women between 40 to 59 years of age to be 36.0% and 66.0%, respectively (75). A decrease in age-adjusted death rates for CHD of nearly 50% was observed between 1980 and 2000 (76). This was explained by reductions in traditional risk factors and implementation of evidence-based therapies. The decrease in CHD-related deaths would have been greater, however, but increasing BMI and diabetes rates during that time contributed to additional CHD deaths.

Our findings provide more evidence for abnormalities in inflammation and hemostasis as potential mechanisms underlying the association between elevated BMI and CHD. As such, they support the current recommendation of weight maintenance or reduction to achieve an appropriate body weight (77).

4.2 BASELINE CRP AND BASELINE CAC IN BLACK WOMEN

Adjusting for BMI accounted for the associations between CAC and all four novel CV risk factors with the exception of CRP and CAC presence in black women. Prior results from SWAN demonstrated that black women had greater odds of CRP levels >3 mg/L when compared with white women (OR, 1.37; 95% CI, 1.07-1.75), even after adjusting for age, socio-economic status BMI, and other CV risk factors (78). Black women have less visceral adipose tissue, but paradoxically have higher CRP levels than white women (79). In human umbilical vein endothelial cells from blacks and whites, cells from blacks had higher nitric oxide levels, higher interleukin-6 levels, and lower superoxide dismutase activity (80). It strengthens the theory that genetic differences in oxidative stress and inflammation are involved in the difference in clinical CHD rates. At present, the precise mechanism for the racial difference in CHD development is unknown, but it may signify the presence of non-adipose mediated inflammatory conditions.

Our observation may have important clinical implications. In a study of greater than 22,000 women and men of black and white race who had established CHD, black women had the poorest unadjusted survival rates (81). The 15-year survival for black women remained worse compared with white women even after adjusting for traditional CV risk factors, socioeconomic status, treatment factors, and other significant predictors of mortality (41.5% vs. 45.5%, $p<0.0001$). Black women have been noted to have a significantly higher burden of traditional CV risk factors in those with and without prior CHD (82). Primary prevention is therefore of particular importance.

The writing committee for the 2010 guidelines for CV risk in asymptomatic adults, published by the American College of Cardiology Foundation and the American Heart Association (AHA), did not recommend selective race-based risk assessment approaches despite

the recognition of disparities between races with regards to traditional risk factors, CHD incidence, and outcomes (83). In the Reasons for Geographic and Racial Differences in Stroke (REGARDS) prospective cohort study that enrolled subjects from 2003 to 2007, black women without prior CHD had higher age-standardized rates of acute CHD events than white women (84). These were also accounted for by higher traditional CV risk burden. The study, however, enrolled those 45 years and older, and thus had a mean age of 64.1 years. Preventative measures targeted towards non-traditional risk factors earlier in life may be important for certain subgroups, particularly those who are younger.

Pharmacotherapy guideline recommendations from the AHA for primary prevention in non-diabetic women without high-risk features for CV disease focus on control of blood pressure and dyslipidemia, as well as aspirin (77). Medications for blood pressure control are recommended based on measured blood pressure values irrespective of the presence or absence of clinical CHD. In contrast, recommendations for aspirin and pharmacotherapy for dyslipidemia in the absence of established CHD are largely dependent upon CHD risk classification. Statins, but not aspirin, are associated with lower CRP levels in patients with stable CHD (85). This suggests that inflammatory mechanisms that promote atherogenesis may be particularly responsive to statins.

Despite the existence of current AHA recommendations, statins in women without known CHD and <65 years of age have not been shown to impact total or CHD mortality (86). In fact, the only randomized placebo-controlled study to demonstrate significant reduction in total or CHD mortality for women has been the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) that included 6,801 women (87). Inclusion criteria for JUPITER women were age 60 years or older, LDL-C less than 130 mg/dL,

and CRP of 2.0 mg/L or greater. It is reasonable to hypothesize that elevated CRP levels identify women who would benefit the most from statin medication. Routine screening for CRP, however, is not recommended by the AHA expert panel (77).

Our finding that CRP was associated with the presence of CAC in black women with a mean age of 51 years, even after adjusting for traditional CV and socio-economic risk factors, suggests that CRP may have a role in CHD prevention in this particular subgroup. Inflammatory mechanisms of atherogenesis may have a more significant role in the development of subclinical CHD in black women at this age. At present, the study of CRP in blacks has been limited (88). Existing data is not substantial enough to recommend routine screening of CRP in middle-aged black women. One of the goals of risk prediction refinement is the potential to reclassify individuals based on low-, intermediate-, and high-risk estimation (89). For primary prevention of CHD, a major consideration is the threshold for recommending statin therapy. For non-diabetics with LDL-C levels <190 mg/dL, the current threshold is an estimated 10-year atherosclerotic CV risk ≥7.5% (90). Based on our data, black women undergoing the menopausal transition who are of intermediate risk, but who do not have a 10-year risk ≥7.5%, may be considered for CRP screening. Those with an elevated CRP may benefit from statin use. Ultimately, a prospective trial on this strategy is warranted.

4.3 BASELINE PAI-1 AND CAC PROGRESSION IN WOMEN

In our study, baseline levels of PAI-1 were significantly associated with the presence and extent of CAC progression, independent of traditional CV risk factors and socio-economic status. To the best of our knowledge, there are no studies to date that have assessed the relationship

between PAI-1 and CAC progression. Interestingly, PAI-1 was not associated with presence or extent of baseline CAC. These findings are complimentary with a previous study that did not find a correlation of PAI-1 level with presence of CAC on a single measurement in non-diabetics, as our population had few diabetics (57). Thus, PAI-1 may be an important temporal marker for individuals who are at elevated risk for aggressive atherosclerotic development at a particular time of life. Some have postulated that a single measure of CAC is a representation of long-term, or lifetime, atherosclerotic plaque burden whereas CAC progression is more indicative of short-term, or current, disease activity (91).

Increased PAI-1 concentration as a marker for impaired fibrinolysis has been investigated as a potential contributor to ACS. PAI-1 has not been significantly associated with ACS after adjustment for traditional CV risk factors and insulin resistance (92, 93). The types of coronary lesions that are typically involved with ACS are not calcified (19). Inhibition of fibrinolysis through PAI-1 has been postulated as a potential mechanism to increase arterial fibrin deposition and thrombosis (54). PAI-1 may thus be an important factor in CAC progression in late stage atherosclerotic lesions as altered healing of ruptured atheromas may contribute to increasing CAC. CAC progression has been correlated with mortality and morbidity, and CAC magnitude has been correlated with atherosclerotic burden.

To date, statins have failed to demonstrate significant decreases in CAC progression (22). Lipid regulation may not adequately affect the calcification process in coronary atheromas, which occurs late in the atherosclerotic process. Therefore, CHD events and CAC progression continue despite optimization of traditional CV risk factors, including statin use. The mechanisms that prevent worsening CAC burden may ultimately improve upon current state-of-the-art CHD prevention recommendations. Our findings suggest that targeting hemostatic

abnormalities, as evidenced by elevated PAI-1 levels, may theoretically slow CAC progression and decrease CHD events.

Increased activation of the renin-angiotensin-aldosterone system (RAAS) has been known to be associated with abnormalities in the fibrinolytic system, particularly endothelial expression of PAI-1 by angiotensin (94). Brown and colleagues demonstrated that angiotensin-converting enzyme (ACE) inhibition using ramipril could block increases in PAI-1 levels that were stimulated by hydrochlorothiazide activation of the RAAS for the study duration of 6 weeks. In contrast, angiotensin II type I (AT_1) receptor blockade with losartan was efficacious for only 1 week (95). These investigators then demonstrated that furosemide-induced increases in PAI-1 levels could be inhibited by a combination of AT_1 receptor and aldosterone receptor blockade using candesartan and spironolactone over a 2 week period. (96). Candesartan or spironolactone in isolation could not inhibit furosemide-induced PAI-1 expression. These results suggest that ACE inhibition alone or the combination of AT_1 receptor and aldosterone receptor inhibition have the potential to chronically reduce elevated levels of PAI-1. This may theoretically relieve PAI-1 mediated suppression of fibrinolysis.

Decreasing PAI-1 activity has also improved vascular structure in mouse models. AT_1 receptor blockade with azilsartan decreased PAI-1 expression in the aortic wall of mice fed a high fat diet (97). In addition, mice fed azilsartan had more stable plaques as evidenced by greater cellularity and collagen than mice that were fed placebo. Administration of apelin to mice has been shown to attenuate angiotensin II-induced perivascular fibrosis in coronary artery sections (98). The observed effect was postulated to be at least in part due to decreased PAI-1 levels by blocking angiotensin II-mediated PAI-1 gene expression. These findings add evidence

to the potential histologic stability, and prevention of calcification, that may be conferred to atherosclerotic plaques by controlling PAI-1 expression.

The association of PAI-1 with CAC progression in our study generates several interesting hypotheses. First, abnormalities in the fibrinolytic system may play an important role in CAC progression in middle-aged women undergoing the menopausal transition. Recent results from SWAN demonstrated that complement protein C3 was associated with PAI-1 and tPA-ag, and that C3 is higher in post-menopausal women compared to pre-menopausal women (68). Thus, the complement system may have an important role in the elevated risk of CHD in post-menopausal women. There does not appear to be a significant contribution on PAI-1 level from gender (99). BMI and waist-to-hip ratio, however, have been shown to independently predict PAI-1 levels in women but not men (100). It is therefore important to limit our results to subjects who are similar to those in our study population.

Second, midlife women with elevated PAI-1 levels with hypertension may benefit from using non-diuretic anti-hypertensive medications as these activate the RAAS and PAI-1 expression. Third, blocking PAI-1 expression through the RAAS may prevent or decrease CAC progression. This in turn may be decrease CHD-related morbidity and mortality that is currently not prevented by optimization of traditional risk factors. Futures studies should evaluate the association of PAI-1 levels with subclinical atherosclerosis in a broader population. If similar relationships are present, then a randomized controlled trial on RAAS inhibition to decrease CAC progression in those with elevated PAI-1 levels may be warranted.

4.4 CHANGE IN NOVEL RISK FACTORS AND CAC PROGRESSION

Finally, we demonstrated that changes in novel CV risk factors were not associated with CAC progression. The magnitude of change between baseline and follow-up CAC measurements was small, relative to baseline values. Thus our findings suggest that CRP, fibrinogen, PAI-1, and tPA-ag levels are relatively stable from year-to-year in midlife women, and repeat measures are unlikely to yield benefit. Due to limited sample size and a relatively short follow-up time, these results should be interpreted with caution.

4.5 STRENGTHS AND LIMITATIONS

Our study had several notable strengths. To the best of our knowledge, this is the first study to evaluate the relationships of CRP, fibrinogen, PAI-1, and tPA-ag to CAC in women undergoing the menopausal transition. This period is recognized as a critical time for the change in CHD risk in women. Although the analysis was retrospective, the data collection was performed in a systematic and prospective manner. By design, there were a significant proportion of black women in our study population. In addition, our analyses evaluated the presence and extent of baseline CAC, and the presence and extent of CAC progression over a follow-up period that averaged over 2 years.

Our study also had several limitations. We only had women of white and black ethnicity, so the results may not be generalizable to other races. The number with diabetes was exceedingly low, so diabetes was not included as a separate covariate in our analyses. The follow-up time was on average less than 3 years so differences seen on the progression analyses may not be as great

compared to longer follow-up times. The majority of the subjects with CAC had low CAC scores so these results may not be applicable in subjects with higher CAC scores. Finally, there is not a universally accepted definition of CAC progression. Our definitions, however, have been used before in publications from other large cohort studies.

4.6 CONCLUSIONS

Our results demonstrate that the associations of inflammation and hemostasis with CAC presence are virtually all mediated through BMI in women undergoing the menopausal transition. The exception is the association of CRP with CAC presence in black women, which is independent of traditional CV risk factors and socio-economic factors. Additionally, PAI-1 is associated with CAC progression and suggests a potential new pathway to decrease CHD-related morbidity and mortality that is not accounted for with traditional CV risk factors and prevention strategies. Finally, changes in CRP, fibrinogen, PAI-1, and tPA-ag do not appear to be significant from year-to-year. This will, however, need to be replicated in a larger sample with longer follow-up.

APPENDIX: LITERTURE REVIEW

A Review of the Association of Coronary Artery Calcification with C-Reactive Protein, Fibrinogen, Plasma Activator Inhibitor-1, and Tissue Plasminogen Activator: Implications for Further Study of Middle-Aged Women Undergoing the Menopausal Transition

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ABSTRACT

Novel cardiovascular (CV) risk factors associated with inflammation and hemostasis may be related to coronary artery calcification (CAC) and CAC progression as measured by cardiac computed tomography. Inflammatory markers that have been subjects of recent investigation include C-reactive protein and fibrinogen. Plasminogen activator inhibitor type 1 and tissue plasminogen activator are hemostatic markers which also be involved in the pathophysiologic process of coronary artery calcification. A literature search was performed of the Ovid MEDLINE database from 1948 to January Week 3 2013. The first search was performed using Medical Subject Headings (MeSH) “coronary artery calcification” or “coronary artery calcium”. The second search was performed using MeSH “C-reactive protein” or “fibrinogen” or “plasminogen activator inhibitor type 1” or “tissue plasminogen activator”. Articles were finally

reviewed by title, abstract, and text for inclusion. The search yielded 77 articles. There are few studies evaluating the association between novel CV risk factors and CAC.

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death in the United States (1). Coronary artery calcification (CAC), a manifestation of subclinical CVD, is of particular importance as it may predict risk of non-fatal myocardial infarction and cardiac death (2). On a pathophysiologic level, this represents calcium deposition within fibroatheromas (3). Detection is typically made by cardiac computed tomography (CT) (4). There has been suggestion that increasing levels of CAC seen on serial measurements with cardiac CT, referred to as CAC progression, may be more predictive of future events than a single measurement (4). Traditional risk factors for CVD established by the Adult Treatment Panel III have been related to CAC (5). These include age, gender, total and high-density lipoprotein cholesterol levels, smoking, blood pressure, and hypertension. It has been established that inflammatory processes increase the risk of development of coronary artery disease (6-8). In addition, hemostatic factors are potential emerging risk factors given the role of thrombosis in atherogenesis and acute coronary syndromes (8-11).

It has become evident that evaluating individuals during middle age can predict their lifetime risk of CVD (12). Focusing on long-term prevention of CVD events is of particular interest in women. Women presenting with acute myocardial infarction are more likely to have atypical symptoms and less likely to receive evidence-based therapies (13,14). It is accepted that differences in the benefits and risks associated with preventative measures exist between men

and women (15). Some of this may be related to differences in the pathophysiologic processes between men and women. Evidence has shown that associations between certain inflammatory markers and calcification are more pronounced in women compared to men (16-19). The menopausal transition is also a time of particular interest, given that the risk for CVD increases substantially in the post-menopause period (20). Adverse lipid increases occur within the one year period around the final menstrual period (21). Potential associations of inflammatory and hemostatic biomarkers with CAC in peri-menopausal women are largely unknown.

The goal of the proposed literature review is to the evaluate the potential association between CAC, CAC progression and several factors related to inflammation and hemostasis, specifically C-reactive protein (CRP), fibrinogen, plasminogen activator inhibitor type 1 (PAI-1), and tissue plasminogen activator (tPA). Focus was given towards studies consisting of patients with no known heart disease or other significant chronic illnesses. Particular attention was directed towards women going through the menopausal transition with no known cardiac history and no significant co-morbid illnesses. The potential importance for evaluating the relationship between these risk factors and CAC and CAC progression using the Study of Women's Health Across The Nation (SWAN) will be addressed.

METHODS

A literature search was performed of the Ovid MEDLINE database from 1948 to January Week 3 2013. The first search was performed using Medical Subject Headings (MeSH) "coronary artery calcification" or "coronary artery calcium" or "CAC". The second search was performed using MeSH "C-reactive protein" or "fibrinogen" or "plasminogen activator inhibitor type 1" or

"tissue plasminogen activator". The intersection of the searches was used to find articles pertaining to the emerging risk factors of interest with CAC and CAC progression.

The search was limited to the English language and humans. Articles were finally reviewed by title, abstract, and text for inclusion. Review articles were excluded. Inclusion criteria consisted of studies in which subjects had undergone assessment of coronary artery calcium score via computed tomography, and if analysis included at least one of the novel cardiovascular risk factors of interest.

RESULTS

The first search using Medical Subject Headings (MeSH) "coronary artery calcium" or "coronary artery calcification" yielded 995 articles (**FIGURE 1**). The second search performed using MeSH "c-reactive protein" or "fibrinogen" or "plasminogen activator inhibitor type 1" or "tissue plasminogen activator" yielded 75,545 articles. There were 105 articles which intersected both searches. The titles and abstracts were reviewed for relevance and review articles were excluded. The search ultimately yielded 77 pertinent articles. Of these 77 articles, 26 directly addressed the association of one of the four risk factors of interest with CAC and/or CAC progression. Studies relating to baseline CAC are listed on **TABLE 1** (16-19, 22-38). Studies pertaining to CAC progression or development are listed on **TABLE 2** (39-43). The other studies included those with established CVD, specific significant co-morbid conditions (i.e. dialysis), or did not directly address associations between the risk factors of interest and CAC. These are listed on **TABLE 3** (39-94). Brief summaries of the findings of the 26 citations that are directly related to the current study research question are list below. Of these 26 studies, only 4 addressed CAC progression.

Studies addressing inflammatory markers were the most common. CRP was assessed in 25 of the 26 studies (96%). Fibrinogen was the next most common and analyzed in 9 studies (35%). There was only one study (4%) that included PAI-1 as a variable, and zero studies included TPA.

Association of Baseline Novel CV Risk Factors to Baseline CAC

Bielak et al. (2000, Table 1, Study #1) examined the relationship between fibrinogen and high quantities of CAC using 288 participants from the Epidemiology of Coronary Artery Calcification Study (18). This case-control study compared 57 men and 57 women with CAC that was 80th percentile or greater for their sex and 10-year age group, to sex-matched controls with no detectable CAC. They found in a univariate logistic regression model that an increase of 1 standard deviation in fibrinogen concentration was associated with statistically significant odds ratio for high quantities of CAC in men (1.6, 95% CI 1.1-2.5) and women (2.5, 95% CI 1.6-4.1). Women 50 to 59 years of age with high fibrinogen were more likely to have high quantities of CAC than women with low fibrinogen. The multivariate model accounting for all major risk factors demonstrated that only fibrinogen and an interaction variable for fibrinogen and age were significant predictors of high CAC (<0.0001). A notable limitation of this study was excluding patients with low and intermediate quantities of CAC.

Hunt et al. (2001, Table 1, Study #2) found that CRP was unrelated to CAC in a nest case-control study in 188 men in the Prospective Army Coronary Calcium study (22). Cases were those with any detectable CAC, or >0 CAC score via the Agatston method, whereas controls were those with a CAC score of 0. Subjects were active-duty Army personnel between

the ages of 40-45 years who participated at the time of a routine, periodic Army physical exam and were free of known CV disease. They were not typical of the general population, so the results may not be generalizable.

Wang et al. (2002, Table 1, Study #3) used a sample of 321 individuals (48% women) without known CVD from the Framingham Heart Study to demonstrate that increasing CRP, analyzed as quintiles, was significantly correlated with the raw CAC score determined by the Agatston method (23). The majority of women were post-menopausal as the mean age ± standard deviation was 61 ± 9 years. Those with clinically apparent CVD were excluded. The age-adjusted Spearman correlation was 0.25 for men and 0.26 for women. This was significant even after adjustment for age and Framingham risk score for both men and women (P=0.01). The main limitation was that CRP levels were obtained 4 to 8 years prior to the CT scans. Subjects with high CRP may have had significant CAC progression prior to CT scan, which would in turn lead to a higher strength of association.

Colhoun et al. (2002, Table 1, Study #4) analyzed 196 type 1 diabetics and 195 controls between the ages of 30-55 years (24). They grouped patients by diabetic status and sex. Menopausal status was not considered. In the univariate analysis, the raw CAC score and CRP were significantly and positively correlated in all groups except diabetic women. The investigators also calculated odds ratios for the relationship between CRP and the presence of any CAC (CAC score >0 via Agatston score). After adjustment for other CV risk factors and body-mass index, the association was significant in men but not women. A limitation of the study was that the average age was relatively young with a mean ± standard deviation of 37.9 ± 0.3 years in nondiabetic women and 37.5 ± 0.5 years in diabetic women. The majority of these women were therefore likely to be pre-menopausal.

Kullo et al. (2003, Table 1, Study #5) examined 354 (58% women) non-Hispanic white hypertensive patients from Rochester, Minnesota (19). CAC was transformed to log(CAC + 1) to reduce skewness. The majority of women were post-menopausal as the mean age ± standard deviation was 66 ± 7 years, and menopause status was not analyzed. Approximately half the women were on estrogen. CRP, evaluated in quintiles, was not associated with Log(CAC + 1) in men or women. Increasing fibrinogen levels, also evaluated by quintiles, were positively associated with increasing Log(CAC + 1) in women. The association was attenuated after adjustment for conventional risk factors.

Reilly et al. (2003, Table 1, Study #6) performed a cross-sectional study on 914 (45% women) predominantly Caucasian subjects recruited due to a family history of premature CVD (25). The median age for women was 50 years (range 34 to 71) and menopausal status was not accounted for. Ln(CAC + 1) was analyzed as a continuous variable and raw CAC scores as quintiles. Increasing levels of CRP categorized as tertiles was not associated with higher Ln(CAC + 1) in men. There was a positive association between CRP and CAC in women with all approaches performed in this study, even after adjusting for traditional risk factors. This was no longer significant, however, when BMI was included.

Huang et al. (2005, Table 1, Study #7) studied 124 (37% women) subjects with chest pain in Taiwan who were referred for CAC determination (26). The mean age ± standard deviation was 67 ± 1 years. Menopausal status was not reported. Subjects were divided into three groups based on raw CAC scores via Agatston: 0, 1-199, and ≥200. There was no significant difference in CRP among the three groups.

Bowden et al. (2005, Table 1, Study #8) demonstrated no association between CRP and CAC in subjects from the Diabetes Heart Study (27). CAC was analyzed as an outcome as both

log(CAC) if the CAC score was >0, and as a the binary outcome of presence of CAC (CAC score >0 via Agatston). The 666 subjects (59% women) were participating in a study of families with type 2 diabetes mellitus and had a mean age of 60.8 years. In the overall population, 83% of patients were affected with diabetes. Menopausal status was not reported.

Khera et al. (2006, Table 1, Study #9) examined CRP levels in 3373 subjects between 30 and 65 years of age who were in the Dallas Heart Study (28). Women represented 56% of the subjects and approximately half were black. The mean age by quartile ranged from 42.8 to 46.3 years. Estrogen use was included as a covariate, but menopausal status was not. CAC was evaluated as a binary outcome (raw CAC score >10 via Agatston) and by three raw CAC score thresholds: >10, >100, and >400. There was no statistically significant association between CRP and CAC in either sex after adjusting for traditional CV risk factors, BMI, estrogen use, and statin use.

Godsland et al. (2006, Table 1, Study #10) failed to demonstrate a positive correlation between CRP and CAC in 573 (36% women) type 2 diabetic subjects in the Prospective Evaluation of Diabetic Ischaemic Heart Disease by Computed Tomography (PREDICT) study (29). In fact, CRP tended to decrease with increasing CAC score. The Spearman correlation coefficient was rho = –0.07 (p=0.07) for the entire sample and rho = –0.12 (p=0.006) for the subgroup with detectable calcification. CAC was evaluated in six categories based on raw CAC scores via Agatston, and as a binary outcome based on detectable (>10) or undetectable CAC. CAC was also evaluated as log(CAC + 1). The mean ± standard deviation of age in women was 63 ± 7 years and menopausal status was not reported. The race distribution was 70% white and 17% Asian Indian.

Ye et al. (2007, Table 1, Study #11) evaluated 32 (44% women) subjects with LDL-C levels >190 mg/dl and a history of familial hypercholesterolemia against 34 healthy control subjects (30). There was no report of menopausal status. The mean age ± standard deviation in the group with hypercholesterolemia was 36.0 ± 17.8 years. CAC was assessed as a binary (CAC score >0 via Agatston) outcome as detectable or undetectable. CRP was significantly higher in the CAC detectable group compared to the undetectable group (0.29 ± 0.08 vs. 0.07 ± 0.08 mg/dl, p=0.001). In the multivariable analysis, CRP was the only independent predictor of detectable CAC (relative risk 5.034, 95% confidence interval 1.525-16.613, p=0.04).

Wilund et al. (2008, Table 1, Study #12) studied 13 (6 women) highly trained, endurance athletes aged 60 to 80 years and 12 (6 women) sedentary controls to assess relationships between cardiorespiratory fitness, CAC, and bone mineral density (31). CAC score was determined by the Agatston method and this was log-transformed. CRP was no significantly correlated with logCAC when calculated using Pearson correlation coefficient. The main limitations of this study are the small sample size and the atypically high physical fitness of the subjects.

Erbel et al. (2008, Table 1, Study #13) demonstrated that Adult Treatment Panel III risk categories were significantly and sex-dependently altered when CAC and CRP were included in a risk model (16). The 4345 (53% women) subjects were randomly selected from 3 cities in Germany and 6.8% of the subjects had a history of coronary artery disease. The raw CAC score via Agatston was used to partition subjects into categories: <100, 100-399, and ≥400. CRP was likewise partitioned into three categories. ≤1 mg/L, 1-3 mg/L, and >3 mg/L. CAC was of most utility in men whereas CRP was of most use in women. The correlation between CAC and CRP was weak in the univariate analysis (R^2=0.02), but statistically significant (p<0.0001). An adjusted analysis of this relationship was not performed.

Ramadan et al. (2008, Table 1, Study #14) sought to assess the relation of CAC with endothelial function and inflammatory markers in 177 (42% women) asymptomatic individuals of intermediate risk with an average age of 50.6 ± 5.9 years (32). Raw CAC scores calculated via the Agatston method were categorized into four groups: 0, 1-99, 100-399, and ≥400. It was also transformed as log(CAC + 1). There was no significant difference in the mean CRP across CAC grades, although CRP was higher in the group that demonstrated positive CAC. This was no longer significant after adjusting for gender and body mass index (p=0.221).

Michos et al. (2009, Table 1, Study #15) evaluated the relationship between several CV risk factors and measurements of subclinical vascular disease from the Multi-Ethnic Study of Atherosclerosis (33). Subjects were ≥70 years of age in this cross-sectional substudy. CAC score was calculated by the Agatston method and low subclinical disease was defined as CAC <25th percentile. There was no relationship between CRP and CAC, or other measures of subclinical CVD. The limitation of this study is that the average age was greater than 70 years of age, so the results are may not be applicable to middle-aged women.

Pratte et al. (2009, Table 1, Study #16) evaluated 560 type 1 diabetes patients and 693 non-diabetes patients from the Coronary Artery Calcification in Type 1 Diabetes (CACT1) Study, of which 51% were women (34). CAC was evaluated as a binary outcome based on the presence or absence of any CAC. PAI-1 levels were independently associated with CAC presence in type 1 diabetics between 20 and 55 years of age, but not non-diabetic subjects. In the univariate analysis, the mean average CRP level was higher in the CAC positive group in the non-diabetes subgroup ($p<0.05$). The mean average fibrinogen level was higher in the CAC positive group among diabetics ($p<0.05$). The main limitation of this study was the focus on young diabetic patients. Thus, the mean ± standard deviation of age was 37 ± 9.1 years in

diabetics and 39.1 ± 9.1 years in non-diabetics. The results may therefore not be applicable to older individuals.

Jenny et al. (2010, Table 1, Study #17) evaluated the association of inflammatory biomarkers, including CRP and fibrinogen, with the presence of CAC as defined by Agatston score >0 (35). The study included 6783 (53% women) subjects from the Multi-Ethnic Study of Atherosclerosis (MESA). Menopausal status was not included. The relative risks in multivariable adjusted models were 1.05 (95% CI, 0.99-1.12, p=0.63) for CRP and 1.09 (1.02-1.16, p=0.01) for fibrinogen. An important limitation was that subjects were partitioned based on the finding of any CAC, since prognosis is dependent upon degree of CAC when it is present.

Qasim et al. (2011, Table 1, Study #18) studied the relationship between CRP and CAC in 1299 subjects with type 2 diabetes from the Penn Diabetes Heart Study and 860 without diabetes from the Study of Inherited Risk of Coronary Atherosclerosis (17). Women accounted for 41% of the overall study population. CAC was analyzed with Tobit regression of log(CAC + 1). CRP was associated with higher CAC in diabetic women (Tobit ratio 1.60, 95% confidence interval 1.03-2.47) even after adjustment for age, race, medications, Framingham risk score, and body mass index. The result was not significant in non-diabetic women (Tobit ratio 1.29, 95% confidence interval 0.98-1.69), and there was no association in CRP to CAC in men. The combined diabetic and non-diabetic women sample was significant (Tobit ratio 1.44, 95% confidence interval 1.13-1.83)

Freitas et al. (2011, Table 1, Study #19) evaluated 178 (79% women) healthy subjects between the ages of 80 and 102 years who were enrolled in the GEROS study, a prospective cohort to identify CV risk markers in the primary prevention setting (36). CAC was examined as a raw CAC score via Agatston as well as a binary outcome with a CAC score cutoff of >100

considered as significant disease. Those above the 75th percentile of CRP had a significantly higher mean raw CAC scores than the first three quartiles. Binary logistic regression demonstrated an independent association between a CAC score of >100 as the dependent variable and CRP >75th percentile.

Blaha et al. (2011, Table 1, Study #20) studied the relationship between obesity, CRP, and subclinical atherosclerosis in the Multi-Ethnic Study of Atherosclerosis using a CRP cut-off of ≥2 mg/L, which was suggested by the JUPITER trial as a means to guide statin recommendation (37). There were 950 (51% women) subjects who met the JUPITER criteria with a mean age of 66.7 years. The race composition of the subjects was 41% white, 31% black, 5% Chinese, and 23% Hispanic. CAC was evaluated as a binary outcome with a CAC score via Agaston of >0, and in three groups: 0, 1-100, and >100. There was no association between CRP and CAC when anthropomorphic variables were included.

Gupta et al. (2012, Table 1, Study #21) investigated the relationship of CRP and CAC across a range of body mass index categories in 2685 (53% women) subjects enrolled in the Dallas Heart Study (38). A CAC score calculated via Agatston of >10 was used to define the presence of CAC. CRP was divided into three groups of <1 mg/l, 1-3 mg/l, and >3 mg/l. Increasing CRP was associated with increased CAC prevalence in those with a normal BMI category women, but not overweight (<30 mg/m^2) or obese (>30 mg/m^2). In contrast, the relationship was significant in both normal and overweight men, but not obese men.

Association of Baseline Novel CV Risk Factors to CAC Progression or Subsequent Development

Anand et al. (2007, Table 2, Study #22) examined subjects with type 2 diabetes who were between 30 and 65 years of age and without known CVD (39). The study population was of 54% South Asian origin. The mean age was 52 years and menopause status was not accounted for. CAC was calculated by Agatston and volumetric scores. Volumetric scores were categorized into 1-10 mm^3, 11-100 mm^3, 101-400 mm^3, and 100-400 mm^3. CAC progression was defined as a change of $\geq$2.5 between the square root transformed values of the CAC volumetric measurements. The mean follow-up interval was 2.5 ± 0.4 years. CRP was neither related to extent of CAC at baseline, nor CAC progression.

Green et al. (2009, Table 2, Study #23) measured fibrinogen in 1396 young black and white participants from the Chicago and Minneapolis sites in the Coronary Artery Risk Development in Young Adults (CARDIA) study (40). Subjects were ages 18-30 years at baseline. Hemostatic factors were drawn at the year 7 follow-up visit and CAC was determined 13 years later at the 20 year follow-up visit. The presence of CAC was determined by an Agatston CAC score of >0. An elevated fibrinogen level in those between the ages of 25 and 37 years at the year 7 visit was independently associated with the presence of CAC. The main limitations of this study are the very young age of the study population at the time of initial enrollment, and analysis of CAC using only a binary variable based on presence or absence of CAC.

Saremi et al. (2009, Table 2, Study #24) evaluated the association of risk factors to baseline and follow-up CAC in 197 and 189 subjects, respectively (41). Individuals were enrolled in Veterans Affairs Diabetes Trial (VADT). Sex distribution was not reported, but subjects were 94% male in the main study. Progression was defined as an increase in the square root volumetric scores of $\geq$2.5 mm^3, as well as a continuous variable. The average follow-up was

4.6 years. CRP was not significantly related to CAC progression. Correlation with baseline CAC was not analyzed.

Green et al. (2010, Table 2, Study #25) repeated their analysis of the relationship between fibrinogen and CAC in the CARDIA study, this time including the Birmingham and Oakland sites with the Chicago and Minneapolis sites (42). They noted that fibrinogen increased from a mean of 3.32 g/L to a mean of 4.05 g/L over the 13-year study. CAC was evaluated as a binary outcome based on the presence of CAC as determined by an Agatston CAC score of >0. Fibrinogen was positively associated with CAC incidence longitudinally (P=0.037), but not in the cross-sectional analysis (p=0.147).

Rodrigues et al. (2010, Table 2, Study #26) evaluated the relationship of fibrinogen to CAC progression over 2.4 ± 0.4 from the CACT1 prospective cohort (43). Progression was defined as an increase in the square-root transformed CAC volume of >2.5 mm^3 or the development of clinical coronary artery disease. This study included 546 subjects with type 1 diabetes and 640 controls without diabetes. Women accounted for 55% of the population, but menopausal status was not addressed. In the combined population of diabetics and non-diabetics, baseline fibrinogen levels were higher in progressors when compared to non-progressors (276 ± 61 mg/dl vs. 259 ± 61 mg/dl, p=0.0003).

DISCUSSION

The major findings from this literature review is that 1) there are a number of articles that addressed the association of inflammatory biomarkers, CRP and fibrinogen, with CAC, 2) there is little data on the association between PAI-1 and CAC, 3) there are no manuscripts addressed

potential associations between TPA and CAC, 4) there is a paucity of data with any of these novel CV risk factors on CAC progression, and 5) few of these studies evaluated middle-aged women and none took menopausal status into account.

The relationship of CRP to CAC in asymptomatic subjects was generally not consistent. Wang et al. found an association between CRP levels by quintile and raw CAC scores in healthy men and women (23). The delay of 4 to 8 years between CRP and CAC determination makes it unclear if higher CAC in this group was based on a greater baseline CAC presence at the time of CRP analysis. Qasim et al. found a positive association between CRP and CAC among diabetic women, but not in men or non-diabetic women (17). It is difficult to make conclusions based on these results as subjects were drawn from two cross-sectional studies. One study recruited subjects based on a history of premature CVD and the other based on a confirmed diagnosis of type 2 diabetes. A study of individuals with type 2 diabetes mellitus recruited from diabetes clinics by Godsland et al. actually demonstrated that CRP decreased with increasing CAC (29). The authors felt that this was a chance observation. These discrepancies may be related to the populations studied, the technique of analyzing the relationship between CRP and CAC, or a combination. Ultimately, the relationship of CRP to CAC among healthy middle-aged women is yet to be conclusively established and more study is warranted in subjects more representative of the general population.

Early studies on the association of fibrinogen with CAC suggested a positive correlation, particularly in women (18,19). More recently, longitudinal studies demonstrated that high fibrinogen levels were associated with the development of CAC more than a decade later in life, and that patients with type 1 diabetes may be at particular risk (41,42). Fibrinogen has been cited, along with CRP, as a possible means by which CV event risk prediction may be enhanced

in those judged to be at intermediate risk (44). As with CRP, the understanding of the relationship of fibrinogen to CAC should be improved to gain insights into potential mechanisms for disease progression. Due to early studies demonstrating that fibrinogen may play an important role in CAC development in women, understanding more of this process taking into account the menopausal transition may uncover important insights into the relationship.

The lone study that addressed the relationship between PAI-1 and CAC was performed in a relatively young group of subjects enrolled in a prospective cohort study designed to examine the development of subclinical atherosclerosis in those with and without type 1 diabetes (34). Subjects enrolled were between the ages of 20 and 55 years and otherwise healthy. Interestingly, only the diabetes subjects ages 35 years and younger maintained a positive relationship between PAI-1 and CAC. The association was weakened and no longer statistically significant after controlling for variables associated with insulin resistance. Weaknesses of the study included the relatively young average age of the study population, lack of accounting for race and menopause status, and assessment of CAC as a binary outcome based on presence or absence of any CAC. Further elucidation of the relationship between PAI-1 level and CAC, as well as CAC progression, are therefore needed.

Only 5 studies evaluated the association of the four novel risk factors (CRP, fibrinogen, PAI-1, TPA) with CAC progression or CAC development. Of these, two studies by Green et al. were from the CARDIA study and only included one CAC measurement obtained 13 years after the baseline biomarker measurement (40, 42). Thus, only three studies addressed CAC progression between CT scans obtained at two different time points. A study performed in military veterans demonstrated no correlation between CRP and CAC progression (41). The patient population, however, was 94% men with a mean average age of 61.2 years. The study by

Anand et al., that did not find an association between CRP and CAC progression, was conducted entirely in subjects with type 2 diabetes and 54% were of South Asian ethnicity, so the results may not be relevant to healthy individuals of other ethnicities (39). Those with CAC progression examined in the CACT1 study demonstrated a significant association with fibrinogen in those with type 2 diabetes, but not healthy controls (43). This may be due to the fact that 67% of 206 individuals with CAC progression were diabetics and only 33% were otherwise healthy individuals. In short, there are no significant data for these risk factors and CAC progression in healthy middle-aged women.

Menopause is a critical time period as risk assessment during this age predicts the lifetime risk of developing CVD (5). As CAC detected on a CT scan represents distinct stages in the atherosclerotic process, insights into the relationship between novel CV risk factors and CAC may enhance our understanding of the underlying pathophysiologic mechanisms and direct future treatment efforts. Thus, analysis of the subjects enrolled in SWAN Heart may offer significant insight into these potential relationships.

CONCLUSION

Additional studies are needed to determine subgroups that may have the highest correlation between CRP and fibrinogen with CAC and CAC progression. Data regarding potential associations between PAI-1 and TPA with CAC are virtually non-existent. Women progressing through the menopausal transition are at an age where identifying their lifetime CVD risk may modify the ultimate development of CVD (5). The SWAN Heart study provides an ideal

opportunity to study potential associations between inflammatory and hemostatic markers with CAC and CAC progression.

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FIGURE 1. Schematic diagram of search strategy used to identify articles.

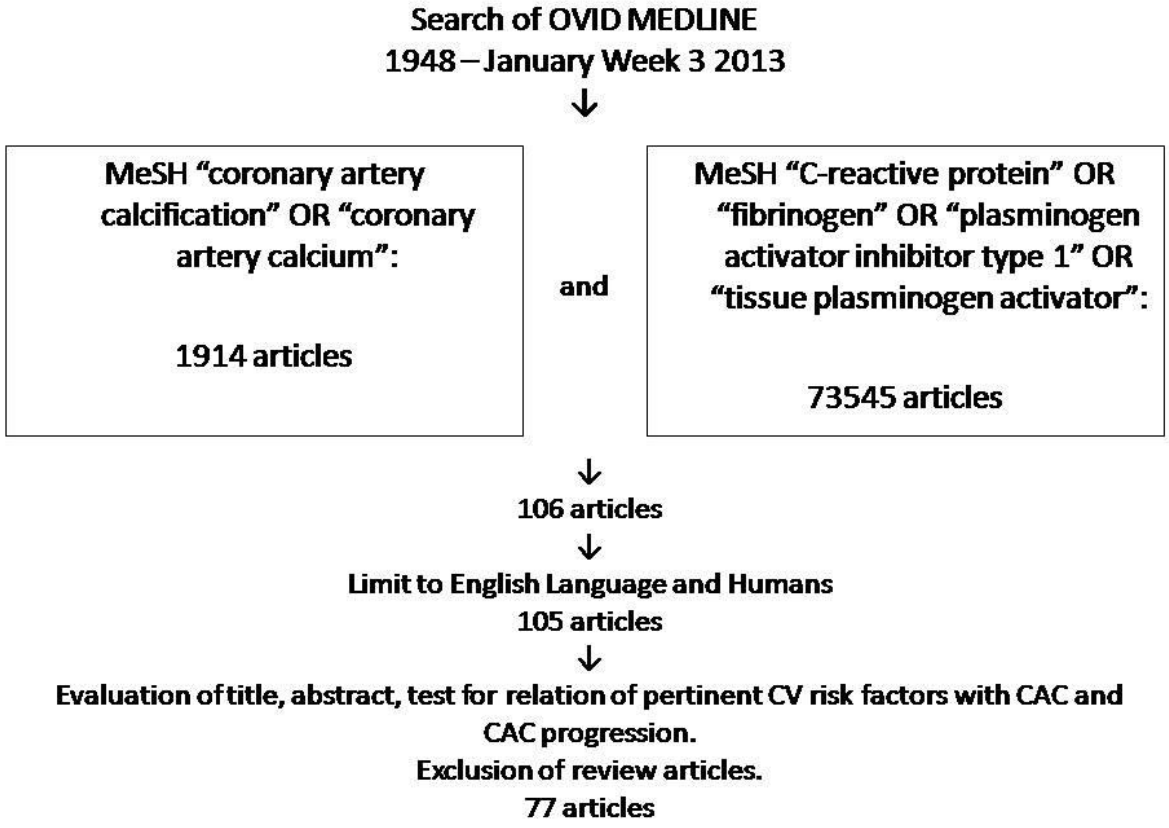

TABLE 1. Characteristics of Studies Examining Novel Cardiovascular Risk Factors with Baseline CAC.

Study #	1st Author (Year, reference) - Journal	Study Design	Population / Aim	Sample	CAC Measure(s)	Novel CV Risk Factor	Major Findings
1	Bielak (2000, 18) – ATVB	Case-control – Epidemiology of Coronary Artery Calcification (ECAC) Study	Participants <70 yrs (but ≥50 yrs women, ≥40 yrs men) without CAD / Assess relation of fibrinogen with high CAC (≥80th percentile for sex and age)	227, 113 (50%) women; race not specified	Binary (80th percentile) using raw CAC score via Agatston	CRP, Fibrinogen	*High fibrinogen was associated with high quantities of CAC, particularly in young women. *CAC presence was not associated with elevated CRP
2	Hunt (2001, 22) – Am Heart J	Nested case-control – Prospective Army Coronary Calcium (PACC)	Active-duty Army personnel ages 40-45 yrs / Assess relation of CRP with CAC presence	188, 100% men; race not specified	Binary (>0) CAC score and logCAC via Agatston	CRP	*The mean hsCRP level was unrelated to the log-transformed CAC score.
3	Wang (2002, 23) – Circulation	Cross-sectional – Framingham	Subjects aged 5-70 yrs / Assess relation between CRP and CAC	321; 154 (48%) women; race not specified	Raw CAC score via Agatston	CRP	*High CRP levels were associated with increased coronary calcification as measured by raw CAC score
4	Colhoun (2002, 24) – Diabetes Care	Cross-sectional	T1DM and non-T1DM subjects aged 30-55 yrs	196 T1DM, 195 non-T1DM; 199 (51%) women; race not specified	Raw CAC and binary (>0) CAC presence via Agatston	CRP	*The association of CRP and CAC was present in men even after adjustment. *CRP was not associated with CAC in women.
5	Kullo (2003, 19) – Am J Cardiol	Cross-sectional – Genetic Network of Arteriopathy (GENOA)	Hypertensive siblings / Assess relation of CRP and fibrinogen with CAC in hypertensive pts	354; 206 (58%) women; 100% white	Log (CAC score via Agatston + 1)	CRP, fibrinogen	*CRP was not associated with CAC in either gender. In women, fibrinogen was associated with CAC.
6	Reilly (2003, 25) – ATVB	Cross-sectional - SIRCA	Non-DM 30-75 yrs with FH of premature CVD but no CAD / Assess relation of CRP and CAC	914; 414 (45%) women; 95% white	CACS via Agatston; linear reg. of ln (CAC + 1), tobit reg. of ln (CAC + 1),	CRP	*CRP not associated with CAC in men, but weakly associated in women. This was lost after adjustment for BMI.

					binary (>0) CAC presence, Ordinal reg. by quintiles		
7	Huang (2005, 26) – Chest	Cross-sectional	Pts with suspected CAD / Assess relation of CAC to endothelial dysfunction	124; 46 (37%) women; race not specified	Raw CAC score via Agatston by tertiles	CRP	*Higher CAC scores strongly predicted endothelial dysfunction *CRP was not associated with degree of CAC
8	Bowden (2005, 27) – Am Heart J	Cross-sectional – Diabetes Heart Study	Siblings concordant for T2DM without renal dysfunction / Assess relation between CRP and CAC	666; 393 (59% women); 84% white, 16% black	Binary (>0) CAC score and raw CAC score if >0 via modified Agatston	CRP	*CRP was not significantly associated with CAC.
9	Khera (2006, 28) – Circulation	Cross-sectional – Dallas Heart	Pts 30-65 yrs without prior CVD / Assess relation between CRP and CAC	3373; 1885 (56%) women; 52% black	Binary (>10) CAC score via Agatston and by tertiles	CRP	*Subjects with higher CRP had a modest increase in prevalence of CAC, but this was not independent of traditional risk factors.
10	Godsland (2006, 29) – Diabetic Med	Cross-sectional – PREDICT	T2DM adults / Assess relation of novel CV risk factors and CAC	573; 207 (36%) women; 70% white, 17% Asian Indian	Binary (>10) CAC score, raw CAC score, and log (CAC + 1) via Agatston	CRP, fibrinogen	*Age was the major contributing factor for CAC; other risk factors with little effect. *Negative association between CRP and CAC.
11	Ye (2007, 30) – Am J Cardiology	Case-control	Pts with LDL-C levels >190 mg/dl and positive family history of hyperlipidemia in Taiwan / Assess relation between CAC to FMD and CRP	66 (32 case, 34 controls); 21 (47%) women; 100% Chinese	Binary (>0) CAC score and raw CAC score via modified Agatston	CRP	*Familial hyperlipidemia pts with positive CACS have decreased flow-mediated dilation and increase CRP. *Hs-CRP was the only independent predictor of the presence of CAC.
12	Wilund (2008, 31) – Metabolism	Case-control	Pts 60-80 yrs with no prior CAD / Assess relation between cardiorespiratory fitness, CAC, and BMD	13 athletes, 12 controls; 6 (46% women); race N/A	Raw CAC score and logCAC via Agatston	CRP	*Chronic exercise may simultaneously inhibit CAC and increase BMD. *CRP was not significantly correlated to logCAC (Pearson -.151)
13	Erbel (2008, 16) -	Prospective	Participants in the Heinz	4066; 2148	Raw CAC score	CRP	*Adding CAC and hs-CRP to

	Atherosclerosis	cohort – Heinz Nixdorf Recall Study	Nixdorf Recall study / estimate the sex dependent effect of risk factors and CAD on the presence of CAD, calculate RR for history of CAD, evaluate effect of CAD and CRP on NCEP ATP III class	(53%) women; race N/A	via Agatston by tertile and binary (75^{th} percentile)		NCEP ATP risk changed distribution of considerably with strong differences between sexes. *although statistically significant ($p<0.0001$), the correlation between CAC and hs-CRP was weak ($R^2=0.02$).
14	Ramadan (2008, 32) Circulation J	Cross-sectional	Asymptomatic Middle Eastern Egyptians 40-59 yrs with intermediate risk / Assess relation between CAC and FMD, NMD, CRP, IL-6, and OxLDL	177; 74 (42%) women; 100% Egyptian	Raw CAC score via Agatston by tertile	CRP	*FMD correlated negatively with CAC *IL-6 was correlated with CAC *CRP was not correlated with CAC
15	Michos (2009, 33) – Preventive Cardiology	Cross-sectional - MESA	Asymptomatic people without known CAD ≥70 yrs/ assess relation of risk factors with CAC	1824; 949 (52%) women; white, black, Chinese and Hispanic - % not specified	Binary CAC score via Agatston ($<25^{th}$ percentile)	CRP	*No association CRP and CAC
16	Pratte (2009, 34) – J Diabetes and it's Complications	Cross-sectional – CACT1	Diabetes type I with no known CAD aged 19-56 yrs / assess relation between PAI-1 and CAC	1253 (560 T1DM, 693 non-T1DM); 640 (51%) women; race N/A	Binary CAC score via Agatston	PAI-1, CRP, fibrinogen	*PAI-1 was significantly associated with the presence of CAC (OR 1.24, 95% CI 1.13-1.36, P<.001); significant post-adjustment. *CRP associated with CAC in non-T1DM. Fibrinogen associated with CAC in T1DM.
17	Jenny (2010, 35) – Atherosclerosis	Cross-sectional - MESA	Asymptomatic people without known CAD / assess relation between inflammatory markers and CAC	6783; 3578 (53%) women; 39% white, 28% black, 12% Chinese, 22% Hispanic	Binary (0 vs. >100) CAC score via Agatston	CRP, fibrinogen	* CRP, IL-6, fibrinogen were weakly associated with CAC
18	Qasim (2011, 17) – Clin Endocrinol	Cross sectional – Penn	Pts at risk for CAD (FH or T2DM) aged 35-75 yrs	1299 PDHS – T2DM, 860	Tobit regression of Ln(CAC + 1)	CRP	*female gender was associated with higher plasma

		Diabetes Heart Study and Study of Inherited Risk of Coronary Atherosclerosis	/ assess whether CRP has gender differences in association with CAC in DM and non-DM pts	SIRCA – non-DM; 883 (41%) women; 75% white, 20% black	score via Agatston		CRP in DM and non-DM pts *in DM and non-DM women, CRP was associated with higher CAC
19	Freitas (2011, 36) – Atherosclerosis	Cross-sectional	Healthy, asymptomatic individuals between 80 and 102 years / assess relation between mediators of inflammation and subclinical CVD	178; 140 (79%) women; race N/A	Binary (>100) and raw CAC score via Agatston	CRP, fibrinogen	*Those above the 75th percentile for CRP had higher CAC scores
20	Blaha (2011, 37) – ATVB	Cross-sectional - MESA	Asymptomatic people without known CAD / assess relation between obesity, high hsCRP (≥2 mg/dl), and CAC/cIMT	6760; 53% female; 39% white, 27% black; 12% Chinese; 22% Hispanic	Binary (>0) and raw CAC score via Agatston	CRP	*hsCRP not associated with CAC when anthropomorphic variables were included *when hsCRP and obesity were both present, no evidence of multiplicative interaction
21	Gupta (2012, 38) – J Am Coll Cardiol	Cross-sectional – Dallas Heart	Asymptomatic people without known CAD / assess if relation between CRP and atherosclerosis (i.e. CAC) differs by BMI	2899 (2685 with CAC measurement); 1600 (55%) women; 31% white, 50% black, 17% Hispanic	Binary CAC (>10) score via Agatston	CRP	*Increasing CRP was associated with increased CAC prevalence in normal and overweight men and normal women, but not obese men or women

TABLE 2. Characteristics of Studies Examining Novel Cardiovascular Risk Factors with CAC Progression.

Study #	1st Author (Year, reference) - Journal	Study Design	Population / Aim	Sample	CAC Measure(s)	Novel CV Risk Factor	Major Findings
22	Anand (2007, 39) – J Am Coll Cardiol	Prospective cohort	T2DM 30-65 yrs without CHD – Assess relation between CV risk factors, select novel risk factors and CAC progression	398; 154 (39%) women; 21% white, 55% South Asian, 23% Afro-Carib.	Difference in sq root transformed raw CAC score via Agatston; progression 2.5 yrs; >2.5 mm^3 significant	CRP	*Baseline CAC and suboptimal glycemic control of strong risk factors for progression. *CRP was not a predictor of CAC progression.
23	Green (2009, 40) – Atherosclerosis	Prospective cohort - CARDIA	Healthy black and white adults aged 18-30 yrs / assess relation of hemostatic factors to subclinical atherosclerosis 13 years later	1396; 753 (54%) women; 62% white, 38% black	Binary (>0) CAC score via Agatston	CRP, fibrinogen	*increasing quartiles of fibrinogen were associated with CAC and cIMT 13 years later *did not assess CRP to CAC
24	Saremi (2009, 41) – Atherosclerosis	Cross-sectional – Veterans Affairs Diabetes Trial (VADT)	Age >40 yrs with T2DM enrolled in Veterans Affairs Diabetes Trial / assess relation between CRP, IL-6, and Lp-PLA2	306;17 (6%) women; 66% non-Hispanic white	Raw CAC score and ln(CAC + 1) via Agatston	CRP	*IL-6 remained significantly associated with CAC S, particularly in those with lower abdominal artery calcium. *CACS did not increase with CRP and Lp-PLA2.
25	Green (2010, 42) – J Thrombosis & Hemostasis	Prospective cohort – Coronary Artery Risk Development in Young Adults (CARDIA)	Healthy black and white adults aged 18-30 yrs / assess relation of fibrinogen to subclinical atherosclerosis	2969 for longitudinal; 1636 (55%) women; 56% white, 44% black	Binary (>0) CAC score via Agatston	CRP, fibrinogen	*higher levels of fibrinogen during young adulthood are positively associated with incidence of CAC *fibrinogen increased 22% over 13 yrs
26	Rodrigues (2010, 43) – Atherosclerosis	Prospective cohort – CACT1	Diabetes type I with no known CAD / assess if fibrinogen predicts CAC in adults with type 1 diabetes	546 T1DM, 640 controls; 650 (55%) women; race N/A	Difference in sq root transformed raw CAC score via Agatston; progression 2.4 yrs; >2.5 mm^3 significant	fibrinogen	*higher levels of fibrinogen predicted CACS progression in T1DM, independent of standard CV risk factors

TABLE 3. Characteristics of Studies Examining Novel Cardiovascular Risk Factors with Coronary Artery Calcification (CAC) and CAC Progression in Subjects with Established Cardiovascular Disease, Significant Co-Morbid Illnesses, and/or Without Specific Assessment of Risk Factors with CAC.

Study #	1st Author (Year, reference) - Journal	Study Design	Population / Aim	Sample	CAC Measure(s)	Novel CV Risk Factor	Major Findings
27	Meng (2002, 44) – Am Heart J	Cross-sectional –ALIVE	African-American men aged 25-45 yrs with HIV / Assess relation of HIV PIs on SCD	98; 29 (30%) women; 100% black	Raw CAC score via Agatston	CRP	*The use of PIs are associated with CAC. *no systematic assessment of CRP to CAC
28	Meng (2003, 45) – Int J Cardiol	Cross-sectional – ALIVE	African-American men aged 25-45 yrs with cocaine use / Assess relation of CRP and endothelial function with CAC, lipid profile, and cardiac changes	53; 16 (30%) women; 100% black	Raw CAC score via Agatston	CRP	*Elevated CRP is associated with CAC.
29	Lai (2003, 46) – AIDS Patient Care	Cross-sectional – AIDS Links to the Intravenous Experience (ALIVE) study	African-American men aged 25-45 yrs with HIV / Assess relation of individual protease inhibitors on CV risk factors	98; 29 (30%) women; 100% black	Raw CAC and binary (>5) CAC presence via Agatston	CRP	*Those taking nelfinavir, ritonavir, or saquinavir were more likely to have higher CACS than those on non-PI regimens. *no systematic assessment of CRP to CAC
30	Grahame-Clarke (2003, 47) – Circulation	Cross-sectional	Cohort of DM and non-DM / Assess relation of anti-IgG antibodies to CMV and HSV to endothelial dysfunction	400; 194 (51%) women; race not specified	CACS via Colhoun	CRP	*CMV-seropositive individuals have endothelial dysfunction. *no systematic assessment of CRP to CAC
31	Tong (2004, 48) – Am J Cardiol	Cross-sectional	African-American men aged 25-45 yrs with HIV or cocaine use / Assess relation of LV mass and CAC	159; 52 (33%) women; 100% black	Binary (>0) CAC presence via Agatston	CRP	*LV mass indicies were larger in CAC-positive groups than CAC-negative groups. *no systematic assessment of CRP to CAC
32	Reilly (2004, 49) – Circulation	Cross-sectional - SIRCA	Non-DM 30-75 yrs with FH of premature CVD but no CAD / Assess relation of insulin resistance and metabolic syndrome with CAC	840; 397 (47%) women; 95% white	Ln (CAC + 1) via Agatston, Ordinal reg. by quintiles	CRP	*Metabolic syndrome and homeostasis model assessment were associated with CAC, independent of traditional risk factors. *no systematic assessment of

							CRP to CAC
33	Deo (2004, 50) – J Am Coll Cardiol	Cross-sectional – Dallas	Pts 30-65 yrs without prior CVD / Assess relation between monocyte chemoattractant protein-1 and CAC	3499; 1915 (55%) women; 52% black, 29% white, 17% Hispanic	Binary (>10) CAC presence via Agatston	CRP	*no systematic assessment of CRP to CAC
34	Ferramosca (2005, 51) – Am Heart J	Randomized trial	ESRD on HD / Assess sevelamer vs. calcium acetate on CAC progression at 1 yr and risk factor measurements	108; 38 (35%) women; 36% black, 64% non-black	Raw CAC score via Agatston	CRP	*CACS did not progress in sevelamer group but did in Ca-acetate groiup (P<0.001). *CRP did not change significantly. *no systematic assessment of CRP to CAC
35	Burdon (2006, 52) – Am Heart J	Cross-sectional – Diabetes Heart Study	Siblings concordant for T2DM without renal dysfunction / Assess relation between CD40 gene and CAC	620; 345 (56%) women; 100% white	Ln (CAC) via modified Agatston	CRP	*Genetic variation in CD40 is associated with CAC in diabetic families. *no systematic assessment of CRP to CAC
36	Steffes (2006, 53) – Obesity	Cross-sectional – CARDIA	Healthy black and white adults aged 18-30 yrs / assess relation of adiponectin to CV risk factors and CAC	2483; 1348 (40%) women; 43% black, 57% white	Binary (>0) CAC presence via Agatston	CRP	*Adiponectin correlated positively with CAC. *no systematic assessment of CRP to CAC
37	Anand (2006, 54) – J Am Coll Cardiol	Cross-sectional	T2DM / Assess relation of CRP, IL-6, osteogrotegerin with CAC and near-term CV events	510; 201 (39%) women; 22% white, 54% Asian; 23% black	Raw CAC score via Agatston by quintiles	CRP	*Osteoprotegerin predicted CAC. *CRP was not related to extent of CAC.
38	Jung (2006, 55) – Nephrology Dialysis Transpl	Prospective cohort	>18 yrs on HD / Assess relation of CAC progression over 2 yrs with risk factors	40; 14 (35%) women; 100% Korean	Difference in sq root transformed raw CAC score via Agatston; progression 2 yrs; >2.5 mm^3 significant	CRP	*Degree of CAC progression was associated with the time-integrated level of CRP.
39	Coutinho (2007, 56) – Am J Hypertension	Cross-sectional	Pts with ≥2 siblings with HTN before age 60 yrs /	1107; 657 (59%)	Log (CAC + 1) score via	CRP, fibrinogen	*Serum UA was associated with 10-year probability of

			Assess relation of serum uric acid with 10-yr probability of CAD and CAC	women; race not specified	Agatston		CHD, metabolic syndrome, log CRP, and fibrinogen. *no systematic assessment of CRP to CAC
40	Reilly (2007, 57) – J Investigative Med	Cross-sectional - SIRCA	Non-DM 30-75 yrs with FH of premature CVD but no CAD / Assess relation of IL-6 and TNFR2 with metabolic syndrome, CRP, and CAC	875; 402 (46%) women; 95% white	Raw CAC score and log (CAC + 1) via Agatston	CRP	*IL-6 and TNFR2 were independently associated with CAC. *no systematic assessment of CRP to CAC
41	Mehta (2007, 58) – Hypertension	Cross-sectional - Dallas Heart	Pts 30-65 yrs without prior CVD / Assess relation between LVH and CAC and CRP	2633; sex not specified; ≈50% black, ≈30% white, ≈20% Hispanic	Binary (>10) CAC score and log (CAC + 1) via Agatston	CRP	*LV wall thickness is associated with CAC, but not CRP in multivariable adjusted model. *no systematic assessment of CRP to CAC
42	Nettleton (2007, 59) – Am J Clin Nutrition	Cross-sectional – MESA	Pts 45-84 yrs free of CVD at baseline / Assess relation between diet and CVD	5089; ≈50% women	Binary (>0) CAC presence via Agatston	CRP, fibrinogen	*Subtle differences in dietary pattern composition affect associations with markers of SCD. *no systematic assessment of CRP or fibrinogen to CAC
43	Lutsey (2007, 60) – Br J Nutrition	Cross-sectional - MESA	Pts 45-84 yrs without CVD / Assess relation between whole grain intake, CVD risk factors and measures of SCD	5496; ≈50% women	Binary (>0) CAC presence via Agatston	CRP	*whole grain intake was unrelated to subclinical CVD *no systematic assessment of CRP to CAC
44	Lakoski (2007, 61) – Atherosclerosis	Cross-sectional – MESA	Pts 45-84 yrs without CVD / estimate the proportion of intermediate-risk participants who might be reclassified as high risk based on CRP or CACS	1450 intermediate risk; 195 (8%) women; 31% white, 30% Chinese, 29% black, 30% Hispanic	Binary (>100) CAC presence via Agatston, as well as age-gender-specific 75th percentile	CRP	*Differences in reclassification was based on the screening test used, cut points selected, and demographics of the individual. *no systematic assessment of CRP to CAC
45	Mohler (2007, 62) – J	Prospective	Pts with moderate-to-	61; sex and	N/A	CRP	*No significant reduction in

	Heart Valve Disease	cohort	severe aortic stenosis / assess treatment with statin on CAC and AC	race N/A			calcification with statin treatment. *Baseline CRP predicted CAC progression.
46	Dotsenko (2007, 63) – J Thrombosis and Hemostasis	Nested substudy of Peripheral artery disease in South Asian and European men with CAD (PARSEC)	Men >45 years from European and South Asia / Assess relation between platelet/leukocyte activation and atherosclerosis, also between races	54; 0% women; 56% white, 44% South Asian	Raw CAC score (using proprietary softward from Philips MxView) by tertiles	CRP	*Increased PMC are related to aortic and femoral, but not coronary or carotid, atherosclerosis. CRP related to PMC. *no systematic assessment of CRP to CAC
47	Reiner (2007, 64) – ATVB	Prospective cohort - CARDIA and Cardiovascular Health Study (CHS)	Pts 18-30 yrs / assess relation between USF1 tagSNPs, CVD risk factors, and aging-related phenotypes	1875 CARDIA, 4536 CHS; sex N/A; 100% white	Not described	CRP	*Complex relationship between USF1 genotype, atherosclerosis phenotypes, and CVD risk. *no systematic assessment of CRP to CAC
48	Nettleton (2008, 65) – Am J Clin Nutrition	Cross-sectional – MESA	Pts 45-84 yrs free of CVD at baseline / Assess relation between diet and CVD	5042; 2601 (52%) women; white, black, Chinese and Hispanic - % not specified	Raw CAC and binary (>0) CAC presence via Agatston	CRP, fibrinogen	*The Comprehensive Healthy Dietary Pattern was associated with favorable changes in a variety of CVD risk factors. *no systematic assessment of CRP to CAC
49	Qasim (2008, 66) – J Am Coll Cardiol	Cross-sectional - SIRCA	Non-DM 30-75 yrs with FH of premature CVD but no CAD / Assess relation between adiponectin and leptin with CVD risk factors and CAC	860; 403 (47%) women; 95% white	CAC score via Agatston; tobit regression of ln (CAC + 1) and binary (70^{th} percentile)	CRP	*Leptin was a significant predictor of CAC *no systematic assessment of CRP to CAC
50	Chung (2008, 67) – Arthritis & Rheumatism	Cross-sectional	Pts >18 yrs with rheumatoid arthritis / Assess relation between decreased insulin sensitivity and inflammation, by studying SLE and RA	103 SLE (73 women, 89% white), 124 RA (91% women, 68 white)	Raw CAC score via Agatston	CRP	*RA and SLE are associated with insulin resistance, but different mechanisms *no systematic assessment of CRP to CAC

51	Kao (2008, 68) – Am J Cardiology	Case-control	Non-DM women with SLE and RA / assess rates of CAC relative to healthy controls and investigate relation to inflammatory markers	157 SLE, 181 RA, 157 controls; 100% women, 96% white	Raw CAC score by quartile and binary (>0) CAC presence via Agatston	CRP, fibrinogen	*Women with SLE/RA have increased odds of CAC compared with controls *the highest CRP quartile was associated with CAC outcomes
52	Solus (2008, 69) – Arthritis & Rheumatism	Case-control	Pts >18 yrs with rheumatoid arthritis / Assess relation between NT-proBNP and CAC and inflammation	159 RA, 88 controls; 165 67% women; 87% white	Raw CAC score via Agatston	CRP	*NT-proBNP is not associated with CACS *no systematic assessment of CRP to CAC
53	Elkeles (2008, 70) – Eur Heart J	Prospective cohort – Prospective evaluation of diabetic ischaemic heart disease by computed tomography (PREDICT)	Pts with T2DM aged 50-75 yrs recruited from an outpatient clinic / assess relation between CACS, events, and risk factors	589; 216 (37%) women; 71% white; 20% Asian Indian	Raw CAC via Agatston as continuous and by quintile	CRP	*CACS is a powerful predictor of CV events *no systematic assessment of CRP to CAC
54	Hosseinsabet (2008, 71) – Cardiology Journal	Cross-sectional	Pts referred for CABG / assess relation between CRP and CACS	143; 26% women	Log(CAC + 1) via Agatston	CRP	*no relation between CRP and CACS
55	Michos (2009, 72) – Calcified Tissue International	Cross-sectional; Amish Family Calcification	Old Order Amish people / assess relation between serum 25(OH)D levels and CAC, cIMT, or CRP	654; 370 (57%) women; 100% white Amish	Raw CAC score and log(CAC + 1) via Agatston	CRP	*No association between 25(OH)D and CAC, cIMT, or CRP. *no systematic assessment of CRP to CAC
56	Tanaka (2009, 73) – Atherosclerosis	Cross-sectional	Pts with suspicion of CAD who received angiography / assess relation between CV risk factors and characteristics on angiography	424; 137 (32%) women; race N/A but probably 100% Japan.	Binary (≥400) CAC score and log(CAC + 1) via Agatston	CRP	*Positive remodeling by CT correlates to an increase in CRP.
57	Fensterseifer (2009, 74) – Nephrology	Cross-sectional	Hemodialysis for >3 months, age >18 yrs, sinus rhythm / assess CACs in HD pts and correlate	59; 24 (41%) women; 76% white	Raw CAC score via Agatston by tertiles	CRP	*CAC detected in 64.5% of pts. *Correlation between CACS and advanced age, but not

			CACS with clinical parameters and mortality				mortality. *Trend towards higher CRP in severe CACS
58	Gutiérrez (2009, 75) – Circulation	Case-control	Pre-dialysis chronic kidney disease / assess relation between FGF-23 and LVH and CAC	162 CKD, 58 controls; 76 (35%) women; 30% black	Binary (≥100) and raw CAC score via Agatston	CRP	*FGF-23 is independently associated with LV mass index and LVH in patients with CKD, but not CAC *no systematic assessment of CRP to CAC
59	Shea (2009, 76) – Am J Clin Nutrition	Randomized controlled trial	Men or post-menopausal women aged 60-80 yrs; Determine effect of vitamin K1 supplementation on CAC progression (after 3 yrs) in older adults	388; 235 (61%) women; 93% white	Raw CAC score via Agatston; change in CACS between 1st and 2nd CT (after 3 yrs)	CRP	*Vitamin K1 supplementation slows CAC progression in older adults with pre-existing CAC. *no systematic assessment of CRP to CAC
60	Ohtake (2010, 77) – Hemodialysis International	Cross-sectional and prospective cohort	Hemodialysis patients / evaluate risk factors for CAC and impact of CAC on CV events in HD pts	74 cross-sectional, 56 prospective cohort; 27 (36%) women; Japanese?	Raw CAC score via Agatston; change in CACS between 1st and 2nd EBCT (15 months)	CRP, fibrinogen	*CRP and fibrinogen associated with baseline CAC and CAC progression in the univariate analyses; CRP associated with baseline CAC and CAC progression in multiple regression analysis
61	Park (2010, 78) – Respiratory Med	Cross-sectional	Korean males without CVD participating in annual medical check-up program / assess relation between decreased lung function and CAC and other variables	4905; 0% women; 100% Korean	Raw CAC score via Agatston	CRP	*Metabolic syndrome, insulin resistance, coronary atherosclerosis, and systemic inflammation are closely related to the impaired lung function. *no systematic assessment of CRP to CAC
62	Roe (2010, 79) – Atherosclerosis	Prospective cohort	Renal transplant recipients / assess relation between inflammatory markers and CAC; also CAC and CV events	112; 48% women; 63% white	Raw CAC score via Agatston by quintiles	CRP	*CRP was predictive of CAC severity at time of transplant *CAC is predictive of CV events and mortality
63	Rodrigues (2010, 80) – Diabetes Technol Therapeutics	Prospective cohort – Coronary Artery	Diabetes type I with no known CAD aged 19-56 yrs / assess relation between heart rate	1416; 764 (54%) women; race N/A	Difference in sq root transformed raw CAC score via Agatston;	CRP, fibrinogen	*reduced HRV predicted progression of CAC in adults with and without T1D *only univariate comparison

		Calcification Type 1 Diabetes Study (CACT1)	variability and CAC progression		progression 6.0 yrs; >2.5 mm^3 significant		of CRP and fibrinogen to CAC progression
64	Sposito (2011, 81) – Atherosclerosis	Cross-sectional – Brasilia Heart	STEMI / assess frequency that STEMI pts would be candidates for intensive lipid-lowering therapy based on current guidelines	355; 25% women; race N/A	CACS via Agatston	CRP	*more than half would not be candidates for intensive lipid lowering therapy by current clinical algorithms *no correlation between CRP and CAC
65	Banks (2011, 82) – JACC: Cardiovasc Imaging	Cross-sectional – Dallas Heart	Women 30-65 yrs / assess relation between angina and SCA and assess factors associated with angina in women without CAC	1480; 100% women; 30% white, 49% black, 20% Hispanic	Binary (>10) CAC score via Agatston	CRP	*angina is not associated with CAC * no difference in CRP for women with and without angina
66	Salem (2011, 83) – Acta Diabeologica	Cross-sectional	Adolescents 12-18 yrs with DM type I / assess relations between CAC and hsCRP, dyslipidemia, glycemic control, and microvascular complications	60 patients, 60 controls; 30 (50%) girls; race N/A	Raw CAC score via Agatston by quartile	CRP	*12 pts (20%) with DM have CAC *hsCRP was higher in CAC-positive DM pts compared to CAC-negative DM pts, not significant
67	Mathews (2011, 84) – Am J Nephrol	Prospective cohort - feasibility	Chronic hemodialysis patients with CACS >50 / assess the feasibility and safety of sodium thiosulfate, and potential efficacy on CAC and AC	22; 36% women; 86% black	Difference in sq root transformed raw CAC score via Agatston; progression 5.0 mos; >2.5 mm^3 significant	CRP	*STS is safe and feasible *no systematic assessment of CRP to CAC
68	Möhlenkamp (2011, 85) – J Am Coll Cardiol	Prospective cohort – Heinz Nixdorf Recall Study	Healthy volunteers between 45 and 75 years / assess the value of hsCRP and CAC in addition to traditional factors in predicting incident CV events	4814; 2096 (44%) women; race N/A	Log(CAC + 1) score via Agatston, and CAC by quartile	CRP	*hsCRP appears to improve CV risk prediction especially in persons with very low CAC scores *no systematic assessment of CRP to CAC
69	Turkmen (2011, 86) – Clin J Am Soc Nephrol	Cross-sectional	ESRD receiving PD or HD for ≥6 months / assess relation between epicardial	80 ESRD,31 (39%) women; 27	Binary (>10) and raw CAC score via Agatston	CRP	*significant relationship between EAT and components of MIAC syndrome in ESRD

			adipose tissue (EAT) and malnutrition, inflammation, atherosclerosis / calcification (MIAC)	control; race N/A			pts *no systematic assessment of CRP to CAC
70	Blaha (2011, 87) – Lancet	Prospective cohort – MESA	JUPITER pop.: men ≥50 yrs, women ≥60 yrs, LDL <3.37 mmol/L,no lipid therapy, no DM, TRIG <5.65 mmol/L, Cr <176.8 μmol/L, hsCRP ≥2 mg/L	950; 486 (51%) women; 41% white, 31% black, 5% Chinese, 23% Hispanic	Binary (>0) and raw CAC via Agatston	CRP	*CAC further stratifies JUPITER pts *increased CAC burden led to similar increases in absolute CHD and CVD events in both the low (<2) and high (≥2) hsCRP groups *no systematic assessment of CRP to CAC
71	Baker (2011, 88) – J Rheumatology	Case cohort	Non-pregnant women with SLE / assess relation between resistin and systemic inflammation, disease measures and CAC	159 lupus, 70 control; 100% women; 39% white; 51% black	Binary (>0) CAC score via Agatston	CRP	*higher resistin level correlated with CRP *SLE with CAC had higher levels of resistin than SLE without CAC *no systematic assessment of CRP to CAC
72	Cao (2011, 89) – Cardiology	Cross-sectional	Symptomatic people with suspected CAD and eGFR 30 mL/min/1.73 m^2 or greater / assess relations between kidney function and CAC	1572; 439 (28%) women; race N/A, but likely high in Chinese	Raw CAC score via Agatston	CRP	*kidney dysfunction was an independent predictor of CAC *no systematic assessment of CRP to CAC
73	Huang (2012, 90) – Atherosclerosis	Cross-sectional from randomized trial	Asymptomatic women age 42-58 years randomized to placebo or hormone therapy / assess relation between fat deposition with CVD risk factors & CAC	650; 100% women; 74% white	Binary (>0) CAC score via Agatston	CRP	*hepatic fat is associated with CRP and insulin *cardiac fat is associated with CAC *no systematic assessment of CRP to CAC
74	Martin (2012, 91) – Obesity	Cross-sectional	Asymptomatic people with BMI 25 or greater / assess relation between leptin and CAC, in high and low CRP	1460; sex N/A, race N/A	CAC score N/A	CRP	*leptin associated with increased CAC in the presence of high CRP levels *no systematic assessment of CRP to CAC

75	Zhang (2012, 92) – Clinical Cardiology	Cross-sectional	Men presenting for routine medical checkup in Korea / assess relation between bilirubin and CAC	3408; 0% women; race N/A, but likely high in Korean	Raw CAC score, presumed Agatson (not specified), by quintiles and log(CAC + 1)	CRP	*inverse relation between CAC and CRP *no systematic assessment of CRP to CAC
76	Yeboah (2012, 93) – JAMA	Prospective cohort – Multi-Ethnic Study of Atherosclerosis (MESA)	Asymptomatic people without known CAD or DM / predict incident CVD with 6 novel risk markers	1330 intermediate risk; 443 (33%) women; 36% white, 22% black, 16.9% Chinese, 25% Hispanic	Ln(CAC + 1) score via Agatston	CRP	*CAC superior to other risk markers *no systematic assessment of CRP to CAC
77	Park (2012, 94) – Atherosclerosis	Retrospective cohort	Asymptomatic people without known CAD / all CV events by plaque type	4690; 1892 (40%) women; race N/A, but likely high in Korean	Raw CAC score via Agatston by quartiles	CRP	*hsCRP >3 mg/L was an independent predictor of adverse CV events with non-calcified plaques after adjusting for FHS and CACS *did not assess CRP to plaque presence or type

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