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Determinants of Progression of Coronary Artery Calcification in Type 2 Diabetes

Role of Glycemic Control and Inflammatory/Vascular Calcification Markers

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Objectives This study prospectively evaluated the relationship between cardiovascular risk factors, selected biomarkers (high-sensitivity C-reactive protein [hs-CRP], interleukin [IL]-6, and osteoprotegerin [OPG]), and the progression of coronary artery calcification (CAC) in type 2 diabetic subjects.

Background Coronary artery calcification is pathognomonic of coronary atherosclerosis. Osteoprotegerin is a signaling molecule involved in bone remodeling that has been implicated in the regulation of vascular calcification and atherogenesis.

Methods Three hundred ninety-eight type 2 diabetic subjects without prior coronary disease or symptoms (age 52 ± 8 years, 61% male, glycated hemoglobin [HbA_1c] 8 ± 1.5) were evaluated serially by CAC imaging (mean follow-up 2.5 ± 0.4 years). Progression/regression of CAC was defined as a change ≥2.5 between the square root transformed values of baseline and follow-up volumetric CAC scores. Demographic data, risk factors, glycemic control, medication use, serum hs-CRP, IL-6, and plasma OPG levels were measured at baseline and follow-up.

Results Two hundred eleven patients (53%) had CAC at baseline. One hundred eighteen patients (29.6%) had CAC progression, whereas 3 patients (0.8%) had regression. Age, male gender, hypertension, baseline CAC, HbA_1c >7, waist-hip ratio, IL-6, OPG, use of beta-blockers, calcium channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, statins, and Framingham/UKPDS (United Kingdom Prospective Diabetes Study) risk scores were univariable predictors of CAC progression. In the multivariate model, baseline CAC (odds ratio [OR] for CAC >400 = 6.38, 95% confidence interval [CI] 2.63 to 15.5, $p < 0.001$), HbA_1c >7 (OR 1.95, CI 1.08 to 3.52, $p = 0.03$), and statin use (OR 2.27, CI 1.38 to 3.73, $p = 0.001$) were independent predictors of CAC progression.

Conclusions Baseline CAC severity and suboptimal glycemic control are strong risk factors for CAC progression in type 2 diabetic subjects. (J Am Coll Cardiol 2007;50:2218–25) © 2007 by the American College of Cardiology Foundation

Coronary artery calcification (CAC) is pathognomic of coronary atherosclerosis. In recent years, there has been considerable interest in CAC for several reasons. It is a characteristic that can be noninvasively and reproducibly quantified at a population level. Furthermore, multiple large cross-sectional and prospective studies have confirmed that it can be treated as an independent risk factor for improving cardiovascular risk prediction beyond that provided by conventional risk factors (1–3). More recently, some studies suggest that serial assessment of CAC scores might be helpful in monitoring the evolution of coronary atherosclerotic plaque and assessing the effectiveness of medical therapies for reducing cardiovascular risk (4).

Type 2 diabetes is associated with accelerated atherosclerosis and considered to be a coronary heart disease (CHD) equivalent (5). Experimental, histopathological, and clinical studies have shown that it affects the process of atherogenesis at multiple levels, increasing the risk of atherothrombotic clinical sequelae (6). At this time, however, there are

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few studies specifically focusing on CAC in this important patient subgroup. However, recent data suggest that there is a substantial prevalence of CAC, even in type 2 diabetic subjects who are asymptomatic (7,8), and that CAC predicts both abnormal myocardial perfusion and incident cardiovascular disease.

In this article, we provide the first prospective report of the progression of CAC specifically in type 2 diabetic subjects without prior CHD or symptoms at baseline. Additionally, we report on the association between both established cardiovascular risk factors as well as 3 selected biomarkers (high-sensitivity C-reactive protein [hs-CRP], interleukin [IL]-6, and osteoprotegerin [OPG]) and the progression of CAC. High-sensitivity CRP and IL-6 are markers of systemic inflammation, whereas OPG is a signaling molecule involved in bone remodeling that has recently been implicated in the regulation of vascular calcification and atherogenesis (9). Osteoprotegerin is a member of the tumor necrosis factor superfamily that functions as a decoy receptor for the receptor activator of nuclear factor-kappa beta ligand preventing osteoclast activation/bone resorption (10). Osteoprotegerin is upregulated in calcified coronary plaques (11). Plasma OPG measurements have been correlated with angiographic disease severity and cardiovascular events independent of conventional risk factors in recent publications (12,13).

Methods

The study was approved by the local institutional ethics review committees, and all subjects provided written informed consent.

Patients. This is a planned extension of a prospective study of type 2 diabetic subjects without prior CHD that we have previously reported. In brief, 510 asymptomatic patients with type 2 diabetes were enrolled between August 2002 and February 2004. Study participants were recruited from 4 diabetes clinics in secondary care (Northwick Park and Central Middlesex Hospitals, London, United Kingdom). Inclusion criteria were: 1) type 2 diabetes >1 year duration; and 2) age between 30 and 65 years. Exclusion criteria were: 1) typical angina pectoris or anginal equivalent symptoms such as dyspnea on exertion; 2) history of positive stress test, myocardial infarction, heart failure, or coronary revascularization; 3) electrocardiographic evidence of Q-wave myocardial infarction, ischemic ST-segment or T-wave changes, or complete left bundle branch block; 4) prior history of peripheral vascular disease, intermittent claudication, stroke, or transient ischemic attack; and 5) renal impairment (creatinine >1.4 mg/dl) or serious life threatening illnesses. Prevalence of CAC was determined in this baseline cohort with electron beam computed tomography (EBCT). As part of the current study, subjects were then asked to attend for a follow-up assessment similar to the baseline study. In view of difficulties in accurately estimating progression of CAC in the presence of intracoronary stents and metallic clips used in coronary artery bypass grafting, patients who were revascularized in the intervening period were excluded from follow-up imaging. All other subjects were eligible for participation.

Measurements. CLINICAL ASSESSMENT. Demographic data, risk factors, microvascular disease, medication use, body mass index (BMI), waist/hip ratio (WHR), blood pressure, and the predicted 10-year absolute coronary heart disease risk based on the Framingham risk function (14) and UKPDS (United Kingdom Prospective Diabetes Study) risk engine (15) were recorded at follow-up with the same methods as the baseline assessment.

Abbreviations and Acronyms

CAC = coronary artery calcification
CHD = coronary heart disease
CI = confidence interval
EBCT = electron beam computed tomography
HbA_1c = glycated hemoglobin
hs-CRP = high-sensitivity C-reactive protein
IL = interleukin
IQR = interquartile range
LAD = left anterior descending coronary artery
LDL = low-density lipoprotein
OPG = osteoprotegerin
OR = odds ratio

BIOCHEMICAL MEASUREMENTS. Blood was drawn to determine DCCT (Diabetes Control and Complications Trial) aligned glycated hemoglobin (HbA_1c), lipid profile, urea, and creatinine. Serum hs-CRP (ICN Pharmaceuticals, Orangeburg, New York), IL-6 (R&D Systems Inc., Minneapolis, Minnesota), and plasma OPG levels (Biomedica, Vienna, Austria) (16) were measured by enzyme-linked immunosorbent assay (ELISA). Intra-assay and interassay coefficients of variation for hs-CRP, IL-6, and OPG measurements were 3.3% and 3.7%, 6.4% and 7.8%, and 5% and 7.5%, respectively. Urine samples were also obtained to determine the urine albumin/creatinine ratio.

CAC IMAGING. The EBCT scanner that was used in the baseline study was employed in this follow-up study to reduce interstudy variability (GE Imatron C-150, San Francisco, California). The same operators and workstation were also used. In brief, 40 contiguous 3-mm slices were obtained during a single breath-hold starting at the carina and proceeding to the level of the diaphragm. Scan time was 100 ms/slice, synchronized to 40% of the R-R interval. Agatston and volumetric calcium scores were calculated to quantify the extent of CAC by a single experienced investigator blinded to the clinical data on an Aquarius workstation (TeraRecon Inc., San Mateo, California). For determining progression, volumetric CAC scores were used in view of their superior reproducibility (17). The CAC scores were classified into 4 categories on the basis of their severity: 1 to 10 mm^3 (minimal), 11 to 100 mm^3 (mild), 101 to 400 mm^3 (moderate), and >400 mm^3 (severe).

Statistical analysis. Analyses were carried out with STATA version 8 (Stata Corp. LP, College Station, Texas) or SPSS version 12.0 (SPSS Inc., Chicago, Illinois). Con-

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tinuous variables were summarized as mean ± SD or median ± interquartile range (IQR). Variables were examined for a linear relationship with progression of CAC and categorized if necessary. Continuous variables were compared by the Mann-Whitney *U* test and categorical variables by the chi-square test. Bivariate correlations between biomarker values and extent of CAC were performed with Spearman rank correlation. A p value <0.05 (2-sided) was considered statistically significant.

DEFINITION OF PROGRESSION OF CAC. A square root transformation of the baseline and follow-up volumetric CAC measurements was made to stabilize the variance across all ranges of CAC (18). Significant progression/regression of CAC was defined as a change ≥2.5 between the square root transformed values of baseline and follow-up volumetric coronary calcium scores, because a change exceeding this magnitude most likely represents a real change in CAC rather than interscan variability.

Demographic and other clinical/laboratory variables of interest were then evaluated in a univariable logistic regression model to determine their relationship to progression of CAC. A backward selection procedure was applied to identify candidate variables for the multivariable model. Candidate variables included, from the pool of historical risk factors and laboratory variables, those univariable predictors with $p \leq 0.2$. For the multivariable model, only those variables with a $p < 0.05$ were retained for the final variable selection.

Results

Patients. Five hundred ten subjects were enrolled as part of the baseline study. Four patients died during follow-up (median volumetric CAC score = 961 [IQR: 531 to 3,481; range: 316 to 4,201 mm^3]). Sixteen subjects underwent coronary revascularization (median CAC = 680 [IQR: 314 to 1,324; range: 42 to 3,056 mm^3]) and were excluded from follow-up imaging. Of the remaining 490 subjects, 402 attended and completed the follow-up evaluation (response rate 82%). Table 1 summarizes the characteristics of those patients who returned for follow-up imaging versus those who did not. Apart from lower serum triglyceride and IL-6 levels in patients who returned for follow-up imaging, no other statistically significant differences were seen. Image quality was suboptimal in 4 patients, owing to breathing artefacts, and they were excluded from further analysis. Mean follow-up interval was 2.5 ± 0.4 years. Mean age was 52 ± 8 years, and 61% were men. Baseline and follow-up characteristics of the study population and details regarding medical therapy are outlined in Table 2. There was a high proportion of South Asians (n = 217 [54%]) compared with Caucasians (n = 82 [21%]) and Afro-Caribbeans (n = 88 [22%]), due to the geographical location of the study population and the high prevalence of type 2 diabetes in South Asians (Table 3). Among those who underwent follow-up imaging, 8 patients (median CAC at baseline = 491 [IQR 313 to 1,029; range 41 to 1,345 mm^3]) were hospitalized with either unstable angina or acute myocardial infarction during the follow-up period.

Table 1 Characteristics of Patients Who Attended Follow-Up Imaging Versus Those Who Did Not

Characteristic	Did Not Attend (n = 88)	Attended Follow-Up (n = 402)	p Value
Age (yrs)	53 (9)*	52 (8)*	0.36
Male gender (%)	48 (55%)	244 (61%)	0.23
Baseline volumetric CAC (mm^3)	0 (0–93.9)†	3.4 (0–57.9)†	0.87
Mean HbA_1c (%)	8.4 (1.7)*	8.1 (1.5)*	0.24
Mean total cholesterol (mg/dl)	191 (43)*	187 (35)*	0.07
Mean LDL cholesterol (mg/dl)	109 (34)*	105 (31)*	0.22
Mean HDL cholesterol (mg/dl)	47 (11)*	51 (16)*	0.12
Mean triglycerides (mg/dl)	195 (142)*	160 (89)*	0.002
hs-CRP (mg/l)	5.4 (1.7–8.5)†	4.2 (1.8–7.8)†	0.37
IL-6 (pg/ml)	2.6 (1.2–4)†	1.9 (1.2–3)†	0.01
OPG (pmol/l)	6.8 (5.3–11.6)†	6.6 (4.6–10.3)†	0.36
Current smoker (%)	18 (20%)	75 (19%)	0.93
BP >140/90 mm Hg or antihypertensive therapy (%)	64 (73%)	300 (75%)	0.40
BMI (kg/m^2)	28.9 (5)*	28.5 (5.1)*	0.49
WHR	0.95 (0.08)*	0.94 (0.07)*	0.14
Duration of diabetes (yrs)	8.3 (7)*	7.9 (6)*	0.53
Statin therapy (%)	32 (36%)	153 (38%)	0.80

*Mean (SD); †median (interquartile range).
BMI = body mass index; BP = blood pressure; CAC = coronary artery calcification; HbA_1c = glycated hemoglobin; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IL = interleukin; LDL = low-density lipoprotein; OPG = osteoprotegerin; WHR = waist/hip ratio.

Distribution of CAC. One hundred eighty-seven patients (47%) had no CAC at baseline. The severity of CAC was minimal in 36 patients (9%), mild in 93 patients (23%), moderate in 55 patients (14%), and severe in 27 patients (7%). Table 4 compares the distribution of patients in volumetric CAC score categories (No CAC, minimal [1 to 10], mild [11 to 100], moderate [101 to 400], severe [401 to 1,000], and extensive [>1,000] CAC) at baseline and follow-up and illustrates the number of patients who actually shifted risk categories during the course of the study.

Relationship between biomarkers and CAC severity at baseline/follow-up. At baseline, plasma OPG measurements were correlated with the extent of CAC ($r = 0.32$, $p < 0.0001$). In contrast, serum hs-CRP and IL-6 were not related to atherosclerotic plaque burden ($r = 0.02$, p = NS for hs-CRP, and $r = 0.09$, $p = 0.03$ for IL-6). The OPG measurements were related to CAC burden similarly at follow-up ($r = 0.24$, $p < 0.001$).

Progression of CAC. Mean CAC score change/year of follow-up was 16.1 (SD: 44.8) mm^3. Progression of CAC was observed in 118 patients (29.6%), whereas significant regression was seen only in 3 subjects (0.8%). Progression was most frequent in those with pre-existing CAC (Fig. 1A). Among those with no calcification at baseline, progression was relatively infrequent (12%, 22 patients). The absolute change in CAC was small in those with little pre-existing CAC but much greater in those with signifi-

Table 2 Baseline and Follow-Up Characteristics of Study Subjects

Variable	Baseline	Follow-Up
Volumetric CAC score (mm^3)	3.4 (0–57.9)*	13 (0–111.2)*
Mean HbA_1c (%)	8.1 (1.5)†	8 (1.3)†
Total cholesterol (mg/dl)	187 (35)†/183 (160–207)*	175 (31)/176 (152–195)*
LDL cholesterol (mg/dl)	105 (31)†/105 (87–125)*	97 (27)†/97 (76–113)*
HDL cholesterol (mg/dl)	51 (16)†/47 (40–55)*	51 (12)†/49 (42–58)*
Triglycerides (mg/dl)	160 (89)†/142 (93–196)*	151 (116)†/123 (82–189)*
Statin therapy (%)	153 (38%)	199 (50%)
Antihypertensive therapy (%)	194 (49%)	229 (58%)
Insulin alone (%)	31 (8%)	31 (8%)
Insulin + oral agent (%)	55 (14%)	87 (22%)
Oral agent (%)	288 (71%)	267 (66%)
Diet therapy (%)	28 (7%)	17 (4%)
Beta-blocker therapy (%)	30 (8%)	44 (11%)
Calcium channel antagonist (%)	72 (18%)	79 (20%)
ACE inhibitor/AT_2 antagonist (%)	152 (38%)	183 (46%)
Current smoker (%)	75 (19%)	43 (11%)
BMI (kg/m^2)	28.5 (5.1)†	29.4 (6.5)†
WHR	0.94 (0.07)†	0.95 (0.07)†
hs-CRP (mg/l)	4.2 (1.8–7.8)*	2.1 (1.1–5.1)*
IL-6 (pg/ml)	1.9 (1.2–3)*	NA
OPG (pmol/l)	6.6 (4.6–10.3)*	5.9 (4.1–8.2)*
Retinopathy (%)	88 (22%)	96 (24%)
Peripheral neuropathy (%)	73 (18%)	76 (19%)
Microalbuminuria (%)	60 (15%)	67 (17%)

*Median (interquartile range); †mean (SD).
ACE = angiotensin-converting enzyme; AT_2 = angiotensin 2; other abbreviations as in Table 1.

Table 3 Baseline Characteristics of Progressors/Nonprogressors

Variable	Progressors (n = 118)	Nonprogressors (n = 277)
Age (yrs)	54 (7.4)	52 (8.6)
Gender (% male)	67.8%	58.1%
Diabetes duration (yrs)	8 (4.9)	7.7 (5.8)
Systolic/diastolic BP (mm Hg)	140 (16)/85 (12)	135 (16)/83 (12)
Antihypertensive therapy (%)	72 (61%)	120 (43%)
Ever a smoker/current smoker (%)	41%/22%	38%/18%
BMI (kg/m^2)	28.6 (5.3)	28.5 (5)
WHR	0.95 (0.08)	0.93 (0.07)
Total cholesterol (mg/dl)	187 (35)	187 (35)
LDL cholesterol (mg/dl)	105 (27)	105 (31)
HDL cholesterol (mg/dl)	51 (14)	51 (20)
Triglycerides (mg/dl)	159 (98)	160 (89)
Statin therapy (%)	56 (47%)	96 (35%)
HbA_1c (%)	8.1 (1.3)	8.1 (1.6)
Glycemic control (HbA_1c >7%)	86 (73%)	204 (74%)
Median volumetric CAC at baseline	58.8 (6–215.2)	0 (0–20)
Baseline volumetric CAC >0	96 (81.4%)	112 (40%)
Ethnicity (%)		
Caucasian	26 (22%)	56 (20%)
South Asian	80 (68%)	137 (49%)
Afro-Caribbean	10 (8%)	78 (28%)
Other	2 (2%)	9 (3%)

Abbreviations as in Table 1.

cant pre-existing CAC (Fig. 1B). Lesions in the left anterior descending coronary artery (LAD) were more likely to progress (median [IQR] change in volumetric score in LAD: 28.8 [56] mm^3, in comparison with 0 [0], 8.7 [31], and 15.3 [47] mm^3 in the left main stem, left circumflex, and right coronary arteries, respectively [$p < 0.0001$]). Patients with numerous calcified lesions were more likely to progress (43 [72%] patients with >5 lesions in the LAD demonstrated CAC progression in comparison with 75 [41.4%] of those with ≤5 lesions in LAD [$p = 0.004$]).

Table 3 summarizes the baseline characteristics of subjects who demonstrated progression of CAC compared with those who did not. One hundred fifty-seven subjects did not have CAC at either the baseline or follow-up evaluation. Figure 2 illustrates the absolute changes in CAC scores between the baseline and follow-up evaluation in the remaining 241 subjects. Thirty subjects (16%) without baseline CAC progressed to minimal/mild CAC categories at follow-up, as shown in Table 4. No statistically significant differences were seen in risk-factor profiles/biochemical parameters between those patients who progressed from a 0 baseline CAC score and those who did not. Among those with minimal baseline CAC, 25 subjects (70%) progressed to mild/moderate CAC categories, whereas 2 subjects had a regression of CAC score to 0. Twenty-one subjects (23%) with mild baseline CAC progressed similarly to moderate/severe CAC categories at follow-up, whereas regression to minimal CAC was seen in 1 subject. Ten subjects (18%)

Table 4 Distribution of Volumetric CAC Scores at Baseline and Follow-Up

Baseline Volumetric CAC, mm^3	Follow-Up Volumetric CAC Score, mm^3					
	0	1–10	11–100	101–400	401–1,000	>1,000
0	157 (84%)*	16 (9%)	14 (7%)	0	0	0
1–10	2 (5%)	9 (25%)	24 (67%)	1 (3%)	0	0
11–100	0	1 (1%)	71 (76%)	20 (22%)	1 (1%)	0
101–400	0	0	0	45 (82%)	10 (18%)	0
401–1,000	0	0	0	0	11 (69%)	5 (31%)
>1,000	0	0	0	0	0	16

*Percentages are quoted for each coronary artery calcification (CAC) category.

with moderate baseline CAC and 5 subjects (31%) with severe baseline CAC progressed to severe and extensive CAC categories, respectively, at follow-up. None of the subjects with moderate/severe baseline CAC regressed to lower CAC categories.

Age (odds ratio [OR] 1.40, 95% confidence interval [CI] 1.07 to 1.84, p = 0.02); hypertension (OR 1.85, 95% CI 1.08 to 3.17, p = 0.03); baseline HbA_1c >7 (OR 1.84, 95% CI 1.09 to 3.13, p = 0.02); baseline volumetric CAC score (OR for CAC >400 mm^3 = 8.09, 95% CI 3.46 to 18.9, p <0.001); serum IL-6 (OR for log IL-6 = 1.71, 95% CI 1.02 to 2.9, p = 0.05); plasma OPG (OR for plasma OPG >9 pg/ml = 2.5, 95% CI 1.39 to 4.10, p = 0.004); use of statins (OR for statin-treated patients with low-density lipoprotein [LDL] cholesterol <100 mg/dl = 3.12, 95% CI 1.89 to 5.15, p < 0.001); beta-blockers (OR 2.18, 95% CI 1.15 to 4.42, p = 0.02); calcium channel antagonists (OR 2.26, 95% CI 1.36 to 3.76, p = 0.02); and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (OR 1.6, 95% CI 1.02 to 2.5, p = 0.04), Framingham (OR 1.43, CI 1.03 to 1.98, p = 0.03), and UKPDS risk scores (OR 1.78, 95% CI 1.27 to 2.47, p = 0.001) were univariable predictors of CAC progression.

Fifty-six percent of the study participants (n = 221) were treated with statins either at baseline or during the follow-up period. The relationship between statin use and follow-up lipid levels, hs-CRP, and OPG is shown in Table 5. Despite achieving lower lipid levels at follow-up, statin-treated patients had a greater degree of CAC progression (% of patients with progression = 38% [n = 85], absolute CAC score change/year = 25 [SD: 55] mm^3) in comparison with untreated patients (% of patients with progression = 19% [n = 33], absolute CAC change/year = 6 [SD: 23] mm^3). In the final multivariate analysis, baseline CAC (OR for CAC >400 mm^3 = 6.38, 95% CI 2.63 to 15.5, p < 0.001), HbA_1c >7 (OR 1.95, 95% CI 1.08 to 3.52, p = 0.03), and statin use (OR 2.27, 95% CI 1.38 to 3.73, p = 0.001) were independent predictors of CAC progression (Fig. 3).

Of the 8 patients who sustained an acute cardiac event during follow-up, 4 demonstrated CAC progression. We performed logistic regression analyses after reclassifying all patients who sustained an acute cardiac event during follow-up as "progressors." Male gender (OR 1.58, 95% CI 1.01 to 2.47, p = 0.05) and waist-hip ratio (OR [for 0.1-U increase] 1.4, 95% CI 1.04 to 1.87, p = 0.03) were additional univariable predictors of progression in this analysis. However, no significant changes were observed in the results of final multivariate analysis.

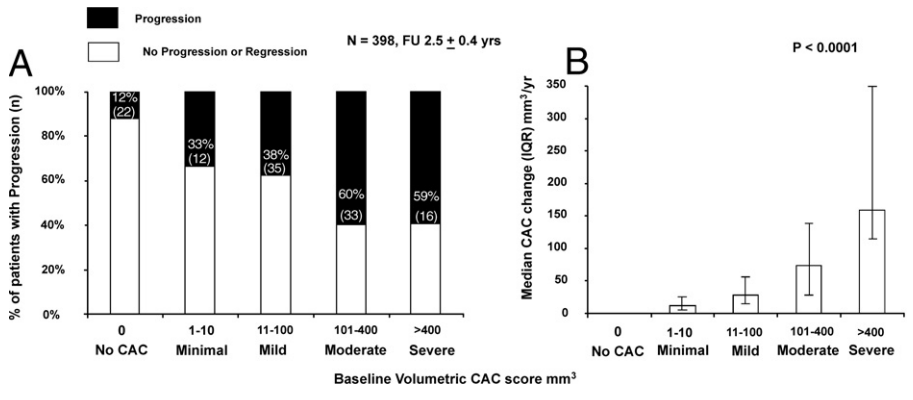

Figure 1 Relationship Between Baseline CAC Severity and CAC Progression

(A) Progression of coronary calcium according to baseline coronary artery calcification (CAC) severity. The number of patients with CAC progression is shown in **parentheses**. **(B)** Median change in volumetric CAC score (mm^3) according to baseline CAC severity. The **error bars** represent interquartile ranges (IQRs). FU = follow-up.

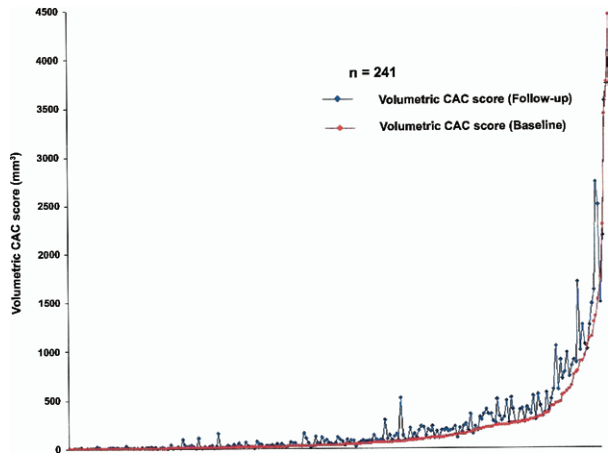

Figure 2 **Absolute Changes in CAC Scores Between the Baseline and Follow-Up Evaluation**

Absolute changes in coronary artery calcification (CAC) scores between the baseline and follow-up evaluation in 241 patients. The remaining 157 patients did not have calcification at the baseline and follow-up evaluation.

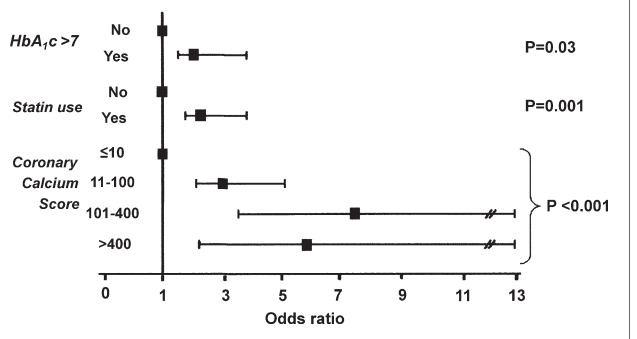

Figure 3 **Multivariate Predictors of Coronary Calcium Progression**

Multivariate predictors of coronary calcium progression. **Black squares** denote odds ratios, and the **horizontal lines** represent 95% confidence intervals. CAC = coronary artery calcification; HbA_1c = glycated hemoglobin.

Discussion

Background and previous work. We have previously reported on the prevalence and predictors of CAC in type 2 diabetic subjects without prior CHD (7). In that report, we verified that majority of the established risk factors (age, male gender, blood pressure, smoking, hyperlipidemia, waist-hip ratio) as well as duration of diabetes, presence of retinopathy, statin use, white race/ethnicity, and Framingham/UKPDS risk scores were univariable predictors of CAC. As part of this previous work, we also evaluated 3 selected biomarkers of inflammation/vascular calcification (hs-CRP, IL-6, and plasma OPG) (19). Neither hs-CRP nor IL-6 were predictive of prevalent CAC (baseline CAC score). In contrast, plasma OPG was a powerful predictor of prevalent CAC (OR 3.08, 95% CI 2.42 to 3.92, $p < 0.001$).

Key findings of the present study. The current report extends our previous work by examining the factors predicting progression of CAC (follow-up: 2.5 years) in the same population. We found that progression was frequent (29.6%), particularly in those with pre-existing CAC. Conversely, those without CAC at baseline rarely showed progression (12%), even after adjusting for age. Regression of CAC was rare, occurring in only 3%. All of the traditional risk factors that were related to baseline calcification remained predictive of CAC progression with the exception of hyperlipidemia and smoking status. Notably, serum HbA_1c, IL-6, and OPG (although not hs-CRP) were univariable predictors of progression of CAC. Plasma OPG levels also correlated with atherosclerotic plaque burden at follow-up. We found that statin therapy failed to inhibit the progression of CAC. On the contrary, statin-treated patients had a greater degree of progression. Similar findings were observed in patients treated with beta-blockers, calcium channel antagonists, and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

Our findings are in accordance with published clinical (20) and experimental (21) studies that suggest that calcification occurring in intimal atherosclerotic lesions might itself induce further inflammation and calcification in a positive feedback loop, driving atherosclerotic disease progression. Our results also imply that subjects without CAC at baseline do not need repeat studies in the short term. We have previously shown that such patients also have normal myocardial perfusion and an excellent short-term prognosis (median follow-up = 2.2 years). It is noteworthy that some

Table 5 **Relationship Between Statin Use and Biochemical Parameters**

	Untreated Patients (n = 177)		Statin Therapy (n = 221)		
Parameter	Baseline	Follow-Up	Baseline	Follow-Up	p Value*
Total cholesterol (mg/dl)	187 (31)	183 (27)	187 (27)	168 (35)	0.007
LDL cholesterol (mg/dl)	109 (27)	105 (27)	107 (31)	98 (27)	0.001
HDL cholesterol (mg/dl)	50 (9)	51 (12)	47 (10)	49 (16)	0.35
Triglycerides (mg/dl)	161 (89)	159 (98)	169 (89)	160 (108)	0.55
hs-CRP (mg/l)	4.2 (1.6–10.1)	3.3 (1.3–5.5)	4 (1.9–7.6)	2.1 (1–5.1)	0.01
OPG (pmol/l)	6.1 (4.2–10)	6 (4.4–8.6)	6.7 (4.7–10.5)	5.65 (4–7.9)	0.11

*Difference between follow-up measurements in untreated and statin-treated patients.
Abbreviations as in Table 1.

traditional risk factors were related to incident CAC but not progression. It is possible that the lack of blinding of patients to their baseline CAC scores might have affected their subsequent behavior (e.g., smoking), thus attenuating risk relationships. An alternative explanation is that the coronary calcium score reflects the lifetime coronary atherosclerotic plaque burden, whereas progression of coronary calcium score is a more proximate measure, reflecting current disease activity. If true, this implies that serum HbA_1c, IL-6, and plasma OPG levels are reflecting current disease activity, consistent with current notions of the biological roles of inflammation in atherogenesis.

OPG and CAC. The findings of our study extend previous evidence suggesting an association between elevated OPG levels and vascular disease. Two previous prospective studies showed the prognostic value of plasma OPG measurements in asymptomatic subjects as well as post-myocardial infarction patients (13,22). Kiechl et al. (13) also showed that OPG was significantly related to the severity and 10-year progression of carotid atherosclerosis. Abedin et al. (23) recently demonstrated that plasma OPG levels were independently associated with CAC in an unselected population. Together, these observations support the value of OPG in identifying patients with or at risk for vascular disease.

Previous studies of progression of CAC. Relatively few studies of progression of CAC have been published to date. In 495 asymptomatic patients who underwent sequential EBCT studies, Raggi et al. (24) found that the risk of first myocardial infarction was linked to progression of CAC. In another large (n = 1,153) but retrospective study, they also reported that diabetic patients with no coronary calcium on baseline scans developed it more often than nondiabetic subjects during follow-up and that CAC progression was greater in diabetic subjects than nondiabetic subjects, particularly in the absence of statin therapy (4). Again, disease progression was significantly greater in those patients who experienced a myocardial infarction during follow-up.

The majority of studies evaluating progression of CAC have not been observational studies but have instead specifically examined the impact of cholesterol levels and lipid-lowering therapy on progression of CAC. Conflicting results have been reported. An early, small (n = 149) retrospective study (25) linked the efficacy of lipid-lowering treatment with change in volumetric CAC scores. A subsequent small prospective study (n = 66) (26) showed that progression of CAC was inhibited at LDL cholesterol levels <130 mg/dl. In contrast, in a retrospective study by Yoon et al. (20), LDL cholesterol levels were not predictive of CAC progression. In another prospective study of 661 patients, Wong et al. (27) reported that only high-density lipoprotein cholesterol (and not LDL cholesterol) was related to progression of CAC. The prospective multicenter EBEAT (EBCT Assessment of Coronary Calcification in High-Risk Patients with Minimal or Moderate Coronary Atherosclerosis Receiving Intensive Lipid Lowering Atorvastatin Therapy) study (n = 366) found no difference in progression of CAC in subjects receiving high-dose (80 mg) versus standard-dose (10 mg) atorvastatin (28). In a small randomized controlled trial of 102 patients with calcific aortic stenosis and CAC, Houslay et al. (29) reported that atorvastatin failed to inhibit progression of CAC. Our findings are consistent with the aforementioned studies and the prospective MESA (Multi-Ethnic Study of Atherosclerosis) published recently (30). The MESA also demonstrated that lipid levels were related to baseline CAC but not its progression. In addition to the risk factors predictive of CAC progression documented in our study, family history of CHD and white race/ethnicity were also predictors in the MESA. It is worthwhile noting that MESA did not include any subjects of South Asian origin. It also had a larger sample size (n = 5,756), which provides a greater power for identifying modest risk associations. Statin-treated patients in the MESA had a greater progression of coronary calcium, a finding similar to ours. There are several possible explanations for the lack of impact of medical therapy on CAC progression. In general, patients receiving medical therapy have a greater cardiovascular risk. In our study, statin-treated patients had a greater baseline plaque burden (median CAC 32 mm^3, IQR 0 to 189) than untreated patients (median CAC 0 mm^3, IQR 0 to 56), supporting this notion. Alternatively, the aforementioned results might have been due to insufficient lowering of LDL cholesterol. The average LDL cholesterol level in statin-treated patients in our study was 98 mg/dl, whereas in the TNT (Treating to New Targets) trial (31), the average on-treatment LDL cholesterol in the intensively treated arm was 77 mg/dl. Finally, data from animal experiments indicate that atherosclerotic calcification can increase in extent, especially in the initial stages of atherosclerotic plaque regression, due to fibrosis, foam cell necrosis, and resultant calcification (32,33).

Study limitations. Not all subjects who participated in the initial baseline study were subsequently enrolled in this study (n = 510 vs. n = 402), either owing to cardiac death/coronary revascularization in the intervening period or because they moved out of the area or declined the invitation. Neither subjects nor their physicians were blinded to their baseline CAC scores, which might have influenced their subsequent behavior and treatment, as discussed previously. Because 54% of study patients were of South Asian origin, our findings might not necessarily be generalizable to other ethnic groups.

Conclusions

We have documented the factors predicting progression of CAC (age, male gender, presence of hypertension, suboptimal glycemic control, waist-hip ratio, serum IL-6, plasma OPG, baseline CAC score, and Framingham/UKPDS risk scores) in asymptomatic type 2 diabetic subjects without prior cardiovascular disease. Our study confirms that pro-

gression of CAC occurs mainly in persons with pre-existing CAC. Plasma OPG measurements were correlated with baseline/follow-up CAC severity and predicted CAC progression. Further prospective studies evaluating the prognostic value of CAC progression are necessary to establish the usefulness of measuring CAC progression in asymptomatic diabetic subjects.

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