Serum Cystatin C Predicts Progression of Subclinical Coronary Atherosclerosis in Individuals With Type 1 Diabetes

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OBJECTIVE—Renal function is an important determinant of coronary atherosclerosis, and serum cystatin C is a novel accurate measure of glomerular filtration rate (GFR) and a predictor of cardiovascular events and mortality. We hypothesized that in individuals with type 1 diabetes, cystatin C would *1*) predict progression of subclinical coronary atherosclerosis (SCA) and *2*) be a stronger predictor of SCA than serum creatinine, GFR (estimated by the Cockcroft-Gault [GFRCG] and Modification of Diet in Renal Disease [GFRMDRD] formulas), and albumin excretion rate.

RESEARCH DESIGN AND METHODS—Coronary artery calcification was measured twice, using Imatron C-150 Ultrafast CT, over a 2.5 ± 0.4-year interval in 509 adults with type 1 diabetes (42% male, age 36 ± 9 years, duration 23 ± 9 years). SCA progression (n = 131) was defined as a >2.5 increase in square root calcium volume score or development of clinical coronary artery disease. Predictors of SCA progression were examined in a model selected by stepwise logistic regression and an a priori–determined model. Next, each measure of renal function was inserted into the stepwise model, one at a time, and Akaike information criterion was used to compare the fit of the competing models.

RESULTS—The stepwise model included cystatin C (odds ratio 1.44, 95% CI 1.00–2.18, P = 0.048), age, baseline coronary artery calcification, sex, diabetes duration, systolic blood pressure, and HDL. The stepwise model had a better fit than any of the competing models with serum creatinine, GFRCG, GFRMDRD, or albumin excretion rate replacing cystatin C.

CONCLUSIONS—In individuals with type 1 diabetes, cystatin C modestly predicts SCA. ***Diabetes*** **56:2774–2779, 2007**

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Received for publication 19 April 2007 and accepted in revised form 23 July 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 27 August 2007. DOI: 10.2337/db07-0539.

AER, albumin excretion rate; AIC, Akaike information criterion; CAC, coronary artery calcification; CACTI, Coronary Artery Calcification in Type 1 Diabetes; CAD, coronary artery disease; CVD, cardiovascular disease; CVS, calcium volume score; GFR, glomerular filtration rate; GFRCG, GFR Cockcroft-Gault; GFRMDRD, GFR Modification of Diet in Renal Disease; SCA, subclinical coronary atherosclerosis.

Extensive literature exists on renal disease as a cardiovascular disease risk factor (1–3), and renal disease is an important complication of type 1 diabetes (1). Additionally, in individuals with type 1 diabetes, renal disease dramatically increases the risk of cardiovascular disease (4–10), which is the major cause of morbidity and mortality in type 1 diabetes (11). The current gold standard estimations of glomerular filtration rate (GFR), a marker of renal function, require infusion of external substances, are time consuming, and are expensive, precluding routine use in clinical settings or in large epidemiologic studies (12). Estimation of GFR from prediction equations based on serum creatinine measurements (Cockcroft-Gault [GFRCG] or Modification of Diet in Renal Disease [GFRMDRD] equations) have imperfections (12,13). Cystatin C is emerging as a biomarker superior to serum creatinine for estimating GFR (14–18) and predicting the risk of death and cardiovascular events (19–23).

Cystatin C is a 13-kDa nonglycosylated basic protein produced by nucleated cells at a constant rate that is freely filtered at the glomerulus. Because it is produced at a steady rate, is freely filtered at the glomerulus, does not return to the bloodstream, and is eliminated by the kidneys, it has been proposed to be closer to an "ideal" endogenous marker of GFR. Unlike serum creatinine, cystatin C has been reported to not be dependent on age, sex, or muscle mass (14,23,24), whereas other studies have reported cystatin C levels are influenced by thyroid function, exogenous glucocorticoids, age, sex, and smoking (25,26). Cystatin C can be measured in serum or plasma and has been shown to be stable after freezer storage and repeated freeze-thaw cycles (14,27,28).

Cystatin C has been proposed to reflect cumulative effects on GFR over time (similar to A1C as a measure of glycemia over time), to have less measurement variability (because of diet or glycemia) than iothalamate, and to better estimate the slope in decline in GFR and therefore better detect trends in change in GFR to allow for clinical intervention (24,29). Cystatin C has also been demonstrated to better predict cardiovascular disease (CVD) events and death than serum creatinine (or serum creatinine–based estimates of GFR) (19–23). However, little data exist on the relationship of cystatin C to GFR in individuals with type 1 diabetes (16,18) and none on the relationship of cystatin C to CVD events, death, or subclinical coronary artery atherosclerosis in individuals with type 1 diabetes.

Therefore, our aim was to *1*) evaluate cystatin C as a predictor of subclinical coronary atherosclerosis and *2*)

compare the predictive value of cystatin C to serum creatinine and estimated GFR to progression of subclinical coronary atherosclerosis (SCA) (measured by coronary artery calcification [30] or a clinical coronary artery disease [CAD] event) in individuals with type 1 diabetes in the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study cohort.

RESEARCH DESIGN AND METHODS

The data presented in this report were collected as part of the baseline examination of 1,416 participants in the CACTI study who were 19–56 years of age and included 652 men and women with type 1 diabetes and 764 nondiabetic control subjects (31). All subjects were asymptomatic for CAD and had no history of coronary artery bypass graft, coronary angioplasty, or unstable angina. Patients with diabetes generally had been diagnosed when <30 years of age, and among those who were ≥30 years at diagnosis, positive antibodies or a clinical course consistent with type 1 diabetes was present. Because few nondiabetic subjects had abnormal renal function at baseline and these participants were less likely to have progression of subclinical coronary artery atherosclerosis, this analysis focuses on the members of the cohort with type 1 diabetes. Of the 652 individuals enrolled at baseline, 606 (93%) had available stored serum to measure cystatin C. Of these, 509 (84%) had data on progression of subclinical coronary artery atherosclerosis (mean follow-up time 2.5 ± 0.4 years), and 498 had complete covariate data for the variables included in the a priori model based on a previous analysis (32) performed on the full cohort, including additional CVD risk factors (with the exception of albumin excretion rate [AER], missing in 68 subjects in which an additional analysis was performed). Subjects not included in the analyses were slightly younger and more likely to be current smokers. All subjects provided informed consent, and the study was approved by the Colorado Combined Institutional Review Board.

Examination and laboratory measurements. Participants completed the baseline examination between March 2000 and April 2002, and a more detailed description of the study and baseline characteristics of this cohort has been published (33). Resting systolic blood pressure and fifth-phase diastolic blood pressure were measured three times while the subjects were seated, and the second and third measurements were averaged (34). Hypertension was defined as current antihypertensive therapy or untreated hypertension (blood pressure ≥140/90 mmHg) at the time of the study visit. Fat measurements using computed tomography were determined, and participants completed a standardized questionnaire including medical history and medication inventory, as previously reported (33).

Imaging. All patients underwent two electron beam computed tomography scans within 5 min without contrast at baseline and two scans at follow-up as previously described (33). Images were obtained of the entire epicardial system using an Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA), with a 100-ms exposure. The standard acquisition protocol was used (35). Scanning started from near the lower margin of the bifurcation of the main pulmonary artery. Images were electrocardiographically triggered at 80% of the R-R interval, and 30–40 contiguous 3-mm slices were acquired. The volume scores were calculated using the volumetric method, which is based on isotropic interpolation (36).

Covariate measurements

Laboratory analyses. After an overnight fast, blood was collected and centrifuged, and separated serum was stored at −70°C until assayed. Cystatin C was measured on stored serum samples in the clinical lab at University of Colorado Hospital in Denver, Colorado, using a commercially available particle-enhanced immunonephelometric assay (Dade-Behring). Stored samples from the subjects' baseline study visit had previously been thawed once. The coefficient of variation was 3.3%. Intra-assay precision is 2.3–4.1% and interassay precision is 2.6–3.3% per the package insert. Results are reported in milligrams per liter, with a sensitivity cutoff of 0.23 mg/l.

Total plasma cholesterol and triglyceride levels were measured using standard enzymatic methods, HDL cholesterol was separated using dextran sulfate, and LDL cholesterol was calculated using the Friedewald formula. High-performance liquid chromatography was used to measure A1C (high-performance liquid chromatography, Bio-Rad variant). Plasma glucose was measured using a standard hexokinase method. Homocysteine was determined by the Abbot IMX automated procedure. C-reactive protein, plasminogen activator inhibitor 1, and fibrinogen were measured in the laboratory of Dr. Russell Tracy at the University of Vermont. C-reactive protein was measured using the BNII nephelometer from Dade Behring, using a particle-enhanced immunonepholemetric assay. Plasminogen activator inhibitor 1 was done as a two-site enzyme-linked immunosorbent assay. Fibrinogen was measured in an automated clot-rate assay using the Sta-r instrument. Urine albumin was measured by radioimmunoassay, and AER was determined by radioimmunoassay; the results of two timed overnight urine collections were averaged.

Anthropometric variables. We measured height and weight and calculated BMI. Minimum waist and maximum hip measurements were obtained in duplicate, and the results were averaged. Intra-abdominal fat and subcutaneous fat were assessed using abdominal computed tomography scan at the L4–L5 levels. The total intra-abdominal fat volume and subcutaneous fat volume in cubic centimeters were measured using the AccuAnalyzer software from AccuImage.

Insulin resistance. Insulin resistance was approximated as the inverse of the estimated glucose disposal rate (EGDR), calculated according to the formula: EGDR = 24.31 − 12.22 × (waist-to-hip ratio) − 3.29 × (hypertension) − 0.568 × (A1C). The equation was derived from hyperinsulinemic-euglycemic clamps performed in 24 type 1 diabetic participants in the Pittsburgh Epidemiology of Diabetes Complications Study (37).

GFR estimation. GFR was estimated by both the Cockcroft-Gault formula (GFRCG) (38) and the Modification of Diet in Renal Disease equation (GFRMDRD) (39), both based on measurement of serum creatinine.

Interview variables. Duration of diabetes was determined by patient self-report. Current and former smoking status was obtained by questionnaire, and for smokers, the total number of pack-years was calculated.

Definition of subclinical coronary atherosclerosis progression and coronary artery calcification progression. In this study, we chose to define coronary artery calcification (CAC) progression as reported by Hokanson et al. (40), who noted that bias in the interscan variability of calcium volume scores (CVSs) exists such that the variability increases as levels of coronary calcium increase. If not accounted for, this may lead to overestimating changes in CVS over time at higher levels of coronary calcium. Alternatively, using percent change in CVS as a potential measure of changes in coronary calcium may underestimate changes at higher levels of coronary calcium. Using paired mean CVS measurements in 1,074 subjects who had two EBCT scans done 5 min apart, Hokanson et al. found that square root transformation of CVS provides a stable estimate of interscan variability across the ranges of coronary calcium observed in the current study, thus allowing investigations of changes in coronary calcium that are not biased by level of coronary calcium. Furthermore, Hokanson et al. suggested using a difference between baseline and follow-up square root transformed CVS of ≥2.5 to signify significant change in CVS, since a change of this magnitude is <1% likely to be due to interscan variability. In addition, participants who had a CAD event (myocardial infarction, coronary artery bypass graft, angioplasty with stent, or death attributed to CAD as adjudicated by a three-physician committee) were also considered as having SCA progression.

Statistical analysis. Data are presented as arithmetic means and SDs for continuous variables (geometric means and ranges for log-transformed variables) and percentages for categorical variables.

Correlation coefficients between cystatin C and other renal-related measures (serum creatinine, GFRCG, GFRMDRD, and AER) were calculated. The relationships of each of these renal-related measures to cystatin C were examined graphically. To evaluate cystatin C as a predictor of SCA progression, stepwise multiple logistic regression analysis was performed with a P value <0.1 as the criteria for entry and removal from the model. Age, baseline CVS, and sex were forced into all models. The following variables were considered for inclusion in the stepwise model predicting SCA progression: cystatin C, follow-up years, type 1 diabetes duration, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, hypertension (yes/no), smoking status (current and ever versus never), A1C, total cholesterol, LDL, HDL, triglycerides, C-reactive protein, plasminogen activator inhibitor 1, fibrinogen, homocysteine, serum creatinine, AER, GFRCG, and GFRMDRD. Once the final stepwise logistic regression model was chosen (which included cystatin C as a significant predictor of SCA progression), a series of additional models was fit, replacing cystatin C with each of the other renal-related measures. The Akaike information criterion (AIC) was used to determine which renal-related measure best predicted SCA progression, where lower AIC values indicate a better model fit. Next, cystatin C was entered into an a priori model (32) that included age, sex, baseline CVS, duration of diabetes, HDL, hypertension, LDL, smoking status (current and ever versus never), waist circumference, and A1C. Finally, cystatin C was again replaced with other renal-related measures and AIC values compared across the competing models. These models were repeated excluding the type 1 diabetic subjects with CAD events (n = 11).

Human subjects. The study protocol was reviewed and approved by the Colorado Combined Institutional Review Board, and informed consent was obtained from all participants before enrollment.

TABLE 1
Baseline characteristics of CACTI type 1 diabetic subjects with cystatin C measurements (n = 509), stratified by CAC progression status

	Progressors	Nonprogressors	P
n	131	378	—
Age (years)	43.4 ± 7.8	34.8 ± 8.4	<0.0001
Female/male (%)	42/58	57/43	0.002
Race (non-Hispanic white) (%)	94	95	0.55
Duration of diabetes (years)	29.3 ± 8.5	21.2 ± 8.2	<0.0001
Baseline square root CVS	7.7 ± 9.9	1.0 ± 3.0	<0.0001
Cystatin C (mg/l)	1.01 ± 0.64	0.78 ± 0.16	<0.0001
Serum creatinine (mg/dl)	1.3 (0.8–7.9)	1.2 (0.8–2.3)	<0.0001
AER (μg/min)	17 (1–3,468)	8 (1–1,885)	<0.0001
GFRCG (ml/min per 1.73 m^2)	80.6 ± 33.3	88.9 ± 23.9	0.01
GFRMDRD (ml/min per 1.73 m^2)	59.2 ± 17.9	66.5 ± 12.8	<0.0001
A1C (%)	8.1 ± 1.2	7.9 ± 1.3	0.09
Insulin dose (units · kg^{-1} · day^{-1})	0.57 ± 0.27	0.61 ± 0.26	0.14
BMI (kg/m^2)	26.7 ± 4.6	25.9 ± 4.1	0.08
Average waist (cm)	88.6 ± 12.3	83.7 ± 11.9	<0.0001
Visceral fat at L4–L5 (cm^2)	10.5 ± 0.7	10.3 ± 0.6	0.0001
Subcutaneous fat at L4–L5 (cm^2)	11.7 ± 0.7	11.7 ± 0.6	0.69
1/estimated glucose disposal rate	0.15 ± 0.05	0.12 ± 0.05	<0.0001
Systolic blood pressure (mmHg)	125 ± 14	115 ± 13	<0.0001
Diastolic blood pressure (mmHg)	79 ± 9	77 ± 8	0.009
Hypertension (yes/no) (%)	64	33	<0.0001
Total cholesterol (mg/dl)	176 ± 32	173 ± 33	0.27
LDL cholesterol (mg/dl)	102 ± 26	99 ± 28	0.30
HDL cholesterol (mg/dl)	55 ± 17	57 ± 16	0.42
Triglycerides (mg/dl)	89 (32–368)	78 (25–357)	0.007
C-reactive protein (μg/ml)	2.2 ± 3.0	1.9 ± 2.0	0.28
Plasminogen activator inhibitor 1 (ng/ml)	19.1 ± 25.1	16.1 ± 22.0	0.20
Fibrinogen (mg/dl)	276 ± 63	258 ± 62	0.005
Homocysteine (μmol/l)	9.1 (5.0–50.0)	7.4 (3.8–44.3)	<0.0001
Alcohol drinks/month	13.2 ± 24.4	13.7 ± 25.0	0.83
Smoking (current) (%)	15	10	0.12
Smoking (ever) (%)	25	18	0.09

Data are means ± SD or geometric means (range) unless otherwise indicated.

RESULTS

The baseline characteristics of study participants with type 1 diabetes are displayed in Table 1 stratified by SCA progression status. Progressors were more likely to be male, were older, and had longer diabetes duration than nonprogressors. Progressors had higher baseline CVS, waist circumference, waist-to-hip ratio, visceral fat, 1/estimated glucose disposal rate (a marker of insulin resistance), systolic blood pressure, diastolic blood pressure, triglycerides, fibrinogen, and homocysteine than nonprogressors. Cystatin C, serum creatinine, AER, GFRCG, and GFRMDRD all indicated worse renal function in progressors than nonprogressors.

As expected, cystatin C correlated with other renal-related measures of renal function: serum creatinine (r = 0.82), GFRCG (r = −0.38), GFRMDRD (r = −0.58), and AER (r = 0.48) ($P < 0.0001$ for all).

In a stepwise multiple logistic regression analysis, cystatin C was a significant predictor of SCA progression (odds ratio [OR] 1.44, 95% CI 1.00–2.08, P = 0.048) while adjusted for other covariates that also entered the model (duration, systolic blood pressure, and HDL) in addition to covariates forced into the model (age, sex, and baseline CVS) (Table 2). Excluding subjects (n = 11) who had CAD events, cystatin C had a similar relationship to CAC progression but had decreased power and was no longer statistically significant (OR 1.39, 95% CI 0.97–1.99, P = 0.075). In the a priori–defined model, cystatin C was also a significant predictor of SCA progression (OR 1.50, 95% CI 1.06–2.14, P = 0.024), when adjusted for additional CVD risk factors that were not independent predictors of SCA progression in this study population (Table 3). Again, after excluding subjects who had CAD events, cystatin C had a similar relationship to CAC progression, but it was no longer statistically significant (OR 1.39, 95% CI 0.96–2.20, P = 0.08).

Next, to compare how well each renal-related variable predicts SCA progression, cystatin C was replaced in the final stepwise model individually with each of the other

TABLE 2
Predictors of SCA progression: final model from stepwise multiple logistic regression analysis (n = 509)

	OR (95% CI)*	P
Age	1.71 (1.22–2.39)	0.002
Baseline CVS	2.46 (1.71–3.54)	<0.001
Sex	1.36 (0.79–2.34)	0.27
Cystatin C	1.44 (1.00–2.08)	0.048
Type 1 diabetes duration	1.59 (1.16–2.17)	0.004
Systolic blood pressure	1.66 (1.25–2.21)	0.0005
HDL	0.66 (0.51–0.86)	0.003

OR and 95% CIs are per SD of each variable: cystatin C = 0.37 mg/l, age = 9 years, CVS = 6.4 Agatston units, sex = male, type 1 duration = 9 years, systolic blood pressure = 14 mmHg, HDL = 16 mg/dl.

TABLE 3
Predictors of SCA progression: a priori model in multiple logistic regression analysis (n = 498)

	OR (95% CI)*	P
Age	1.78 (1.24–2.54)	0.002
Baseline CVS	2.39 (1.64–3.50)	<0.001
Sex	1.65 (0.93–2.93)	0.09
Cystatin C	1.46 (1.02–2.07)	0.038
Type 1 diabetes duration	1.65 (1.18–2.30)	0.003
Hypertension (yes/no)	1.55 (0.89–2.68)	0.12
HDL	0.76 (0.56–1.02)	0.07
LDL	0.94 (0.72–1.23)	0.64
Smoking ever	1.30 (0.67–2.52)	0.43
Smoking current	1.41 (0.64–3.11)	0.39
Waist circumference	1.27 (0.95–1.69)	0.11
A1C	1.16 (0.86–1.57)	0.32

*OR and 95% CIs are per SD of each variable: cystatin C = 0.36 mg/l, age = 9 years, CVS = 6.4 Agatston units, sex = male, type 1 duration = 9 years, hypertension = yes, HDL = 16 mg/dl, LDL = 28 mg/dl, smoking = yes, waist circumference = 12 cm, A1C = 1.3%. Note: Compared with the best model (Table 2), 11 subjects were excluded because of missing data.

renal-related measures (serum creatinine, GFRCG, and GFRMDRD) in the best model with the same subjects (n = 509) and AIC values were compared (Table 4). Cystatin C had the lowest AIC of all renal-related measures while adjusting for other variables included in the stepwise model (age, sex, baseline CVS, duration, systolic blood pressure, and HDL), indicating that cystatin C was a slightly better predictor of SCA progression than serum creatinine, GFRCG, or GFRMDRD, although the 95% CIs do overlap considerably. Furthermore, in addition to having the lowest AIC, it was the only renal-related variable to be statistically significant (P = 0.048) while adjusting for the other variables in the final stepwise model. Excluding the subjects with CAD events did not change the relationship of AIC values between models (data not shown).

This model was then repeated in a smaller dataset (n = 430) that included all subjects with two timed overnight baseline urine measurements. Similar results were obtained for cystatin C (OR 1.45, 95% CI 1.05–2.00, P = 0.026). In this analysis, AER was of borderline significance when it replaced cystatin C (OR 1.28, 95% CI 0.99–1.65, P = 0.064). The analysis was also repeated with renal-related variables defined categorically (cystatin C and serum creatinine by quartiles and the GFR measurements as >90, 90–60, 60–30, and <30 ml/min per 1.73 m^2, and albuminuria [yes/no]). However, the AIC values were superior for the continuous compared with the categorized variables, indicating a better fit for the renal-related measures as continuous variables (data not shown). Excluding the seven subjects who reported being on oral glucocorticoids at baseline did not change the relationship of cystatin C to SCA progression (OR 1.45, 95% CI 1.01–2.10, P = 0.045). Finally, the final stepwise model was fit for the subset of subjects with no CAC at baseline (n = 319) to predict incident CAC, and the point estimate for cystatin C was slightly larger but no longer statistically significant (OR 1.59, 95% CI 0.90–2.82, P = 0.11).

TABLE 4
Comparison of renal function markers as predictors of CAC progression (n = 509), adjusted for age, baseline CAC, sex, type 1 duration, systolic blood pressure, and HDL

	OR (95% CI)*	P	AIC
Cystatin C	1.44 (1.00–2.08)	0.048	404.2
Serum creatinine	1.34 (0.92–1.95)	0.12	406.0
GFRCG	1.22 (0.90–1.65)	0.20	407.3
GFRMDRD	0.93 (0.69–1.25)	0.62	408.6

*OR and 95% CI are per SD of each variable: cystatin C = 0.37 mg/l, age = 9 years, CVS = 6.2 Agatston units, sex = male, type 1 duration = 9 years, systolic blood pressure = 14 mmHg, HDL = 16 mg/dl, serum creatinine = 0.47 mg/dl, GFRCG = 26.9 ml/min per 1.73 m^2, GFRMDRD = 14.6 ml/min per 1.73 m^2.

DISCUSSION

The main finding of this paper is that increasing serum cystatin C, a marker of worsening GFR, predicts progression of SCA, even while adjusting for other CVD risk factors. Furthermore, cystatin C better predicted SCA progression than serum creatinine and serum creatinine–derived estimates of GFR (GFRCG and GFRMDRD) or AER, although the difference was modest and the 95% CIs overlap considerably. This is the first article comparing cystatin C to other measures of renal function as a predictor of SCA in individuals with type 1 diabetes and, as such, is consistent with and an addition to previous literature relating cystatin C to CVD events and mortality in other populations (19–23). Further verification is needed to determine whether cystatin C has a role in clinical care or epidemiologic research as a marker of renal function and a predictor of outcomes such as CVD and death. Previous studies have suggested that cystatin C might change earlier than serum creatinine–based estimates of GFR and therefore holds promise to be an early marker of impaired renal function for more timely clinical intervention (24). Both GFRMDRD and GFRCG are limited at detecting early change in renal function, specifically in young adults with type 1 diabetes (12). Should further studies relate cystatin C to health outcomes such as CVD and mortality, then the role of cystatin C would be bolstered as a routine measure of renal function in patients with type 1 diabetes who are at high risk of diabetic nephropathy. Previously, the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study has reported that CAC score increased 1.3-fold per 10 mg/24 h increase in AER, but evaluation of other measures of renal function was not reported (41). Additionally, in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study, A1C was related to CAC, as the CACTI study has reported previously in pilot data in a subset of subjects (42), although A1C was not a significant predictor in this analysis similar to other reports in people with type 1 diabetes (43,44).

Our data are similar to previous articles relating elevated cystatin C levels to CVD. Cystatin C has been shown to be a stronger predictor of CVD events and death than serum creatinine or estimated GFR in the elderly in three large epidemiologic studies (21–23). In contrast to cystatin C, a J-shaped relationship between serum creatinine and mortality has been described (23) and serum creatinine–based equations estimating GFR may be inaccurate at higher GFRs (12) and therefore limited in detecting early compromise in GFR. Cystatin C has also been significantly associated with a first ischemic coronary event (20) and for risk of secondary CVD events (19). Our data add to the current literature, in which cystatin C better predicts CVD events and mortality, by finding that in a young type 1

diabetes cohort at high risk for both kidney disease and CVD, cystatin C predicts progression of SCA, independent of known CVD risk factors.

Previously, cystatin C also has been shown to be a better predictor of chronic kidney disease (21) and to accurately detect early changes in renal function in individuals with type 2 diabetes (24). Most (16,18,45,46), but not all (47), studies have found cystatin C to be a better marker of GFR in individuals with diabetes than serum creatinine.

Some limitations in our data need to be acknowledged and addressed in future studies. First, we do not compare cystatin C values to gold standard measures of GFR such as inulin or iothalamate. The CACTI study's objective is to investigate CAD in individuals with type 1 diabetes, not renal disease, except as a factor in CAD. Second, currently, we do not have longitudinal measurements of cystatin C in the CACTI study but plan to do so in the future. Third, we use a surrogate marker of CAD instead of health outcomes such as CAD events or death, since the CACTI cohort is relatively young and was asymptomatic for CAD at enrollment and has had few CAD events as of this writing ($n = 15$ for the cohort, with 11 type 1 diabetic subjects in this analysis); data on patient outcomes are being collected prospectively, and analyses are presented both with and without CAD events. However, extensive methodologic detail has been taken to carefully define CAC progression in the CACTI cohort (40), and this methodology has been used in other datasets (48,49). Finally, we are unable to tease out the roles of cystatin C (as an estimator of GFR) compared with AER as a predictor of subclinical coronary artery atherosclerosis because of limited statistical power. Previously, the UK Prospective Diabetes Study has suggested that renal disease, as defined by albuminuria versus impaired GFR in individuals with type 2 diabetes, might have different risk factors and a different relationship to health outcomes (50). A paradigm shift in ascertainment of renal disease in individuals with diabetes has even been suggested with measurement of cystatin C in serum replacing measurement of AER in urine samples (29), although much more data are needed for the routine use of cystatin C in clinical care and research studies in lieu of or as a complement to AER. However, should data support increased cystatin C as a marker of negative health outcomes, the ease of measurement on a single blood sample instead of overnight urine collection is appealing. On the other hand, estimates of GFR, which is the putative role of cystatin C, may provide alternate and complementary data to AER (50). Also, GFR estimates based on cystatin C may be superior to serum creatinine–based estimates, and a number of articles have investigated this (51–53). Excluding subjects reporting glucocorticoid usage did not change the relationship of cystatin C to SCA progression, similar to a previous report in which cystatin C concentrations were not affected by high-dose corticosteroid therapy in children (54).

In conclusion, we demonstrate that cystatin C, a marker of GFR, independently and significantly predicts progression of SCA in individuals with type 1 diabetes. Although promising, future validation, including longitudinal data, on the role of cystatin C to CAD, renal function, and mortality are needed before routine implementation in clinical care.

ACKNOWLEDGMENTS

Support for this study was provided by the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) Grants R01 HL61753 and HL79611 and Diabetes and Endocrinology Research Center Clinical Investigation Core Grant P30 DK57516. The study was performed at the Adult General Clinical Research Center at the University of Colorado at Denver and Health Sciences Center (supported by NIH Grant M01 RR00051), at the Barbara Davis Center for Childhood Diabetes, and at the Colorado Heart Imaging Center in Denver, CO. D.M.M. was supported by the K12 training grant DK063722-05 and K23 DK075360.

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