Plasma Connective Tissue Growth Factor (CTGF/CCN2) Levels Predict Myocardial Infarction in the Veterans Affairs Diabetes Trial (VADT) Cohort

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OBJECTIVE

Connective tissue growth factor (CTGF), also known as CCN2, is a potent chemotactic and extracellular matrix-inducing matricellular protein that has been implicated in progression of inflammatory and fibroproliferative disorders. An emerging role of CTGF/CCN2 is that of a prosclerotic factor implicated in the development of cardiac disease. Our objective was to determine the role of CTGF/CCN2 as a predictor of cardiovascular events in type 2 diabetes in the Veterans Affairs Diabetes Trial (VADT) cohort.

RESEARCH DESIGN AND METHODS

Levels of CTGF/CCN2 were measured in 952 VADT patients a median of 1.9 years after entry into the study. Participants were followed for an average of 3.3 years for vascular outcomes. CTGF/CCN2 categories were defined as below the detectable limit (referent, 54.5%), lower half of detectable values (22.8%), and upper half of detectable values (22.7%). Hazard ratios (HRs) for cardiovascular end points in relation to CTGF/CCN2 categories were calculated by Cox proportional hazards models.

RESULTS

During follow-up, 4.8% had a myocardial infarction (MI), 6.9% had an MI or cardiovascular death, and 6.9% died. After adjustments by conventional risk factors, individuals in the highest category of CTGF/CCN2 were at higher risk of MI (HR 2.43 [95% CI 1.15, 5.14]), MI or cardiovascular death (HR 2.71 [95% CI 1.44, 5.08]), and all-cause mortality (HR 2.70 [95% CI 1.43, 5.08]) relative to individuals with CTGF below the detectable limit.

CONCLUSIONS

Our study indicates that high levels of CTGF/CCN2 predict future MI and cardiovascular death in patients with type 2 diabetes.

Diabetes is associated with a number of metabolic and cardiovascular risk factors that contribute to a high rate of vascular events. The risk factors and mechanisms that contribute to the development of these complications are inadequately defined. In the clinical setting, we strive to maintain glycemic control, promote smoking cessation, and monitor and treat hypertension and hyperlipidemia to well-established goals. Nonetheless, it is clear that our current tools for risk assessment do not provide a complete

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picture, and as a result our treatment strategies are suboptimal, particularly with respect to macrovascular events in patients with established type 2 diabetes. This fact is underscored by recently released outcomes from the CSP-465 Veterans Affairs Diabetes Trial (VADT) (1,2), the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (3), and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial (4), all of which failed to show a significant benefit of tight glycemic control on cardiovascular events (5). In fact, the intensive glycemic control arm of ACCORD was stopped early owing to excess mortality (3). The mechanisms responsible for the initiation and progression of atherosclerosis are not completely defined. Early atherosclerotic lesions are characterized by endothelial dysfunction, accumulation of inflammatory cells, vascular smooth muscle cell proliferation, and transendothelial migration, as well as extracellular matrix deposition in the vessel wall (6). Moreover, the accelerated vascular pathology associated with diabetes is not fully explained by coexistence of traditional cardiovascular risk factors such as hypertension, dyslipidemia, and smoking.

Our data implicate a role for connective tissue growth factor (CTGF), also known as CCN2, as a mediator of diabetic vascular injury. CTGF is a member of the CCN family of matricellular proteins that has been implicated in the progression of the inflammatory process, response to injury, and wound healing (7,8). Dysregulation of CTGF/CCN2 expression has been linked to various fibroproliferative disorders including diabetes complications and atherosclerosis (8,9). An emerging role of CTGF/CCN2 is that of a prosclerotic factor implicated in the development of cardiac disease (10–15). CTGF/CCN2 expression is upregulated in cardiac tissue after myocardial infarction (MI) and has been shown to modulate cardiac function and tissue remodeling (16,17). Furthermore, CTGF/CCN2 was shown to be an effector of transforming growth factor (TGF)-β–mediated cardiac fibrosis, and the expression of CTGF in cardiac fibroblasts and cardiac myocytes was shown to be mediated via TGF-β (18–20). Moreover, CTGF/CCN2 has been shown to modulate the adverse effects of high glucose and free fatty acids on cardiomyocytes function such as hypertrophy and apoptosis (21). It is of interest to note here that CTGF/CCN2 promotes deposition of extracellular matrix proteins through interaction with other factors. For instance, CTGF regulates the fibrosis-associated renal lymphangiogensis in obstructed kidneys via induction and direct interaction with vascular endothelial growth factor C (22). In addition, the increased collagen deposition in lung fibrosis as a result of deletion of protein phosphatase and tensin homologue gene is mediated via increased expression of CTGF (23). At the molecular level, a number of factors have been shown to regulate the expression of CTGF/CCN2, including advanced glycation end products, sphingosine-1-phosphate, bradykinin, and LDL (24–29).

In earlier work, we reported that in type 1 diabetes, higher plasma CTGF/CCN2 levels were associated with increased common and internal carotid intima-media thickness, an established marker of subclinical atherosclerosis, as well as macroalbuminuria and hypertension (9). In the current study, we build on our prior work and examine the ability of CTGF to predict MI in patients with type 2 diabetes in the VADT.

RESEARCH DESIGN AND METHODS

VADT Design and Population

The details of the VADT design have previously been described (1). Briefly, 1,791 veterans with type 2 diabetes and suboptimal glucose control were randomized in 20 participating sites to receive either intensive or standard glucose control. The goal for HbA_{1c} levels was an absolute reduction of 1.5% in the intensive therapy group compared with the standard therapy group. All other modifiable cardiovascular risk factors were treated aggressively and uniformly in both arms of the study. All patients were treated according to guidelines of the American Diabetes Association for blood pressure, hypertension, diet, exercise, and diabetes education (30). All patients were prescribed aspirin, and all patients with elevated lipid levels were prescribed statins unless contraindicated. The study was approved by the institutional review board at each of the participating sites. All patients provided written informed consent.

Of the 1,791 VADT study participants, 995 patients from 17 of the participating sites, or approximately half from the standard arm and half from the intensive treatment arm, agreed to participate in a substudy focused on determining the association between specific biomarkers and macrovascular disease. The biochemical, physical, and demographic profiles of the 995 patients in the substudy did not differ significantly from those of the 796 not included in the substudy, with the exception of slightly lower age and LDL cholesterol and slightly higher triglyceride levels, as well as a higher prevalence of aspirin use at baseline in substudy participants compared with non–substudy participants (31). The study population for the current report consists of 952 of the 995 participants for whom samples were available for measurement of CTGF. In 43 patients, not enough plasma was collected to perform the measurements. Supplementary Fig. 1 includes a flow diagram of study participants.

Enrollment for the VADT study occurred from December 2000 to May 2003. Measurement of CTGF was performed on samples collected during a routine follow-up between July 2002 and March 2006, a median of 1.94 years (range 0–5.03) after participants' baseline examination. Plasma samples were obtained after an overnight fast and stored at −80°C. Patients were followed until lost to follow-up, death, or May 2008. The median follow-up time after measurement of CTGF was 3.76 years (range 0–5.75). CTGF measurement for the current analysis occurred after all samples were collected. The baseline VADT cohort examination was standardized and included interviews, blood pressure measurements, anthropometric measurements, and fasting venipuncture (1).

CTGF/CCN2 Measurement

CTGF in plasma was measured with a sandwich ELISA that detects both intact CTGF and CTGF that has been proteolytically cleaved in the hinge region to release the N-fragment of CTGF (N+W-CTGF assay). The capture antibody is human anti-human CTGF–domain 1 (FibroGen, Inc., San Francisco, CA). A standard curve was prepared with rhCTGF (CTGF expressed in CHO cells and affinity purified with an anti-CTGF antibody column; FibroGen). After plate washing, 50 μL samples, standards, and controls was added along with 50 μL detection antibody (mouse anti-human CTGF–domain 2 antibody, 500 ng/mL; FibroGen, Inc.) for >1 h. Secondary antibody (100 μL,

goat anti-mouse IgG(H+L)-AP, 1:2,000; Thermo Fisher Scientific, Waltham, MA) was added for 1 h. Stationary incubation at 37°C in 100 μL light-sensitive substrate buffer (0.1% diethanolamine in 1.1 mmol/L $MgCl_2$ with 1 mg/mL *para*-nitrophenylphosphate substrate) for 5–10 min (depending on yellow color development) was followed by addition of 100 μL stop solution (10 N NaOH) to stabilize the colored product. Absorbance at 405 nm was acquired on a SpectraMax 340PC spectrophotometer and analyzed with SoftMax Pro 4.8 software (Molecular Devices, Sunnyvale, CA).

End Points

The primary end point for the VADT was the time to the first occurrence of any one of a composite of cardiovascular events. Each VADT event was adjudicated by an end point committee that used strict algorithms to define and document each event. The composite end point included documented MI; stroke; death from cardiovascular disease (CVD); new or worsening congestive heart failure; surgical intervention for cardiac, cerebrovascular, or peripheral vascular disease; inoperable coronary artery disease (CAD); and amputation for ischemic gangrene. Secondary outcomes included MI, CAD, death from CVD, and death from any cause. CAD included MI, coronary revascularization procedures, and clinically identified inoperable CAD. Cardiovascular death included sudden death as defined by the Framingham Heart Study, CAD, cerebrovascular accident, and other cardiovascular events (i.e., cardiomyopathy).

Statistical Analyses

Prospective analyses were carried out in which the plasma level of CTGF, as a biomarker, and cardiovascular end points, including MI and the composite end point, were the outcomes of interest. Since the CTGF assay was designed to measure levels above median values, slightly more than half (54.5%) of VADT participants had CTGF levels below the detectable level; therefore, for purposes of statistical analyses, individuals were categorized based on their CTGF level into one of three groups: those below the detectable limit, <7.8 ng/mL (54.5%); those with levels in the lower half of the detectable range, 7.8–13.4 ng/mL (22.8%); and those with levels in the upper half of the detectable range, >13.4–106 ng/mL (22.7%). Baseline clinical and demographic characteristics of the cohort are shown in Table 1, stratified by the three categories of CTGF level. Differences across categories of CTGF were tested using χ^2 for categorical characteristics, while means, adjusted for age, ethnic minority status, and treatment arm, were determined for continuous variables using linear regression.

For cardiovascular time-to-event outcomes, Kaplan-Meier survival curves and Cox proportional hazards models were used to calculated hazard ratios (HRs) for end points of interest in relation to CTGF categories. Because CTGF levels were measured not at the baseline VADT examination but, instead, a median of 1.94 years later, left truncation was used to account for differences in time at risk; hence, a participant was considered at risk for a given event between measurement of CTGF

Table 1—Clinical and demographic characteristics of the VADT population (n = 952) stratified by CTGF category

		CTGF categories (cut points), ng/mL			
	Total population	Below detectable limit <7.8 (n = 519)	≥7.8–13.4 (n = 217)	>13.4–106 (n = 216)	P**
Measured at VADT baseline					
Age (years)*	59.7 (59.2, 60.3)	57.9 (57.2, 58.6)	61.1 (60.0, 62.2)	62.7 (61.6, 63.8)	<0.0001
Diabetes duration (years)	11.4 (10.9, 11.9)	10.5 (9.9, 11.1)	11.5 (10.5, 12.5)	13.5 (12.5, 14.5)	<0.0001
Male*	97.1	95.95	98.62	98.15	0.0835
Non-Hispanic white*	60.0	54.91	66.82	65.28	0.0021
Intensive treatment*	49.7	46.63	53.46	54.17	0.0866
Prior vascular event*	38.5	33.53	39.63	49.07	0.0004
Current smoker*	17.3	19.85	12.56	15.74	0.0472
Hypertension*	73.1	69.19	76.96	78.70	0.0105
Exercise*	44.5	43.91	45.83	44.44	0.8918
Adherence to diet*	46.7	45.95	47.69	47.69	0.8667
Aspirin*	91.8	92.26	90.74	91.63	0.7885
Measured at time of CTGF measurement					
Statin*	79.0	78.72	77.31	81.40	0.5662
ACE*	68.5	68.09	67.59	70.23	0.8099
ARB*	12.6	10.83	15.74	13.49	0.1681
HbA_{1c} (%)	8.0 (7.9, 8.1)	8.0 (7.9, 8.1)	7.9 (7.8, 8.1)	8.1 (7.9, 8.2)	0.6426
ACR‡	19 (17, 21)	13 (11, 15)	23 (18, 29)	38 (30, 48)	<0.0001
eGFR	79.6 (78.1, 81.1)	84 (83, 86)	77 (74, 80)	70 (67, 73)	<0.0001
BMI (kg/m^2)	32.4 (32.0, 32.7)	31.7 (31.3, 32.2)	32.9 (32.2, 33.5)	33.4 (32.7, 34.0)	<0.0001
SB pressure (mmHg)	127 (126, 128)	126 (125, 128)	128 (125, 130)	130 (128, 132)	0.0244
DB pressure (mmHg)	72.8 (72, 73)	73 (72, 74)	72 (71, 73)	73 (71, 74)	0.3449
HDL cholesterol (mg/dL)	38 (37, 39)	39 (38, 40)	37 (35, 38)	37 (36, 39)	0.0547
LDL cholesterol (mg/dL)	96 (94, 98)	95 (92, 98)	94 (89, 98)	100 (96, 105)	0.0487
Triglycerides (mg/dL)‡	153 (148, 159)	145 (138, 153)	156 (145, 168)	172 (160, 186)	0.0012

Continuous characteristics are shown as means with associated 95% CIs, while categorical characteristics are shown as percentages. Characteristics of the study population adjusted for age, minority status, and treatment arm of the study. ACR, albumin-to-creatinine ratio; ARB, angiotensin II receptor blockers; DB, diastolic blood; SB, systolic blood. *Unadjusted. ‡Owing to nonnormal distributions, geometric means are presented. **χ^2 for categorical variables and F test for continuous variables.

levels and the end of VADT follow-up. Initial models examining the association between the three CTGF categories and each cardiovascular event of interest were assessed after age, ethnic minority, and treatment arm were controlled for. Secondary models additionally adjusted for prior cardiovascular event, hypertension status, and smoking status at date of randomization, as well as LDL cholesterol level, triglycerides, use of ACE inhibitors, and use of statins at time of CTGF measurement. The final model also adjusted for estimated glomerular filtration rate (eGFR) at time of CTGF measurement. These covariates were chosen a priori, since they represent either study design variables or established cardiovascular risk factors.

Appropriate interaction terms were used to determine whether treatment arm, ethnic minority status, or prior CVD event modified the relationship between CTGF categories and outcomes of interest. Potential effect modifiers were chosen a priori, since they represent either study design variables or established cardiovascular risk factors. The assumption of proportional hazards was evaluated by testing for interaction between the two CTGF index variables (i.e., defining the three CTGF categories) and continuous time variables. Reported *P* values are two sided with a type I error rate significance level of $\alpha = 0.05$. HRs (95% CI) are displayed. All analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

RESULTS

At VADT baseline, the mean age of the study population was 59.7 years and the mean duration of diabetes was 11.4 years. Of the 952 participants studied, 97.1% were male, 60% were non-Hispanic white, 38.5% had experienced a prior event, and 49.7% were assigned to the VADT intensive treatment group. HbA_{1c} levels, diastolic blood pressure, and HDL cholesterol remained similar across the three categories of CTGF after adjustment for age, ethnic minority status, and treatment arm of the study. Also, there was no association of CTGF categories with treatment for statins, ACE inhibitors, or aspirin. BMI, systolic blood pressure, albumin-to-creatinine ratio, eGFR, LDL cholesterol, and triglycerides were statistically significantly different among the three CTGF groups (Table 1). During the follow-up period, 46 had an MI and 66 experienced an MI or cardiovascular death after measurement of CTGF (Table 2).

Figure 1 depicts Kaplan-Meier survival curves for MI (Fig. 1*A*), MI combined with cardiovascular death (Fig. 1*B*), and all-cause mortality (Fig. 1*C*). Cox proportional hazards models were used to examine the ability of CTGF to predict VADT macrovascular end points (Table 3). After adjustment for age, minority status, treatment arm, prior cardiovascular event, hypertension status and smoking status at date of randomization, as well as LDL cholesterol level, triglycerides, use of ACE inhibitors, and use of statins at time of CTGF measurement, individuals with CTGF level >13.4 ng/mL, compared with individuals with CTGF level <7.8 ng/mL, had significantly higher risk of MI (HR 2.36 [95% CI 1.16, 4.84]), MI or cardiovascular death (HR 2.76 [95% CI 1.51, 5.04]), and death from any cause (HR 3.02 [95% CI 1.66, 5.50]). This significantly increased risk was not observed in comparison of individuals with CTGF level between 7.8 and 13.4 ng/mL with individuals with CTGF level <7.8 ng/mL (Table 3). Moreover, additional adjustment for eGFR at time of the biomarker measurement had only a minimal impact on the HR for MI (HR 2.43 [95% CI 1.15, 5.14]) and MI or cardiovascular death (HR 2.71 [95% CI 1.44, 5.08]) and a slightly larger impact on the HR for all-cause mortality (HR 2.70 [95% CI 1.43, 5.08]). There was no evidence that study treatment arm, minority status, or prior history of a cardiovascular event modified the association between CTGF level and any of the macrovascular end points examined.

Table 2—VADT cardiovascular events after measurement of CTGF (total *N* = 952)

Cardiovascular end points	*N* (%)
MI	46 (4.83)
MI, procedure or inoperable	105 (11.03)
MI or cardiovascular death	66 (6.93)
Composite end point*	166 (17.44)
Death	66 (6.93)

*Composite end point includes documented MI; stroke; death from cardiovascular causes; new or worsening congestive heart failure; surgical intervention for cardiac, cerebrovascular, or peripheral vascular disease; inoperable CAD; and amputation for ischemic gangrene.

CONCLUSIONS

We previously reported that in type 1 diabetes, increased plasma CTGF levels were associated with increased common and internal carotid intima-media thickness, an established marker of subclinical atherosclerosis, as well as macroalbuminuria and hypertension (9). In the current study, we report CTGF is a strong predictor of MI, MI combined with cardiovascular death, and all-cause mortality. The ability of CTGF to predict these end points remained after established cardiovascular risk factors were controlled for, including age, minority status, VADT treatment arm, history of CVD, history of hypertension, lipid levels, smoking status, use of ACE inhibitors, use of statins, and eGFR. Moreover, when incident MI was combined with procedure or inoperable disease (i.e., not limited to acute events), the HR declined and was not significant, indicating that CTGF may be related to acute events through a mechanism other than its association with atherosclerotic burden, for which CTGF has been postulated to be a plaque-stabilizing factor (12).

A major difference between the VADT and DCCT/EDIC (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) cohorts besides diabetes type (i.e., type 1 vs. type 2 diabetes) is the level of CVD at the time of measurement of CTGF. Patients enrolled in the VADT study were older, had a longer duration of diabetes and a high comorbidity burden, and were likely to have a history of CVD (i.e., 38.5%). DCCT/EDIC participants, on the other hand, were younger, had a shorter duration of diabetes and a low comorbidity burden, and had not had a cardiovascular event prior to enrollment. Results from both the DCCT/EDIC and VADT, which collectively include participants across the continuum of diabetes disease burden, indicate that CTGF may play a role not only in development of atherosclerosis but also in prediction of acute events and, as such, may have substantial value both as a biological marker of inflammation-induced tissue injury and as a therapeutic target.

The findings of the current study also point to a relationship between plasma CTGF levels and renal function. Our data indicated that the mean eGFR levels declined across increasing categories of CTGF. This is in line with our previous findings in which increased levels of plasma CTGF N-fragment were associated

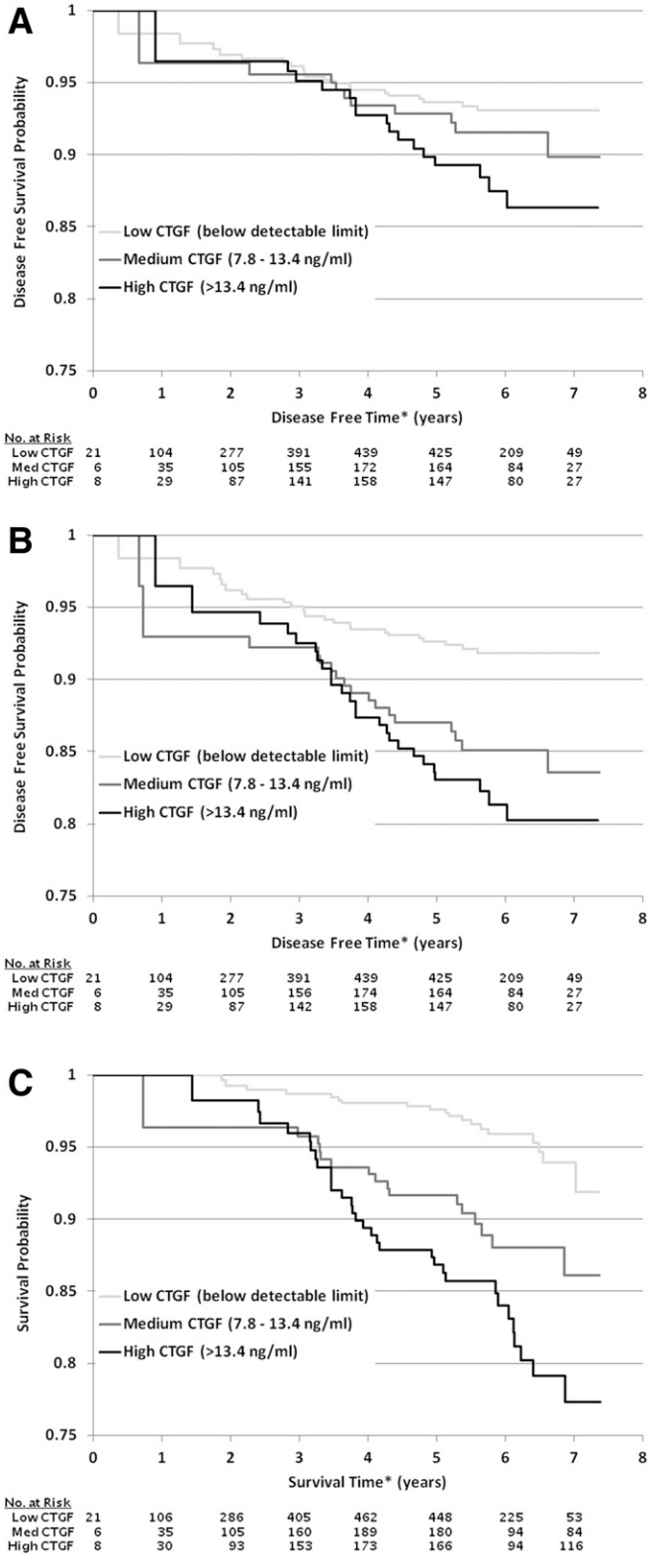

Figure 1—Kaplan-Meier survival curves for incident MI (*A*), MI combined with cardiovascular death (*B*), and all-cause mortality (*C*). *The time axis starts at time of randomization, and left truncation is used to account for differences in time at risk, since CTGF was measured on samples collected a median of 1.94 years after randomization. Med, medium.

with decreased eGFR in patients with type 1 diabetes (9). The increase in plasma CTGF levels observed in patients with type 1 diabetes with overt albuminuria was not the result of a decrease in renal clearance of CTGF but, rather, was a result of increased production (9). It is important to point out that adjustment for eGFR had little impact on the HRs of CTGF between model 2 and model 3 (Table 3), indicating that renal function is not a confounder of the relationship between CTGF and outcomes of interests (i.e., MI, MI and cardiovascular death, all-cause mortality).

The increased risk of cardiovascular events seen in patients with type 2 diabetes is associated with a cluster of risk factors for cardiovascular and metabolic disorders that tend to coexist in these patients, including central adiposity, hypertension, and dyslipidemia (32). However, the complex signaling networks through which diabetes, hypertension, and dyslipidemia accelerate vascular damage and trigger acute vascular events are not well-defined. The modifiable factors engaged in these processes have yet to be identified, but there is evidence for promotion of chronic low-grade inflammation, oxidative stress, endothelial dysfunction, stimulation of proliferative/apoptotic pathways, and deposition of extracellular matrix. Importantly, inflammatory mediators and growth factors are increasingly recognized as key players in the pathogenesis of macrovascular disease (33).

Emerging studies indicate that CTGF/CCN2 is a pathogenic risk determinant acting along a causal pathway for development of CVD. CTGF is a secreted proadhesive matricellular protein that has been implicated in modulating inflammatory and fibroproliferative disorders (34). The expression of CTGF mRNA and protein levels were increased in vascular cells of advanced atherosclerotic lesions and were found to be aggregated predominantly in areas near the fibrous cap of the plaque, suggesting potential involvement in atherosclerotic plaque production (35,36). In addition, inhibition of CTGF in the cardiovascular system was shown to reverse tissue remodeling and the process of fibrosis (37). Moreover, plasma CTGF was linked to an increased risk of cardiovascular events and mortality in patients with atherosclerotic disease and was associated with plaque stabilization after stroke (12,15). Although the exact mechanisms through which CTGF mediates its inflammatory and fibrotic effects on the vasculature are not fully defined, engagement of all six members of integrin receptors and

Table 3—Adjusted HRs (95% CI) from Cox proportional hazards regression models for CTGF in relation to various outcomes* in the total population

	CTGF		
	Model 1**	Model 2†	Model 3‡
MI			
Below detectable limit	1.00	1.00	1.00
7.8–13.4 ng/mL	1.28 (0.59, 2.75)	1.34 (0.61, 2.95)	1.36 (0.62, 3.02)
>13.4 ng/mL	2.24 (1.13, 4.45)	2.36 (1.16, 4.84)	2.43 (1.15, 5.14)
MI, procedure or inoperable disease			
Below detectable limit	1.00	1.00	1.00
7.8–13.4 ng/mL	0.77 (0.45, 1.31)	0.79 (0.46, 1.36)	0.79 (0.46, 1.37)
>13.4 ng/mL	1.40 (0.88, 2.23)	1.32 (0.82, 2.13)	1.32 (0.80, 2.17)
MI or cardiovascular death			
Below detectable limit	1.00	1.00	1.00
7.8–13.4 ng/mL	1.78 (0.95, 3.33)	1.63 (0.84, 3.18)	1.62 (0.83, 3.17)
>13.4 ng/mL	2.79 (1.55, 5.01)	2.76 (1.51, 5.04)	2.71 (1.44, 5.08)
Composite end point			
Below detectable limit	1.00	1.00	1.00
7.8–13.4 ng/mL	1.02 (0.69, 1.53)	0.94 (0.62, 1.42)	0.93 (0.61, 1.42)
>13.4 ng/mL	1.61 (1.11, 2.33)	1.44 (0.99, 2.10)	1.43 (0.97, 2.11)
All death			
Below detectable limit	1.00	1.00	1.00
7.8–13.4 ng/mL	1.76 (0.90, 3.46)	1.39 (0.68, 2.87)	1.30 (0.62, 2.71)
>13.4 ng/mL	3.30 (1.82, 5.99)	3.02 (1.66, 5.50)	2.70 (1.43, 5.08)

*The number of events for each outcome for each model are as follows: MI, 46, 45, and 45 for model 1, model 2, and model 3, respectively; MI, procedure or inoperable disease, 105, 104, and 104; MI or cardiovascular death, 66, 63, and 63; composite end point, 166, 162, and 162; and all death, 66, 63, and 63. **Adjusted for age, minority, and treatment arm. †Additionally adjusted for prior cardiovascular event, hypertension status, and smoking status at date of randomization, as well as LDL, triglycerides, use of ACE inhibitors, and statin use at time of CTGF measurement. ‡Additionally adjusted for eGFR at time of CTGF measurement.

binding to heparin sulfate proteoglycans such as syndecan-4, LDL-related receptor protein, and the cation-independent mannose-6-phosphate receptor have been implicated as key factors through which CTGF transduces its cellular effects (38). It is of interest to point out here that CCN3 (nov), another member of the CCN family, has been shown to act downstream of TGF-β to inhibit the expression of CCN2 and, hence, attenuate deposition of extracellular matrix proteins (39,40). CCN3 functions as an endogenous negative modulator of the profibrotic actions of CCN2 in vivo to promote deposition of matrix proteins, thus providing a mechanistic pathway in which the activity of CCN2 can be regulated in disorders of fibrosis (39,40).

One limitation of our study is that the assay used to measure CTGF was designed to measure CTGF levels above median values in the population; hence, slightly more than half of the VATD population had CTGF levels below the detectable limit of the assay. Thus, we were forced to group all individuals below the detectable limit into a single category and were therefore unable to examine CTGF across a continuum of levels. Second, because the vascular disease burden was high among patients at enrollment into the VADT study, our measurement of CTGF, while occurring prior to development of acute cardiovascular events, likely represents CTGF measured when atherosclerotic burden was high. Hence, it is difficult to determine the temporal relationship between high plasma CTGF levels and development of atherosclerosis and whether CTGF levels were elevated because of the high burden of atherosclerosis or whether they played a causal role in the development of atherosclerosis. It is also difficult to know whether the association between CTGF and acute events only reflects an association between high atherosclerotic burden and increased risk of acute events or whether CTGF levels could also be related mechanistically to acute events. Our analysis, which reports an association with MI and MI combined with cardiovascular death, but not with MI combined with procedure or inoperable disease, suggests that CTGF levels may be related mechanistically to acute events. A third limitation is the somewhat low number of end points, with 46 events for MI and 66 events when MI is combined with cardiovascular death.

In summary, this study demonstrates for the first time that high levels of CTGF/CCN2 predict future MI and cardiovascular death in patients with type 2 diabetes. Plasma CTGF/CCN2 levels warrant further study as a potential biomarker to be used for risk stratification with respect to prediction of cardiovascular events. Moreover, further understanding of the mechanisms responsible for the reported relationship between CTGF/CCN2 and acute vascular events may identify novel targets for therapeutic interventions.

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