ORIGINAL RESEARCH

Association of Circulating Tissue Inhibitor of Metalloproteinases-1 and Procollagen Type III Aminoterminal Peptide Levels With Incident Heart Failure and Chronic Kidney Disease

Wolfgang Lieb, MD, MSc; Rebecca J. Song, MPH; Vanessa Xanthakis, PhD; Ramachandran S. Vasan, MD

***Background*—**Tissue inhibitor of metalloproteinases-1 (TIMP-1) and procollagen type III aminoterminal peptide are established circulating markers of extracellular matrix remodeling and associated with cardiovascular disease. The association of both biomarkers with incident congestive heart failure and chronic kidney disease (CKD) in the community is not well studied.

***Methods and Results*—**We measured plasma total TIMP-1 and procollagen type III aminoterminal peptide levels in 922 Framingham participants (mean age, 57 years; 57% women) and related both biomarkers to the risk of incident CKD and congestive heart failure in multivariable-adjusted Cox regression models. Plasma total TIMP-1 levels were positively associated with risk of incident CKD (164 events; hazard ratio per 1 SD in log-biomarker, 1.90; 95% CI, 1.53–2.37) in multivariable models, including adjustments for left ventricular mass, C-reactive protein, and B-type natriuretic peptide levels. The association of total TIMP-1 with risk of congestive heart failure was statistically significant in an age- and sex-adjusted model, but was attenuated upon adjustment for conventional risk factors. Blood procollagen type III aminoterminal peptide levels were not related to the risk of CKD or congestive heart failure.

***Conclusions*—**Higher baseline levels of total TIMP-1 conferred an increased risk for incident CKD, independent of conventional risk factors and circulating biomarkers of chronic systemic inflammation and neurohormonal activation. Our prospective observations in a large community-based sample support the role of matrix remodeling in the pathogenesis of CKD. **(*J Am Heart Assoc.* 2019;8e011426. DOI: 10.1161/JAHA.118.011426.)**

Key Words: biomarker • chronic heart failure • chronic kidney disease

Progressive structural remodeling and increased fibrotic activities in the heart and the kidneys are associated with chronic organ function decline,[1] which may ultimately lead to congestive heart failure (CHF)[2] and chronic kidney disease (CKD).[3] Increased collagen/connective tissue deposition in the extracellular matrix is a hallmark of myocardial and renal fibrosis and antedate overt disease.[2,3]

Collagen turnover and extra cellular remodeling are regulated by matrix metalloproteinases and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs).[4–6] Another interesting group of candidate biomarkers for extracellular matrix remodeling are procollagen peptides, which represent molecules that are cleaved from collagen precursors.[4] Accordingly, circulating concentrations of metalloproteinases, TIMPs, and procollagen peptides might represent useful biomarkers for the prediction of the risk of developing those clinical disease conditions that are characterized by increased fibrotic activity—CHF and CKD are prototypical conditions.

There are several circulating biomarkers of extracellular matrix remodeling and tissue fibrosis, including several propeptides such as procollagen types I, II, and III aminoterminal peptide (PINP, PIINP, PIIINP, respectively) and type 1

From the Framingham Heart Study, Framingham, MA (W.L., V.X., R.S.V.); Section of Preventive Medicine and Epidemiology, Boston University School of Medicine, Boston, MA (V.X., R.S.V.); Departments of Epidemiology (R.J.S., R.S.V.), and Biostatistics (V.X.), Boston University School of Public Health, Boston, MA; Institute of Epidemiology, Kiel University, Kiel, Germany (W.L.).

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Correspondence to: Ramachandran S. Vasan, MD, Framingham Heart Study, 73 Mount Wayte Ave, Framingham, MA 01702-5803. E-mail: vasan@bu.edu and Wolfgang Lieb, MD, MSc, Institute of Epidemiology, UKSH, Haus 1, Niemannsweg 11, 24105 Kiel, Germany. E-mail: wolfgang.lieb@epi.uni-kiel.de

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Clinical Perspective

What Is New?

- Tissue inhibitor of metalloproteinases-1 and procollagen type III aminoterminal peptide are biomarkers of extracellular matrix remodeling.
- We observed in our community-based sample with almost 20 years of follow-up that baseline plasma concentrations of tissue inhibitor of metalloproteinases-1 were associated with new-onset chronic kidney disease even after adjusting for standard risk factors and markers of systemic inflammation and neurohormonal activation; furthermore, baseline circulating tissue inhibitor of metalloproteinases-1 concentrations were associated with an increased risk of incident congestive heart failure in age- and sex-adjusted models, but the association was attenuated upon adjustment for standard risk factors.
- Blood procollagen type III aminoterminal peptide levels were not associated with the risk of incident chronic kidney disease or congestive heart failure in our sample.

What Are the Clinical Implications?

- Our data provide additional evidence that higher circulating concentrations of markers of extracellular matrix remodeling, especially tissue inhibitor of metalloproteinases-1, may reflect a greater propensity for developing chronic kidney disease in the future.

procollagen C-terminal propeptide. In the present investigation, we focused on the relations of blood concentrations of tissue inhibitor of metalloproteinases-1 (TIMP-1) and procollagen type III aminoterminal peptide (PIIINP) to the risk of developing CHF and CKD. These biomarkers were assayed between 2002 and 2004 and at a time when preliminary evidence linked both biomarkers to cardiac and renal structure and function, as detailed below; other biomarkers of tissue fibrosis were not assayed and hence were unavailable for analyses. The clinical correlates of TIMP-1 and PIIINP in our sample have been reported previously.[7,8] Circulating TIMP-1 concentrations were associated with age, sex, body mass index, the total/HDL-cholesterol ratio, alcohol intake, smoking, diabetes mellitus, and the use of antihypertensive medication,[8] whereas circulating PIIINP levels were correlated with age and body mass index in our sample.[7]

Furthermore, TIMP-1 concentrations have been comprehensively studied in patients with clinical heart failure (HF) and were related to HF stages and prognosis.[9,10] Furthermore, associations of cardiac remodeling traits, for example, left ventricular (LV) mass,[8,11,12] with blood TIMP-1 levels have been reported. On a parallel note, PIIINP levels were associated with indices of cardiac structure and function in some[13] but not all reports.[7] However, it is not well known whether circulating TIMP-1 or PIIINP levels are associated with incident CHF in the general population.

Both biomarkers have also been evaluated in prior studies in relation to renal diseases. For example, *urinary* levels of PIIINP were associated with CKD progression in the elderly,[14] and in a relatively small sample of children, serum and urinary PIIINP levels were evaluated in the context of obstructive nephropathy.[15] Furthermore, serum PIIINP levels correlated with CKD stage in a moderate-sized clinical sample (n=242).[16] Similarly, in other moderate-sized referral samples, both serum and urine TIMP-1 levels have been associated with CKD.[17,18]

Notwithstanding the aforementioned intriguing evidence, it is not clear if circulating TIMP-1 and PIIINP levels are associated with new-onset CKD in the general population. Most prior reports were limited by relatively small samples,[11,15] referral bias,[9,10] and cross-sectional design.[7,8] We hypothesized that plasma levels of total TIMP-1 and PIIINP are associated with incident HF and CKD prospectively. We tested these hypotheses in a moderate-sized community-based sample followed up for nearly 2 decades.

Methods

Study Sample

Analyses were conducted using data obtained on the Framingham Offspring cohort.[19] Participants of this cohort are examined at the Framingham Research Center approximately every 4 years. At the sixth examination cycle, plasma total TIMP-1 (sum of circulating and metalloproteinase-bound TIMP-1) and PIIINP levels were measured in a subsample of participants —those with echocardiographic evidence of increased LV wall thickness and/or increased LV end-diastolic diameter (LVEDD) (in the top sex-specific decile) and in a reference sample with both LV wall thickness and LVEDD below the 50th percentile (detailed below; see Echocardiographic Measurements).[8,20] Therefore, examination cycle 6 was considered the baseline examination for the present investigation.

We restricted TIMP-1 and PIIINP measurements to these 2 subgroups: (1) individuals with evidence of structural cardiac alterations; and (2) a reference group with no evidence of cardiac remodeling,[20] because initial evidence from other studies suggested that circulating levels of these markers are altered in patients with clinical HF or in individuals with evidence of cardiac remodeling (eg, with LV hypertrophy).[20–22] There were 3532 Offspring participants who attended examination cycle 6; among those, 924 had measurements of both TIMP-1 and PIIINP, and we excluded people with a serum creatinine value >2 mg/dL (n=2), resulting in a sample of n=922 (base sample). For the CHF analyses, we additionally excluded those with prevalent CHF at baseline (n=12),

resulting in a sample size of 910 participants (Sample 1). For the CKD analyses, we excluded those without a serum creatinine measurement at baseline (n=3), with an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m² at baseline (n=68), or those who did not have a serum creatinine measurement during follow-up (n=44), resulting in a sample size of 807 participants (Sample 2). The study protocol was approved by the Institutional Review Board at Boston University Medical Center, and all participants provided written informed consent. Anonymized data from the Framingham Heart Study have been made publicly available at the database of Genotypes and Phenotypes.[23]

Biomarker Measurements

Plasma total TIMP-1 and PIIINP levels were measured in duplicate in plasma samples from the sixth examination cycle using an ELISA assay (Amersham Pharmacia Biotech, Piscataway, NJ) and a radioimmunoassay (Orion Diagnostica, Espoo, Finland), respectively.[24] The coefficients of variation were <5% (TIMP-1) and 6% (PIIINP), respectively.[24]

Echocardiographic Measurements

Based on echocardiographically obtained values for LV wall thickness and for LVEDD (please see Data S1 for details), participants were classified into a referent group (with both LV wall thickness and LVEDD below the 50th percentile) and into a remodeled group (with values for at least one of LVEDD or LV wall thickness above the 90th percentile), generating a binary variable labeled "LV sampling group" as described in detail elsewhere.[20,24]

Definition and Adjudication of Clinical End Points

CHF was defined according to predefined published epidemiologic criteria[25] and considered present if 2 major or if 1 major and 2 minor criteria were present.[25] Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology formula.[26] Incident CKD was defined as an eGFR ≥60 mL/min per 1.73 m² at examination cycle 6[27] and an eGFR <60 mL/min per 1.73 m² at examination cycle 7, 8, or 9. The adjudication of clinical events was conducted by a panel of experienced physicians, who reviewed all pertinent medical records (Framingham Research Center data, physician office visits, and hospitalizations).

Statistical Analyses

Pairwise Spearman rank correlations of TIMP-1 and PIIINP levels with each other and with CRP (C-reactive protein) and B-type natriuretic peptide (BNP) levels were estimated adjusting for age and sex.[28] We natural-logarithmically transformed TIMP-1 and PIIINP to normalize their skewed distributions and standardized the values. To relate TIMP-1 and PIIINP to incident clinical events, Cox proportional hazards regression models were estimated treating TIMP-1 and PIIINP as independent variables (separate model for each) and time to incident CHF (Sample 1) and CKD (separate model for each; Sample 2) as outcome variables after confirming that the assumption of proportionality of hazards was met for both. For the analyses related to incident CKD, Cox proportional hazard models with discrete time intervals were applied because we defined incident CKD at defined time points (the Framingham examination cycles), based on eGFR estimations obtained in the Framingham Heart Study research clinic.

We estimated an age- and sex-adjusted model (Model 1) as well as multivariable-adjusted models including age, sex, body mass index, systolic blood pressure, antihypertensive treatment, current smoking, diabetes mellitus, and LV sampling group (Model 2). A third model (Model 3) additionally included LV mass, and Model 4 also adjusted for blood CRP and BNP concentrations, established markers of chronic systemic inflammation, and neurohormonal activation, respectively. To assess linearity of the association between TIMP-1 and incident CKD, we estimated restricted cubic splines, adjusting for all covariates used in Model 4. In secondary analyses, we also adjusted Model 4 additionally for biomarkers of liver function [AST (aspartate transaminase), ALT (alanine transaminase), and GGT (gamma-glutamyltransferase)] or for the intake of lipid-lowering and antidiabetic medications.

Results

The clinical, biochemical, and echocardiographic characteristics of our study sample are provided in Table 1. Sample characteristics stratified by tertiles of TIMP-1 are displayed in Table S1. Age- and sex-adjusted pairwise correlations of plasma total TIMP-1, PIIINP, CRP, and BNP are displayed in Table S2. Over the course of the follow-up period, 71 individuals developed CHF (median follow-up time, 17.3 years) and 164 participants developed incident CKD. We assessed cardiac function after the onset of CHF in 69 of 71 individuals with incident CHF. Thirty-three (48%) of those who developed HF over the course of the follow-up period, developed HF with reduced ejection fraction (<50%).

Association of Plasma Total TIMP-1 and PIIINP With Incident CKD and CHF

Plasma total TIMP-1 levels were positively associated with incident CKD and CHF in age- and sex-adjusted models (Table 2). Upon multivariable adjustment for standard cardiovascular disease (CVD) risk factors (Model 2), the associations

Table 1. Baseline Characteristics of the Sample, Stratified by Men and Women

Characteristic	Men (n=395)	Women (n=527)
Age, y	57.3±9.9	57.6±9.8
Body mass index, kg/m²	27.7±3.9	26.6±5.6
Systolic blood pressure, mm Hg	128±18	125±20
Total/HDL cholesterol ratio	4.8±1.4	3.9±1.3
Total cholesterol, mg/dL	196.6±35.0	212.9±40.1
Triglycerides, mg/dL	137.1±92.6	130.4±82.7
Hypertension treatment, n (%)	106 (26.8)	120 (22.8)
Lipid-modifying treatment, n (%)	48 (12.2)	52 (9.9)
Diabetes mellitus treatment, n (%)	34 (8.6)	19 (3.6)
Prevalent CVD, n (%)	57 (14.4)	43 (8.2)
Diabetes mellitus, n (%)	51 (12.9)	32 (6.1)
Estimated glomerular filtration rate, mL/min per 1.73 m²	85.5±17.4	87.2±18.6
Current smoking, n (%)	46 (11.6)	92 (17.5)
AST, U/L	22.0 (19.0, 22.0)	19.5 (17.0, 23.0)
ALT, U/L	22.0 (17.0, 29.0)	16.0 (13.0, 21.0)
GGT, total fraction, U/L	25.6 (18.8, 34.9)	18.2 (13.8, 26.5)
LV sampling group		
Referent, n (%)	226 (57.2)	307 (58.3)
Remodeled, n (%)	169 (42.8)	220 (41.7)
Echocardiographic traits		
LV mass, g	189.5±56.6	135.7±40.4
LV enddiastolic diameter, cm	5.0±0.6	4.5±0.5
LV wall thickness, cm	2.0±0.3	1.8±0.3
Fractional shortening, %	35.2±0.1	38.5±0.1
Biomarkers		
PIIINP, ng/mL	3.4 (2.6, 4.2)	3.0 (2.4, 4.0)
TIMP-1, ng/mL	804.8 (723.5, 899.7)	754.0 (674.8, 851.3)

Data are presented as mean±SD or median (Q1, Q3), unless otherwise noted. AST indicates aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyltransferase; CVD, cardiovascular disease; HDL, high-density lipoprotein; LV, left ventricular; PIIINP, procollagen type III aminoterminal peptide; TIMP-1, tissue inhibitor of metalloproteinases-1.

of plasma total TIMP-1 with CKD remained statistically significant. Additionally, in models with adjustments for standard risk factors, LV mass, BNP, and CRP, the association of TIMP-1 with incident CKD was maintained (Table 2; Models 3 and 4). Additional adjustments for biomarkers of liver function (AST, ALT, and GGT) or for the intake of lipid-lowering and antidiabetic medications still revealed highly statistically significant associations for TIMP-1 ($P<0.001$) with incident CKD (data not shown). The restricted cubic spline showed a linear relation between TIMP-1 and CKD (Figure; P for nonlinearity=0.074).

However, the association of TIMP-1 with CHF was rendered statistically nonsignificant upon multivariable adjustment for standard risk factors (Table 2; Models 2 and 3). Additional adjustment for biomarkers of liver function (AST, ALT, and GGT) or for the intake of lipid-lowering and antidiabetic medications did not alter the results (data not shown).

Circulating PIIINP concentrations were not related to incident CKD or CHF in age- and sex-adjusted and multivariable-adjusted models (Table 2).

Discussion

In a moderate-sized community-based sample, we examined the associations of plasma total TIMP-1 and PIIINP levels with new-onset (incident) CKD and CHF. Our main observations were 3-fold. First, plasma total TIMP-1 concentrations were positively associated with risk of incident CKD in multivariable-adjusted models. Second, plasma total TIMP-1 levels were positively associated with incident CHF in age- and sex-adjusted models, but not in multivariable models that included standard CVD risk factors. Third, we did not observe an association of circulating PIIINP levels with incident CKD or CHF in our sample.

Association of Plasma Levels of Total TIMP-1 With Incident CKD

TIMP-1 plasma levels displayed a strong linear and consistent association with incident CKD that was robust even after adjustment for biomarkers of chronic systemic inflammation (CRP) and neurohormonal activation (BNP), biomarkers of liver function, or intake of lipid-lowering and antidiabetic medications. This is in line with examinations on smaller samples in clinical settings. In a cross-sectional analysis, serum TIMP-1 levels were elevated in patients with advanced CKD and were highest in patients on dialysis (n=217).[29] Likewise, studies have shown that blood TIMP-1 levels were higher in hypertensive patients with (n=52) as compared with those without CKD (defined as eGFR<60 mL/min per 1.73 m²; n=335) and

Table 2. Associations of Circulating Levels of Total TIMP-1 and PIIINP With CKD and CHF

	HR per 1 SD Increment in Log-TIMP-1	*P* Value	HR per 1 SD Increment in Log-PIIINP	*P* Value
Incidence of CKD (164 events)				
1) Age- and sex-adjusted model	1.96 (1.61–2.39)	<0.001	1.03 (0.87–1.22)	0.74
2) Multivariable-adjusted model*	1.98 (1.61–2.43)	<0.001	1.03 (0.86–1.23)	0.77
3) Model 2+LV mass	1.97 (1.61–2.42)	<0.001	1.03 (0.86–1.23)	0.76
4) Multivariable-adjusted model*+LV mass+CRP+BNP	1.90 (1.53–2.37)	<0.001		
Incidence of CHF (71 events)				
1) Age- and sex-adjusted model	1.41 (1.09–1.82)	0.008	1.20 (0.94–1.53)	0.153
2) Multivariable-adjusted model*	1.21 (0.92–1.59)	0.174	1.28 (0.98–1.67)	0.076
3) Multivariable-adjusted model*+LV mass	1.20 (0.91–1.57)	0.200	1.27 (0.97–1.67)	0.086

BNP indicates B-type natriuretic peptide; CHF, congestive heart failure; CKD, chronic kidney disease; CRP, C-reactive protein; HR, hazard ratio; LV, left ventricular; PIIINP, procollagen type III aminoterminal peptide; TIMP-1, tissue inhibitor of metalloproteinases-1.

*The multivariable-adjusted model was adjusted for age, sex, body mass index, systolic blood pressure, antihypertensive treatment, current smoking, diabetes mellitus, and LV sampling group.

inversely associated with eGFR, even after controlling for standard risk factors.[30] Furthermore, TIMP-1 levels were higher in renal transplant recipients as compared with a healthy reference group and correlated with kidney function after kidney transplantation (n=150).[31] In a small study, control individuals (n=24) displayed statistically significantly higher TIMP-1 levels as compared with patients with stage I/II CKD (n=20) and patients with stage V CKD (n=20).[32] TIMP-1 levels did not differ between controls (n=45) and patients with CKD (n=132) in another report.[33]

We extend these prior predominantly cross-sectional analyses of referral samples. We observed a positive association of baseline plasma levels of TIMP-1 with new-onset CKD in multivariable-adjusted models including traditional CVD risk factors (several of which were correlated with TIMP-1 levels)[8] as well as LV mass, CRP, and BNP. This suggests that a relevant part of the association of TIMP-1 concentrations with incident CKD might be independent of traditional CVD risk factors. Indeed, cell experimental data underscore the significance of TIMP-1 for renal function and aging,[34] and

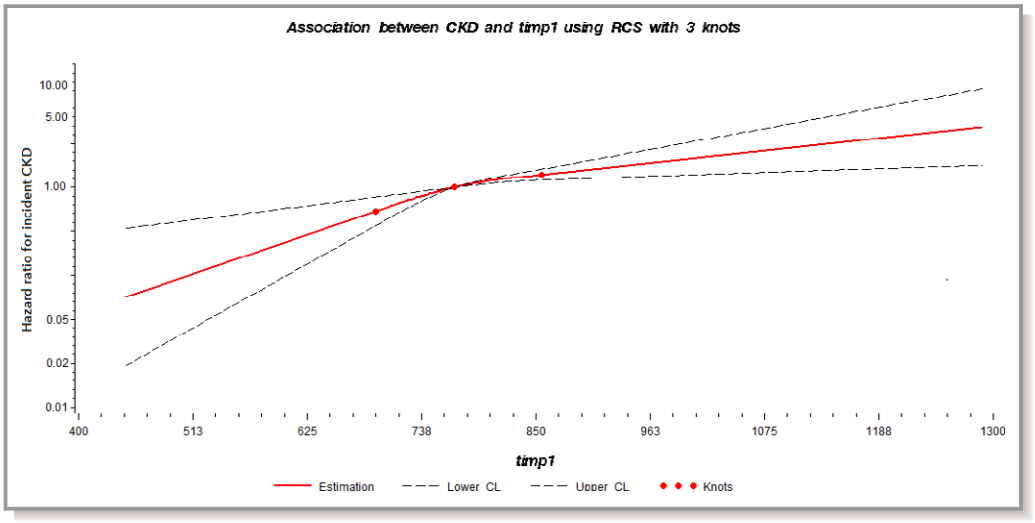

Figure. Restricted cubic splines (RCS), displaying the association of plasma total tissue inhibitor of metalloproteinases-1 (TIMP-1) with incident chronic kidney disease (CKD), adjusted for age, sex, body mass index, systolic blood pressure, intake of antihypertensive medication, smoking, diabetes mellitus, left ventricular sampling group, left ventricular mass, C-reactive protein, and B-type natriuretic peptide; with knots placed at 25th, 50th, and 75th percentile (*P* for nonlinearity=0.074). The reference value for TIMP-1 was 769.93.

various links between the different biological functions of TIMP-1 and CKD have recently been summarized,[35] including, for example, effects of TIMP-1 on apoptosis, an important feature of CKD.[35]

Association of PIIINP Levels With Incident CKD

Data on the association of PIIINP with CKD are limited. In the community-based Cardiovascular Health Study, *urine* levels of PIIINP in elderly participants (mean±SD age, 78±5 years at baseline) were associated with renal function decline and incident end-stage CKD, but the association with incident end-stage CKD was rendered statistically nonsignificant in multivariable analyses.[14] In smaller clinical samples, however, *serum* PIIINP levels correlated with the stage of CKD (total n=242)[16] and the urinary ratio of PIIINP and creatinine correlated with renal function (eGFR) and the degree of interstitial fibrosis in patients with different CKD stages (n=199).[36] In our larger middle-aged community-based sample, however, plasma PIIINP levels were not associated with incident CKD. It is possible that urinary PIIINP levels more closely reflect fibrotic activities in the kidneys and might, therefore, better predict incident CKD or kidney function decline, as compared with plasma PIIINP levels.

Plasma Levels of Total TIMP-1 and PIIINP in Relation to Incident Heart Failure

In prior analyses, we have reported that both markers, plasma total TIMP-1 and PIIINP, conferred a higher risk of all-cause mortality in the Framingham cohort.[24] Plasma PIIINP levels were also positively associated with the risk of incident CVD (a combined end point, including coronary insufficiency, angina pectoris, myocardial infarction, transient ischemic attack, stroke, intermittent claudication, and HF).[24] At the time of the latter analysis,[24] we lacked statistical power to evaluate the associations of these biomarkers with individual CVD end points.[24] Meanwhile, other community-based studies confirmed the associations of circulating/urinary TIMP-1 and PIIINP with all-cause mortality,[14,37,38] and some of them also with incident CVD,[37,39–41] even though some of the associations were substantially attenuated upon multivariable adjustment.[37,41]

With respect to HF, the observations in other community-based cohorts have been conflicting. In the MESA (Multi-Ethnic Study of Atherosclerosis), high serum PIIINP levels were associated with HF with preserved ejection fraction but not with HF with reduced ejection fraction.[42] In the Cardiovascular Health Study, *plasma* and *serum* PIIINP levels were positively associated with incident CHF[39,43] and with hospitalization for CHF,[43] respectively, in multivariable-adjusted models. *Urinary* PIIINP levels were not associated with HF risk in the Cardiovascular Health Study[14] or the Health ABC (Health, Aging, and Body Composition) study.[44] Our analyses of plasma PIIINP concentrations are in agreement with these latter studies.

Furthermore, associations of blood TIMP-1 with CHF have been reported, in most cases in cross-sectional settings in smaller clinical samples.[21,45] We extend these observations by examining the association between plasma TIMP-1 and new-onset (incident) CHF in the community. We observed a positive association between circulating TIMP-1 levels and risk for incident CHF in age- and sex-adjusted models, an association that was rendered statistically nonsignificant upon adjustment for standard CVD risk factors. This may indicate that the association between total TIMP-1 levels and CHF risk may be mediated by conventional risk factors, a premise we did not evaluate (due to modest number of incident CHF events). Indeed, prior analyses in our sample revealed that plasma TIMP-1 levels were associated with several CVD risk factors, including age, sex, body mass index, smoking, diabetes mellitus, lipid levels, and the intake of antihypertensive treatment.[8]

Strengths and Limitations

Strengths of the present investigation include the community-based prospective design, the careful assessment of 2 important clinical end points (chronic renal insufficiency and HF) and the prospective evaluation of baseline TIMP-1 and PIIINP levels with these end points over a time period of almost 20 years. Limitations include the moderate sample size (n=922) and the assessment of only 2 of several potential markers of extracellular matrix remodeling, even though we focused on markers previously implicated in CVD and renal disease. Furthermore, the sample, although community-based by design, selected individuals based on the distributions of echocardiographic measures, as noted above. Finally, our sample consisted of middle-aged white individuals of European ancestry. Therefore, our findings may not be generalizable to other age groups or racial groups/ethnicities.

Conclusion

We report a consistent positive association of plasma total TIMP-1 with incident CKD. This observation in a large, community-based, prospective setting supports a role of matrix remodeling in the pathogenesis of CKD. Replication of this observation in larger samples with a broader panel of extracellular matrix remodeling markers is warranted.

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Disclosures

None.

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Supplemental Material

Data S1.

Echocardiographic measurements

Ultrasonographic examinations of the heart were performed using the leading edge technique[1] on a Hewlett-Packard Sonos 1000 machine. Parameters of left ventricular structure and function were obtained at end-diastole, including LV end-diastolic diameter (LVEDD), LV posterior wall thickness (PWT), and inter-ventricular septum thickness (IVST), with the sum of the latter 2 defining LV wall thickness. Left ventricular mass was estimated as LVM (in gram) = $0.8[1.04((LVEDD+IVST+PWT)^3 - LVEDD^3)] +0.6$.[2]

Table S1. Baseline characteristics of the sample, stratified by tertiles of circulating TIMP-1 concentrations.

Characteristic	TIMP-1 Tertile 1 (446-720 ng/mL)	TIMP-1 Tertile 2 (>720-838 ng/mL)	TIMP-1 Tertile 3 (>838-1411 ng/mL)
Age, y	53.8 ± 8.3	56.5 ± 8.9	62.0 ± 10.4
Women, n (%)	92 (30)	141 (46)	162 (53)
Body Mass Index, kg/m²	25.6 ± 4.1	27.5 ± 5.3	29.1 ± 5.2
Systolic blood pressure, mm Hg	122 ± 19	126 ± 18	131 ± 20
Total/HDL Cholesterol ratio	3.8 ± 1.2	4.4 ± 1.4	4.6 ± 1.5
Total Cholesterol, mg/dL	205.0 ± 35.8	210.3 ± 39.2	202.6 ± 41.2
Triglycerides, mg/dL	117.0 ± 29.1	135.2 ± 93.4	147.6 ± 94.0
Hypertension treatment, n (%)	32 (10.4)	73 (23.7)	121 (39.4)
Lipid-modifying treatment, n (%)	18 (5.9)	36 (11.7)	36 (15.0)
Diabetes treatment, n (%)	4 (1.3)	12 (3.9	37 (12.1)
Prevalent CVD, n (%)	12 (3.9)	26 (8.4)	62 (20.2)
Diabetes mellitus, n (%)	7 (2.3)	20 (6.5)	56 (18.2)
Estimated glomerular filtration rate, mL/min/1.73 m²	91.0 ± 15.5	87.2 ± 18.6	80.8 ± 19.4
Current smoking, n (%)	52 (16.9)	34 (11.0)	52 (16.9)
Asparate aminotransferase, U/L	20.0 (17.0, 24.0)	20.0 (17.0, 25.0)	21.0 (18.0, 26.0)
Alanine aminotransferase, U/L	18.0 (14.0, 23.0)	18.0 (14.0, 25.0)	18.0 (14.0, 26.0)
Gamma-glutamyltransferase, total fraction, U/L	18.8 (13.9, 25.7)	21.0 (15.4, 31.3)	24.5 (17.5, 32.5)
LV sampling group			
Referent, n (%)	214 (69.7)	180 (58.4)	139 (45.3)
Remodeled, n (%)	93 (30.3)	128 (41.6)	168 (54.7)

Echocardiographic traits			
LV mass, g	139.5 ± 41.7	161.2 ± 57.2	175.5 ± 58.2
LV end-diastolic, cm	4.6 ± 0.5	4.8 ± 0.6	4.8 ± 0.6
LV wall thickness, cm	1.8 ± 0.3	1.9 ± 0.3	2.0 ± 0.3
Fractional shortening, %	38.1 ± 0.1	37.2 ± 0.1	35.9 ± 0.1
Biomarkers			
TIMP-1 ng/mL	667.1 (631.3, 695.3)	778.6 (753.3, 802.6)	915.7 (868.8, 995.5)
PIIINP, ng/mL	2.8 (2.2, 3.5)	3.2 (2.5, 3.9)	3.7 (2.8, 4.6)

Data are presented as mean±sd or median (Q1, Q3), unless otherwise noted.

HDL indicates high-density lipoprotein; LV, left ventricular; CVD, cardiovascular disease; TIMP-1, tissue inhibitor of metalloproteinases; PIIINP, procollagen type III aminoterminal peptide; sd, standard deviation

Table S2. Pair-wise Spearman correlation coefficients for total TIMP-1, PIIINP, CRP and BNP, adjusted for age and sex (n=860).

	Spearman Partial Correlation Coefficients			
	total TIMP-1	**PIIINP**	**CRP**	**BNP**
total TIMP-1	r=1	r=0.22 p<0.001	r=0.16 p<0.001	r=0.13 p<0.001
PIIINP	r=0.22 p<0.001	r=1	r=0.08 p=0.013	r=0.08 p=0.015
CRP	r=0.16 p<0.0001	r=0.08 p=0.013	r=1	r=-0.05 p=0.126
BNP	r=0.13 p<0.001	r=0.08 p=0.015	r=-0.05 p=0.12	r=1

TIMP-1 indicates Tissue Inhibitor of Metalloproteinases-1; PIIINP, Procollagen type III aminoterminal peptide; CRP, C-reactive protein; BNP, B-type natriuretic peptide

Supplemental References:

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