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Serum Fractalkine (CX3CL1) and Cardiovascular Outcomes and Diabetes: Findings From the Chronic Renal Insufficiency Cohort (CRIC) Study

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Abstract

Background—Cardiometabolic disease is a major cause of morbidity and mortality in persons with chronic kidney disease (CKD). Fractalkine (CX3CL1) is a potential mediator of both atherosclerosis and metabolic disease. Studies on the relationship of CX3CL1 with risk of CVD events and metabolic traits are lacking, particularly in the high-risk setting of CKD.

Study Design—Cross-sectional and longitudinal observational analysis.

Setting & Participants—Adults with CKD from 7 US sites participating in the Chronic Renal Insufficiency Cohort (CRIC) Study.

Predictor—Quartiles of plasma CX3CL1 levels at baseline.

Outcomes—Baseline estimated glomerular filtration rate (eGFR) from a creatinine- and cystatin C–based equation, prevalent and incident CVD, diabetes, metabolic syndrome and its criteria, homeostatic model assessment of insulin resistance, hemoglobin A1C, myocardial infarction, all-cause mortality, and the composite outcome of myocardial infarction/all-cause mortality.

Results—Among 3687 participants, baseline CX3CL1 levels were positively associated with several CVD risk factors and metabolic traits, lower eGFR, and higher levels of inflammatory cytokines as well as prevalent CVD (OR, 1.09; 95% CI, 1.01–1.19; $p=0.03$). Higher CX3CL1 was also associated with prevalent diabetes (OR, 1.26; 95% CI, 1.16–1.38; $p<0.001$) in adjusted models. During a mean follow up of 6 years, there were 352 deaths, 176 myocardial infarctions, and 484 with composite outcomes. In fully-adjusted models, 1-SD higher CX3CL1 increased the hazard for all-cause mortality (1.11; 95% CI, 1.00–1.22; $p=0.02$) and the composite outcome (1.09; 95% CI, 1.00–1.19; $p=0.04$).

Limitations—Study design did not allow evaluation of changes over time, correlation with progression of phenotypes, or determination of causality of effect.

Conclusions—Circulating CX3CL1 may contribute to both atherosclerotic CVD and diabetes in a CKD cohort. Further studies are required to establish mechanisms through which CX3CL1 affects pathogenesis of atherosclerosis and diabetes.

INDEX WORDS

cardiometabolic disease; chronic kidney disease (CKD); cardiovascular disease (CVD); atherosclerosis; metabolic syndrome; diabetes; fractalkine (CX3CL1); Chronic Renal Insufficiency Cohort (CRIC) Study

Chronic kidney disease (CKD), regardless of etiology, confers a markedly elevated risk for morbidity and mortality from cardiometabolic disease¹. Elucidating novel pathophysiologic pathways of these diseases may lead to more effective therapies. Fractalkine (CX3CL1) is a possible mediator of atherogenic cardiovascular disease (CVD) and diabetes mellitus (DM)^{2,3}. This chemokine promotes leukocyte adhesion and migration to vascular lesions in animal models of atherosclerosis⁴; similar effects are hypothesized to occur in obese adipose tissue³ in the pathogenesis of DM.

CX3CL1 is an inflammatory chemokine that promotes monocyte adhesion to endothelial cells⁵ and adipocytes and is elevated in persons with diabetes³. Humans with coronary artery disease (CAD) have increased CX3CR1-expressing peripheral blood mononuclear cells as well as higher serum CX3CL1 levels⁶; elevated levels are also found in patients with unstable angina pectoris⁴. Nonsynonymous variants of CX3CR1 (the CX3CL1 receptor), namely, a valine to isoleucine substitution at amino acid 249 (V249I) and a threonine to methionine substitution at amino acid 280 (T280M), which decrease binding of CX3CL1 to monocytes in functional assays, have been associated with reduced atherosclerosis and cerebrovascular disease^{7,8}. Polymorphisms in the CX3CR1 gene have also been associated with obesity and metabolic traits^{9,10}.

Animal and *in vitro* models and cross-sectional studies in humans suggest that CX3CL1 is associated with both atherogenesis^{4,6–8} and diabetes³, however, the relationship between plasma CX3CL1 with incident myocardial infarction (MI) and death as well as diverse metabolic traits have not been described in humans. To address whether CX3CL1 may contribute to the development of atherogenic CVD or associate with insulin resistance and diabetes, we performed a cross-sectional analysis of CX3CL1 with prevalent CVD and metabolic phenotypes in a population with CKD and determined whether CX3CL1 levels predict longitudinal outcomes. We examined in the Chronic Renal Insufficiency Cohort (CRIC) Study whether baseline plasma levels of CX3CL1 were associated with CVD risk factors, diabetes, metabolic traits, and the outcomes of MI and all-cause mortality in adults with CKD.

Methods

Study Population

The CRIC Study is an ongoing prospective, observational study of CKD¹⁰. The design of this cohort study and baseline characteristics of the participants have been reported¹¹. Briefly, 3939 individuals were recruited at seven US sites and followed up annually for an average of 6 years. Participants were aged 21–74 years, 46% female, ethnically diverse (45% white, 46% black, 5% Hispanic, 4% Asian/Pacific Islander/Native American), with a broad span of kidney function (estimated glomerular filtration rate [eGFR] range of ~15–90

[mean, 43.4±13.5 (standard deviation)] ml/min/1.73 m²) and ~48% having DM¹². We measured plasma CX3CL1 in 3,869 of the 3,939 (98.2%) participants. Among the participants with plasma CX3CL1 values, 182 were excluded from the analysis because of missing values for other variables, resulting in a sample of 3,687. The institutional review boards of all participating institutions approved the study protocol and all participants provided written informed consent.

Exposures

Levels of CX3CL1 were measured in duplicate using a commercial ELISA (Quantikine Immunoassay (R&D Systems Inc, Minneapolis, MN)^{13,14}. Intra-assay and inter-assay coefficients of variation were 9.9% and 12.0%, respectively, for low-concentration and 6.3% and 8.2% for high-concentration controls. High-sensitivity plasma C-reactive protein (hsCRP) was assayed by nephelometry and interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) were measured by high-sensitivity ELISA¹⁵ (R&D Systems Inc, Minneapolis, MN).

Cross-sectional Outcomes

Demographic factors and clinical data were obtained at baseline and annually by interview and questionnaire. Blood and urine laboratory tests were measured at a central laboratory using standard assays. Lipids, including total and high- and low-density cholesterol and triglycerides, were measured enzymatically (Hitachi 912, Roche Diagnostic Systems Inc., NJ, USA). An equation derived from CRIC Study data (including serum creatinine, cystatin C, age, sex, and race) was used to determine eGFR¹⁶. Diabetes was defined as fasting glucose ≥ 126 mg/dl, random glucose ≥ 200 mg/dl, or use of insulin or anti-diabetic medication. Metabolic syndrome was defined by accepted guidelines¹⁷, requiring at least three of the following: (1) history of hypertension, systolic blood pressure (SBP) > 130 mm Hg, or diastolic blood pressure (DBP) > 85 mm Hg, (2) history of diabetes or plasma glucose ≥ 100 mg/dl, (3) waist circumference > 102 cm for men and > 88 cm for women, (4) triglycerides ≥ 150 mg/dl, and (5) HDL < 40 mg/dl for men or < 50 mg/dl for women. Homeostatic model assessment of insulin resistance (HOMA-IR) was estimated as follows: $[\text{Glucose (mmol/L)} \times \text{Insulin (mU/L)}] / 22.5$ ¹⁸. Hypertension was defined as a SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or use of antihypertensive medications. Hyperlipidemia was defined as use of cholesterol-lowering medications or total serum cholesterol > 200 mg/dL.

Our primary metabolic outcome was diabetes at baseline. The presence of HOMA-IR and other metabolic syndrome components were examined in secondary analyses to provide greater insight into the relationships with metabolic traits.

Longitudinal Outcomes

Our primary longitudinal outcomes were the occurrence of probable or definite MI, all-cause mortality, and a composite of these endpoints during the average six years of follow-up. The MI and mortality events were assessed as previously described¹⁹, including review of detailed hospital records by two physician adjudicators. Participants were followed annually in person with interim telephone calls until death, withdrawal from study, lost to follow-up, or June 30, 2009, when data were locked for this analysis. More than 90% of the participants

were retained during this period. Prevalent CVD at enrollment (defined as self-reported prior diagnosis of CVD, stroke or peripheral vascular disease) was analyzed as a secondary outcome.

Statistical Analysis

One-way analysis of variance (continuous variables) and Pearson's chi-squared tests (categorical variables) were used to compare characteristics across baseline quartiles of plasma CX3CL1 levels. We used logistic regression to examine unadjusted and multivariable adjusted relationships between plasma CX3CL1 level and DM, and prevalent CVD at enrollment. The CX3CL1 values were standardized by subtracting the sample mean and dividing by the standard deviation (SD). Odds ratios (ORs) are reported per 1 SD increment of standardized CX3CL1.

Statistical models with prevalent CVD as the outcome were adjusted sequentially as follows: Model 1, for demographic factors (age, sex and race); Model 2, for demographic factors and traditional CVD risk factors (body mass index [BMI] and binary indicators for DM, hypertension, hyperlipidemia, and tobacco use); Model 3, for demographic factors, traditional CVD risk factors, and inflammatory biomarkers (log transformed IL-6, TNF- α , and hsCRP levels); Model 4, for demographic factors, traditional CVD risk factors, inflammatory biomarkers, and kidney function measures (eGFR and urinary albumin-creatinine ratio); and Model 5, for demographic factors, traditional CVD risk factors, inflammatory biomarkers, kidney function measures, and the binary indicator for metabolic syndrome.

For longitudinal outcomes there was an additional Model 6, which included Model 5 above plus prevalent CVD at baseline. Cox proportional hazards regression was used to examine unadjusted and multivariable adjusted relationships between CX3CL1, per 1-SD increment of standardized CX3CL1, and each of the following incident outcomes: (1) probable or definite MI, (2) all-cause mortality, and (3) a composite outcome of probable or definite MI and all-cause mortality (MI/all-cause mortality). Adjusted survival curves were generated using quartiles of CX3CL1 to illustrate findings fully adjusted for age, sex, race, traditional CVD risk factors, inflammatory markers, kidney function measures, and metabolic syndrome (Model 5). We explored potential impact of plasma CX3CL1 on the prognostic performance in risk prediction models: adjusted for age, sex, and race (Model 2) or fully adjusted (Model 5) with change in the area under the curve (AUC) for MI, all-cause mortality and combined outcomes using approaches described by Pencina et al²⁰.

Statistical models with diabetes or HOMA-IR as outcomes were identical to those of prevalent CVD except Model 2 contained only BMI. Model 5 was included only for the analysis with diabetes as an outcome. Analyses were performed using the R statistical software, version 2.15.2 (R Foundation for Statistical Computing). All statistical tests were 2-sided, with p values <0.05 considered statistically significant.

Results

Association of Plasma CX3CL1 Levels With Demographic and Clinical Factors

Baseline characteristics of study participants by plasma CX3CL1 quartiles are presented in Table 1. In unadjusted analyses, increasing CX3CL1 quartiles were associated with female and non-White participants. Higher plasma CX3CL1 was found among persons with diabetes, hypertension, and hyperlipidemia; higher BMI; higher plasma levels of the inflammatory markers IL-6, TNF- α , and hsCRP; and history of CVD. Persons with lower eGFR, higher plasma cystatin C and FGF-23, lower calcium, higher phosphorous, and higher urinary albumin-creatinine ratio had higher levels of CX3CL1. Because of the association noted, we looked further at direct correlation between inflammatory markers and CX3CL1 levels and found the following correlations by Spearman's ρ : TNF- α , 0.24 ($p<0.001$); hsCRP, -0.04 ($p=0.03$); and IL-6, 0.68 ($p<0.001$).

Plasma CX3CL1 and Self-reported Prevalent CVD at Baseline

Increased baseline levels of CX3CL1 were associated with a higher prevalence of self-reported CVD at enrollment (overall prevalence in study population, 33%) in models adjusting for demographic factors, known CVD risk factors (including DM) and inflammatory biomarkers (IL6, TNF- α and hsCRP): the OR per 1-SD increase in standardized CX3CL1 was 1.17 ($p<0.001$). This effect was attenuated but remained statistically significant after adjusting further for baseline eGFR and urinary albumin-creatinine ratio (OR, 1.09; $p=0.03$) (Table 2) as well as presence of the metabolic syndrome (OR, 1.09; $p=0.03$).

Plasma CX3CL1 and Occurrence of MI and All-Cause Mortality

During a median 6 years' follow-up, a total of 176 participants were identified as having a probable MI (127 definite MI), 352 died, and 484 had either an MI or died, leading to a cumulative incidence of these outcomes of 4.7% for probable or definite MI, 9.6% for death, and 13.2% for the composite outcome of MI and death. In Table 3, multivariable-adjusted hazard ratios (HRs) are presented for probable MI, all-cause mortality, or the composite outcome for a 1-SD difference in the standardized CX3CL1 concentration. Higher CX3CL1 levels were associated with all-cause mortality (HR, 1.23; $p<0.001$) and the MI/all-cause mortality composite outcome (HR, 1.22; $p<0.001$) in models adjusted for demographic factors, known risk factors (including diabetes) and inflammatory biomarkers (IL-6, TNF- α , and hsCRP) and remained significant for all-cause mortality (HR, 1.13; $p=0.02$) and the composite outcome (HR, 1.11; $p=0.01$) once additional adjustment for study entry eGFR and albumin-creatinine ratio had been done. For MI alone, trends were similar but were not statistically significant in fully adjusted models. Further adjustment for prevalent CVD somewhat attenuated the estimates (HRs for all-cause mortality and for the MI/all-cause mortality composite outcome of 1.11 and 1.09 [$P=0.02$ and $p=0.04$], respectively). Adjusted survival curves with fully adjusted associations between quartiles of plasma CX3CL1 and MI, all-cause mortality, or the composite outcome are shown in Figure 1. Similar to the results shown in Table 3, increasing quartiles of CX3CL1 were associated with decreased event-free survival curves for all-cause mortality as well as the composite outcome.

We assessed the AUC to explore the predictive performance of plasma CX3CL1 when added to traditional and novel risk factors. The change in AUC for MI, all-cause mortality and the composite outcome was evaluated in incremental models (Models 2 and 5) (Table S1, available as online supplementary material). Plasma CX3CL1 enhanced the AUC modestly when added to demographic characteristics and traditional risk factors for all three outcomes (e.g., 0.68 to 0.70 for all-cause mortality [$P<0.001$]) and modestly increased the AUC for all-cause mortality (0.74 to 0.76 [$p<0.001$]) and the composite outcome in the fully adjusted models.

Plasma CX3CL1, Metabolic Syndrome, and DM

Diabetes and metabolic syndrome at baseline were each strongly associated with increasing CX3CL1 quartiles (Table S2). Blood glucose and blood pressure were positively associated with increasing levels of CX3CL1. However, waist circumference, triglycerides, high-density lipoprotein cholesterol components and HOMA-IR levels were not.

In multivariable models, higher CX3CL1 levels at study entry were significantly associated with higher baseline prevalence of diabetes (overall prevalence in study population of 48%) after adjusting for demographic factors, BMI, and inflammatory biomarkers (IL-6, TNF- α and hsCRP): OR of 1.36 per 1-SD increase in standardized CX3CL1 ($p<0.001$). This association persisted after further adjustment for measures of kidney disease (OR, 1.22; $p<0.001$) and metabolic syndrome (OR, 1.26; $p<0.001$) (Table 4). Analysis of HOMA-IR with multivariable models showed no significant association between baseline CX3CL1 levels and this surrogate marker of insulin resistance (Table S3).

Discussion

Among a large cohort of subjects with CKD and diverse clinical and demographic characteristics, baseline levels of CX3CL1 were associated not only with CVD at enrollment but also with incident events including death and the composite of MI and death during approximately six years of follow-up. Levels of CX3CL1 also were independently associated with prevalent diabetes but not with measures of insulin resistance (HOMA-IR). These associations remained after adjustment for indices of kidney function and damage in addition to multiple demographic and CVD risk factors, suggesting complex cardiometabolic associations of the CX3CL1-CX3CR1 system in persons with CKD.

To our knowledge, this is the first large study of the association of circulating CX3CL1 with CVD in CKD and the largest prospective study of incident events to date. In a study of 46 patients with unstable angina pectoris, increased plasma CX3CL1 and CX3CR1-expressing monocytes were found in subjects with plaque rupture vs. those without²¹. Multiple studies in rodent models suggest causality for CX3CL1-CX3CR1 signaling in promoting monocyte recruitment and progression of atherosclerosis^{22–24,25}. Circulating CX3CL1 levels have also been reported to predict all-cause and cardiovascular mortality in a small study of advanced heart failure patients²⁶, although the mechanism underlying this relationship in heart failure remains uncertain.

Our analysis of the association between CX3CL1 and CVD outcomes and all-cause mortality provide several novel insights. First, higher CX3CL1 levels were directly related to increased prevalent CVD as well as the composite outcome of incident MI and all-cause mortality in fully adjusted analyses. It is not surprising that the strength of prediction for incident MI and all-cause mortality was weaker in adjusted models since other inflammatory and metabolic risk factors may be acting upon the same pathway as CX3CL1. It appears unlikely, though, that CX3CL1's association with diabetes explains the prediction of longitudinal outcomes, as adjustment for metabolic factors and diabetes status had minimal impact on the risk estimates for mortality and MI. In our analysis, plasma CX3CL1 was a stronger predictor of all-cause mortality than MI. Although trends were in the same direction for prediction of MI, the association did not meet significance in adjusted analysis, raising the question of whether CX3CL1 relates to death via a mechanism distinct from the modulation of atherosclerosis, as might also be the case in heart failure patients²⁶.

Prior studies have suggested that CX3CL1 may modulate glucose homeostasis and insulin resistance. We have reported that CX3CL1 is induced in inflamed human adipose and that human adipocytes support monocyte adhesion via CX3CL1³. Sirois-Gagnon et al. reported an association of genetic variants in CX3CR1 with obesity in humans; we also found modest association of CX3CR1 genetic variation with obesity and DM type 2^{3,9}. Findings from rodent studies are conflicting. In obese mice fed a high-fat diet, knockout of the CX3CR1 gene does not attenuate adipose inflammation or the development of peripheral insulin resistance²⁷; these observations are consistent with the lack of an association of CX3CL1 with metabolic syndrome and HOMA-IR among CRIC Study participants. Recently, Lee et al. reported another strain of CX3CR1 knockout mice had a defect in pancreatic β -cell insulin secretion²⁸ and developed glucose intolerance with overt hyperglycemia on high-fat diet, suggesting a protective role for CX3CL1-CX3CR1 in β -cell function. In contrast, we found an apparent opposite pattern in humans with higher levels of plasma CX3CL1 associating with higher prevalence of DM. Further validation of mice model findings and characterization of human CX3CL1 effects on pancreatic β -cell function are warranted²⁹.

We evaluated association of CX3CL1 with metabolic traits, CVD, and all-cause mortality in the CRIC Study, a large, prospective, well-phenotyped cohort of subjects with CKD and a high prevalence of diabetes and risk for CVD. The study was limited by CX3CL1 levels having been assessed only at baseline, as we were thus unable to evaluate change in levels over time, correlate with progression of CVD or metabolic disease, or assess levels near the time of incident events. While we were unable to observe an association of CX3CL1 with HOMA-IR, we cannot definitively exclude an association with insulin resistance given the lack of gold-standard measures of hepatic and peripheral insulin sensitivity. We acknowledge that cross sectional analysis does not allow determination of directionality and we cannot exclude the possibility that DM itself leads to elevation of CX3CL1, rather than CX3CL1 contributing to development of DM. Further, the components of all-cause mortality were not available, thus the association of CX3CL1 to specific causes of death could not be evaluated.

We also recognize the possibility that plasma CX3CL1 accumulates with kidney failure as a function of reduced GFR. Our analysis did show positive association of plasma CX3CL1

with reduced GFR, although association with death and myocardial infarction persisted after correction for kidney function measures. While several studies report CX3CL1 expression in the kidney and suggest a role in kidney inflammation and disease^{30–32}, none directly address the question of whether plasma CX3CL1 levels increase with increasing GFR. One group described positive association of plasma CX3CL1- and CX3CR1-positive CD4 T cells with carotid intima-media thickness in CKD patients³³, a finding that supports a functional role of CX3CL1 in CVD, rather than simple accumulation due to reduced filtration.

In conclusion, higher levels of plasma CX3CL1 were associated with traditional cardiovascular risk factors, prevalent CVD, and diabetes in adults with CKD. Levels of CX3CL1 also predicted all-cause mortality after adjustment for traditional risk. These findings, in the context of our current mechanistic knowledge of CX3CL1-CX3CR1, suggest that CX3CL1 may contribute to both atherosclerotic CVD and diabetes. This is consistent with cell-specific, context- and time-dependent roles for other chemokine systems, e.g., CXCL12¹⁹, in cardiometabolic biology and disease. Mechanistic and human genomic studies are needed to establish the precise role of CX3CL1-CX3CR1 in diverse metabolic and cardiovascular disorders, determine whether mechanisms in atherosclerosis and diabetes are discrete or overlapping, and establish if these are amenable to therapeutic targeting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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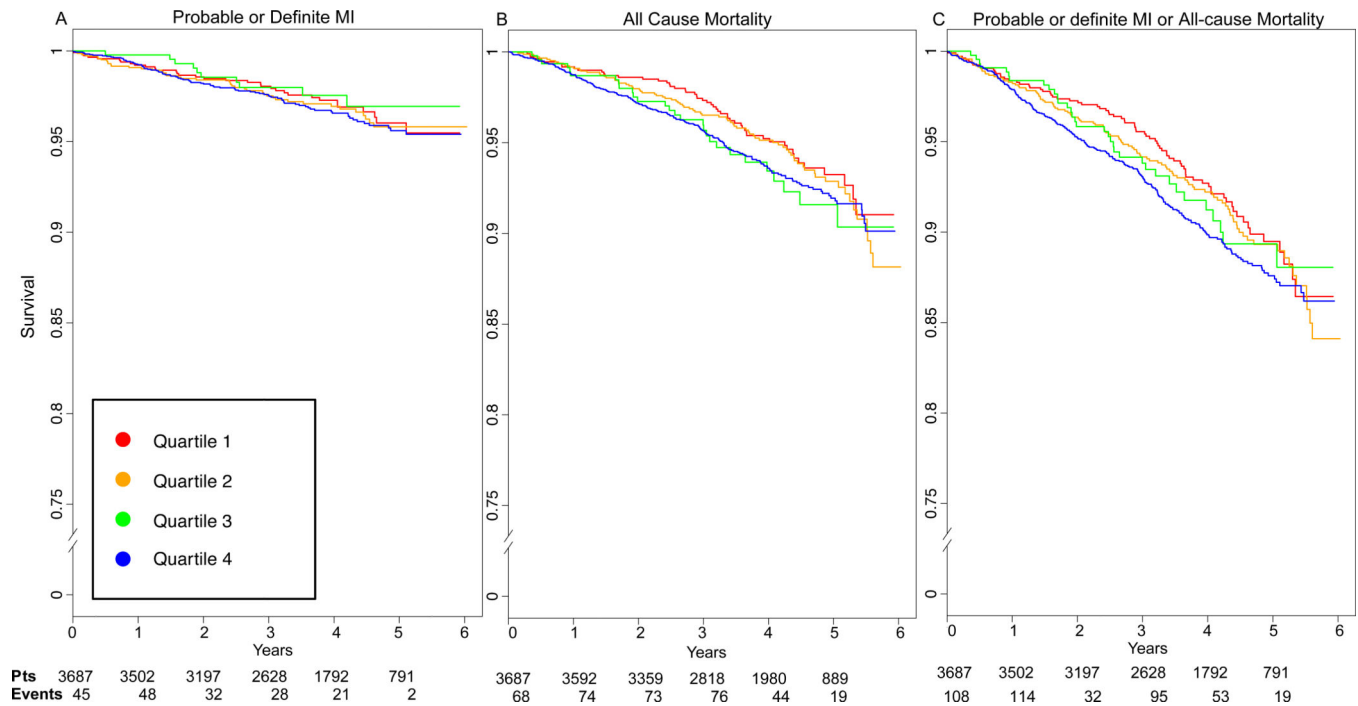


Figure 1.

Adjusted survival curves for effect of plasma CX3CL1 (fractalkine) quartiles on incident events. Fully adjusted for A) probable and definite MI, B) all-cause mortality, and C) composite probable and definite MI / all-cause mortality.

Table 1

Baseline characteristics by quartiles of plasma CX3CL1 (fractalkine)

Characteristics	Total (N=3,687)	Q1: <0.66 ng/ml (n=923)	Q2: 0.66–0.85 ng/ml (n=923)	Q3: 0.86–1.08 ng/ml (n=921)	Q4: >1.08 ng/ml (n=920)	P [†]
Demographics						
Age (y)		60 (52, 65)	61 (54, 67)	61 (53, 67)	59 (51, 66)	0.09
Female Sex	1655 (44.9)	391 (42.4)	406 (44.0)	407 (44.2)	451 (49.0)	0.03
Race						<0.001
White	1568 (42.5)	450 (48.8)	426 (46.2)	391 (42.5)	301 (32.7)	
Black	1518 (41.2)	329 (35.6)	357 (38.7)	388 (42.1)	444 (48.3)	
Hispanic	454 (12.3)	88 (9.5)	105 (11.4)	115 (12.5)	146 (15.9)	
Other	147 (4.0)	56 (6.1)	35 (3.8)	27 (2.9)	29 (3.2)	
Traditional CV Risk Factors						
Tobacco Use [^]	475 (12.9)	98 (10.6)	126 (13.7)	122 (13.2)	129 (14.0)	0.1
Family history CAD	583 (15.8)	136 (14.7)	146 (15.8)	149 (16.2)	152 (16.5)	0.7
Hypertension	3170 (86.0)	740 (80.2)	782 (84.7)	806 (87.5)	842 (91.5)	<0.001
Prior CVD	1227 (33.28)	242 (26.2)	309 (33.5)	311 (33.8)	365 (39.7)	<0.001
Diabetes	1778 (48.22)	323 (35.0)	412 (44.6)	484 (52.6)	559 (60.8)	<0.001
BMI (kg/m ²)		30.3 [26.7–34.8]	31.2 [27.1–36.4]	30.8 [26.8–36.1]	31.2 [26.6–37.3]	0.002
High Cholesterol	3032 (82.2)	725 (78.6)	756 (81.9)	777 (84.4)	774 (84.1)	0.003
Kidney function/damage measures						
eGFR (ml/min/1.73 m ²)		53.6 (42.8, 65.3)	45.6 (35.6, 56.2)	39.9 (31.0, 51.0)	34.5 (26.1, 44.2)	<0.001
Urinary ACR (µg/mg) [#]		16.5 (5.4, 102)	41 (7.9, 259)	100 (11.4, 622)	265 (22.7, 1245)	<0.001
Cystatin C (mg/L)		1.2 (1, 1.5)	1.3 (1.1, 1.7)	1.5 (1.2, 1.9)	1.7 (1.4, 2.1)	<0.001
Measures of Mineral Metabolism						
FGF-23 (RU/ml)		107 (75.8, 166)	138 (94.1, 213)	158 (107, 252)	195 (126, 314)	<0.001
Serum phosphorous (mg/dl)		3.5 (3.2, 3.9)	3.6 (3.2, 4.0)	3.7 (3.3, 4.1)	3.8 (3.4, 4.3)	<0.001
Serum calcium (mg/dL)		9.2 (8.9, 9.5)	9.2 (8.9, 9.5)	9.2 (8.9, 9.5)	9.1 (8.8, 9.4)	<0.001

Characteristics	Total (N=3,687)	Q1: <0.66 ng/ml (n=923)	Q2: 0.66–0.85 ng/ml (n=923)	Q3: 0.86–1.08 ng/ml (n=921)	Q4: >1.08 ng/ml (n=920)	<i>p</i> [†]
Inflammatory biomarkers						
hsCRP (mg/l) [‡]		2.6 (1.1, 6.6)	2.7 (1.1, 6.7)	2.6 (1.1, 6.3)	2.3 (0.9, 5.9)	0.1
TNF-α (pg/ml) [‡]		1.8 (1.2, 2.7)	2.0 (1.4, 3.1)	2.3 (1.6, 3.2)	2.7 (1.9, 3.8)	<0.001
IL-6 (pg/ml) [‡]		1.6 (0.9, 2.5)	1.9 (1.2, 3.1)	2 (1.2, 3.2)	2.2 (1.4, 3.5)	<0.001

Note: Values for categorical variables are given as number (percentage of column category), values for continuous variables are given as median [interquartile range]. Conversion factors for units: phosphorous in mg/dl to mmol/L, ×0.3229; calcium in mg/dl to mmol/L, ×0.2495.

[†] *p*-value by ANOVA (continuous variables) and Chi-squared test (categorical variables)

[‡] log-transformed to meet normality assumption

Abbreviations and definitions: BMI, body mass index; CAD, coronary artery disease; CV, cardiovascular; CVD, cardiovascular disease (defined as self-reported prior diagnosis of CVD, stroke or peripheral vascular disease); eGFR, estimated glomerular filtration rate (using equation from Chronic Renal Insufficiency Cohort Study data)¹⁶; FGF, fibroblast growth factor; hsCRP, high-sensitivity C-reactive protein; IL-, interleukin; TNF, tumor necrosis factor; ACR, albumin-creatinine ratio; Q, quartile

[^] tobacco use defined as self-reported current smoker

Table 2

Association of plasma CX3CL1 (fractalkine) with self-reported CVD at baseline

	OR (95% CI)[†]	P value
Model 1	1.29 (1.20–1.38)	<0.001
Model 2	1.21 (1.13–1.31)	<0.001
Model 3	1.17 (1.08–1.26)	<0.001
Model 4	1.09 (1.01–1.19)	0.03
Model 5	1.09 (1.01–1.19)	0.03

Note: Prevalent CVD is defined as prior myocardial infarction or coronary revascularization at baseline (n with any CVD=1227). Model 1: CX3CL1 + Demographic factors (age, sex, race); Model 2: CX3CL1 + Demographic factors + Traditional risk factors (diabetes, hypertension, hyperlipidemia, tobacco use, body mass index); Model 3: CX3CL1 + Demographic factors + Traditional risk factors + plasma inflammatory biomarkers (log transformed IL6, tumor necrosis factor α and high-sensitivity C-reactive protein); Model 4: CX3CL1 + Demographic factors + Traditional risk factors + plasma inflammatory biomarkers + kidney function measures (estimated glomerular filtration rate using equation from Chronic Renal Insufficiency Cohort Study data¹⁶ and log transformed urinary albumin-creatinine ratio); Model 5: CX3CL1 + Demographic factors + Traditional risk factors + plasma inflammatory biomarkers + kidney function measures + metabolic syndrome

CI, confidence interval; CVD, cardiovascular disease; OR, odds ratio

[†] for one standard deviation increase in standardized CX3CL1

Table 3

Multivariable association of plasma CX3CL1 (fractalkine) with incident MI and all-cause mortality

Failure event	HR *(95% CI)	P value
Probable or definite MI (n=176)		
Model 1	1.21 (1.06, 1.38)	0.01
Model 2	1.17 (1.02, 1.34)	0.03
Model 3	1.14 (1.00, 1.31)	0.06
Model 4	1.05 (0.90, 1.22)	0.6
Model 5	1.05 (0.90, 1.22)	0.5
Model 6	1.03 (0.89, 1.20)	0.7
All-cause mortality (n=352)		
Model 1	1.32 (1.21, 1.45)	<0.001
Model 2	1.29 (1.17, 1.41)	<0.001
Model 3	1.23 (1.13, 1.35)	<0.001
Model 4	1.13 (1.02, 1.24)	0.02
Model 5	1.13 (1.02, 1.24)	0.02
Model 6	1.11 (1.01, 1.22)	0.04
Any MI or all-cause mortality (n=484)		
Model 1	1.30 (1.21, 1.40)	<0.001
Model 2	1.26 (1.17, 1.36)	<0.001
Model 3	1.22 (1.13, 1.32)	<0.001
Model 4	1.11 (1.02, 1.21)	0.01
Model 5	1.11 (1.02, 1.21)	0.01
Model 6	1.09 (1.00, 1.19)	0.04

* for one standard deviation increase in standardized CX3CL1

Note: N=3,687. Please see Table 2 for definitions of models 1–5; Model 6: CX3CL1 + Demographic factors + Traditional risk factors + plasma inflammatory biomarkers + kidney function measures + metabolic syndrome + prevalent cardiovascular disease at baseline.

CI, confidence interval; HR, hazard ratio; MI, myocardial infarction;

Table 4

Association of plasma CX3CL1 (fractalkine) with diabetes mellitus at baseline

	OR (95% CI) *
Model 1	1.45 (1.35–1.56)
Model 2	1.46 (1.36–1.58)
Model 3	1.36 (1.26–1.47)
Model 4	1.22 (1.16–1.38)
Model 5	1.26 (1.16–1.38)

*
for one standard deviation increase in standardized CX3CL1

Note: N=3,687. P<0.001 for all rows. Please Model 1: CX3CL1 + Demographic factors (age, sex, race); Model 2: CX3CL1 + Demographic factors + body mass index (BMI); Model 3: CX3CL1 + Demographic factors + BMI + plasma inflammatory biomarkers (log transformed IL6, TNF α and hsCRP); Model 4: CX3CL1 + Demographic factors + BMI + plasma inflammatory biomarkers + kidney function measures (CRIC-defined estimated glomerular filtration rate¹⁶ and log transformed urinary albumin: creatinine ratio); Model 5: CX3CL1 + Demographic factors + BMI + plasma inflammatory biomarkers + kidney function measures (CRIC-defined estimated glomerular filtration rate¹⁶ and log transformed urinary albumin: creatinine ratio) +Metabolic syndrome.

CI, confidence interval; OR, odds ratio