ORIGINAL RESEARCH

Cystatin C Predicts Incident Cardiovascular Disease in Twins

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***Background*—**Cystatin C is associated with both renal function and atherosclerotic cardiovascular disease (ASCVD). We have previously shown a genetic correlation between cystatin C and prevalent ASCVD. The objective of this article is to study whether variation in cystatin C or creatinine predicts incident ASCVD when controlled for genetic factors.

***Methods and Results*—**The predictive value of cystatin C and creatinine for incident ASCVD was studied in 11 402 Swedish twins, free of CVD at baseline, in an adjusted Cox-regression model during a median follow-up of 71 months. Twin pairs discordant for incident stroke, myocardial infarction and ASCVD during follow-up were identified and within-pair comparisons regarding cystatin C and creatinine levels were performed. We also investigated whether contact frequency and degree of shared environment influences were associated with similarity in cystatin C levels. In univariate analysis, cystatin C predicted incident ASCVD hazard ratio 1.57, 95% CI 1.47–1.67. When adjusted for traditional Framingham risk factors as covariates, cystatin C remained a predictor of incident stroke hazard ratio 1.45, 95% CI (1.25–1.70), ASCVD hazard ratio 1.26, 95% CI (1.13–1.41), and myocardial infarction hazard ratio 1.16, 95% CI (1.01–1.33). In twins discordant for incident stroke, cystatin C at baseline was higher in the twin who experienced a stroke compared to the healthy co-twin (1.11±0.3 mg/L versus 1.06±0.3 mg/L), whereas creatinine was lower in the twin who developed CVD compared to their healthy co-twins (76.1±16.9 μmol/L versus 79.4±20.3 μmol/L).

***Conclusions*—**Variation in cystatin C relates to incident ASCVD and to stroke when adjusted for genetic confounding. In identical twins, cystatin C may be a sensitive marker of early hypertensive end-organ damage and small-vessel disease, whereas creatinine level may reflect nutritional status. The findings in disease-discordant monozygotic twins indicate that unique, possibly preventable, environmental factors are important. **(*J Am Heart Assoc*. 2016;5:e003085 doi: 10.1161/JAHA.115.003085)**

Key Words: cardiovascular disease • co-twin-control study • cystatin C • genetic epidemiology • myocardial infarction • stroke

Cystatin C is a 13.3-kDa protein, well known and commonly used in the clinic as a marker of kidney function.[1] It is also involved in extracellular matrix remodeling[2] and may be directly associated with the development of atherosclerotic cardiovascular disease (ASCVD).[3,4] There is a well-established relation between chronic kidney disease (CKD) and ASCVD,[5–7] which is evident even in patients with only mild renal impairment,[8] and a lowered glomerular filtration rate (GFR) is a risk factor for incident ASCVD.[9] Like ASCVD, CKD is a complex disease derived from a combination of multiple genetic and environmental factors.[10] Previous studies have estimated the heritability of GFR in the range between 0.36 and 0.82,[11,12] which indicates that additive genetic effects explain 30% to 80% of the interindividual variation of GFR. Although there is a phenotypical association between CKD and ASCVD with pathophysiological similarities, especially regarding small-vessel disease in the kidney and brain,[13] as well as common risk factors such as diabetes mellitus and hypertension, the reported genetic overlap between CKD and ASCVD is low.[14] However, in a recent study a possible polygenic overlap between renal dysfunction and ischemic stroke was suggested.[15] Some genetic polymorphisms that affect cystatin C levels independently of kidney function have been reported, but there is no evidence that these polymorphisms are related to cardiovascular risk.[16]

We have previously shown a moderate heritability for variations in both cystatin C and kidney function according to GFR calculated by modification of diet in renal disease and

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CKD-epi formulas. We have also shown a genetic correlation between cystatin C and prevalent ASCVD, indicating that cystatin C and ASCVD share genetic influences.[17] However, it has not been studied previously whether variation of renal function as measured by levels of cystatin C or creatinine predicts incident ASCVD when controlling for genetic factors. Thus, it is currently unknown whether, when accounting for genetic factors, variation in kidney function caused by individual specific environmental factors remains an important predictor of incident ASCVD.

Methods

Participants

Study participants were all obtained from the TwinGene project. TwinGene is a Swedish population-based cohort of twins born between 1911 and 1958, contacted and enrolled for testing between the years 2004 and 2008.[18] All eligible participants had previously participated in a computer-assisted telephone interview called SALT (Screening Across The Life Span Twin Study).[19] Furthermore, both twins within the pairs had to be alive and provide their informed consent for study participation. The zygosity of the twins was based on self-reported childhood resemblance, or by DNA markers (54% of the study sample). According to a recent independent test of the validity of similarity-based zygosity assignments among the adults in the TwinGene study, there is a dizygotic (DZ) to monozygotic (MZ) error rate of 2.56%, corresponding to an accuracy of 97.4% (95% CI: 96.6–98.2).[18] Participants who had previously donated DNA for studies in the Swedish Twin Registry and participants who had declined participation in further studies or had a record of hepatitis were excluded. During enrollment in the TwinGene project, participants were asked to fill out a questionnaire about common diseases such as cardiovascular disease and diabetes mellitus.[18] These statements were thereafter verified through the national inpatient registry, which was the source of the data used in the study. Furthermore, the participants were asked to make an appointment at their local healthcare facility for blood sampling and anthropometry measurements.[18] In total, 12 645 individuals donated blood to the study.

Ethical Approval

The study was approved by the Regional Ethical Review Board in Stockholm.

Sampling

Participants were instructed to fast from 8:00 PM on the night before the blood sampling. A total sample volume of 50 mL of venous blood was drawn from each participant. Tubes with serum and whole blood for clinical chemistry analyses and DNA extraction were sent by overnight mail to Karolinska Institute Biobank. Serum samples were aliquoted by Tecan-robot into 1-mL fractions and placed in 1.8-mL cryotubes that were stored in liquid nitrogen tanks at the Karolinska Institute Biobank. Clinical blood chemistry assessments were performed from fresh blood samples at baseline by the Karolinska University Laboratory for the following biomarkers: triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (by Friedewald formula), C-reactive protein, glucose, apolipoprotein A-I, apolipoprotein B, hemoglobin, and hemoglobin A1c[18] (data regarding biomarkers relevant for this study are shown in Table 1). Serum and plasma were stored frozen at −80°C, or in liquid nitrogen, at Karolinska Institute Biobank before they were thawed and sent for laboratory analysis. For this project, serum aliquots from a total of 12 570 subjects were withdrawn, thawed, and directly shipped to a laboratory for clinical blood analysis. Of these, 257 (2%) were excluded due to bad or missing sample, insufficient sample volume, hemolysis, lipemia, or missing donor ID. Also, an additional 911 (7%) subjects with prevalent cardiovascular disease (CVD) on enrollment were excluded, leaving a total of 11 402 (91%) individuals for the final analysis (see Table 1 for descriptive statistics).

Cardiovascular Disease Assessment

Data regarding prevalent CVD at baseline examination were collected from the Swedish National Inpatient Register and was defined as previous hospitalization with any of the following primary diagnoses: acute myocardial infarction (MI) (ICD-10: I21, I22. ICD-9: 410. ICD-8: 410), unstable angina (ICD-10: I20.0. ICD-9: 411.8. ICD-8: 411), and stroke (ICD-10: I60, I61, I62, I63. ICD-9: 430, 431, 432, 433, 434. ICD-8: 430, 431, 432, 433, 434) or the surgical codes: FNG02, FNG05 percutaneous transluminal coronary angioplasty or FNC, FND, FNE coronary artery bypass graft.These diagnoses were defined according to the primary diagnosis as recorded in the Patient Register. The Patient Register includes hospitalized cases, as well as outpatient visits, but not visits to primary care. The positive predictive value (ie, validity) of the MI diagnosis in the Swedish Patient Register has been demonstrated to be 95% when only primary diagnoses are considered.[20]

Follow-Up

A prospective follow-up of participants for a median time of 71 (SD±16) months was made. Information regarding incident cardiovascular morbidity and mortality during follow-up was collected from the Swedish National Inpatient

Table 1. General Characteristics of Study Participants

	All	Women	Men
Total number of individuals*, n	11 402	6455 (57%)	4947 (43%)
Complete pairs	4127	4781 (58%)	3473 (42%)
Monozygotic twins, n	2879	1680 (58%)	1199 (42%)
Same-sex dizygotic twins, n	4299	2511 (58%)	1788 (42%)
Opposite-sex dizygotic twins, n	4194	2255 (54%)	1939 (46%)
Unknown zygosity, n	30	9 (30%)	21 (70%)
Age, y	64.5 (±8.0)	64.3 (±8.1)	64.7 (±7.9)
Height, cm	169.1 (±10.7)	163.2 (±8.0)	176.4 (±9.0)
Weight, kg	74.2 (±13.8)	68.5 (±12.1)	81.7 (±12.2)
Body mass index, kg/m^2	25.9 (±4.1)	25.7 (±4.4)	26.2 (±3.7)
Systolic blood pressure, mm Hg	138.6 (±19.7)	137.9 (±20.0)	139.5 (±19.2)
Glucose mmol/L (serum)	5.5 (±1.1)	5.4 (±1.0)	5.7 (±1.3)
HbA1c % (serum)	4.8 (±0.6)	4.8 (±0.6)	4.8 (±0.7)
HDL cholesterol mmol/L (serum)	1.4 (±0.4)	1.6 (±0.4)	1.3 (±0.3)
LDL cholesterol mmol/L (serum)	3.8 (±1.0)	3.9 (±1.0)	3.8 (±0.9)
Total cholesterol mmol/L (serum)	5.9 (±1.1)	6.0 (±1.1)	5.6 (±1.1)
Cystatin C mg/L (plasma)	1.00 (±0.25)	0.99 (±0.23)	1.03 (±0.27)
Creatinine μmol/L (plasma)	76.5 (±18.1)	69.5 (±12.6)	85.8 (±20.0)
eGFR mL/min per 1.73 m^2 (CKD-epi)†	86.3 (±15.6)	80.7 (±13.8)	93.6 (±14.8)
Current smoker, n	1861 (16%)	1098 (17%)	763 (15%)
Antihypertensive treatment, n	2459 (21.5%)	1426 (22%)	1033 (21%)
Antilipids treatment, n	1046 (9%)	582 (9%)	464 (9%)
Diabetes mellitus‡, n	933 (8%)	421 (6.5%)	512 (10%)

Values are in means±SD or percentage. CKD indicates chronic kidney disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*Number of individuals.
†Derived from the CKD-epi formula based on creatinine.
‡According to Swedish Diabetes Registry.

Register records and the Swedish Cause of Death Register. End points were cardiovascular mortality, nonfatal MI, need for revascularization, and stroke.

Blood Tests

The blood tests were performed at the Department of Clinical Chemistry and Pharmacology, University Hospital, Uppsala, Sweden. Serum samples were analyzed according to IDMS-standard on Abbott Architect ci8200 and ci16200 instruments (Abbott Park, IL). Reagents for the enzymatic creatinine method were from Abbott. Reagents for the immunoturbidimetric cystatin C method, which follows the IFCC-standard, were from Gentian (Moss, Norway). The total analytical imprecision of creatinine measurements were 3% and 2% at 70 and 350 μmol/L, respectively. Corresponding figures for cystatin C were 3% and 2% at 0.86 and 3.3 mg/L.

Calculations of estimated glomerular filtration rate (eGFR) were performed with the CKD-epi formula according to Levey et al[21].

Statistics

The predictive value per SD increase of logarithmized cystatin C for incident stroke, incident MI, and incident ASCVD was studied in a Cox-regression survival analysis adjusted for systolic blood pressure, diabetes mellitus (yes/no), current smoking (yes/no), eGFR (creatinine-based CKD-epi), total cholesterol, high-density lipoprotein, and antihypertensive medication (yes/no). A robust sandwich covariance matrix estimate was incorporated into the model to account for any intracluster dependence, which otherwise may inflate precision estimates due to correlated (twin-ships) data. Any outliers were windsorized to ±5 SD from mean. The Cox

proportional hazard model assumption was checked through Wald statistics.

Same-sexed twin pairs discordant for ASCVD, MI, and stroke during follow-up were identified. Independent 2-sample and paired *t* tests were performed in order to verify significant differences regarding cystatin C levels on group- and pair level between twins with incident ASCVD and twins without incident ASCVD. Thereafter, a conditional stepwise logistic regression analysis was performed in order to verify significant differences regarding cystatin C, firstly when adjusted for the same covariates as stated above (Table 2, Model 2) and subsequently with C-reactive Protein added to the model (Table 2, Model 3). The conditional logistic regression inherently adjusted for all variables that were the same among the twins. Further subanalyses were performed on twins discordant for MI and stroke.

Twin Contact and Age at Separation

Data on self-reported intrapair contact frequency, meaning the frequency by which the twins in a pair met each other, and age at separation was obtained from the SALT interviews.[19] Contact frequency data were coded into 4 levels; (1) twins met each other less than once a year; (2) twins met on a yearly basis; (3) twins met on a monthly basis; and (4) twins met on a weekly basis. Where both twins had reported age at separation, average value was used for further analysis. By computing the rank-order correlation (Spearman) between contact frequency and the absolute intrapair difference in trait levels adjusted for age and sex and log-transformed where applicable, we explored whether contact frequency and the degree of shared-environment influences, such as age at separation from co-twin, were associated with similarity in trait levels (Table 3).

Results

Participants were followed for a median time of 71 (SD±16) months. One hundred nineteen MZ and 155 same-sexed DZ twin pairs became discordant for incident ASCVD, stroke, or MI during follow-up.

The results of Cox regression analysis in the whole cohort are shown in Table 2. In univariate analysis, cystatin C was a predictor of incident stroke (hazard ratio, 95% CI 1.69, 1.56–1.84), MI (1.49, 1.39–1.60), and ASCVD (1.57, 1.47–1.67). No association between MI and creatinine-based CKD-epi was observed. When adjusted for all covariates including CKD-epi calculated eGFR (Model 2, Table 2), cystatin C remained a predictor of incident stroke (hazard ratio 1.45, CI 1.25–1.70), MI (hazard ratio 1.16, CI 1.01–1.33), and ASCVD (1.26, 1.13–1.41). When adding C-reactive Protein to the multivariate model (Model 3, Table 2), the association between cystatin C and MI did not remain significant (hazard ratio 1.24, CI 0.99–1.32), while it remained for the other outcomes.

A total of 116 MZ and 155 same-sexed DZ twin pairs became discordant for incident ASCVD during follow-up. In twins who became discordant for stroke, cystatin C at baseline was higher in the twin who experienced a stroke compared to the twin who remained healthy in both MZ (1.11±0.27 mg/L versus 1.06±0.26 mg/L, $P<0.05$) and DZ pairs (1.2±0.37 mg/L versus 1.07±0.23 mg/L, $P<0.01$). Conversely, creatinine was lower in the twin who developed ASCVD compared to the co-twin who remained healthy (76.7±16.7 μmol/L versus 80.1±19.4 μmol/L, $P=0.02$) (Table 4).

The results of conditional regression analysis in pairs discordant for incident ASCVD are shown in Table 5. In univariate analysis, cystatin C was significantly associated with stroke and ASCVD but not MI in same-sexed DZ twins, whereas in MZ the association was of borderline insignificance ($P=0.052$). When adjusted for the same covariates as in the Cox regression model and stratified by zygosity, cystatin C did not remain significantly associated with any outcome in MZ but to stroke in same-sexed DZ. However, in multivariate analysis when eGFR was added as a covariate, cystatin C was significantly associated with incident stroke in MZ.

Data on contact frequency by at least 1 of the twins in a pair was available for 11 040 (97%) of the study participants. The intrapair correlation on contact frequency was high ($\rho=0.80$) for the 3954 pairs where both responded. Data on age at separation were available for 11 145 (98%) individuals, and correlation was somewhat lower compared to contact frequency ($\rho=0.65$) for 3206 responding pairs. MZ twins reported a higher contact frequency and higher mean age at separation than DZ twins. None of these measures was significantly related to the absolute intrapair difference in adjusted trait levels in MZ, but contact level was related to intrapair difference in cystatin C and eGFR levels in DZ (Table 3).

Discussion

Here we studied the association of cystatin C and creatinine-based eGFR to incident ASCVD in a prospective co-twin control design and confirm findings from previous studies on the predictive value of cystatin C in a large subset of Swedish twins. The novel findings of this study were that in identical twins a high level of cystatin C related to incident stroke, whereas a low level of creatinine was associated with incident ASCVD.

The association between cystatin C level and all-cause mortality was first reported by Shlipak et al,[22] and the

Table 2. Hazard Ratios for Incident ASCVD in Unadjusted and Adjusted Cox Prediction Models in 11 402 Twins

	Univariate		Adjusted Model 1*		Adjusted Model 2†		Adjusted Model 3‡	
Variable	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Log cystatin C								
Stroke	1.69 (1.56–1.84)	<0.001	1.31 (1.17–1.46)	<0.001	1.45 (1.25–1.70)	<0.001	1.44 (1.23–1.68)	<0.001
MI	1.49 (1.39–1.60)	<0.001	1.11 (1.00–1.24)	0.05	1.16 (1.01–1.33)	0.04	1.24 (0.99–1.32)	0.06
ASCVD	1.57 (1.47–1.67)	<0.001	1.19 (1.10–1.29)	<0.001	1.26 (1.13–1.41)	<0.001	1.24 (1.12–1.39)	<0.001
CKD-epi (crea)								
Stroke	0.78 (0.69–0.87)	<0.001	0.90 (0.80–1.03)	0.13				
MI	0.94 (0.85–1.04)	0.25	0.96 (0.86–1.08)	0.54				
ASCVD	0.99 (0.99–1.00)	<0.001	0.94 (0.86–1.03)	0.17				
Age								
Stroke	1.11 (1.09–1.12)	<0.001						
MI	1.07 (1.06–1.08)	<0.001						
ASCVD	1.09 (1.08–1.10)	<0.001						
Sex								
Stroke	0.51 (0.41–0.63)	<0.001						
MI	0.38 (0.31–0.46)	<0.001						
ASCVD	0.44 (0.37–0.51)	<0.001						
Smoking								
Stroke	1.03 (0.96–1.12)	0.39						
MI	1.10 (1.03–1.18)	0.003						
ASCVD	1.07 (1.10–1.31)	0.006						
HDL								
Stroke	0.67 (0.51–0.89)	0.006						
MI	0.33 (0.26–0.43)	<0.001						
ASCVD	0.48 (0.39–0.59)	<0.001						
LDL								
Stroke	0.93 (0.82–1.04)	0.21						
MI	1.13 (1.02–1.26)	0.02						
ASCVD	1.05 (0.97–1.14)	0.20						
Total cholesterol								
Stroke	0.91 (0.82–1.02)	0.09						
MI	1.01 (0.92–1.12)	0.81						
ASCVD	0.98 (0.91–1.06)	0.60						
Anti-HT treatment								
Stroke	0.91 (0.82–1.02)	0.09						
MI	1.01 (0.92–1.12)	0.81						
ASCVD	0.98 (0.91–1.06)	0.60						
Diabetes mellitus								
Stroke	0.91 (0.82–1.02)	0.09						
MI	1.01 (0.92–1.12)	0.81						
ASCVD	0.98 (0.91–1.06)	0.60						

Continued

Table 2. Continued

Variable	Univariate		Adjusted Model 1*		Adjusted Model 2†		Adjusted Model 3‡	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Systolic BP								
Stroke	1.02 (1.02–1.03)	<0.001						
MI	1.02 (1.01–1.02)	<0.001						
ASCVD	1.02 (1.02–1.02)	<0.001						
CRP								
Stroke	1.02 (1.02–1.03)	<0.001						
MI	1.01 (1.01–1.02)	<0.001						
ASCVD	1.02 (1.01–1.02)	<0.001						

Age and sex inherent in all models. Anti-HT indicates antihypertensive; ASCVD, atherosclerotic cardiovascular disease; CKD-epi, glomerular filtration rate according to the CKD-epi formula based on creatinine; crea, creatinine; CRP, C-reactive Protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; Log, logarithmized; MI, myocardial infarction; SBP, systolic blood pressure.

*Adjusted model 1 includes SBP, serum cholesterol, HDL, treatment for hypertension (yes/no), diabetes mellitus (yes/no), and smoking status (yes/no).

†Adjusted model 2 includes SBP, serum cholesterol, HDL, treatment for hypertension (yes/no), diabetes mellitus (yes/no), smoking status (yes/no), and eGFR (CKD-epi).

‡Adjusted model 3 includes SBP, serum cholesterol, HDL, treatment for hypertension (yes/no), diabetes mellitus (yes/no), smoking status (yes/no), eGFR (CKD-epi), and C-reactive Protein.

prognostic value of cystatin C for cardiovascular morbidity as a biomarker for CKD has also been thoroughly investigated.[23–26] Thus, the finding of the current study that cystatin C is superior to creatinine for prediction of incident ASCVD confirms findings from previous population-based studies.[27–29] However, since our study is the first of this topic in a twin cohort, it allows us to control for genetic confounding.

The finding that cystatin C is related to incident stroke in identical twins is novel and indicates that individual specific environmental factors that affect cystatin C are also associated with incident stroke. However, our study design does not allow us to draw conclusions about what constitutes these unique environmental factors. Previously reported environmental factors that are associated with cystatin C or mild eGFR reduction are smoking, occupational exposure to lead and arsenic, use of corticosteroids, and thyroid dysfunction.[30–35] When we adjusted for the traditional risk factors serum cholesterol, diabetes mellitus, antihypertensive treatment, systolic blood pressure, smoking, and decreased kidney function, the association between cystatin C and stroke remained. This indicates that other external factors are important for the strong association between cystatin C and incident ASCVD and may also be possible to prevent if identified.

Table 3. Correlation Between Absolute Intra-Pair Difference of Adjusted Trait Values and (A) Co-Twin Contact Frequency and (B) Age at Separation From Co-Twin

Phenotype	MZ†			ssDZ‡		
	*r**	*P* Value	N (Pairs)	*r**	*P* Value	N (Pairs)
(A) Contact frequency						
Cystatin C	−0.04	0.16	1173	−0.06	0.03	1527
CKD-epi	−0.01	0.65	1165	−0.03	0.22	1530
(B) Age at separation						
Cystatin C	−0.01	0.70	1177	−0.03	0.25	1495
CKD-epi	−0.01	0.65	1116	−0.003	0.90	1499

Trait values are Log-transformed (where applicable) and *z*-score standardized, age and sex adjustment inherent in model. CKD-epi indicates glomerular filtration rate according to the CKD-epi formula based on creatinine; DZ, dizygotic twin; MZ, monozygotic twin.

*Spearman correlation coefficient.

P values remained insignificant for opposite sex dizygotic when stratified by sex,

†Monozygotic twins, ‡Same-sex dizygotic twins.

Another novel and interesting finding of the current study was that in the identical twin pairs that later became discordant for CVD, a lower creatinine value at baseline was observed in the twin who developed CVD during the follow-up compared to the co-twin who remained healthy. Twins with previous CVD were excluded and the twin-control study design adjusts for age and sex, all of which are determinants of creatinine on a population level.[36] An important determinant of creatinine is muscle mass and in the twin model, which to a large extent adjusts for length, interindividual differences in creatinine may be even more closely related to interindividual differences in muscle mass.[37] Thus, the finding that the healthy twin had higher creatinine and lower creatinine-based eGFR may be a marker of increased muscle mass possibly due to increased physical activity, less malnutrition, and a healthier lifestyle.[38] A similar relation was observed to both stroke and MI, although not significant, which may be due to power issues.

Still it remains to be determined whether it is unique properties of cystatin C that are independently and causally

Table 4. Paired *t* Tests in Twin-Pairs Discordant for Incident Stroke, ASCVD, and MI

Stroke						
	MZ (n=59)			DZ (n=79)		
Variable	Sick	Healthy	*P* Value	Sick	Healthy	*P* Value
Cystatin C	1.11±0.27	1.06±0.26	0.044	1.20±0.37	1.07±0.23	0.002
Creatinine	76.12±16.87	79.39±20.32	0.128	85.05±20.19	81.16±13.42	0.141
CKD-epi (crea)	85.56±17.32	83.51±16.91	0.190	79.73±18.15	82.52±16.32	0.124
HDL	1.40±0.48	1.34±0.35	0.143	1.36±0.38	1.43±0.42	0.138
Total cholesterol	5.98±1.30	5.77±1.26	0.268	5.90±1.29	5.91±1.37	0.945
BMI*,†	26.66±3.93	26.09±4.49	0.149	25.84±3.86	25.58±3.75	0.581
SBP‡,§	149.2±21.92	146.1±20.22	0.543	151.6±25.79	147.0±21.32	0.128
MI						
	MZ (n=71)			DZ (n=84)		
Variable	Sick	Healthy	*P* Value	Sick	Healthy	*P* Value
Cystatin C	1.06±0.22	1.06±0.22	0.936	1.11±0.32	1.08±0.24	0.579
Creatinine	77.28±16.86	79.97±18.32	0.127	81.66±16.32	80.57±16.98	0.519
CKD-epi (crea)	86.02±16.03	83.42±15.88	0.131	84.38±16.52	83.55±16.69	0.646
HDL	1.29±0.35	1.30±0.38	0.727	1.30±0.38	1.38±0.38	0.097
Total cholesterol	5.78±1.06	5.62±1.06	0.314	6.11±1.22	5.92±0.99	0.259
BMI#,¶	26.13±3.83	26.56±3.84	0.182	26.51±3.86	25.82±4.05	0.186
SBP‖,**	143.3±20.27	149.0±19.68	0.048	147.6±23.98	146.2±20.63	0.714
ASCVD						
	MZ (n=116)			DZ (n=149)		
Variable	Sick	Healthy	*P* Value	Sick	Healthy	*P* Value
Cystatin C	1.08±0.25	1.07±0.24	0.333	1.13±0.31	1.06±0.22	0.018
Creatinine	76.71±16.72	80.11±19.43	0.021	81.66±18.46	80.68±15.58	0.713
CKD-epi (crea)	85.86±16.63	83.19±16.37	0.031	82.70±16.78	84.31±19.45	0.556
HDL	1.33±0.36	1.31±0.30	0.326	1.33±0.34	1.40±0.39	0.050
Total cholesterol	5.92±1.16	5.69±1.14	0.069	5.98±1.25	5.88±1.18	0.443
BMI††,‡‡	26.45±4.21	26.46±3.93	0.891	26.08±3.58	25.69±3.82	0.301
SBP††,‡‡	146.6±21.19	148.5±19.74	0.412	147.6±23.98	146.2±20.63	0.328

Data regarding BMI and blood pressure are lacking for a small group of participants, possibly because they were overlooked in the health examination at baseline. ASCVD indicates atherosclerotic cardiovascular disease; BMI, body mass index; CKD-epi, glomerular filtration rate according to the CKD-epi formula based on creatinine; crea, creatinine; DZ, dizygotic twin; HDL, high-density lipoprotein; MI, myocardial infarction; MZ, monozygotic twin; SBP, systolic blood pressure.

*MZ (n=54).
†DZ (n=75).
‡MZ (n=52).
§DZ (n=74).
‖MZ (n=61).
¶DZ (n=77).
#MZ (n=67).
**DZ (n=80).
††MZ (n=107).
‡‡DZ (n=139).

associated with vascular remodeling and the development of ASCVD. Findings from a recently performed Mendelian randomization study (yet only published as an abstract) contradicts such a causal relation;[39] thus it is plausible that the strong association between cystatin C and CVD primarily is a reflection of cystatin C being a better marker of early hypertensive end-organ damage in different vascular beds, that is, a more sensitive marker of early GFR-reduction. Our results could also be in alignment with the hypothesis of a "shrunken pore syndrome" as defined by Grubb et al,[40]

Table 5. Odds Ratios Per 1 SD Increase for ASCVD in Discordant MZ and Same-Sex DZ Twin Pairs

	MZ						DZ					
	Univariate		Adjusted Model 1*		Adjusted Model 2†		Univariate		Adjusted Model 1*		Adjusted Model 2†	
Variable	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Logarithmized cystatin C												
Stroke	2.33 (0.99–5.47)	0.052	2.33 (0.72–7.58)	0.16	4.93 (1.01–24.09)	0.049	2.06 (1.25–3.39)	0.005	1.88 (1.11–3.19)	0.019	1.86 (1.01–3.43)	0.046
MI	0.91 (0.09–9.51)	0.935	0.34 (0.01–16.19)	0.58	2.03 (0.01–352.6)	0.79	1.61 (0.30–8.60)	0.755	0.81 (0.07–9.14)	0.87	0.78 (0.06–9.72)	0.84
ASCVD	1.18 (0.76–1.83)	0.469	1.03 (0.39–2.69)	0.96	1.24 (0.37–4.16)	0.73	1.39 (1.05–1.85)	0.021	1.45 (0.91–2.30)	0.12	1.41 (0.85–2.35)	0.19

Age and sex inherent in all models. ASCVD indicates atherosclerotic cardiovascular disease; CKD-epi, glomerular filtration rate according to the CKD-epi formula based on creatinine; DZ, dizygotic twin; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; Log, logarithmized; MI, myocardial infarction; MZ, monozygotic twin; OR, odds ratio; SBP, systolic blood pressure.

*Adjusted model 1 includes SBP, serum cholesterol, HDL, diabetes mellitus (yes/no), antihypertensive treatment (yes/no), and smoking status (yes/no).

†Adjusted model 2 includes SBP, serum cholesterol, HDL, treatment for hypertension (yes/no), diabetes mellitus (yes/no), smoking status (yes/no), and eGFR (CKD-epi).

suggesting that a reduction in pore diameter of the glomerular membrane, which reduces permeability of cystatin C but not creatinine, is responsible for the increased association of cystatin C–based eGFR with end-stage renal disease, hospitalization, MI, and premature death. In this regard cystatin C might be a marker of early vascular aging, and as such detect subclinical manifestation of features such as small-vessel degeneration, left ventricular heart load, arterial calcification, and matrix remodeling and intima alterations.[41,42]

Previous studies have reported on genetic overlap between kidney function and ASCVD.[15] Although these overlaps were quite modest, ranging from 0.1% to 0.26% and associated with different stroke subtypes, they confirm earlier epidemiological studies on the matter suggesting such genetic overlaps.[43] Although both creatinine- and cystatin C–based kidney function was used in the study by Holliday et al, creatinine was more commonly used. We have, in a previous study, observed a stronger genetic association between cystatin C and prevalent ASCVD compared to that between creatinine and ASCVD.[17] Since cystatin C is superior for risk prediction, further investigations on the possible genetic overlap between stroke and cystatin C are warranted.

In our previous study, referred to above, we observed that nonshared environment mediates phenotypic correlation between cystatin C and prevalent ASCVD. In the current study a similar association was observed between cystatin C and incident stroke. Since previous studies have shown that only a small fraction of stroke variance is explained by genetic overlap with renal function estimates, it is important to further investigate the other part of the association, which is related to nonshared environment and indisputably also better suited as a target for preventive measures. This is also supported by the findings of Olden et al,[14] who state that nongenetic factors in the causal pathway are responsible for the major part of the association between ASCVD and kidney function. In this study we have also found that the intrapair contact frequency and age at separation were not significantly associated with trait-level similarity in MZ twins (Table 3), lending further support to the hypothesis of a plausible unique (ie, nonshared within pairs) environmental factor.

We observed a stronger association between cystatin C and incident stroke compared to incident MI. Svensson-Färbom and colleagues were able to demonstrate a significant association between cystatin C and ASCVD morbidity that was not present for creatinine-based eGFR until eGFR was below 45 mL/min, corresponding to less than 1% of the study population. However, they did not study the associations with MI and stroke separately. A plausible explanation, especially if we assume that cystatin C is a marker of small-vessel disease and hypertensive end-organ damage, is that in normal kidney function cystatin C captures the risk of ASCVD through small-vessel disease but when renal function declines, this

discrimination gets distorted and cystatin C instead captures the risk of ASCVD due to renal dysfunction. The link to hypertension would also explain the stronger association between cystatin C and stroke compared to MI since blood pressure level is more strongly related to stroke than to MI according to previous literature.[44–46] This may also explain why we did not observe a significant difference in cystatin C levels in twins discordant for MI.

Conclusions

Variation in cystatin C relates to incident ASCVD and stroke when adjusted for genetic confounding. In identical twins, cystatin C may be a sensitive marker of early hypertensive end-organ damage and small-vessel disease, whereas creatinine level may reflect nutritional status. The findings in disease-discordant monozygotic twins indicate that unique, possibly preventable, environmental factors are important.

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Disclosures

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