ORIGINAL RESEARCH

Comparison of Heritability of Cystatin C- and Creatinine-Based Estimates of Kidney Function and Their Relation to Heritability of Cardiovascular Disease

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***Background*—**Decreased renal function is an established risk factor for cardiovascular disease (CVD). Causal mechanisms between estimates of renal function and CVD are intricate and investigation of the relative importance of genetic and environmental factors for the variability of these phenotypes could provide new knowledge.

***Methods and Results*—**Cystatin C and creatinine levels in 12 313 twins were analyzed. Uni- and bivariate heritability for these traits and CVD was estimated through structured equation modelling and genome-wide complex trait analysis (GCTA) in order to independently confirm additive genetic effects. Twin model-estimated heritability of Cystatin C was 0.55 (95% confidence interval [CI], 0.49 to 0.60) in men, 0.63 (0.59 to 0.66) in women, and 0.60 (0.56 to 0.63) in both sexes combined. For creatinine, heritability estimates were in the same range. Heritability of CVD was 0.39 (0.02 to 0.67) in men and 0.20 (0.00 to 0.61) in women. The phenotypic correlation between Cystatin C and CVD correlation was 0.16 (0.12 to 0.20) in men and 0.17 (0.13 to 0.21) in women, whereas the genetic correlation in males was 0.41 (0.21 to 0.62) while it was non-significant in females. Trough GCTA, the heritability of Cystatin C and creatinine in both sexes combined was estimated to 0.40 (SE 0.07, P=8E^{-9}) and 0.19 (SE 0.07, P=0.003), respectively.

***Conclusions*—**Twin model-based heritability of Cystatin C was higher compared to previous studies. Co-variation between Cystatin C and CVD in males was partly explained by additive genetic components, indicating that Cystatin C and CVD share genetic influences. The GCTA provided independent evidence for significant contribution of additive genetics to trait variance of Cystatin C.
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Key Words: cardiovascular diseases • cystatin C • genetic epidemiology • heritability • kidney

Serum concentrations of Cystatin C, a protein involved in extracellular matrix remodelling,[1,2] reflects glomerular filtration rate (GFR) as precisely as, or better than, creatinine without the need to take factors such as age, race, body mass, and gender into account.[3–5] Decreased renal function is a well established risk marker for cardiovascular disease (CVD),[6,7] but it has been proposed that Cystatin C, in addition to its function as a marker of GFR, could be an independent predictor of CVD.[8–11] Both genetic and environmental factors, in a complex interplay, contribute to a risk profile prone to develop atherosclerotic CVD[12,13] and Cystatin C may thus be one component of such a risk profile.[14–16] The relationship between Cystatin C- and creatinine-based estimations of renal function and CVD is intricate and causal mechanisms are difficult to study.[17,18] No previous study have directly compared the heritability of creatinine and Cystatin C or commonly used estimates of renal function based on these biomarkers. Here, we perform such comparisons using both the classical twin model as well as an SNP-based method (genome-wide complex trait analysis [GCTA]) to assess heritability. The primary aim was to estimate the relative importance of genes for the phenotypic variability of Cystatin C and creatinine levels, as well as for the variability in commonly used estimates of renal function based on these biomarkers, in a well-powered twin study. A secondary aim was to study the relation of

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heritability of Cystatin C and creatinine to heritability of cardiovascular disease.

Method

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 2004–2008.[19] All eligible participants had previously participated in a computer-assisted telephone interview called SALT (Screening Across The Life Span Twin Study).[20] Further, 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 DZ to MZ error rate of 2.56%, corresponding to an accuracy of 97.4% (95% CI: 96.6 to 98.2).[19] Participants who had previously donated DNA for studies in the Swedish Twin Registry (STR) and participants who had declined participation in further studies or had a record of hepatitis were excluded. In total 12 645 individuals donated blood to the study.

Ethical Approval

The study was approved by the regional ethical review board of Stockholm.

Sampling

Participants were instructed to fast from 8 PM on the night before the blood sampling. A sample volume of totally 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 KI 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 KI Biobank. For this project serum aliquots from a total of 12 570 subjects were withdrawn, thawed and directly shipped off to laboratory for clinical blood analysis. Of these 257 were excluded due to bad or missing sample, non-sufficient sample volume, hemolysis, lipemia, missing donor ID, leaving a total of 12 313 individuals for the final analysis.

Blood Tests

Clinical blood assessments for Cystatin C and creatinine were performed by the Akademiska Laboratory at Akademiska University hospital in Uppsala. Particle reinforced immuno-turbidimetric analysis of plasma Cystatin C was performed using an Architect ci8200 immunoassay analyzer (Abbott Laboratories, Abbott Park, IL). Plasma creatinine was analyzed using Arcitect c8000 and Arcitect c16000 (Abbott Laboratories) through an enzymatic method.

Calculations

Cystatin C values expressed as mg/L were automatically converted into GFR (mL/min per 1.73 m^2) in the laboratory using the formula[21]:

$$y(\mathrm{GFR}) = 79.901 \times (\text{Cystatin C mg/L})^{-1.4389}.$$

For calculating GFR from creatinine and Cystatin C separately and creatinine and Cystatin C combined the CKD-epi formulas according to Inker et al[22] were used. eGFR using the MDRD (Modification of Diet in Renal Disease)-formula was calculated from creatinine according to the formula described by Levey et al[23] In order to assess the heritability for decreased kidney function specifically, omitting variations of eGFR in the normal range, the heritability for being in the lowest quintile of eGFR for each equation was also calculated.

Cardiovascular Disease Assessment

As Cystatin C is a risk marker for different atherosclerotic manifestations,[14–16] a composite trait indicating CVD was chosen in order to capture a phenotype that reflects the general atherosclerotic burden in the study group. Information regarding prevalent CVD at the baseline examination was collected from the Swedish National Inpatient Register and was defined as hospitalization with any of the following primary diagnoses or surgical codes: acute myocardial infarction (ICD-10: I21, I22; ICD-9: 410, 411.8; ICD-8: 410, 411), coronary revascularization by coronary artery bypass surgery or percutaneous coronary angioplasty (FNG02, FNG05, FNC, FND, FNE), or stroke (ICD-10: I60, I61, I62, I63; ICD-9: 430, 431, 432, 433, 434; ICD-8: 430, 431, 432, 433, 434). Univariate heritability analysis of the separate phenotypes stroke and coronary artery disease (CAD) was performed. In the bivariate analysis these phenotypes showed similar results as the primary phenotype (CVD) and therefore only the relation to the CVD phenotype was reported. 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 the primary care. The positive predictive value (ie, validity) of the myocardial infarction diagnosis in the Swedish Patient register has been demonstrated to be 95% when only primary

diagnoses are considered.[24] The validity of the stroke diagnosis in the inpatient registry has been reported to be 92%.[25]

Twin Contact and Age at Separation

Data on self-reported intra pair 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.[20] Data on contact frequency by at least one of the twins in a pair was available for 11 920 (97%) of the study participants. Contact frequency data was 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; (4) twins met on a weekly basis. The intra pair correlation on contact frequency was high (ρ=0.80) for the 4599 pairs where both responded. Data on age at separation was available for 12 023 (98%) individuals, correlation was somewhat lower compared to contact frequency, (ρ=0.66) for 4615 responding pairs. Where both twins had reported age at separation, average value was used for analysis. By computing the rank-order correlation (Spearman) between contact frequency and the absolute intra-pair difference in adjusted trait-levels, we explored if contact frequency and the degree of shared-environment influences, such as age at separation from co-twin, was associated with similarity in trait levels.

DNA Extraction and Genotyping

DNA extraction was made using Puregene extraction kit (Gentra systems, Minneapolis, MN) on a 7 mL EDTA tube of blood. Subsequently DNA was stored at −20°C. Subjects in whom the DNA concentration in the stock-solution was below 20 ng/μL, as well as subset of 302 female monozygous twin pairs participating in a previous genome-wide effort was excluded. Thereafter, DNA from all available DZ twins+1 twin from each available MZ twin pair (n=9896) was sent to Uppsala, Sweden for genome-wide genotyping using the Illumina OmniExpress bead chip. Genotyping results for 9836 subjects and 731 442 autosomal SNPs passed the initial lab-based quality control (QC).

In further QC SNPs with missing information exceeding 3% (GENO>0.03) (n=3922), a minor allele frequency of less than 1% (n=79 893) or a Hardy-Weinberg equilibrium (HWE) test *P* value ≤1E-07 (n=3071), were excluded. Individuals with low genotyping success (MIND>0.03) (n=10), male heterozygosity of X-chromosomes (n=36), deviations in heterozygosity of more than 5 standard deviations (SD) from the population mean (n=49) and/or where unknown (cryptic) relatedness (n=124) was detected, were excluded. After the QC there were 9617 individuals and 644 556 autosomal SNPs remaining.

Methods for Estimating Heritability

Heritability can be measured in 2 different ways, broad-sense (H^2) and narrow-sense (h^2). The broad-sense heritability measures the ultimate ability to predict phenotype from genotype as it measures the full contribution of genes to the phenotype. This can be broken down further into contribution from individual alleles "*additive,*" contributions due to homologous alleles at a locus "*dominance*" and combinations of non-homologus loci "*epistasis.*" The narrow sense heritability captures the "*additive*" contribution of genes to the trait and is the same as the maximum variance that can be explained by a linear combination of the allele counts.[26,27] To estimate heritability for the chosen phenotypes 2 different methods were used. First a classic quantitative biometrical genetic model fitting method was used. The observed variation of each phenotype was decomposed into the influence of additive genetic factor (A), common environmental factor (C) or dominance genetic factor (D), and unique environmental factor (E).[28,29] The heritability was estimated as proportion of variance explained by A and D in an ADE model (if the intraclass correlation in MZ twins [rMZ] is larger than twice of the correlation in DZ twins [rDZ]), or only A in an ACE-model (if rMZ $\leq 2 \times$ rDZ). The classical twin model relies on assumptions of random mating, equal environment sharing between MZ and DZ, absence of gene-environment interaction and correlation and no difference for the traits between twins and general population.[30] The second method used to estimate heritability in this study was through genome-wide complex trait analysis (GCTA). GCTA is a recently developed method whereby the proportion of variance of a complex trait that is explained by common genetic variation is estimated using SNP markers.[31] Although, it is not possible to directly compare the heritability estimates from twin model-analysis with the heritability estimates from the GCTA, the GCTA estimate can provide independent evidence of additive genetic effects on the trait variance. While GTCA-estimated heritability is limited to capture the additive effects of common SNPs, and is insensitive to interactions such as epistasis or dominance it also represents a lower bound for heritability estimates in twin studies. In this way the use of both methods on the same complex traits may independently and collectively reveal essential information about the genetic architecture of the trait.[32]

When relating genotype to phenotype in large heterogenic populations, there is a risk for bias arising from population stratification, ie, variance due to systematic ancestry differences due to migration, for example.[33] To avoid such bias, adjustment for genetic principal components (PCs) was performed.[34] PCs of the genotype data significantly correlated to the phenotypes were identified through a multiple stepwise regression analysis. As data on PCs for all individuals were not

available (not all phenotyped subjects had been genotyped), a sub-analysis of all phenotypes adjusted for significant PCs were then made in order to investigate the magnitude of the influence from them.

Statistics

Initial data handling and descriptive statistics were performed in SAS version 9.3 (SAS Institute, Cary, NC). To examine differences in variability and means between monozygotic (MZ) and dizygotic (DZ) twins a proc *t* test was performed. The distributions of Cystatin C, creatinine and MDRD were skewed and these variables were log transformed in order to achieve approximate normal distributions. Before further investigations, traits for logarithmized Cystatin C and creatinine together with machine estimated-GFR were adjusted for age and sex by linear regression models. In order to estimate GFR according to MDRD and the different CKD-epi formulas, age and sex were included in the calculations and thus no further adjustment was made for those covariates. After these adjustments, the residuals were z-score transformed and the influence of outliers were restrained through winsorizing outliers to −4 and +4 SDs.

SEM-modelled heritability estimation

In order to estimate variance components for each phenotype, maximum likelihood estimation and model fitting were performed using the structural equation statistical package OpenMx in R (http://openmx.psyc.virginia.edu). In univariate twin analyses the adjusted values of the investigated

Table 1. General Characteristics of Study Participants

	All	Men	Women
Total no of individuals, n	12 313	5585	6728
Complete pairs	4794	2133	2661
MZ, n	3155	1353	1804
OSDZ, n	4534	2191	2343
SSDZ, n	4588	2018	2570
UKZ, n	34	23	11
Age, y	64.9 (±8.1)	65.2 (±8.0)	64.6 (±8.2)
Weight, kg	74.5 (±13.9)	81.8 (±12.3)	68.5 (±12.1)
Height, cm	169.2 (±9.2)	176.3 (±6.9)	163.2 (±6.2)
Body mass index	26.0 (±4.1)	26.3 (±3.7)	25.7 (±4.4)
Current smoker, n	2021 (16.4%)	866 (15.5%)	1155 (17.2%)
Previous smoker, n	4870 (39.6%)	2504 (44.8%)	2366 (35.2%)
Never smoker, n	5335 (43.3%)	2170 (38.9%)	3165 (47%)
Diabetes	1202 (9.8%)	687 (12.3%)	515 (7.7%)
Hypertension*	5901 (47.9%)	2799 (50.1%)	3102 (46.1%)
Systolic blood pressure, mm Hg	138.7 (±19.7)	139.6 (±19.3)	138 (±20.0)
Diastolic blood pressure, mm Hg	81.9 (±11.0)	83.1 (±10.6)	80.9 (±11.3)
Pulse pressure, mm Hg	56.8 (±16.3)	56.5 (±15.5)	57.1 (±16.3)
Hyperlipidemia†	9094 (73.9%)	3654 (65.4%)	5440 (80.9%)
Anti-hypertensive treatment	2675 (21.7%)	1320 (23.6%)	1355 (20.1%)
Statin treatment	1648 (13.4%)	911 (16.3%)	737 (11%)
CVD‡	960 (7.8%)	663 (11.9%)	297 (4.4%)
Waist circumference, cm	91.3 (±12.2)	97.0 (±10.2)	86.6 (±11.6)
Waist/hip ratio	0.89 (±0.13)	0.94 (±0.13)	0.84 (±0.11)

Values are in means±SD or percentage. MZ indicates monozygotic; OSDZ, opposite-sexed dizygotic; SSDZ, same-sexed dizygotic; UKZ, unkonown zygosity.
*Systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg.
†Total cholesterol >5.0 mmol/L.
‡Cardiovascular disease (defined as ICD10=I20.0, I21, I22, I63; ICD9=410, 411B, 433, 434; ICD8=410, 411, 432, 433, 434; Surgical codes=FNG02, FNG05, FNC, FND, FNE. Diagnosed before study enrollment).

phenotypes were fitted into an ACE or ADE model,[35] as described above.

We conducted a bivariate heritability analysis to estimate the relative importance of genetic, common, and unique environmental influence to the phenotypic correlation between Cystatin C and creatinine. We also tested whether the genetic influence on Cystatin C and creatinine were correlated to the genetic influence on cardiovascular morbidity in terms of manifest CVD. Based on the univariate models, an ACE model was preferred for CVD, whereas an ADE was preferred for Cystatin C and creatinine. Because we cannot estimate the effect of A, D, C, E simultaneously with data from MZ and DZ twins only, ACE models were fitted for all bivariate twin analyses to keep consistency. Liability threshold model was applied to dichotomous variable (CVD) by assuming that the ordered categories reflect an imprecise measurement of an underlying normal distribution of liability.[36] The variance of CVD was constrained to one for calculating its correlation with Cystatin C/creatinine. Parameter estimates from a bivariate ADE model between Cystatin C and creatinine can be accessed upon request. The genetic correlation (rA) was calculated as: $cor_A/(\sqrt{(A\%_{trait1})})\times r_A\times(\sqrt{(A\%_{trait2})})$ where corA was standardized additive genetic covariance, $A\%_{trait1}$ and $A\%_{trai2}$ were the proportions of additive genetic variance for the respective traits. The common and unique environment component correlation was calculated similarly: $cor_C/(\sqrt{(C\%_{trait1})})\times r_C\times(\sqrt{(C\%_{trait2})})$ and $cor_E/(\sqrt{(E\%_{trait1})})\times r_E\times(\sqrt{(E\%_{trait2})})$. Through this the phenotypic correlation could be estimated to $cor_A+cor_C+cor_E$. Finally the bivariate heritability (h^2biv) was calculated as: $cor_A/(cor_A+cor_C+cor_E)$, which is the proportion of phenotypic correlation explained by genetic correlation.

Genome-wide complex trait analysis

Variance explained by all SNPs was estimated by restricted maximum likelihood (REML) modeling of the genetic relationship matrix (GRM) with phenotype-levels as implemented in the GCTA version 1.11 software package.[31] Since GCTA relies on comparisons between subjects that are not closely related, the sample was filtered for close relations. For complete monozygotic (MZ) twin pairs, 1 twin was randomly selected to be genotyped. For complete DZ twin-pairs 1 member of each pair was randomly selected rendering the sample reduced to 6634 participants. A further restriction was implemented by only considering pair-wise combinations of unrelated subjects with relatedness less than 0.025 which led to exclusion of 999, leaving n=5635 in the final sample on which GCTA analysis was conducted. The analyses were adjusted for genetic PCs displaying significant association to Cystatin C-, Creatinine levels, and estimated GFR. The following phenotypes were analyzed: Cystatin C, creatinine, eGFR (Cys C),

Table 2. Clinical Chemistry Characteristics of Study Participants

	All	Men	Women
N	12 313	5585	6728
Glucose, mmol/L	5.6 (±1.2)	5.8 (±1.3)	5.4 (±1.1)
Hba1c, %	4.8 (±0.7)	4.8 (±0.7)	4.8 (±0.6)
LDL, mmol/L	3.8 (±1.0)	3.7 (±1.0)	3.9 (±1.0)
HDL, mmol/L	1.4 (±0.4)	1.2 (±0.3)	1.6 (±0.4)
Triglycerides, mmol/L	1.3 (±0.8)	1.4 (±0.9)	1.3 (±0.7)
Total cholesterol, mmol/L	5.8 (±1.1)	5.5 (±1.1)	6.0 (±1.1)
CRP, mg/L	3.22 (±6.5)	3.37 (±7.6)	3.08 (±5.0)
Creatinine, μmol/L	77.5 (±23.6)	86.9 (±28.8)	69.7 (±14.2)
Cystatin C, mg/L	1.02 (±0.3)	1.05 (±0.3)	0.99 (±0.3)
Estimated GFR, MDRD*	83.1 (±18.1)	85.6 (±19.8)	81.1 (±16.2)
eGFR CysC, mL/min per 1.73 $m^{2\dagger}$	83.6 (±21.9)	81.2 (±21.8)	85.7 (±21.6)
CKD-epi Crea, mL/min per 1.73 $m^{2\ddagger}$	86.1 (±16.0)	92.9 (±15.5)	80.4 (±14.0)
CKD-epi CysC, mL/min per 1.73 $m^{2\ddagger}$	76.4 (±19.5)	77.2 (±20.2)	75.8 (±18.8)
CKD-epi Crea+CysC, mL/min per 1.73 $m^{2\ddagger}$	77.4 (±16.0)	76.8 (±16.2)	77.9 (±15.9)

All values are means±standard deviations. CKD indicates Chronic Kidney Disease; CRP, C-Reactive Protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease; N, number of participants, CKD indicates Chronic Kidney Disease.

*GFR according to the MDRD formula.

†Machine calculated GFR.

‡GFR according to the CKD-epi formula.

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Table 3. Univariate ACE/ADE Estimates for all the Phenotypes, and Test for Sex Difference

Phenotype	Sex	h^2	A	C	D	E	Qualitative Sex Diff	Quantitative Sex Diff
Cystatin C	M	0.55 (0.49 to 0.60)	0.26 (0.14 to 0.43)		0.29 (0.11 to 0.43)	0.45 (0.40 to 0.51)	N	Y
	F	0.63 (0.59 to 0.66)	0.56 (0.37 to 0.65)		0.06 (0.00 to 0.26)	0.37 (0.34 to 0.41)		
	Both	0.60 (0.56 to 0.63)	0.37 (0.24 to 0.49)		0.23 (0.09 to 0.37)	0.40 (0.37 to 0.44)		
Creatinine	M	0.56 (0.51 to 0.61)	0.24 (0.12 to 0.41)		0.32 (0.14 to 0.46)	0.44 (0.39 to 0.49)	N	Y
	F	0.62 (0.58 to 0.65)	0.61 (0.49 to 0.65)		0.01 (0.00 to 0.13)	0.38 (0.35 to 0.42)		
	Both	0.59 (0.56 to 0.62)	0.38 (0.25 to 0.51)		0.21 (0.08 to 0.35)	0.41 (0.38 to 0.44)		
eGFR* (Cys C)	M	0.55 (0.50 to 0.60)	0.28 (0.14 to 0.47)		0.28 (0.65 to 0.42)	0.45 (0.40 to 0.50)	N	Y
	F	0.63 (0.59 to 0.66)	0.60 (0.41 to 0.66)		0.03 (0.00 to 0.23)	0.37 (0.34 to 0.41)		
	Both	0.60 (0.57 to 0.63)	0.39 (0.26 to 0.52)		0.21 (0.07 to 0.35)	0.40 (0.37 to 0.43)		
MDRD† (crea)	M	0.62 (0.57 to 0.66)	0.39 (0.26 to 0.58)		0.22 (0.03 to 0.37)	0.38 (0.34 to 0.43)	N	Y
	F	0.65 (0.62 to 0.68)	0.65 (0.58 to 0.68)		0.00 (0.00 to 0.07)	0.35 (0.32 to 0.38)		
	Both	0.63 (0.60 to 0.66)	0.56 (0.43 to 0.64)		0.07 (0.00 to 0.21)	0.37 (0.34 to 0.40)		
CKD-epi‡ (Cys C+crea)	M	0.56 (0.46 to 0.62)	0.56 (0.46 to 0.62)	0.17 (0.11 to 0.25)		0.28 (0.25 to 0.31)	N	Y
	F	0.36 (0.27 to 0.44)	0.36 (0.27 to 0.44)	0.39 (0.31 to 0.47)		0.25 (0.23 to 0.28)		
	Both	0.48 (0.42 to 0.54)	0.48 (0.42 to 0.54)	0.26 (0.20 to 0.31)		0.26 (0.24 to 0.28)		
CKD-epi‡ (crea)	M	0.62 (0.50 to 0.68)	0.62 (0.50 to 0.68)	0.06 (0.02 to 0.16)		0.32 (0.28 to 0.36)	N	Y
	F	0.37 (0.27 to 0.48)	0.37 (0.27 to 0.48)	0.30 (0.21 to 0.39)		0.32 (0.29 to 0.36)		
	Both	0.54 (0.47 to 0.61)	0.54 (0.47 to 0.61)	0.14 (0.08 to 0.19)		0.32 (0.30 to 0.35)		
CKD-epi‡ (Cys C)	M	0.51 (0.39 to 0.58)	0.51 (0.39 to 0.58)	0.21 (0.15 to 0.31)		0.28 (0.25 to 0.32)	N	Y
	F	0.38 (0.30 to 0.47)	0.38 (0.30 to 0.47)	0.38 (0.30 to 0.46)		0.24 (0.21 to 0.26)		
	Both	0.46 (0.40 to 0.52)	0.46 (0.40 to 0.52)	0.28 (0.23 to 0.33)		0.25 (0.24 to 0.28)		
CVD§	M	0.39 (0.02 to 0.67)	0.39 (0.02 to 0.67)	0.17 (0.05 to 0.45)		0.44 (0.32 to 0.58)	N	N
	F	0.20 (0.00 to 0.61)	0.20 (0.00 to 0.61)	0.27 (0.00 to 0.51)		0.53 (0.37 to 0.70)		
	Both	0.41 (0.13 to 0.62)	0.41 (0.13 to 0.62)	0.12 (0.00 to 0.31)		0.47 (0.38 to 0.59)		
CAE‖	M	0.48 (0.08 to 0.77)	0.48 (0.08 to 0.77)	0.19 (0.00 to 0.49)		0.33 (0.22 to 0.48)	N	N
	F	0.30 (0.00 to 0.72)	0.30 (0.00 to 0.72)	0.25 (0.00 to 0.58)		0.45 (0.27 to 0.66)		
	Both	0.51 (0.21 to 0.73)	0.51 (0.21 to 0.73)	0.12 (0.00 to 0.33)		0.37 (0.27 to 0.49)		

Continued

Table 3. Continued

Phenotype	Sex	h^2	A	C	D	E	Qualitative Sex Diff	Quantitative Sex Diff
Stroke[¶]	M	0.24 (0.00 to 0.61)	0.24 (0.00 to 0.61)	0.17 (0.00 to 0.47)		0.60 (0.38 to 0.80)	N	N
	F	0.48 (0.02 to 0.70)	0.48 (0.02 to 0.70)	0.00 (0.00 to 0.33)		0.52 (0.30 to 0.78)		
	Both	0.45 (0.07 to 0.59)	0.45 (0.07 to 0.59)	0.00 (0.00 to 0.00)		0.55 (0.41 to 0.72)		

A indicates added genetic component; C, common environmental component; CAE, Coronary artery event; CVD, cardiovascular disease; D, dominance genetic component; E, unique environment component; eGFR, estimated glomerular filtration rate; h^2, heritability, defined as A in ACE-model and A+D in ADE-model; MDRD, Modification of Diet in Renal Disease; qualitative sex-difference, genetic correlation of less than 0.5 among opposite-sex twin pairs suggesting different genetic factors operating for males and females; quantitative sex-difference, significant difference in correlation between male and females.

*Machine calculated GFR.

[†]GFR according to the MDRD formula.

[‡]GFR according to the CKD-epi formula.

[§]Cardiodascular disease (definition in method).

[‖]Coronary artery event (definition in method).

[¶]Stroke defined in method.

MDRD (crea), CKD-epi (Cys C+crea), CKD-epi (Cys C), and CKD-epi (crea).

Results

Overall, 12 313 individuals were available for analysis, whereof 9588 were in complete twin pairs. For the GCTA 5635 individuals were included. General characteristics of the overall study group are summarized in Table 1. The mean age was 64.9 years, 55% of the sample was female, and 7.8% had a history of cardiovascular disease prior to enrollment. Mean Cystatin C level was 1.02 mg/L and mean creatinine level was 77.5 μmol/L. The estimates of GFR based on Cystatin C were in general lower compared with estimates based on creatinine (Table 2 and stratified by zygosity in Data S1).

The estimated heritability (h^2) of Cystatin C with the twin model was 0.55 (95% CI, 0.49 to 0.60) in men, 0.63 (95% CI, 0.59 to 0.66) in women and 0.60 (0.56 to 0.63) for both sexes combined. For creatinine h^2 was 0.56 (95% CI, 0.51 to 0.61) in men, 0.62 (95% CI, 0.58 to 0.65) in women, and 0.59 (0.56 to 0.62) for both sexes combined. For these traits a dominant genetic component was significant. The additive and dominant genetic variance components are presented in Table 3. For the phenotypes derived from Cystatin C and creatinine the dominance component was significant as well, whereas for CKD-epi estimated GFR and prevalent cardiovascular disease the correlation between MZ was less than twice the correlation for DZ and hence the ACE model was used. Effect of non-shared environment was significant for all phenotypic traits. MZ twins reported a higher contact frequency and higher mean age at separation than DZ twins. Mean contact level was 3.01 for MZ twins while it was 2.59 for DZ twins (t test, $P<0.0001$). Mean age at separation 20.0 years and 18.6 years for MZ and DZ, respectively (*t* test, $P<0.0001$). None of these measures were significantly related to the absolute intra-pair difference in adjusted trait levels (Data S1).

Through GCTA we found the estimate of the proportion of genetic variance to total phenotypic variance, V(g)/V(p), captured by all investigated markers to be significant for all traits (Table 4). GCTA heritability for Cystatin C was 0.40 (SE 0.07, $P=8E^{-9}$) and for creatinine 0.19 (SE 0.07, $P=0.003$). As there were sex-differences in heritability observed in the classical twin-model, we tested a GCTA model that included gene by sex interaction without finding any significant interaction term ($P>0.05$) for any of the 7 tested phenotypes.

In the twin model, heritability for decreased kidney function as a dichotomized variable was higher compared with eGFR as a continuous varaiable for eGFR (Cyst C) and CKD-epi (Cys C) (0.46 versus 0.73 and 0.60 versus 0.82), while for creatinine-based measurements, ie, MDRD and

Table 4. Genome-Wide Complex Trait Analysis (GCTA) of the 7 Phenotypes Investigated

Phenotype	Source	Variance	SE	*P* Value
Cystatin C	V genotypic (g)	0.404	0.0763	
	V environmental (e)	0.608	0.0749	
	V phenotypic (p)	1.012	0.0192	
	V (g)/V (p)*	0.399	0.0743	$8E^{-9}$
Creatinine	V genotypic (g)	0.190	0.0727	
	V environmental (e)	0.798	0.0733	
	V phenotypic (p)	0.988	0.0186	
	V (g)/V (p)	0.192	0.0733	0.003
eGFR (Cystatin C)†	V genotypic (g)	0.418	0.0766	
	V environmental (e)	0.594	0.0751	
	V phenotypic (p)	1.012	0.0192	
	V (g)/V (p)	0.413	0.0644	$3E^{-9}$
MDRD (Creatinine)‡	V genotypic (g)	0.186	0.0720	
	V environmental (e)	0.791	0.0726	
	V phenotypic (p)	0.977	0.0184	
	V (g)/V (p)	0.191	0.0734	0.004
Cdk-epi (Cystatin C+Creatinine)§	V genotypic (g)	0.192	0.0737	
	V environmental (e)	0.821	0.0744	
	V phenotypic (p)	1.013	0.0191	
	V (g)/V (p)	0.190	0.0725	0.003
Cdk-epi (Cystatin C)§	V genotypic (g)	0.247	0.0733	
	V environmental (e)	0.769	0.0735	
	V phenotypic (p)	1.016	0.0192	
	V (g)/V (p)	0.243	0.0717	$9E^{-5}$
Cdk-epi (Creatinine)§	V genotypic (g)	0.084	0.0707	
	V environmental (e)	0.918	0.0724	
	V phenotypic (p)	1.002	0.0189	
	V (g)/V (p)	0.084	0.0705	0.1

All values adjusted for age, sex and correlated principal components. eGFR indicates estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; SE, standard error; V, variance.

*$V(g)/V(p)=h^2$ (heritability).

†Glomerular filtration rate (GFR) according to the modification of diet in renal disease (MDRD)-formula.

‡Machine calculated GFR.

§GFR according to the CKD-epi formula.

CDK-epi (crea) it was lower (0.63 versus 0.30) (0.54 versus 0.00), respectively. The GCTA "chip-heritability" was lower for all dichotomized phenotypes but remained significant for cystatin C-based equations, while it was almost zero and non-significant for the creatinine-based equations (data shown in Data S1).

The results of the bivariate heritability analysis are shown in Table 5. The phenotypic correlation between Cystatin C and creatinine was estimated to 0.63 (95% CI, 0.61 to 0.65) in men and 0.55 (95% CI, 0.53 to 0.57) in women. The proportion of this correlation explained by additive genetic components (the bivariate heritability, h^2biv) was 0.52 (95% CI, 0.44 to 0.59) in men and 0.51 (95% CI, 0.36 to 0.65) in women. For Cystatin C versus CVD the correlation was 0.16 (95% CI, 0.12 to 0.20) in men and 0.17 (95% CI, 0.13 to 0.21) in women and the genetic correlation in males was 0.41 (0.21 to 0.62) while it was non-significant in females. The results of the bivariate analysis on creatinine and Cystatin C versus CVD are presented in Table 5 (Further data also shown in Data S1).

Table 5. Bivariate Heritability Analysis

	Male	Female	Combined
Cystatin C vs creatinine			
Bivariate correlations			
Genetic (r_a)	0.64 (0.58 to 0.70)	0.53 (0.43 to 0.62)	0.60 (0.57 to 0.63)
Shared environmental (r_c)	−0.99 (−1 to 1)	0.99 (−1 to 1)	0.95 (−1.00 to 1.00)
Non-shared environmental (r_e)	0.63 (0.58 to 0.67)	0.52 (0.47 to 0.57)	0.57 (0.53 to 0.60)
Phenotypic correlation	0.63 (0.61 to 0.65)	0.55 (0.53 to 0.57)	0.59 (0.58 to 0.60)
Mediated by:			
Bivariate heritability (biv h^2)	0.52 (0.44 to 0.59)	0.51 (0.36 to 0.65)	0.59 (0.54 to 0.63)
Bivariate shared environment (biv c^2)	0.00 (−0.02 to 0.04)	0.12 (−0.00 to 0.25)	0.00 (−0.01 to 0.02)
Bivariate non-shared environment (biv e^2)	0.48 (0.41 to 0.56)	0.37 (0.32 to 0.42)	0.41 (0.37 to 0.46)
Cystatin C vs CVD			
Bivariate correlations			
Genetic (r_a)	0.41 (0.21 to 0.62)	0.05 (−0.24 to 0.43)	0.30 (0.14 to 0.59)
Shared environmental (r_c)	−0.99 (−1 to 1)	0.99 (−1 to 1)	−0.99 (−1.00 to 1.00)
Non-shared environmental (r_e)	0.01 (−0.12 to 0.13)	0.17 (0.06 to 0.28)	0.08 (−0.01 to 0.16)
Phenotypic correlation	0.16 (0.12 to 0.20)	0.17 (0.13 to 0.21)	0.16 (0.14 to 0.19)
Mediated by:			
Bivariate heritability (biv h^2)	1.13 (0.59 to 1.72)*	0.10 (−0.49 to 0.88)	0.84 (0.40 to 1.28)
Bivariate shared environment (biv c^2)	−0.15 (−0.49 to 0.17)	0.43 (−0.20 to 0.89)	−0.05 (−0.34 to 0.24)
Bivariate non-shared environment (biv e^2)	0.02 (−0.35 to 0.39)	0.47 (0.17 to 0.77)	0.21 (−0.04 to 0.46)
Creatinine vs CVD			
Bivariate correlations			
Genetic (r_a)	0.32 (0.12 to 0.54)	−0.19 (−0.36 to 0.06)	0.21 (0.06 to 0.48)
Shared environmental (r_c)	−0.99 (−1 to 1)	0.43 (−0.38 to 1)	−0.99 (−1.00 to 1.00)
Non-shared environmental (r_e)	−0.11 (−0.23 to 0.02)	0.09 (−0.33 to 0.21)	−0.03 (−0.12 to 0.06)
Phenotypic correlation	0.09 (0.05 to 0.13)	0.05 (0.01 to 0.09)	0.07 (0.05 to 0.10)
Mediated by:			
Bivariate heritability (biv h^2)	1.64 (0.64 to 3.03)*	−1.16*†	1.34 (0.37 to 2.57)
Bivariate shared environment (biv c^2)	−0.09 (−0.79 to 0.55)	1.37†	−0.14 (−0.89 to 0.49)
Bivariate non-shared environment (biv e^2)	−0.55 (−1.50 to 0.08)	0.79†	−0.20 (−0.89 to 0.35)

Cystatin C and creatinine are sex-, age adjusted and log-transformed. CVD indicates cardiovascular disease.
*If the genetic correlation is in opposite direction with the environmental correlation, the proportion of the phenotypic correlation mediated by genetic component can go beyond 100%, whereas the sum of proportions of the phenotypic correlation mediated by ra, rc, and re, is always between 1.0 and −1.0.
†There were some convergence problems when estimating the confidence interval for creatinine vs CVD in females.

Discussion

In this study we examined the relative importance of genes and environment for several phenotypes with well-known relation to both kidney function and cardiovascular morbidity in a large population-based study of middle-aged to elderly Swedish twins. Both Cystatin C and creatinine were highly heritable, and the heritability estimates were higher in women than men. The GCTA analysis provided independent evidence for significant contribution of additive genetics to trait variance for all phenotypes and indicated that more of the variance of Cystatin C is captured by common SNP's as compared to creatinine. Further, decreased kidney function, defined as having an eGFR in the lowest quintile, was more heritable when estimated from Cystatin C-based equations compared with creatinine-based ones. We also report a significant genetic correlation between levels of Cystatin C and creatinine as well as a genetic correlation between levels of Cystatin C and CVD in males indicating that there is a substantial overlap in genetic factors influencing both traits in males.

By using the classic twin model we estimate the heritability of both Cystatin C and creatinine to just under 0.6 on average for both sexes. For Cystatin C the estimate is higher than what has been found in a previous study that used an extended pedigree model, where the heritability was estimated to be 0.40 when adjusted for age and sex, and attenuated to 0.35 upon further multivariable adjustment.[37] The study by Parikh et al differs from ours regarding methodology, size, and age of the participants, therefore results may not be fully comparable. For serum creatinine, previous studies have reported heritabilities in the range of 0.00 to 0.64,[38–41] whereas the heritability of creatinine-based estimated GFR was 0.31 to 0.63.[41,42] The heritability for creatinine in our study is similar to that found by Jermendy et al,[39] but higher compared with what is described by Nilsson et al[40] In accordance with our results, Nilsson et al found that the genetic influence was higher in women than in men, although under an ACE model. In a Danish population-based twin study Bathum et al[43] no heritability at all was found in men but a substantial dominance component in women. However, the study cohorts in all these studies were 5- to 10-fold smaller than ours, and the Jermendy cohort is also substantially younger, and why comparisons need to be done with some caution. In the present study there were significant differences between the sexes both in terms of heritability and the proportion of the variance explained by additive and dominance genetic factors respectively. In women the broad sense heritability, in the present study consisting of additive and dominance components contributing to the variance (Table 3), was larger than in men, which for creatinine is consistent with findings from Nilsson et al.

The finding of a dominance effect for Cystatin C is, to our knowledge, new. In previous twin studies the ACE model has been preferred, whereas in ours the ADE model was preferred for 4 out of 7 investigated traits. A reason for this might be the large study population, which enhances power and enables detection of weaker variance components underlying the traits. The most striking sex difference in our findings was also related to the dominance component, where in men the dominance component was about the same size as the additive component whereas in women the dominance component was almost absent. For the phenotypes in our study where the ACE model was preferred (CKD-epi formula derived GFR and CVD) heritability was higher in men, but the common environment component was significantly larger in women.

The reported "chip heritability" V(g)/V(p) from the GCTA analysis in the current study was 0.40 for Cystatin C and 0.19 for creatinine. Previous studies using the GCTA method for various traits and diseases has found that the "chip heritability" tends to be in the order of one-quarter to one-half of the twin-based heritability.[44] Here, we found the Vg/Vp to approach 65% of the twin-based sex averaged estimate for Cystatin C and Cystatin C based machine estimate of GFR, but only about 30% for creatinine and MDRD, which indicates that an unusually large proportion of the genetic variability for Cystatin C appears to be captured by the investigated common SNP markers.

Since the GCTA and the twin model represent 2 different methods of estimating heritability, the 2 methods should be regarded as complementary rather than directly comparable. One of the major differences between the methods, which also likely explains the difference in the twin study estimates compared with the GCTA-estimates, is that the GCTA only estimates genetic variability captured by the SNP markers. Non-tagged polymorphism such as rarer single base-pair mutations, copy number variations and other types of rare alleles, which will affect the estimate in the twin-based model, will not contribute to the GTCA. Further, the GCTA model assumes additive genetic variance and thus only captures the narrow-sense heritability, ie, non-additive effects such as dominance, gene-gene interactions (epistasis), and gene-environment interactions are not accounted for. There is also a possibility that the estimates of the twin-model are inflated by a violation of the assumption of equally shared environment, which might further widen the gap between the heritability estimates derived from the 2 models. The equally shared environment assumption stipulates that monozygotic and dizygotic twins are exposed to trait-relevant shared-environmental influences equally. A violation of this assumption so that MZ twins share more trait relevant environmental factors would mimic genetic dominance effects.

The definition of cardiovascular disease used in this study is a commonly used endpoint in cardiovascular interventional studies and in epidemiological cohort studies. However, no previous study has reported on the heritability of this combined phenotype. The heritability of 0.39 respectively 0.20 in men and women for CVD in the current study can be compared with a heritability of 0.57 in men and 0.38 in women for death in coronary heart disease reported by Zdravkovic et al[45] When we divided CVD into its components CAD and stroke, the heritability for CAD increased to 0.48 in men and 0.30 in women. These differences may in part be explained by the fact that the Zdravkovic study only studied mortality from CHD, in which genes might be more apparent than for other manifestations. Due to a larger number of outcomes the power to assess heritability in the Zdravkovic study was better than in the TwinGene study. Further, it may be more difficult to delineate the role of genetic effects for composite endpoints such as CVD as risk factor patterns for the included endpoints may differ.

In the current study an intermediate phenotypic correlation was observed between Cystatin C and creatinine. This correlation was explained by shared genetic- and non-shared environment to approximately the same extent. Since the

variations in both phenotypes reflect renal function, renal function is probably more strongly related to the covariance of the 2 traits than to the individual phenotypes. The observation that shared genes contributed substantially to the covariance of the 2 traits, and thus renal function, is an expected finding, while the small contribution of shared environment is not. The most common risk factors for CKD are overweight, hypertension, and diabetes mellitus[46] for all of which both genes and environmental factors are important. In the current study very few in the study population had CKD and the majority of the variation of the studied phenotypes was in the normal range. The contribution of these classic risk factors to the variation of the studied phenotypes in the normal range is unknown.

A novel finding in this study was an observed genetic correlation between CVD and Cystatin C despite a low phenotypic correlation between the traits. The relation between creatinine and CVD was similar but the phenotypic correlation between these 2 traits was very weak. Although the variation in Cystatin C only explained a small part of the variation in CVD, the covariance between the traits was entirely explained by common genes. This finding was only significant in males and may reflect that CVD was more common in men and thus a low power in the study to detect possible associations in women. It is possible that the shared genetic factor may affect all these phenotypes directly. However, the finding that Cystatin C and CVD partly share common genes may also indicate a possible causal relation between Cystatin C and CVD as defined in this study. This reasoning is in analogy with Mendelian randomization studies in which firstly an instrumental variable that is related to the variance of the studied risk factor is identified (1 or several SNPs from the genome) and secondly the relation of this instrumental variable to the occurrence of the disease in question is studied. A Mendelian randomization study on the role of Cystatin C and CVD is currently ongoing and may shed further light on causality behind this relation.

In conclusion, the heritability of Cystatin C in our study was higher compared with previous studies. The GCTA analysis provided independent evidence for significant heritability indicated by the twin-model for all phenotypes. The heritability captured with GCTA analysis was larger for Cystatin C compared to creatinine. Cystatin C was weakly correlated to CVD, although more strongly than creatinine. The covariation between Cystatin C and CVD in males was explained by additive genetic components indicating that Cystatin C and CVD share genetic influences.

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Disclosures

None.

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