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Association of a Cystatin C Gene Variant With Cystatin C Levels, CKD, and Risk of Incident Cardiovascular Disease and Mortality

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Abstract

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Supplementary Material
Table S1: Genotyping and imputation platforms.
Table S2: Study-specific characteristics of participants.
Table S3: Association between rs13038305 and kidney function measures.
Table S4: NRI of genotype-adjusted $eGFR_{cys}$, stratified by genotype and baseline eGFR.
Table S5: Reclassification of mortality events across eGFR categories.
Table S6: Reclassification of cardiovascular events across eGFR categories.
Item S1: Study-specific methods.
Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

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Background—Carriers of the T allele of the single-nucleotide polymorphism rs13038305 tend to have lower cystatin C levels and higher cystatin C-based estimated glomerular filtration rate ($eGFR_{cys}$). Adjusting for this genetic effect on cystatin C concentrations may improve GFR estimation, reclassify cases of CKD, and strengthen risk estimates for cardiovascular disease (CVD) and mortality.

Study Design—Observational.

Setting & Population—Four population-based cohorts: Atherosclerosis Risk in Communities (ARIC), Cardiovascular Health (CHS), Framingham Heart (FHS), and Health, Aging, and Body Compostion (Health ABC) studies.

Predictors—We estimated the association of rs13038305 with $eGFR_{cys}$ and $eGFR_{cr}$, and performed longitudinal analyses of the associations of $eGFR_{cys}$ with mortality and cardiovascular events following adjustment for rs13038305.

Outcomes—We assessed reclassification by genotype-adjusted $eGFR_{cys}$ across CKD categories: <45, 45–59, 60–89, and ≥90 mL/min/1.73 m^2. We compared mortality and CVD outcomes in those reclassified to a worse $eGFR_{cys}$ category with those unaffected. Results were combined using fixed-effect inverse-variance meta-analysis.

Results—In 14,645 participants, each copy of the T allele of rs13038305 (frequency, 21%), was associated with 6.4% lower cystatin C concentration, 5.5 mL/min/1.73 m^2 higher $eGFR_{cys}$, and 36% [95% CI, 29%–41%] lower odds of CKD. Associations with CVD (HR, 1.17; 95% CI, 1.14–1.20) and mortality (HR, 1.22; 95% CI, 1.19–1.24) per 10- ml/min/1.73 m^2 lower $eGFR_{cys}$ were similar with or without rs13038305 adjustment. In total, 1134 participants (7.7%) were reclassified to a worse CKD category following rs13038305 adjustment, and rates of CVD and mortality were higher in individuals who were reclassified. However, the overall net reclassification index was not significant for either outcome, at 0.009 (95% CI, −0.003 to 0.022) for mortality and 0.014 (95% CI, 0.0 to 0.028) for CVD.

Limitations—rs13038305 only explains a small proportion of cystatin C variation.

Conclusions—Statistical adjustment can correct a genetic bias in GFR estimates based on cystatin C in carriers of the T allele of rs13038305 and result in changes in disease classification. However, on a population level, the effects on overall reclassification of CKD status are modest.

Keywords

Cystatin C; chronic kidney disease; genetics; single nucleotide polymorphism; net reclassification improvement

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Reduced glomerular filtration rate (GFR) is a risk factor for end-stage renal disease, cardiovascular disease (CVD) and all-cause mortality.[1] Cystatin C is an alternative biomarker of kidney function with several physiological advantages over creatinine, including a stable, tightly-regulated rate of appearance in the circulation, complete reabsorption and catabolism by the proximal tubule following free glomerular filtration, and undetectable tubular secretion.[2] Consequently, cystatin C is less affected by age, sex, race or muscle mass,[3] and is a better predictor of adverse outcomes such as CVD, end-stage renal disease and death.[2,4,5] Furthermore, it augments discriminatory power for all-cause mortality and end-stage renal disease when used in conjunction with traditional kidney disease biomarkers.[5] Because cystatin C has been found to distinguish borderline cases of CKD in individuals who are low risk for CKD complications, [5] a specific clinical role for cystatin C to confirm CKD in such cases (estimated GFR [eGFR], 45–60 ml/min/1.73 m^2) is included in the revised 2012 KDIGO guidelines on the detection and staging of Chronic Kidney Disease.[6] Furthermore, a combined creatinine–cystatin C equation has recently been

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shown to perform better than equations based on either marker alone, and has also been proposed as a confirmatory test for CKD.[7] In view of a potentially expanded role for cystatin C in the assessment of kidney function, it is timely and important to consider whether its performance as a kidney disease biomarker requires further refinement.

Genetic variability in cystatin C production or metabolism may have a negative impact on GFR estimation and CKD classification. Cystatin C variability has a modest genetic component with an overall contribution from genetic determinants of approximately 6%.[8] The cystatin superfamily gene cluster on chromosome 20 contains the majority of cystatin genes that encode cystatin proteins, which are characterized by multiple cystatin-like sequences.[9] Single-nucleotide polymorphisms (SNPs) in this region have previously been reported as associated with serum cystatin C concentrations,[10] and are believed to influence serum cystatin C levels through effects on production or metabolism, rather than via direct effects on kidney function.[11] A recent genome-wide association study (GWAS) identified an intergenic SNP, reference SNP identification number 13038305 [rs13038305], in the cystatin superfamily gene cluster between *CST3* (cystatin C) and *CST9* (cystatin 9) that was associated with GFR estimated based on cystatin C (eGFRcys), but not with creatinine-based eGFR (eGFRcr) or CKD defined by reduced eGFRcr (CKD_{cr}).[11] Each copy of the T allele of the index SNP, rs13038305, was found to be associated with a 7.6% increase in eGFR and carriers had, on average, lower circulating cystatin C concentrations and higher associated eGFRcys.

In the present analysis, using data from four large, prospective, population-based studies, we considered the association of rs13038305 with cystatin C levels and eGFRcys using the recently published CKD-EPI GFR estimating equation based on cystatin C.[7] We hypothesized that adjustment for the genetic effect on eGFRcys would improve GFR estimation and meaningfully reclassify individuals across categories of CKD. We additionally sought to evaluate whether genotype-adjusted eGFRcys has stronger associations with important adverse outcomes of CKD, namely incident CVD and death, which would be indicative of a refinement in GFR estimation by eliminating genetic noise.

Methods

Participants

Participants were drawn from four prospective, population-based cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium: the Atherosclerosis Risk in Communities (ARIC), Cardiovascular Health (CHS), Framingham Heart (FHS), and Health, Aging, and Body Composition (Health ABC) studies. Each participant provided written informed consent, and the institutional review boards of the respective institutions approved the study protocols. Detailed information pertaining to the study samples, including participant recruitment, phenotype definition, genotyping, imputation, and data quality control are provided in Item S1 (provided as online supplementary material).

Exposure Definitions

Assay details pertaining to serum creatinine and cystatin C are provided in Item S1. eGFRcr was estimated using both the MDRD (Modification of Diet in Renal Disease) Study and the CKD-EPI equations.[12,13] For use in the MDRD Study equation, creatinine levels were statistically calibrated to age- and sex- group specific means in the NHANES III (Third National Health and Nutrition Examination Survey). For use in the CKD-EPI creatinine equation, calibrated creatinine values were also standardized by multiplying by 0.95.[14]

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eGFRcys was estimated using the 2012 CKD-EPI cystatin C equation (Inker et al[7]) CKD was defined as eGFR <60ml/min/1.73m^2 for all methods of GFR estimation.

Outcome Definitions

Incident CVD was defined as incidence of ischemic stroke, myocardial infarction, or death due to clinically recognized CVD events. Individuals with prevalent myocardial infarction or stroke history were excluded. Ascertainment methods of CVD and mortality events in each study are described in Item S1.

Covariate Assessment

Clinical variables—Systolic and diastolic blood pressure were measured using a sphygmomanometer in the seated position following a standard protocol in each study. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or treatment for hypertension at the time of creatinine and cystatin C assay.

Genotyping—Genotypes of the participants in each cohort were obtained using Affymetrix (Affymetrix Inc) or Illumina (Illumina Inc) microarrays and then imputed with HapMap reference panels (International HapMap Project). The imputation quality of rs13038305 was 0.97 in ARIC, 0.82 in CHS, 0.98 in FHS and 1.0 in Health ABC. Details of genotyping and imputation in each cohort are reported in Table S1.

Statistical Methods

Selection of rs13038305 as representative SNP in cystatin C gene cluster—The cystatin C gene cluster polymorphism, rs13038305, was the index SNP in a recent GWAS of eGFRcys conducted in over 12,000 individuals of European ancestry.[8] Each copy of the T allele of rs13038305 (mean frequency from meta-analysis, 21%) was associated with 7.6% higher eGFRcys using the 2008 CKD-EPI equation without covariates.[16] Hence, rs13038305 was selected for this analysis as it had the largest contributions to cystatin C variance and was the strongest among all SNPs evaluated in the GWAS.

Association analyses of rs13038305 genotype with participant characteristics and kidney function—We grouped participants by most likely rs13038305 genotypes (CC, CT or TT), and compared baseline characteristics by group in univariate analysis, using equality of means for continuous variables and Chi-square tests for categorical variables. Linear regression analyses were performed to evaluate associations of rs13038305 with cystatin C (with natural log transformation), eGFRcys, $eGFR_{cr(MDRD),}$ and $eGFR_{cr(CKD\text{-}EPI),}$ based on the minor allele (T allele) imputed dosage to account for imputation uncertainty. Logistic regression analyses were used to evaluate associations of rs13038305 with $CKD_{cr(MDRD),}$ $CKD_{cr(CKD\text{-}EPI)}$ or CKD_{cys}. All regression analyses were adjusted for age, sex, and study center (where applicable).

Adjustment of $eGFR_{cys}$ for rs13038305 genotype—We conducted association analyses of rs13038305 and eGFRcys, adjusted for age and gender, in each of the participating studies. We conducted a fixed-effect inverse variance weighted meta-analysis to estimate the coefficient (β) of the rs13038305 T allele associated with eGFRcys across studies. We used this coefficient to adjust out the effect of the minor allele (T allele) within each study population: Genotype-adjusted eGFRcys = eGFRcys – β*(rs13038305 T allele dosage).

Genotype-adjusted CKDcys was defined as genotype-adjusted eGFRcys <60 ml/min/1.73 m^2.

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Associations of $eGFR_{cys}$ and rs13038305 with incident CVD and mortality— Proportional hazards regression models were used to assess the associations of rs13038305 with incident CVD and mortality, as well as associations of both unadjusted and genotype-adjusted eGFRcys with each outcome. Analyses were performed adjusting for age, sex, and study center (when applicable). eGFRcys was evaluated both as a continuous and dichotomized variable at an eGFR of 60ml/min/1.73m^2.

Reclassification analyses of genotype-adjusted $eGFR_{cys}$—We calculated the total net reclassification improvement (NRI) for mortality and CVD outcomes in four eGFRcys categories: <45, 45–59, 60–89 and ≥90 mL/min/1.73m^2.[17] We also computed the NRI stratified by eGFRcys category and rs13038305 genotype. To assess further whether reclassification based on genotype-adjusted eGFRcys improved risk stratification, we performed proportional hazard regression to estimate hazard ratios for mortality and CVD comparing those reclassified to a worse CKD stage to those not reclassified.

Meta-analysis—Study-specific *P* values for participant characteristics, stratified by genotype, were combined by the Fisher method. The means and standard deviations were combined as averages, weighted by the study sample sizes, and the counts were combined as arithmetic sums. Study-specific results from NRI analyses, linear regression, or proportional hazard analyses were combined using a fixed-effect inverse-variance weighted method using the R metafor software package.[18]

Results

Study Sample Characteristics

Participant characteristics for the total sample (N = 14,645), stratified by rs13038305 genotype, are presented in Table 1. Study-specific participant characteristics are presented in Table S2. The proportions of individuals in each genotype group were 62.5% (CC), 33.0% (CT), and 4.5% (TT). The mean age of the participants was 66 years, and genotype groups did not differ significantly by age, sex or eGFRcr.

Carriers of the minor (T) allele of rs13038305 had approximately 0.06 mg/dL lower cystatin C concentrations and 6 ml/min/1.73m^2 higher eGFRcys per copy, compared with non-carriers. The proportion of individuals classified as having CKD based on eGFRcys was twice as high for the CC vs. TT genotype (17% vs. 8.4%). Adjusting out the effect of the minor allele resulted in lower GFR estimates (5.4 mL/min/1.73m^2 and 10.8 mL/min/1.73m^2 lower eGFRcys in heterozygotes (CT) and homozygotes (TT), respectively, compared with non-carriers; Table 1).

Associations of rs13038305 With Cystatin C, eGFR, and CKD

In multivariable analyses adjusted for age and sex, each copy of the T allele of rs13038305 was associated with 6% lower cystatin C levels ($P = 4.1\times10^{-113}$), 5.5 mL/min/1.73m^2 higher eGFRcys ($P = 3.1\times10^{-124}$) and a lower odds of CKD based on eGFRcys (odds ratio, 0.64; 95% CI, 0.59–0.71; Table S3). As expected, rs13038305 was not associated with eGFRcr or CKDcr (*P* values > 0.3; Table S3).

We also tested the genetic association of eGFRcys with cystatin C in African Americans from the ARIC study (n = 1894). The index SNP within 1 Mb of rs13038305 was rs4815224, which had a minor allele β of 0.034 (p=2.9e-3, minor allele frequency=0.10). As this effect size is approximately half that of rs13038305 in European Americans (β=−0.06), common variants in cystatin C gene cluster are expected to have a smaller influence in African Americans than European Americans in our study.

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Association of Unadjusted and Genotype-Adjusted $eGFR_{cys}$ With Incident CVD and Death

Table 2 presents the results of survival analyses of unadjusted and genotype-adjusted eGFRcys and CKDcys on incident CVD and mortality. In total, there were 2,351 incident CVD events and 3,700 mortality events during follow-up. The mean follow-up ranged from 7.8 to 13.9 years across studies (Table S2). The association of eGFRcys with CVD and mortality risk was not materially different after adjustment for rs13038305 genotype. Similarly, the associations of CKDcys with incident CVD and mortality were not materially different after adjustment for rs13038305 (Table 2). The genotype itself was not significantly associated with CVD or mortality risk.

Reclassification Analyses

A total of 1134 individuals (7.7%) were reclassified to a worse eGFRcys category following genotype adjustment (Table 3). Reclassification was highest in those with eGFR ≥90 mL/min/1.73m^2 at baseline (15.2%). However, the overall NRI results were not significant: NRIs of 0.009 (95% CI, −0.003 to 0.022) and 0.014 (95% CI, 0.0 to 0.028) for mortality and CVD, respectively (Table 4).

With the exception of those with preserved GFR at baseline (≥90mL/min/1.73m^2), participants reclassified to a worse GFR category by genotype-adjusted eGFRcys had a higher risk for adverse outcomes compared with those who were not reclassified (Table 4). For example, in participants with unadjusted eGFRcys between 60–89 mL/min/1.73m^2, reclassification to the 45–59 mL/min/1.73m^2 category was associated with hazard ratios of 1.54 (95% CI, 1.30–1.83) for death and 1.66 (95% CI, 1.34–2.06) for incident CVD compared to the non-reclassified group (Table 4).

In genotype- and baseline eGFRcys–stratified analyses, we observed slightly higher NRI for mortality in specific subgroups of eGFRcys. For example, the NRIs were 0.033 (95% CI, 0.019–0.046) in those with unadjusted eGFRcys of 60–89 mL/min/1.73m^2 and 0.037 (95% CI, 0.005–0.07) in those with 45–59 mL/min/1.73m^2 (Table S4). Furthermore, there was no significant reclassification of CVD outcomes by GFR category, nor was there when analyses were performed stratified by genotype (CC vs. CT vs. TT; Table S4). Absolute numbers of mortality and cardiovascular events reclassified across eGFR categories by genotype adjustment are presented in Tables S5 and S6.

Discussion

The findings of the present study are threefold. First, rs13038305 results in a genetic bias in eGFRcys such that carriers of the minor allele of rs13038305 have GFR estimates approximately 6 mL/min/1.73m^2 higher than non-carriers, per copy. Second, knowledge of genotype at rs13038305 permits statistical correction of this overestimation of GFR, resulting in a modest reclassification of CKD stages although there was no significant net reclassification overall for clinical outcomes. Third, we observed stronger associations with incident CVD and mortality in those reclassified to a worse CKD stage by genotype adjustment compared to those who were not reclassified. Taken together, these results provide proof-of-principle that GFR estimates may be refined by means of genetic information derived from GWASs. However, the effects appear too small to be clinically meaningful at the present time.

Our findings are consistent with other studies that have attempted to translate the results of GWASs to the clinic by improving biomarker performance. For example, a recent GWAS reported 10 significant genomic loci for HbA1c, a biomarker that reflects recent glycemic control and that is widely used in the diagnosis and management of diabetes mellitus.[19] Of

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these SNPs, the 3 with the largest effect estimates for fasting glucose, *G6PC2*, *MTNR1B*, and *GCK*, are believed to truly reflect glycemic control. It is hypothesized the remaining 7 SNPs are associated with HbA1c levels via non-glycemic mechanisms, such as via effects on erythrocyte and iron metabolism pathways.[19] Thus, analogous to our findings regarding rs13038305 and cystatin C concentrations, these non-glycemic loci would be expected to attenuate the effect of hyperglycemia on HbA1c. Similar to our results, when the authors adjusted for the effect of these non-glycemic loci they found the genetic effect size on HbA1c levels to be small. Furthermore, genotype-adjusted HbA1c resulted in a small but detectable reclassification (~2%) in a population screened for diabetes. Whether a reclassification of this order is of importance at a population level remains to be established.

A key advantage of cystatin C over creatinine is that it depends less on muscle mass, permitting the development of simpler GFR estimation equations.[16] For example, GFR estimates based on cystatin C alone are almost as accurate as those for creatinine adjusted for age, sex, and race.[16] Our study quantifies the misclassification due to a cystatin C variant in Europeans and demonstrates that, in principle, improvements in GFR estimates using individual genetic information are possible. As more genetic variants of different frequencies and effect sizes are identified in additional populations, it will be important to determine whether the aggregate of cystatin C variants might have a greater impact on reclassification at a population level. This might be achieved using a polygenic risk score approach, similar to what has been attempted in CKD and diabetes genetic prediction models. [20,21] Genetic association studies in patients with more advanced CKD may also yield more clinically meaningful results, as cystatin C variants could have a greater impact in that setting. Lastly, similar analyses of genetic variants influencing other kidney function biomarkers, such as β2-microglobulin or β-trace protein,[22,23] are an interesting avenue for future research.

Despite minimal effects on reclassification at a population level, rs13038305 may nonetheless have meaningful effects for the individual. As such, our findings illustrate an inherent paradox in using population-derived data to design personalized medicine and identify an obstacle that must be overcome in order to translate GWAS findings to the bedside. For example, we found GFR estimates in rs13038305 T homozygotes (3% of participants) to differ by 12 ml/min/1.73 m^2 in comparison with non-carriers. This level of misclassification could have important consequences to the individual, such as complications arising from inappropriate renal or thrombolytic drug dosing, or missed opportunities for secondary prevention because of delayed nephrology referral in those with borderline GFR. While the results may be too small to justify genetic testing for rs13038305, they may provide useful information in circumstances where a person's genotype is already known.

Strengths of this analysis include the large sample size and well-characterized data across 4 prospective, population-based studies. We were limited by the fact that rs13038305 explained only a small percentage of cystatin C variation. In addition, as the population used to derive the eGFRcys equation would have contained carriers of the minor allele of rs13038305, this may have biased risk estimates for that subset in our analysis. However, this would be expected to attenuate the observed associations by overestimating the effect of rs13038305 on eGFRcys, which would tend to reclassify carriers to a inappropriately severe CKD category and weaken the observed associations with outcomes. Similarly, as estimates of genetic effect size in discovery analyses tend to be upwardly biased ("winner's curse"),[24] it is possible that our use of the same datasets in which rs13038305 was first identified may have overestimated its impact on cystatin C levels. This would be expected to attenuate the associations with outcomes due to a tendency to reclassify carriers with less severe disease to a worse CKD category.

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In conclusion, statistical adjustment can correct for a genetic bias in GFR estimates based on cystatin C in carriers of the rs13038305 minor allele. Individuals reclassified to a worse CKD stage by genotype are at higher risk for adverse outcomes compared to those not reclassified, consistent with a refinement of GFR estimation. However, the effects on overall reclassification of kidney disease are too small to be useful in the assessment of kidney function in the general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of study participants stratified by rs13038305 genotype.

	CC	CT	TT	*P* value*
No. of participants	9147	4828	670	-
Age (y)	65.6 (6.1)	65.5 (5.9)	65.5 (6.0)	0.8
Female sex	5016 (55%)	2688 (56%)	364 (54%)	0.5
Hypertension	4094 (45%)	2175 (45%)	288 (43%)	0.6
Creatinine (mg/dL)	0.91 (0.3)	0.90 (0.3)	0.90 (0.2)	0.3
Cystatin C (mg/dL)	1.02 (0.3)	0.95 (0.3)	0.89 (0.3)	1.5E-42
$eGFR_{cr(CKD-EPI)}$	79.7 (14.7)	80.0 (14.8)	80.5 (14.9)	0.05
$eGFR_{cr(MDRD)}$	79.4 (17.9)	79.6 (17.9)	80.0 (17.6)	0.1
$eGFR_{cys}$	76.9 (17.4)	82.0 (17.7)	88.7 (18.1)	1.9E-69
Genotype-adjusted $eGFR_{cys}$**	76.8 (17.4)	76.6 (17.6)	77.9 (18.1)	0.1
$CKD_{cr(CKD-EPI)}$	1004 (11.0%)	514 (10.6%)	68 (10.1%)	0.1
$CKD_{cr(MDRD)}$	1144 (12.5%)	586 (12.1%)	78 (11.6%)	0.3
CKD_{cys}	1551 (17.0%)	582 (12.1%)	56 (8.4%)	5.1E-17
Genotype-adjusted CKD_{cys}***	1558 (17.0%)	854 (17.7%)	111 (16.6%)	0.6
Prevalent CVD	937 (10.2%)	483 (10.0%)	68 (10.1%)	0.9

Note: Values for categorical variables are given as number (percentage); values for continuous variables, as mean ± standard deviation. Participant counts are arithmetic sums, and denominator for % is number of individuals with the genotype. Means and standard deviations are weighted averages. eGFRs expressed in mL/min/1.73 m^2. Conversion factor for creatinine in mg/dL to μmol/L, ×88.4.

Abbreviations and definitions: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; $eGFR_{cr(CKD-EPI)}$, creatinine-based eGFR calculated using the CKD-EPI equation; $eGFR_{cr(MDRD)}$, creatinine-based eGFR calculated using the MDRD Study equation; $eGFR_{cys}$, cystatin C–based eGFR calculated using the CKD-EPI equation for cystatin C[7]; $CKD_{cr(CKD-EPI)}$, $CKD_{cr(MDRD}$, and CKDcys defined as eGFR < 60 ml/min,1.73 m^2 based on the eGFR from the corresponding equations; MDRD, Modification of Diet in Renal Disease; CVD, cardiovascular disease.

** adjusted for rs13038305 genotype, was derived from $eGFR_{cys}$ by adjusting out the effect of the minor allele of rs13038305

*** defined as genotype-adjusted $eGFR_{cys}$ < 60 ml/min/1.73m^2.

* *P* values were combined from the studies using Fisher method.

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Table 2

Associations of rs13038305 genotype, $eGFR_{cys}$ and CKD_{cys} with risk of incident CVD and all-cause mortality

Predictor	Incident CVD		All-cause mortality	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
SNP associations				
rs13038305***	0.98 [0.92, 1.06]	0.7	0.98 [0.92, 1.03]	0.4
Associations of $eGFR_{cys}$*				
$eGFR_{cys}$	1.17 [1.14, 1.20]	7.79E-37	1.22 [1.19, 1.24]	3.90E-74
Genotype-adjusted $eGFR_{cys}$**	1.18 [1.15, 1.21]	6.49E-37	1.22 [1.19, 1.24]	6.49E-70
Associations of CKD_{cys}				
CKD_{cys}	1.62 [1.47, 1.78]	7.26E-22	1.89 [1.76, 2.04]	1.31E-62
Genotype-adjusted CKD_{cys}****	1.67 [1.52, 1.84]	8.98E-27	1.90 [1.77, 2.04]	8.72E-68

Note: Analyses in the meta-analyzed sample (N = 14,645).

Abbreviations and definitions: CVD, cardiovascular disease; $eGFR_{cys}$, cystatin C–based estimated glomerular filtration rate calculated using the CKD-EPI equation for cystatin C[7]; CKD_{cys} defined as $eGFR_{cys}$ < 60 ml/min/1.73 m^2. HR, hazard ratio; CI, confidence interval

* Per 10 ml/min/1.73 m^2 decrease.

** derived from $eGFR_{cys}$ by averaging out the association of the T allele

*** per copy of T allele.

**** defined as genotype-adjusted $eGFR_{cys}$ < 60 ml/min/1.73m^2

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Table 3

Reclassification across eGFR categories by genotype-adjusted $eGFR_{cys}$ compared with unadjusted $eGFR_{cys}$ category.

		Genotype-adjusted $eGFR_{cys}$				
		≥ 90	**60–89**	**45–59**	**≤ 45**	**Total**
Unadjusted $eGFR_{cys}$	**≥ 90**	3805 (84.8)*	681 (15.2)	0 (0)	0 (0)	4486
	60–89	0 (0)	7636 (95.8)*	334 (4.2)	0 (0)	7970
	45–59	0 (0)	0 (0)	1458 (92.5)*	119 (7.5)	1577
	≤45	0 (0)	0 (0)	0 (0)	612 (100)*	612
	Total	**3805**	**8317**	**1792**	**731**	**14645** *

Note: Unless otherwise indicated, values given as number (percentage). In total, 1134 participants (7.7%) were reclassified to a worse eGFR category.: eGFRs expressed in mL/min/1.73 m^2.

Abbreviations and definitions: eGFR, estimated glomerular filtration rate; $eGFR_{cys}$, cystatin C–based eGFR calculated using the CKD-EPI equation for cystatin C[7]; Genotype-adjusted $eGFR_{cys}$, derived from $eGFR_{cys}$ by averaging out the association of the T allele.

* individuals not reclassified by genotype adjustment.

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Table 4

Comparison of crude incidence rates and HRs for overall mortality and incident CVD outcomes

	All-cause mortality		Incident CVD	
	No change	**Reclassified worse**	**No change**	**Reclassified worse**
eGFRcys >=90				
Incidence Rate	10.5	14.4	8.6	12.8
HR (95% CI)	-	0.96 [0.78, 1.18]	-	1.16 [0.92, 1.47]
eGFRcys 60–89				
Incidence Rate	21.0	39.4	16.6	34.3
HR (95% CI)	-	1.54 [1.3, 1.83]	-	1.66 [1.34, 2.06]
eGFRcys 45–59				
Incidence Rate	42.7	60.6	31.2	41.7
HR (95% CI)	-	1.42 [1.1, 1.83]	-	1.41 [0.99, 1.99]
NRI (95% CI)	**0.009 [−0.003, 0.022]**		**0.014 [0.0, 0.028]**	

Note: Comparison in those reclassified to a worse $eGFR_{cys}$ category by genotype adjustment and those not reclassified. Incidence rates presented as events per 1000 person-years. eGFRs expressed in mL/min/1.73 m2.

Abbreviations: CVD, cardiovascular disease; HR: hazard ratio; NRI: net reclassification index. CI, confidence interval; $eGFR_{cys}$, cystatin C–based estimated glomerular filtration rate calculated using the CKD-EPI equation for cystatin C[7]