Clinical Epigenetics

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Cadmium exposure, epigenetic modifications, and serum cystatin C: insights into mediated pathways and mortality risks in U.S. adults

Yu-Wei Fang[1,2], Ching-Way Chen[3], Ta-Chen Su[4,5,6,7] and Chien-Yu Lin[2,8*]

Abstract

Background Cadmium exposure has been linked to elevated cystatin C levels, disruptions in epigenetic patterns, and increased mortality risk. However, the role of epigenetic modifications in the relationship between cadmium and cystatin C remains poorly understood. Furthermore, it is unclear how cystatin C and epigenetic changes influence the connection between cadmium exposure and mortality outcomes. The study explored the associations among blood cadmium levels, serum cystatin C, an epigenetic biomarker (DNA methylation-predicted cystatin C, DNAmCystatinC), and mortality outcomes.

Methods We utilized data from 8716 participants aged 18 years and older in the National Health and Nutrition Examination Survey (NHANES, 1999–2002), linked to mortality records from the National Center for Health Statistics (NCHS) through 2019.

Results Our findings revealed that higher natural log-transformed (ln)-blood cadmium was associated with elevated ln-serum cystatin C ($\beta = 0.052$, $P < 0.001$) and higher ln-DNAmCystatinC ($\beta = 0.007$, $P = 0.008$). Compared to the reference group (both blood cadmium and DNAmCystatinC ≤ 50th percentile), those with blood cadmium and DNAmCystatinC > 50th percentile had the highest mean serum cystatin C levels (1.26 mg/L vs. 1.11 mg/L; P for trend = 0.002). Structural equation modeling (SEM) indicated that DNAmCystatinC partially mediated the relationship between cadmium exposure and cystatin C, with a total effect of 0.068, a direct effect of 0.066, and an indirect effect of 0.002. Weighted Cox regression analysis showed higher blood cadmium was associated with an increased risk of all mortality outcomes, with stronger associations observed in individuals whose serum cystatin C was at or above the 50th percentile. These findings were consistent both in the overall population and after excluding individuals with chronic kidney disease. Furthermore, a significant interaction was identified between blood cadmium and serum cystatin C in their influence on all-cause mortality.

Conclusions We found higher blood cadmium is linked to increased serum cystatin C and DNAmCystatinC, with DNAmCystatinC partially mediating the effect on serum cystatin C. Notably, serum cystatin C may modify the relationship between cadmium exposure and mortality outcomes. Further research is warranted to elucidate these complex interactions.

*Correspondence:
Chien-Yu Lin
lin7010@mail2000.com.tw; 00724@km.eck.org.tw
Full list of author information is available at the end of the article

Keywords Cadmium, DNA methylation-predicted cystatin C (DNAmCystatinC), Epigenetics, Mortality, National Health and Nutrition Examination Survey (NHANES), Serum cystatin C

Background

Cadmium is a toxic heavy metal that humans are often exposed to through smoking, industrial processes, and contaminated food sources [1]. It accumulates in the body due to poor excretion and long biological half-life, making it a cumulative toxin [2]. The primary mechanisms of cadmium toxicity involve oxidative stress induction, disruption of cellular signaling pathways, and epigenetic modifications [3]. Chronic exposure to cadmium can lead to various diseases, including kidney disorders, osteoporosis, cardiovascular issues, and cancer [1]. Recently, efforts have been made to reduce exposure [4]. However, recent studies still link even low levels of cadmium to significant health risks and an increased risk of mortality [5–8].

Cystatin C is a low-molecular-weight cysteine protease inhibitor produced by all nucleated cells. It is constantly released into the bloodstream and then filtered out by the kidneys. Unlike creatinine, cystatin C levels are less influenced by muscle mass, diet, or gender, making it a reliable biomarker for assessing kidney function [9]. Beyond assessing renal function, cystatin C plays a critical role in regulating protease activity and is gaining attention for its associations with nonrenal conditions, including inflammation, aging, and neurodegenerative diseases, highlighting its potential as a biomarker for broader health outcomes [10, 11]. Serum cystatin C levels have emerged as a reliable predictor of cardiovascular disease (CVD) and mortality, regardless of whether kidney function is normal or impaired [12, 13]. Cystatin C is also emerging as a valuable biomarker for cadmium-induced nephrotoxicity. Studies have shown that urinary cystatin C levels increase significantly before the onset of polyuria and proteinuria in cadmium-exposed rats, suggesting its potential as an early indicator of kidney damage [14]. Environmental cadmium exposure has also been associated with increased kidney damage markers, including serum cystatin C [15]. However, the relationship between cadmium exposure and cystatin C is complex, with some research indicating biomarker-specific effects rather than direct kidney function impacts [16].

Epigenetic modifications modulate gene expression through mechanisms that do not involve changes to the underlying DNA sequence [17]. One key mechanism, DNA methylation, involves the addition of methyl groups to the C5 position of cytosine residues within CpG dinucleotides [18]. This process is heritable but also responsive to environmental influences. Research has shown that cadmium exposure can disrupt DNA methylation in both in vivo and animal models [19–21]. Moreover, epidemiological studies have identified a link between cadmium exposure and alterations in DNA methylation, highlighting its potential role in mediating the relationship between cadmium exposure and adverse health outcomes [22–25]. Among the emerging biomarkers, DNA methylation-predicted cystatin C (DNAmCystatinC) stands out as an epigenetic proxy for serum cystatin C levels. Unlike serum cystatin C, which reflects kidney function and systemic inflammation at a single time point, DNAmCystatinC captures cumulative epigenetic changes influenced by environmental exposures over time. Notably, DNAmCystatinC is integrated into advanced biological aging models, such as GrimAge, improving the accuracy of mortality predictions [26–28]. Our recent study found significant associations between DNAmCystatinC, serum cystatin C, and all-cause mortality, with DNAmCystatinC demonstrating a stronger predictive value for mortality than serum cystatin C alone. The combined use of these biomarkers may thus enhance clinical assessments [29]. Although cadmium is known to disrupt DNA methylation patterns and elevate serum cystatin C levels, the role of epigenetic modifications in these effects remains largely unexplored. Furthermore, given the established link between cadmium exposure and increased mortality, it is critical to investigate how cystatin C and epigenetic changes influence the relationship between cadmium exposure and mortality outcomes. DNAmCystatinC, in particular, shows potential as a biomarker for exploring this relationship.

To bridge this gap, we utilized data from the 1999–2002 National Health and Nutrition Examination Survey (NHANES), linked to mortality records from the National Center for Health Statistics (NCHS) through 2019. These datasets offer comprehensive information on blood cadmium levels, serum cystatin C, DNAmCystatinC, and mortality outcomes. This study seeks to deliver the first epidemiological insights into these associations and their potential implications for health outcomes.

Materials and methods

Study population

The NHANES survey provides a comprehensive, nationally representative snapshot of the U.S. population. This biennial survey uses a complex, multistage sampling method to ensure its findings accurately reflect the broader population. For more detailed information

on the survey's methodology and consent procedures, please visit the NHANES website. Detailed descriptions of the survey methodology and consent procedures can be found on the NHANES website [30]. This research drew from the NHANES 1999–2002 dataset, which initially comprised 26,031 individuals. Among them, 11,441 were 18 years or older, and 10,012 had blood cadmium levels measured. After applying covariate filters, 8716 participants were deemed eligible for the multiple regression analysis. Within this group, additional analyses were conducted on subsets with specific measurements: serum Cystatin C was available for 3899 participants, DNAmCystatinC data were available for 2126 participants, and NCHS 2019 survival status was available for 8712 participants. The selection process, including the breakdown of participant numbers for each analysis, is illustrated in Fig. 1.

Measurement of blood cadmium

This research specifically focused on individuals aged 18 years and above. In NHANES 1999–2002, blood cadmium levels were measured by detecting the absorption of light at a wavelength of 228.8 nm by cadmium atoms. The samples and controls were diluted using a mixture of nitric acid, Triton X-100, and ammonium phosphate. The limit of detection (LOD) for cadmium was set at 0.3 μg/L. For measurements below this threshold, a value equal to the LOD divided by the square root of 2 was assigned. Detailed descriptions of the analytical procedures used are available on the NHANES website [31].

Measurement of serum cystatin C

This study included all participants aged 60 years and older and a randomly selected 25% of individuals aged 12–59 years from the NHANES dataset. The current analysis focused on participants aged 18 years and above. Serum cystatin C levels were measured using the Dade Behring N Latex Cystatin C assay, an automated nephelometric method with established precision and accuracy. The assay exhibited acceptable intra-assay (2.0–3.0%) and inter-assay (3.2–4.4%) coefficients of variation. For values below the limit of detection (LOD), a value of $LOD/\sqrt{2}$ was substituted. Detailed assay procedures are available on the NHANES website [32].

Measurement of DNAmCystatinC

This study included adults aged 50 years and older from the NHANES 1999–2002 surveys with available blood samples for DNA analysis. The sample comprised all eligible participants from racial/ethnic minority groups and approximately half of eligible non-Hispanic White participants. DNA methylation analysis was performed using the Illumina Infinium Methylation EPIC BeadChip on 500 ng of bisulfite-treated DNA. Data processing, including hybridization, amplification, and imaging, was conducted using the Illumina iScan system and RStudio with R (R version 4.3.1). Batch effects (plate, chip, row) were corrected using ComBat, an empirical Bayes method, before biomarker calculation. DNAmCystatinC was calculated from cystatin C-specific probes. Data cleaning involved removing samples with low intensity (< 10.5), mismatches (> 2 SD in age, cell types, or XY ploidy), and filtering probes with p-values $>1\times10^{-16}$, single nucleotide polymorphisms (minor allele frequency $\geq$1%), or cross-hybridization. Missing values were imputed using Horvath's gold standard reference. Normalization included background subtraction, color correction, functional normalization, modified Beta Mixture Quantile normalization (using Horvath's reference). The predicted values of DNAmCystatinC are expressed in pg/mL, consistent with the original plasma cystatin C measurements [26–28]. In the present analyses, we converted DNAmCystatinC to mg/L to ensure consistency with serum cystatin C units, thereby allowing for direct comparison of effect sizes. Detailed analytical procedures are available on the NHANES website [33].

Covariates

This study utilized data from the National Health and Nutrition Examination Survey (NHANES) to assess the association between various sociodemographic, lifestyle, and health factors. Data included age, sex, race/ethnicity, smoking status (current smoker, exposure to environmental tobacco smoke (ETS), nonsmoker) [34], alcohol consumption (defined as consuming at least 12 alcoholic drinks in the past year), body mass index (BMI), hypertension (defined as blood pressure $\geq$140/90 mmHg or use of antihypertensive medication), diabetes (diagnosed by fasting glucose, HbA1c, or medication use), hypercholesterolemia (defined by low-density lipoprotein cholesterol $\geq$130 mg/dL or medication use), chronic kidney disease (CKD) (defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2), and history of CVD and cancer, as self-reported by participants [35].

Outcomes

The NCHS has linked the 1999–2002 NHANES data to national mortality records, enabling long-term follow-up of participant health outcomes. This linkage provides comprehensive data on all-cause, cardiovascular, and cancer-related mortality through 2019. This study utilized data on participant survival status and follow-up duration. Cardiovascular mortality was defined as death due to heart disease or cerebrovascular conditions. Detailed analytical methods are available on the NCHS website [36].

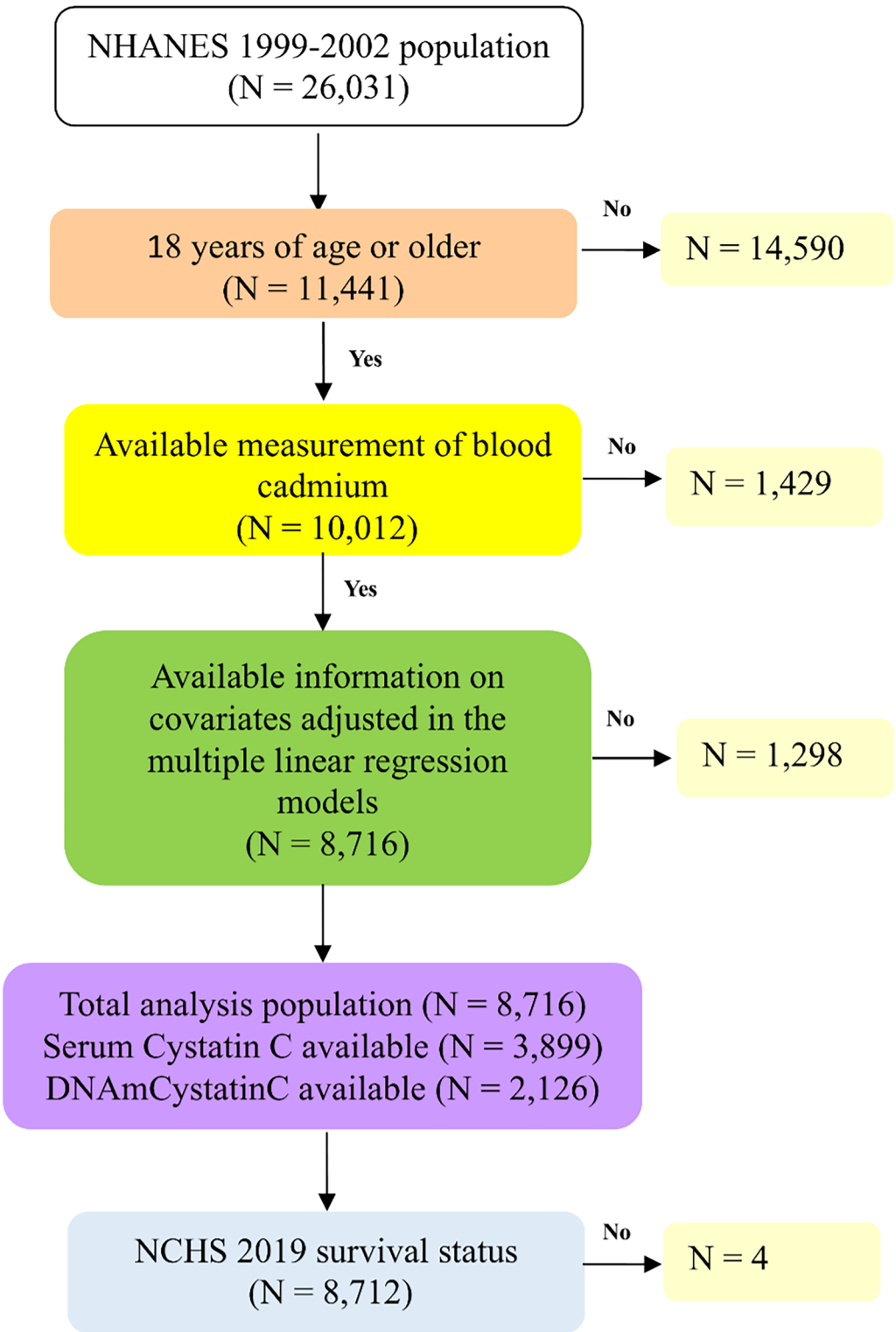

Fig. 1 Flowchart algorithm

Statistics

This study analyzed data using natural log-transformed values (ln-values) of blood cadmium, serum cystatin C, and DNAmCystatinC to address their non-normal distributions. This allowed for the calculation of geometric means and standard errors. Statistical analyses included t-tests, ANOVA, and linear regression models with complex sampling to account for the NHANES survey design [37]. Two regression models were used: Model 1 adjusted for age, sex, ethnicity, family poverty income ratio, smoking, alcohol use, and BMI; Model 2 further adjusted for chronic conditions. Logistic regression analyses were conducted to assess the odds ratios (OR) of chronic diseases associated with each biomarker.

We also utilized Cox proportional hazards models to investigate the association between blood cadmium, serum cystatin C, and DNAmCystatinC with all-cause, cardiovascular, and cancer-related mortality. Hazard ratios (HRs) were calculated for a one-unit increase in the ln of each biomarker, adjusting for covariates. Since NHANES participants aged 85 years and older are top-coded at 85, including chronological age in the DNAmCystatinC calculation may introduce bias. Furthermore, differences in the selection of participants for blood cadmium, serum cystatin C, and DNAmCystatinC measurements could lead to selection bias and limit comparability. Given that serum cystatin C is heavily influenced by kidney function, it is crucial to evaluate these associations in participants without CKD. To address these concerns, we conducted a sensitivity analysis excluding individuals aged 85 years and older, those under 50 years, and participants with CKD. Subgroup analyses and interaction tests were conducted to examine the effects of serum cystatin C and DNAmCystatinC levels on the association between blood cadmium and mortality. In our analysis, the *P* for trend was calculated by assigning the median value of the biomarker within each quartile (or subgroup) to all subjects in that group, then modeling this median as a continuous variable in the weighted multiple linear regression or Cox regression models. All statistical analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA) version 30 with a significance level of 0.05.

In this study, structural equation modeling (SEM) was utilized to examine the connections between blood cadmium, DNAmCystatinC as a mediator, and serum cystatin C as the outcome, with results weighted according to the sampling strategy. The hypothesis proposed that blood cadmium may affect serum cystatin C either directly or indirectly via DNAmCystatinC. The model controlled for potential confounders outlined in Model 2. SEM was built using the CALIS Procedure in SAS, with generalized least squares estimation used to calculate the model coefficients. Model fit was evaluated using the goodness of fit index (GFI), normed fit index (NFI), and root-mean-square residual (RMR). A GFI and NFI above 0.9, along with an RMR below 0.05, indicated a well-fitting model. The parameter estimates and overall model fit results were reported.

Results

Participant characteristics

The participants in the study had an average age of 45.50 years (SD = 19.77), with ages ranging from 18 to 85 years. About 75.9% of individuals had detectable levels of blood cadmium. During a median follow-up period of 219.0 months, data from 4 participants were unavailable. A total of 2153 deaths were documented, of which 670 were due to cardiovascular causes and 462 were cancer-related. Table 1 provides an overview of the demographic characteristics and key biomarkers, including blood cadmium, serum cystatin C, and DNAmCystatinC. Findings indicate that older individuals and those with hypertension, CKD, a history of cardiovascular disease, or a history of cancer exhibit higher levels across all three biomarkers. Higher blood cadmium levels are particularly associated with individuals of other ethnicities, those with lower incomes, lower BMI, active smokers, those consuming 12 or more drinks annually, and individuals with hypercholesterolemia. Elevated serum cystatin C levels are more common among men, non-Hispanic whites, individuals with a family poverty income ratio between 1 and 3, BMI ≥ 30 kg/m^2, ETS, diabetes mellitus, and hypercholesterolemia. Elevated DNAmCystatinC levels are found more frequently in men, Mexican-Americans, those with lower incomes, lower BMI, nonsmokers, individuals consuming fewer than 12 drinks per year, and those with diabetes mellitus.

Biomarker associations with chronic diseases

In Supplemental Table 1, complex samples of logistic regression analysis were performed to examine the OR for chronic diseases with one-unit increases in ln-blood cadmium, ln-serum cystatin C, and ln-DNAmCystatinC, adjusting for Model 1. The logistic regression analysis reveals distinct associations between biomarkers and chronic conditions. A one-unit increase in ln-blood cadmium is significantly associated with higher OR of CKD, a history of CVD, and cancer, but is inversely related to diabetes mellitus. Ln-serum cystatin C is linked to higher OR of hypertension, CKD, and CVD, while inversely associated with hypercholesterolemia. Ln-DNAmCystatinC shows a strong association with CKD and cancer.

Table 1 Basic demographics of the sample subjects, including geometric means (geometric SE) of blood cadmium, serum cystatin C, and DNAmCystatinC

	N	Blood cadmium (μg/L)	N	Serum cystatin C (mg/L)	N	DNAmCystatinC (mg/L)
Total	8716	0.441 (1.007)	3899	0.943 (1.003)	2126	0.614 (1.001)
Sex						
Men	4167	0.439 (1.010)	1971	0.968 (1.007)‡	1082	0.616 (1.002)*
Women	4549	0.443 (1.009)	1928	0.919 (1.007)‡	1044	0.611 (1.002)*
Age (in years)						
18–39	3796	0.381 (1.010)‡	948	0.781 (1.007)‡		
40–59#	2410	0.481 (1.014)‡	632	0.881 (1.013)‡	604	0.576 (1.002)‡
≥ 60	2510	0.507 (1.011)‡	2319	1.037 (1.006)‡	1522	0.629 (1.001)‡
Ethnicity						
Mexican–American	2222	0.419 (1.012)‡	868	0.864 (1.001)‡	604	0.617 (1.002)*
Other Hispanic	443	0.411 (1.029)‡	163	0.916 (1.022)‡	131	0.607 (1.005)*
Non-Hispanic white	4124	0.449 (1.010)‡	2055	0.990 (1.006)‡	882	0.615 (1.002)*
Non-Hispanic black	1630	0.444 (1.017)‡	696	0.930 (1.014)‡	439	0.609 (1.003)*
Other ethnicity	297	0.529 (1.036)‡	117	0.867 (1.026)‡	70	0.611 (1.009)*
Family poverty income ratio						
< 1	1739	0.498 (1.017)‡	692	0.947 (1.013)‡	360	0.621 (1.003)‡
1–3	3648	0.455 (1.011)‡	1738	0.967 (1.007)‡	958	0.620 (1.002)‡
> 3	3330	0.400 (1.010)‡	1469	0.914 (1.007)‡	808	0.602 (1.002)‡
Body mass index (kg/m^2)						
< 25	3025	0.461 (1.013)‡	1268	0.909 (1.009)‡	563	0.619 (1.003)‡
25–30	3047	0.437 (1.011)‡	1433	0.949 (1.008)‡	841	0.614 (1.002)‡
> 30	2644	0.423 (1.012)‡	1198	0.975 (1.008)‡	722	0.609 (1.002)‡
Smoking status						
Nonsmoker	4469	0.361 (1.007)‡	2201	0.945 (1.006)‡	1273	0.617 (1.002)‡
ETS	1794	0.348 (1.011)‡	785	0.949 (1.012)‡	429	0.613 (1.003)‡
Current smoker	2453	0.753 (1.014)‡	913	0.932 (1.010)‡	424	0.605 (1.003)‡
Alcohol consumption (drinks/year)						
< 12	3666	0.404 (1.010)‡	1577	0.949 (1.008)	826	0.618 (1.002)‡
≥ 12	5050	0.469 (1.009)‡	2322	0.939 (1.006)	1300	0.611 (1.002)‡
Hypertension						
No	5944	0.422 (1.009)‡	2026	0.853 (1.005)‡	840	0.603 (1.002)‡
Yes	2772	0.485 (1.011)‡	1873	1.051 (1.008)‡	1286	0.621 (1.002)‡
Diabetes Mellitus						
No	7739	0.440 (1.007)	3239	0.924 (1.005)‡	1622	0.612 (1.002)*
Yes	977	0.449 (1.019)	660	1.045 (1.014)‡	504	0.618 (1.003)*
CKD						
No	8165	0.435 (1.007)‡	3390	0.880 (1.004)‡	1843	0.610 (1.001)‡
Yes	551	0.529 (1.025)‡	509	1.499 (1.018)‡	283	0.640 (1.004)‡
Hypercholesterolemia						
No	6636	0.435 (1.008)‡	2726	0.929 (1.006)‡	1375	0.613 (1.001)
Yes	2080	0.461 (1.014)‡	1173	0.976 (1.008)‡	751	0.614 (1.002)
History of CVD						
No	7939	0.432 (1.007)‡	3310	0.908 (1.005)‡	1723	0.610 (1.002)‡
Yes	777	0.536 (1.022)‡	589	1.168 (1.015)‡	403	0.629 (1.003)‡
History of cancer						
No	8100	0.436 (1.007)‡	3448	0.928 (1.005)‡	1842	0.611 (1.001)‡
Yes	616	0.516 (1.023)‡	451	1.067 (1.013)‡	284	0.628 (1.004)‡

*$P < 0.05$; ‡$P < 0.001$

The category 40–59 should be 50–59 for DNAmCystatinC

Table 1 (continued)

Tested by two-tailed Student's t-tests and one-way analysis of variance

CKD Chronic kidney disease, *CVD* Cardiovascular disease, *DNAmCystatinC* DNA methylation-predicted cystatin C, *ETS* Environmental tobacco smoker

Table 2 Adjusted regression coefficients (S.E.) for differences in ln-cystatin C and ln-DNAmCystatinC relative to a one-unit increase in ln-blood cadmium and ln-DNAmCystatinC, with results weighted for sampling strategy

	Ln-blood cadmium (μg/L)			ln-DNAmCystatinC (mg/L)		
	Unweighted no./ Population size	β coeff (S.E.)	*P value*	Unweighted no./ Population size	β coeff (S.E.)	*P value*
Ln-cystatin C (mg/L)	3899/69,984,036			1662/43,721,652		
Model 1		0.062 (0.011)	< 0.001		0.174 (0.056)	0.004
Model 2		0.052 (0.006)	< 0.001		0.128 (0.042)	0.005
ln-DNAmCystatinC (mg/L)	2126/32,034,886					
Model 1		0.007 (0.002)	0.003			
Model 2		0.007 (0.002)	0.008			

Model 1 adjusted for age, sex, ethnicity, family poverty income ratio, smoking, drinking, and BMI

Model 2 adjusted for hypertension, hypercholesterolemia, diabetes mellitus, CKD, a history of CVD, and a history of cancer

CKD Chronic kidney disease, *CVD* Cardiovascular disease, *DNAmCystatinC* DNA methylation-predicted cystatin C

Relationships between blood cadmium, serum cystatin C, and DNAmCystatinC

Table 2 shows a positive association between ln-blood cadmium and both ln-cystatin C and ln-DNAmCystatinC. The analysis reveals that both ln-cystatin C and ln-DNAmCystatinC are significantly associated with ln-blood cadmium. In Model 1, a one-unit increase in ln-blood cadmium is linked to an increase of 0.062 (S.E. 0.011, $p < 0.001$) in ln-cystatin C and 0.007 (S.E. 0.002, $p = 0.003$) in ln-DNAmCystatinC, indicating a positive association. In Model 2, these associations remain significant, though slightly attenuated, with ln-blood cadmium linked to an increase of 0.052 (S.E. 0.006, $p < 0.001$) in ln-cystatin C and 0.007 (S.E. 0.002, $p = 0.008$) in ln-DNAmCystatinC. Similarly, a one-unit increase in ln-DNAmCystatinC was associated with a higher ln-serum cystatin C across both models ($\beta = 0.128$, SE $= 0.042$, $p = 0.005$ in Model 2). These findings suggest a robust positive relationship between ln-blood cadmium and ln-cystatin C as well as between ln-blood cadmium and ln-DNAmCystatinC, even after adjustments.

Figure 2 presents a summary of serum cystatin C and DNAmCystatinC across quartiles of blood cadmium, DNAmCystatinC, and subgroups of blood cadmium and DNAmCystatinC, based on complex sample multiple linear regression models. The analysis reveals a significant rise in serum cystatin C with increasing quartiles of blood cadmium and DNAmCystatinC. However, the increase in DNAmCystatinC across blood cadmium quartiles is only marginally significant (P for trend $= 0.051$).

Furthermore, the combined effects of blood cadmium and DNAmCystatinC on serum cystatin C levels are shown in Fig. 2D. When using subjects with blood cadmium and DNAmCystatinC levels both ≤ 50th percentile as the reference group, those with blood cadmium > 50th percentile and DNAmCystatinC > 50th percentile demonstrated the highest mean serum cystatin C levels (1.26 mg/L vs. 1.11 mg/L; P for trend $= 0.002$).

SEM analysis

Figure 3 illustrates the associations between blood cadmium, serum cystatin C, and DNAmCystatinC in the SEM analysis, with results weighted for sampling strategy. The SEM revealed a significant positive association between blood cadmium and DNAmCystatinC (Estimate $= 0.003$, $P = 0.012$) and serum cystatin C (Estimate $= 0.066$, $P = 0.001$), and a positive association between DNAmCystatinC and serum cystatin C (Estimate $= 0.075$, $P = 0.001$). Both models demonstrated perfect goodness of fit, with GFI values of 0.79 and NFI values of 0.82 and RMS values of 0.08. The total effect of blood cadmium on the serum cystatin C is 0.068, with a direct effect of 0.066, indicating a positive direct association between blood cadmium and serum cystatin C. Additionally, the indirect effect, mediated through DNAmCystatinC, is 0.002.

Subgroup analysis of biomarker associations

Supplemental Table 2 presents linear regression results for ln-serum cystatin C and ln-DNAmCystatinC per unit increase in ln-blood cadmium across various subpopulations. For serum cystatin C, a positive

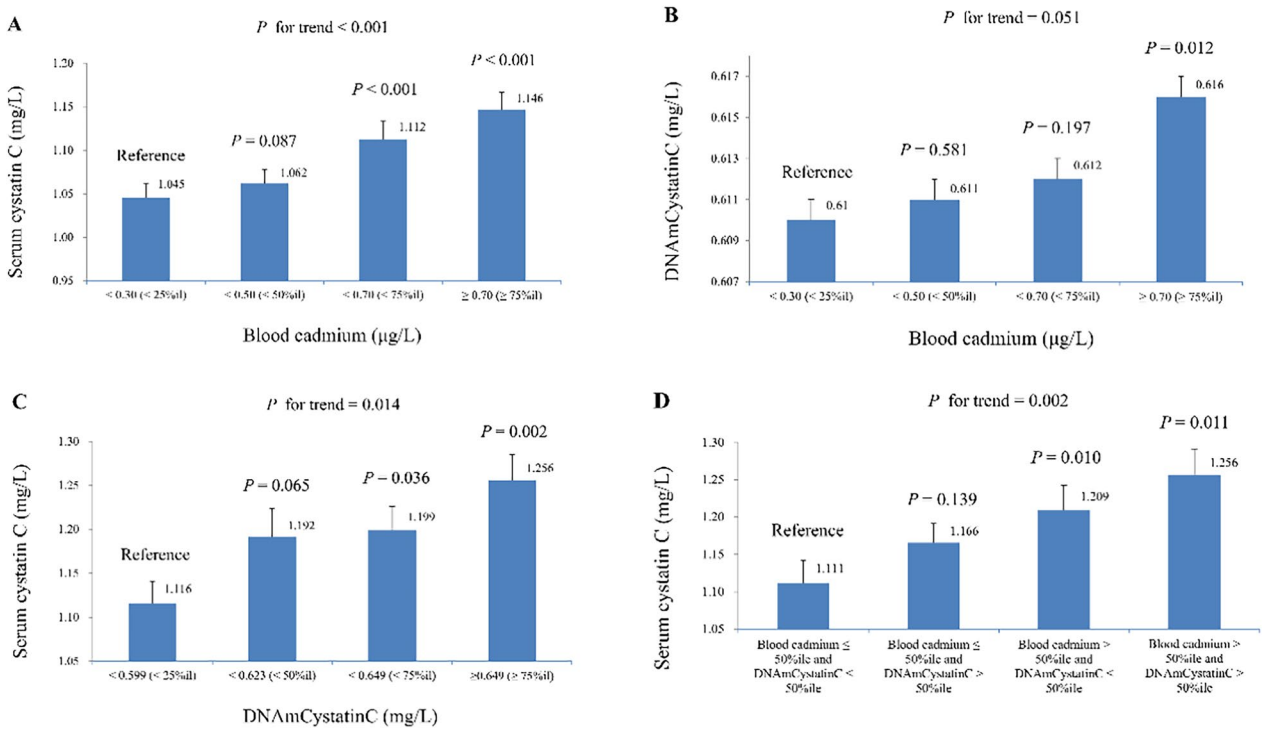

Fig. 2 Geometric means (standard errors) of serum cystatin C and DNAmCystatinC across quartiles of blood cadmium, DNAmCystatinC, and subgroups of blood cadmium and DNAmCystatinC. The results are from complex samples of multiple linear regression models, adjusted for Model 2 and weighted for the sampling strategy. **A** Relationship between blood cadmium and serum cystatin C levels. **B** Relationship between blood cadmium and DNAmCystatinC levels. **C** Relationship between DNAmCystatinC and serum cystatin C levels. **D** Subgroup analysis based on combinations of blood cadmium and DNAmCystatinC

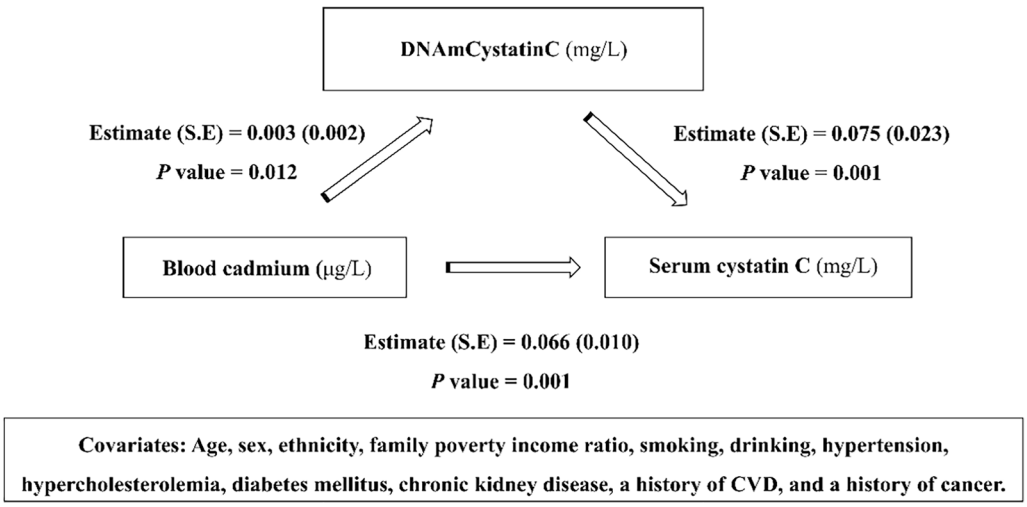

Fig. 3 The relationship between blood cadmium, serum cystatin C, and DNAmCystatinC in the SEM, with results weighted for sampling strategy

association with blood cadmium was observed across all outcomes, with significant interactions detected specifically for age and CKD status. In contrast, the association between blood cadmium and DNAmCystatinC was modest, reaching significance in certain subgroups, including males, younger individuals, non-Hispanic whites, those with lower BMI, active smokers, and higher alcohol consumers. Notably, significant interactions for DNAmCystatinC were found only with smoking status. Overall, the association with blood

Table 3 HR (95% CI) for all-cause, cardiovascular, and cancer mortality associated with a unit increase in ln-blood cadmium, ln-serum cystatin C, and ln-DNAmCystatinC

	Unweighted no./ Population size	HR (95% CI)	*P* value
Ln-blood cadmium (μg/L)	8711/175,136,122		
All-cause mortality		1.436 (1.270–1.625)	< 0.001
Cardiovascular mortality*		1.390 (1.133–1.704)	0.004
Cancer-related mortality		1.619 (1.312–1.998)	< 0.001
Ln-serum cystatin C (mg/L)	3897/176,741,915		
All-cause mortality		3.263 (2.486–4.284)	< 0.001
Cardiovascular mortality*		3.627 (2.335–5.636)	< 0.001
Cancer-related mortality		1.760 (0.964–3.212)	0.064
Ln-DNAmCystatinC (mg/L)	2126/65,561,735		
All-cause mortality		1.936 (1.173–3.201)	0.008
Cardiovascular mortality*		1.662 (0.532–5.173)	0.344
Cancer-related mortality		1.087 (0.194–6.112)	0.887

Results are derived from a weighted Cox regression model accounting for complex sampling design

Adjusted for model 2

*Cardiovascular mortality: death from heart or cerebrovascular disease

DNAmCystatinC DNA methylation-predicted cystatin C, *HRs* hazard ratios

cadmium was generally weaker for DNAmCystatinC than for serum cystatin C, showing greater variability across demographic groups.

Mortality outcomes

Table 3 displays the HR for all-cause, cardiovascular, and cancer-related mortality associated with ln-blood cadmium, ln-serum cystatin C, and ln-DNAmCystatinC, using a weighted Cox regression model. Blood cadmium showed a significant association with elevated risk across all three mortality outcomes. Serum cystatin C emerged as a predictor for both all-cause and cardiovascular mortality, while ln-DNAmCystatinC was a strong predictor of all-cause mortality. In a sensitivity analysis (Supplemental Table 3–5) excluding individuals aged 85 years and older, those under 50 years, and participants with CKD, the associations between the three biomarkers and mortality outcomes remained consistent. The only notable difference was that the association between serum cystatin C and cancer-related mortality was more pronounced in subjects aged 50 years and above.

Stratified mortality analysis

Table 4 presents the HR for all-cause, cardiovascular, and cancer-related mortality associated with a unit increase in ln-blood cadmium, stratified by the 50th percentile of serum cystatin C and DNAmCystatinC. When stratified by serum cystatin C, the HR for cardiovascular mortality remained significant in both subgroups, while significant associations for all-cause and cancer-related mortality were observed only in individuals at or above the 50th percentile. Additionally, a significant interaction was found between cadmium and serum cystatin C in relation to all-cause mortality (P for interaction = 0.029). In the stratification by DNAmCystatinC, only the subgroup below the 50th percentile showed a significant association with all-cause mortality, with no meaningful interactions observed for DNAmCystatinC. Furthermore, our sensitivity analysis in Supplemental Table 6—excluding individuals with CKD—demonstrated that the associations between cadmium exposure and mortality outcomes remained consistent. However, in this analysis, the HR for cardiovascular mortality was significant only in those with serum cystatin C at or above the 50th percentile, and no significant interactions were observed between cadmium and serum cystatin C for all-cause mortality.

Combined effects on mortality

Table 5 shows HR and 95% CI for mortality outcomes across subgroups defined by blood cadmium and serum cystatin C, using weighted Cox regression models. The reference group includes individuals with blood cadmium and serum cystatin C levels at or below the 50th percentile. Participants with both blood cadmium and serum cystatin C levels above the 50th percentile demonstrated the greatest increase in HR for all mortality outcomes. However, a statistically significant trend of rising mortality risk across these subgroups was observed only for all-cause and cardiovascular mortality, not for cancer-related mortality.

Discussion

Using a nationally representative U.S. sample, we identified a positive association between blood cadmium levels and serum cystatin C, with DNAmCystatinC partially mediating this relationship. The combined presence of higher blood cadmium and DNAmCystatinC levels leads to a greater increase in serum cystatin C than either factor individually. Moreover, participants with serum cystatin C levels above the 50th percentile exhibited higher HR for cadmium-related mortality outcomes, indicating

Table 4 HR (95% CI) for all-cause mortality, cardiovascular mortality, and cancer-related mortality associated with a unit increase in ln-blood cadmium across different subgroups of serum cystatin C and DNAmCystatinC

	Unweighted no./ population size	HR	95% CI	*P* value	*P* for interaction
All-cause mortality					
Total	3897/69,972,767	1.398	1.227–1.594	< 0.001	0.029
Serum cystatin C < 50%ile	1969/36,880,868	1.261	0.909–1.750	0.158	
Serum cystatin C ≥ 50%ile	1928/33,091,899	1.402	1.141–1.723	0.002	
Total	2126/32,034,886	1.305	1.132–1.505	0.001	0.206
DNAmCystatinC < 50%ile	1063/20,186,859	1.562	1.226–1.990	0.001	
DNAmCystatinC ≥ 50%ile	1063/11,848,027	1.145	0.944–1.390	0.162	
Cardiovascular mortality*					
Total	3897/69,972,767	1.511	1.252–1.823	< 0.001	0.085
Serum cystatin C < 50%ile	1969/36,880,868	1.376	1.076–1.760	0.013	
Serum cystatin C ≥ 50%ile	1928/33,091,899	2.159	1.495–3.118	< 0.001	
Total	2126/32,034,886	1.248	0.980–1.589	0.071	0.791
DNAmCystatinC < 50%ile	1063/20,186,859	1.480	0.943–2.323	0.086	
DNAmCystatinC ≥ 50%ile	1063/11,848,027	1.174	0.849–1.625	0.320	
Cancer-related mortality					
Total	3897/69,972,767	1.727	1.248–2.391	0.002	0.584
Serum cystatin C < 50%ile	1969/36,880,868	1.533	0.915–2.570	0.101	
Serum cystatin C ≥ 50%ile	1928/33,091,899	1.836	1.266–2.662	0.002	
Total	2126/32,034,886	1.415	0.996–2.010	0.053	0.516
DNAmCystatinC < 50%ile	1063/20,186,859	1.447	0.836–2.505	0.179	
DNAmCystatinC ≥ 50%ile	1063/11,848,027	1.327	0.791–2.225	0.189	

Results derived from a weighted Cox regression model accounting for complex sampling design

Adjusted for Model 2

DNAmCystatinC DNA methylation-predicted cystatin C, *HRs* hazard ratios

* Cardiovascular mortality: death from heart or cerebrovascular disease

Table 5 HR (95% CI) for mortality outcomes in different blood cadmium and serum cystatin C subgroups in complex sample of Cox regression models, with results weighted for sampling strategy

	Serum cystatin C ≤ 50%ile Blood cadmium ≤ 50%ile	Serum cystatin C ≤ 50%ile Blood cadmium > 50%ile	Serum cystatin C > 50%ile Blood cadmium ≤ 50%ile	Serum cystatin C > 50%ile Blood cadmium > 50%ile
Unweighted number/ Population size	1426/27,066,662	544/9,817,386	1092/18,976,334	837/14,123,654
All-cause mortality				
HR (95% CI)	1	1.27 (0.95–1.71)	1.43 (1.17–1.74)	1.80 (1.48–2.19)
P value	Reference	0.104	0.001	< 0.001
P for trend				< 0.001
Cardiovascular mortality				
HR (95% CI)	1	1.81 (1.23–2.65)	1.60 (1.14–2.25)	2.17 (1.54–3.07)
P value	Reference	0.004	0.008	< 0.001
P for trend				< 0.001
Cancer-related mortality				
HR (95% CI)	1	1.15 (0.65–2.01)	1.02 (0.71–1.47)	1.70 (1.08–2.70)
P value	Reference	0.625	0.913	0.024
P for trend				0.111

Adjusted for model 2

that elevated serum cystatin C may heighten vulnerability to cadmium-associated mortality. These findings were consistent both in the overall population and after excluding individuals with CKD. Additionally, a significant interaction between cadmium and serum cystatin C in relation to all-cause mortality suggests potential synergistic effects that increase mortality risk. Our study is the first to explore the relationships between blood cadmium, serum cystatin C, and DNAmCystatinC, as well as their associations with mortality. The findings suggest a potential mechanistic pathway linking cadmium exposure, epigenetic changes, cystatin C, and mortality. Overall, this research offers new insights into how environmental toxins like cadmium influence biological aging and mortality, with broader implications for understanding the health impacts of cadmium exposures.

Cadmium tends to accumulate in the kidney's proximal tubular cells, leading to renal toxicity [38]. Research suggests that even low to moderate cadmium exposure can increase the risk of CKD [5]. In addition to its association with kidney function, cadmium has a distinct link with cystatin C. Studies in cadmium-exposed rats have shown a significant rise in urinary cystatin C levels even before signs of polyuria and proteinuria appear [14]. In human populations, environmental cadmium exposure—particularly in industrial areas with elevated PM2.5 levels—has been associated with higher urinary cadmium and early markers of kidney damage, including serum cystatin C [15]. However, whether the association between cadmium and cystatin C is due to kidney function or represents an independent relationship has not been well explored in previous studies. Our study demonstrates that cadmium is independently associated with cystatin C, even after adjusting for CKD-related factors, indicating this link is not solely kidney function dependent. Subgroup analysis reveals a significant association between blood cadmium and serum cystatin C in both CKD and non-CKD individuals, with notable interactions between cadmium levels and CKD status affecting this relationship. The independent association between cadmium and cystatin C, along with the interaction between cadmium levels and CKD status, suggests that cadmium influences cystatin C through mechanisms beyond kidney filtration alone. Cadmium exposure induces systemic inflammation and oxidative stress, which could drive cystatin C production as part of a broader cellular stress response [10, 11]. In individuals with CKD, existing kidney damage may heighten vulnerability to cadmium's toxic effects, possibly amplifying cystatin C levels due to the kidneys' reduced ability to counteract cadmium-induced oxidative stress and inflammation. This interaction indicates that cadmium exposure could exacerbate cystatin C elevations in those with kidney dysfunction, underscoring a particular risk for CKD patients in the presence of cadmium.

Cadmium induces oxidative stress and the generation of reactive oxygen species, which damage cellular components, including DNA. Cadmium has been observed to cause epigenetic changes, including decreasing overall DNA methylation by inhibiting DNA methyltransferases, modifying histone markers by blocking histone demethylases, and influencing microRNA expression [19–21]. Numerous epidemiological studies have also found associations between cadmium exposure and DNA methylation alterations, such as research involving adults in high-exposure regions of Thailand [39], a U.S. birth cohort [22], and participants in the Strong Heart Study [15]. A recent NHANES study examining the relationship between 64 environmental exposures and epigenetic age acceleration found that serum cadmium and cotinine levels were significantly associated with increased acceleration in multiple epigenetic clocks [40]. In the present study, we observed a positive association between blood cadmium levels and DNAmCystatinC in the general U.S. population aged 50 years and older. Our results align with earlier studies, reinforcing the notion that cadmium exposure can affect epigenetic markers. Additionally, we found a significant interaction between smoking status and blood cadmium levels for DNAmCystatinC, but not for serum cystatin C. Given that smoking is a major source of cadmium exposure [1], this pattern may not only reflect cadmium's ability to induce epigenetic modifications, but also suggest potential synergistic effects between cadmium and other harmful substances in tobacco smoke. Our findings highlight DNAmCystatinC as a potentially sensitive biomarker for cumulative toxic metal exposure and emphasize the need to account for smoking status when assessing cadmium-related epigenetic changes.

Epigenetic mechanisms may clarify the connection between cadmium exposure and health outcomes. A study in animals demonstrated that cadmium's carcinogenic effects might be partially driven by disruptions in DNA methylation [41]. In humans, previous research suggests a connection between cadmium exposure and changes in DNA methylation, emphasizing its potential role in mediating the health effects associated with cadmium exposure [22–25]. Studies indicate that metals can cause DNA methylation, which modifies gene expression and reduces glutathione activity, leading to increased oxidative stress [25]. While cadmium exposure has been linked to both DNA methylation and serum cystatin C, the role of DNAmCystatinC in mediating the relationship between cadmium and serum cystatin C has not been previously documented. Our SEM analysis indicates that DNAmCystatinC may act as a mediator in this

association. The total effect of ln-blood cadmium on ln-serum cystatin C is 0.068, with a direct effect of 0.066 and a modest indirect effect of 0.002 through ln-DNAmCystatinC. These findings suggest that only a small portion of the positive association between cadmium exposure and serum cystatin C might be mediated by its effect on DNA methylation. This study is the first to propose an epigenetic pathway through which cadmium may influence serum cystatin C, albeit to a limited extent.

We observed elevated blood cadmium levels were significantly associated with an increased risk of all-cause, cardiovascular, and cancer mortality, consistent with findings from previous studies using NHANES data and other cohort research [5, 7, 8, 11]. This association was notably stronger among individuals with serum cystatin C levels at or above the 50th percentile, with a significant interaction observed specifically for all-cause mortality. Sensitivity analyses excluding participants with CKD further supported these findings. In addition, participants with both elevated cadmium levels and high serum cystatin C exhibited the highest HR and clear trends for increased mortality. In contrast, no similar associations were observed with DNAmCystatinC. This suggests a synergistic effect between cadmium exposure and serum cystatin C. Elevated serum cystatin C may reflect cumulative physiological damage or reduced reserve, increasing vulnerability to cadmium-induced oxidative stress and inflammation [10, 11]. While DNAmCystatinC may be a less sensitive biomarker, serum cystatin C provides valuable insights into renal function and systemic inflammation. Targeted strategies are essential to reduce cadmium exposure, monitor kidney function, and encourage healthy lifestyle practices in high-risk populations.

This study has several strengths, including a large, nationally representative sample and a long follow-up period, enhancing the generalizability of the findings. The inclusion of both serum cystatin C and its epigenetic biomarker provided a comprehensive view of underlying biological processes. Advanced statistical methods, such as SEM, allowed for a nuanced analysis of direct and indirect effects. However, the study design limits the ability to infer causality. Additionally, residual confounding from unmeasured variables and the use of single-point exposure measurements may have impacted the observed associations. Furthermore, while cadmium exposure was assessed using whole blood, which reflects recent exposure, urinary cadmium, a better indicator of long-term exposure, was measured in only a subset of the study population. Including urinary cadmium would have reduced statistical power. Future studies should incorporate urinary cadmium to more effectively assess the long-term effects of cadmium exposure. Finally, the biomarkers in this study were measured in different age groups, with DNAmCystatinC assessed only among participants aged 50 years and older. This discrepancy may introduce selection bias, limit comparability, and restrict the generalizability of DNAmCystatinC findings to younger populations.

Conclusions

Using a nationally representative sample from the U.S., we observed that blood cadmium levels are positively associated with serum cystatin C, with this relationship partly mediated by DNAmCystatinC. The combination of higher blood cadmium and DNAmCystatinC contributes more to the elevation of serum cystatin C than either factor alone. Moreover, individuals with elevated serum cystatin C levels appeared more susceptible to cadmium-related mortality. These findings were consistent both in the overall population and after excluding individuals with CKD. The significant interaction between cadmium and serum cystatin C on all-cause mortality further highlights a synergistic mechanism that exacerbates mortality risks. Our results advance our understanding of the pathways connecting environmental toxicants to health outcomes, paving the way for targeted interventions and policies aimed at mitigating the impacts of cadmium exposure on public health.

Abbreviations

BMI	Body mass index
CHD	Coronary heart disease
CKD	Chronic kidney disease
CVD	Cardiovascular disease
ETS	Exposed to secondhand smoke
GFI	Goodness of fit index
DNAmCystatinC	DNA methylation-predicted cystatin C
HRs	Hazard ratios
Ln	Natural logarithm
LOD	Limit of detection
NCHS	National Center for Health Statistics
NFI	Normed fit index
NHANES	National Health and Nutrition Examination Survey
ORs	Odds ratios
RMR	Root-mean-square residual
SEM	Structural equation modeling

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01888-y.

Additional file1

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Author contributions

Yu-Wei Fang conducted the literature review and paper writing. Ching-Way Chen and Ta-Chen Su handled statistical analysis. Chien-Yu Lin contributed significantly to hypothesis development and approved the paper's final revision.

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Availability of data and materials
The datasets analyzed in this study can be accessed on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/default.aspx) (accessed on 20 April 2025).

Declarations

Ethics approval and consent to participate
This study was approved by the NCHS Research Ethics Review Board (NCHS IRB/ERB Protocol Number: # 98-12).

Competing interests
The authors declare no competing interests.

Author details
[1]Division of Nephrology, Department of Internal Medicine, Shin-Kong Wu Ho-Su Memorial Hospital, Taipei 111, Taiwan. [2]School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei 242, Taiwan. [3]Department of Cardiology, National Taiwan University Hospital Yunlin Branch, Yunlin 640, Taiwan. [4]Institute of Environmental and Occupational Health Sciences, College of Public Health, National Taiwan University, Taipei 100, Taiwan. [5]Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan. [6]Department of Internal Medicine, College of Medicine, National Taiwan University, Taipei 100, Taiwan. [7]Department of Internal Medicine, Tungs'Taichung MetroHarbor Hospital, Taichung 435, Taiwan. [8]Department of Internal Medicine, En Chu Kong Hospital, No. 399, Fuxing Rd., Sanxia Dist., New Taipei City 237, Taiwan.

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