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The association between low-concentration heavy metal exposure and chronic kidney disease risk through α-klotho

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Although the association between pollution exposure and chronic kidney disease (CKD) has been explored, previous studies have focused on specific effects observed via in vitro or animal experiments. We first conducted a priority screening of pollutants for population CKD risk by using machine learning approaches. We then used the National Health and Nutrition Examination Survey (NHANES) 2007–2016 data from 2415 adults aged 40 years and over to study the joint effects of low-concentration metal exposure and the mediating effects of α-klotho by using Bayesian kernel machine regression (BKMR) and mediation analyses. Priority screening revealed that cadmium (Cd), mercury (Hg), lead (Pb), and thallium (Tl) were associated with the highest risk of developing CKD. The BKMR model revealed a negative joint effect of mixed-metal exposure on CKD risk. Tl presented the highest posterior inclusion probability (PIP) of 1.0000, followed by Pb, with a PIP of 0.6080. Significant mediating effects of α-klotho on Hg–CKD associations were observed. Mendelian randomization demonstrated that a high level of α-klotho is associated with a decreased risk of developing CKD. This is the first study to reveal the risk prioritization of various pollutants in CKD patients, as well as the coexposure effects of metals. Our study also provides insight into the potential mechanisms underlying the association between metal exposure and CKD risk.

Keywords Low concentration, Metals, Chronic kidney disease (CKD), Joint effect, α-Klotho, Mediation

Background

With the increasing prevalence of chronic kidney disease (CKD) and the associated increase in mortality risk, kidney diseases are gradually emerging as a significant public health issue[1]. According to the World Health Organization's (WHO) Global Health Assessment, kidney diseases are the 10th leading cause of death, with the number of deaths reaching 1.3 million in 2019[2]. CKD affects 10% of the global population, with 800 million cases reported in 2022[3]. The increase in the prevalence of CKD and the changes in the burden of kidney diseases cannot be completely explained by the traditional trends of causative factors (such as diabetes and hypertension). This suggests that other previously overlooked risk factors, including environmental pollutants such as pesticides and heavy metals, induce the development of the disease[4,5]. The accelerated processes of global industrialization and urbanization are causing a severe increase in environmental pollution, posing a global health challenge. According to a WHO report, 12.6 million people died from preventable environmental risks globally, accounting for 22% of the global disease burden in 2012[6]. The kidneys, as the primary excretory organs with the physiological role of blood filtration, receive approximately 20% of the cardiac output, resulting in the concentration of pollutants in these organs. Therefore, kidneys are particularly susceptible to the influence of environmental pollutants.

Human activities such as mining, industrial processes, urban expansion, agricultural fertilizer use, and pesticide application release a significant number of pollutants into the environment and ecosystems. Among

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all pollutants in drinking water and food, heavy metals are considered to be the greatest threat to human health because of their persistence and bioavailability in the environment. From 2010 to 2017, the concentrations of heavy metals in North American rivers and lakes continuously increased. For example, cadmium (Cd), lead (Pb), and mercury (Hg) reached levels of 25.33, 116.13, and 15.93 μg/L, respectively, exceeding the WHO-recommended thresholds (3, 10, and 1 μg/L) by 9–15 times[7]. Consequently, populations in the United States (U.S.) are increasingly exposed to environmental pollutants, particularly heavy metals such as Cd, Pb, and Hg, as evidenced by rising environmental concentrations. The National Biomonitoring Program (NBP) of the U.S. CDC has systematically documented biomarkers of heavy metal exposure in the U.S. population, with urinary metal concentrations widely used for assessing long-term exposure. According to the *Improving the Collection and Management of Human Samples Used for Measuring Environmental Chemicals and Nutrition Indicators*, urinary Hg concentrations are primarily used to evaluate chronic low-level exposure, urinary Pb concentrations reflect long-term Pb accumulation, urinary Cd concentrations are a recognized biomarker of chronic environmental and occupational exposure, and urinary thallium (Tl) concentrations serve as markers of environmental and industrial exposure[8,9]. Additionally, the National Health and Nutrition Examination Survey (NHANES) has collected urinary heavy metal data since 1999 as an internal exposure indicator for individual assessment. Animal studies have indicated that Cd, Pb, and Hg lead to disturbances in calcium (Ca) homeostasis, changes in renal haemodynamics, and the occurrence of glomerulonephritis and tubular damage[10]. Elevated levels of reactive oxygen species (ROS) in kidney tissues lead to increased free radical generation and the suppression of oxidative enzyme activity after lipid peroxidation, triggering cell apoptosis and autophagy. This cascade ultimately induces varying degrees of fibrosis and premature kidney ageing[11]. Epidemiological evidence has indicated that populations with double the Cd exposure of other populations have a 1.3-fold increased prevalence of severe tubular dysfunction[12]. Exposure to Hg, Cd, or Pb is associated with the occurrence and severity of kidney disease in elderly individuals[13]. A cross-sectional survey also indicated that Pb, Cd and Hg exposure potentially affect kidney parameters in 12- to 19-year-old adolescents[14]. The human body is typically exposed to a mixture of various pollutants. While past studies have investigated primarily the effects of single heavy metal on CKD risk, our study extends this knowledge by evaluating the combined effects of metal mixtures. Currently, there has been a shift towards the use of mixture analysis to understand the health impacts of exposure to multiple pollutants[15]. This approach acknowledges the potential joint and interactive impact these pollutants may have on health outcomes.

The Klotho gene was discovered as an antiaging gene in 1997[16]. In addition, klotho also serves as a renoprotective agent and is associated with the severity of kidney damage and the prognosis of kidney disease. Epidemiological evidence indicates that α-klotho levels are negatively associated with the severity and prognosis of CKD[17]. Furthermore, a progressive decrease in the α-klotho mRNA and protein levels in the kidneys was observed from CKD stages 1 to 5 in an observational clinical study[18]. Early intake of recombinant α-klotho protein was found to exert a remarkable renoprotective effect and slow the progression of kidney disease[19]. Therefore, α-klotho may serve as a novel biomarker for assessing the condition and prognosis of CKD patients. With respect to the association between α-klotho and metals, in both rats and in vitro studies, Cd exposure was associated with downregulated expression of the α-klotho protein, whereas α-klotho overexpression was associated with decreased Cd concentrations in animals with life-extending mutations[20]. In cross-sectional studies, Pb and Cd exposure levels were negatively related to serum α-klotho levels[21]. Cd can also increase the methylation of the α-klotho gene, leading to a reduction in α-klotho synthesis[22]. Nevertheless, it remains unclear whether heavy metal exposure increases the risk of developing CKD by reducing α-klotho protein levels. In the field of biomedical research, genome-wide association studies (GWASs), which are powerful genetic research tools, have been widely used to identify susceptibility genes for various diseases. Through GWASs, Gergei et al. revealed that six signals at five loci were associated with circulating α-klotho levels in a genome-wide sense[23]. Christoph et al. used publicly available GWAS aggregated statistical data to transform disease-related klotho variants into a novel mouse model of late-onset Alzheimer's disease to determine the role of klotho in late-onset Alzheimer's disease[24].

The main objectives of this study were as follows: (1) compare the impact of different types of pollutants (51 pollutants) on CKD risk; (2) analyse the association between metal exposure and CKD risk (including single heavy metal exposure effects and mixed heavy metal exposure effects); and (3) explore the mediating effect of α-klotho on the association between heavy metal exposure and CKD risk.

Methods

Data source and description

We obtained data from the NHANES, a national representative study of the U.S. population (https://www.cdc.gov/nchs/nhanes/index.htm). The NHANES uses complex multistage probability sampling and conducts surveys every two years. In Stage 1, primary sampling units (PSUs), which mostly consisted of individual counties or, in a few cases, adjacent county groups, were selected. The probability of selecting PSUs was directly proportional to a measure of size (PPS). In Stage 2, each PSU was divided into multiple segments (usually urban blocks or their equivalents), and sample segments were selected using PPS. In Stage 3, residential units or residents of each subdivision were listed, and a sample was randomly selected. In Stage 4, individuals were selected from the list of all individuals residing in the selected households to participate in the NHANES. The detailed sampling process can be found on the website https://www.cdc.gov/nchs/data/series/sr_02/sr02_160.pdf. The present study included 2415 adults aged 40–79 years from five NHANES cycles (2007 to 2016). The non-CKD population ($n=1997$) served as controls, while cases ($n=418$) were defined as participants with CKD. We excluded participants with missing values for CKD-related information ($n=4853$), urinary metal concentrations ($n=8129$), α-klotho levels ($n=6213$), BMI ($n=411$), education ($n=1$), diabetes history ($n=2$), hypertension history ($n=5$) and smoking and drinking status ($n=1545$). We subsequently excluded pregnant participants

($n = 3$). To address potential selection bias, we created an extended cohort ($n = 3960$) that included 1545 participants with missing smoking and drinking status alongside the final analytic sample ($n = 2415$) (Fig. 1). The procedures conducted in NHANES 2007–2016 received approval from the National Center for Health Statistics Research Ethics Review Board, and written informed consent was obtained from all participants.

Definition

This study focused primarily on the associations between CKD risk and environmental heavy metal concentrations. CKD was defined as an eGFR < 60 mL/min/1.73 m^2 or albuminuria (urine albumin-to-creatinine ratio [UACR] ≥ 30 mg/g)[25]. The eGFR was calculated via the CKD-EPI equation improved by Levey et al.[26], and albuminuria was determined using the UACR.

The CKD-EPI equation was as follows:

$$eGFR = 141 \times \min\left(\frac{SCr}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{SCr}{\kappa}, 1\right)^{-1.209} \times 0.993^{Age} \times 1.018\,[if\ female] \times 1.159\,[if\ black] \quad (1)$$

where SCr represents the serum creatinine value. The calibration coefficients for women were α = −0.329 and κ = 0.7, whereas those for men were α = −0.411 and κ = 0.9. Ultimately, CKD was incorporated into the model as a categorical variable.

Pollution concentration measurements

Urinary heavy metal concentrations were analysed with inductively coupled plasma-mass spectrometry (ICP-MS) at the Division of Laboratory Sciences, National Center for Environmental Health, Atlanta, Georgia. The urine samples were digested with concentrated nitric acid (https://wwwn.cdc.gov/nchs/data/nhanes/public/2007/labmethods/uhm_e_met.pdf). Rhodium (Rh-103), gallium (Ga-71), iridium (Ir-193), Te-130 and Bi209 were used as internal standards. The purities of these internal standards were greater than 99.99%. The purities of Cd, Tl, Pb and Hg were also greater than 99.99% (https://wwwn.cdc.gov/nchs/data/nhanes/public/2007/labmethods/uhm_e_met.pdf; https://wwwn.cdc.gov/nchs/data/nhanes/public/2007/labmethods/uhg_e_met_urinary_mercury.pdf). Metal concentrations below the limit of detection (LOD) were calculated as the LOD divided by the square root of two. With respect to the urine dilution, we corrected the metal concentrations by dividing the creatinine concentration per litre of urine (μg/g creatinine). The quality control data for heavy metals detection has been provided in Table S9.

The pesticides were analysed by high-performance liquid chromatography–tandem mass spectrometry using turbo-ion spray atmospheric pressure ionization. The PAHs and PFASs were analysed by isotope dilution high-performance liquid chromatography-tandem mass spectrometry (online SPE-HPLC-MS/MS). The PAEs were detected by atmospheric pressure chemical ionization-tandem mass spectrometry (APCI-MS/MS) and quantified by isotope dilution. The VOCs were analysed by an automated analytical method based on capillary gas chromatography (GC) and mass spectrometry (MS). The phenols were measured by gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with different detection techniques. The detailed laboratory methodology is described on the official NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm).

α-Klotho measurement

Serum α-klotho concentrations in the fasting blood samples were measured using an ELISA kit (IBL International, Japan) with a sensitivity of 6 pg/mL. Each sample was subjected to duplicate analyses, and the average result was

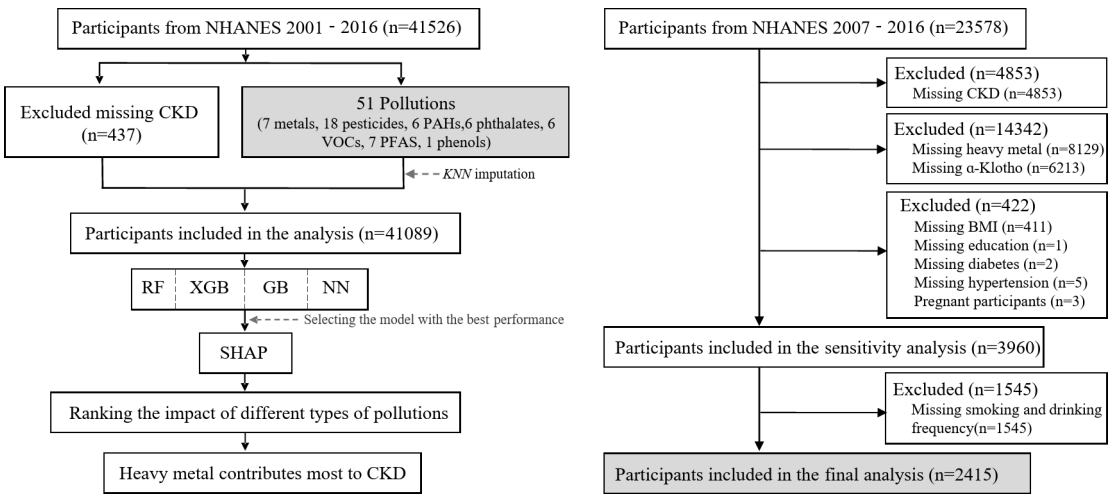

Fig. 1. Data Filtering and Modelling Workflow.

taken as the final value. Samples with duplicate results exceeding 10% were flagged for analyses to be repeated. The entire analysis would require a repeat measurement if the value of the quality control samples exceeded 2 standard deviations (SDs) from the specified value. The laboratory adhered to the manufacturer's protocol and standardized criteria throughout the analysis procedure.

Covariate assessment

Covariates were chosen a priori and included age, sex (male, female), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race/multiracial), education (less than high school, high school or equivalent, more than high school), family poverty-to-income ratio (PIR), BMI, and disease history of diabetes (yes, no), hypertension (yes, no), smoking (yes, no) and drinking frequency (low, moderate, high). Diabetes was defined when patients had an HbA1c level > 6.5% and/or a self-reported physician-diagnosed history of diabetes. Hypertension was defined when there was a self-reported physician-diagnosed history of hypertension. Smoking was defined as self-reported smoking behaviour. Drinking frequency was defined by participants' responses on the alcohol use questionnaire. Pregnant individuals were excluded on the basis of positive laboratory pregnancy tests or self-reported pregnancy during examination. Potential confounding factors were identified on the basis of prior knowledge and directed cyclic graphs (http://www.dagitty.net/dags.html, Fig. S1) and included age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency. In the final group, all covariates were adjusted for, whereas in the extended cohort, smoking and drinking were excluded from the adjustment because of missing data. In our study, diabetes, hypertension, smoking, drinking frequency and demographic information such as sex, race, and education level were used as categorical variables, whereas age, the family PIR, and BMI were used as continuous variables.

Statistical analysis

We applied mean imputation and k-nearest neighbour (KNN) imputation to handle missing values[27]. Then, we compared the mean imputation results and chose the higher-efficacy imputation method by using metrics such as the root mean square error (RMSE), coefficient of determination (R^2), mean absolute error (MAE), and mean squared error (MSE). We used the following four supervised machine learning (ML) models to predict participants' CKD risk: (1) random forest classifier (RF), with default parameters; (2) gradient boosting classifier (GB), with parameters including n_estimators = 50, learning_rate = 0.5, and max_depth = 2; (3) extreme gradient boosting (XGB), with parameters including learning_rate = 0.04, max_depth = 4, n_estimators = 300, gamma = 0.1, subsample = 0.2, and colsample_bytree = 0.4; and (4) neural network (NN), which consisted of two hidden layers. The first hidden layer had 30 neurons with an activation function of "ReLU", whereas the second hidden layer had 20 neurons with an activation function of "sigmoid". The activation function of the output layer was "Softmax", and a dropout layer was added before the output layer, with a dropout rate of 0.6. We evaluated each model's predictive ability in terms of accuracy and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The AUC ranges from 0.5 to 1, where higher values signify better model performance. However, a drawback of ML models is the limited interpretability of complex nonlinear structures. SHAP can provide an explanation for these black-box models[28]. SHAP values originated from cooperative game theory in economics and estimate the average impact of each input feature on the model's output magnitude[28]. The calculated SHAP values were then used to generate feature importance plots to show the most impactive pollutant for CKD risk.

After the ML explanation, we rebuilt a group that contained participants with complete data on CKD, heavy metals, and covariates. The characteristics of the participants are represented as the means ± SDs for continuous variables and as frequencies with proportions for categorical variables. Baseline features and laboratory measurements were compared between CKD patients and controls via the Wilcoxon rank-sum test for continuous variables. For categorical variables, comparisons were made using either Pearson's chi-square test or Fisher's exact test. In the frequency distribution plots, the heavy metal and α-klotho concentrations exhibited right-skewed patterns, with the abscissa representing the concentration values and the ordinate indicating the frequency density. These variables are presented as medians and interquartile ranges (IQRs) in descriptive statistics since the mean can be significantly influenced by extreme values. They were log-transformed for logistic regression, Bayesian kernel machine regression (BKMR) and mediation analysis to improve model fit and reduce the impact of extreme values. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (95% CI) for the association between metal concentrations and CKD risk. Metal concentrations were categorized into tertiles on the basis of their distribution in the control group (low, medium, high). Tests for trends were performed by entering the categorical variables as continuous variables in the model. The associations between concentrations of α-klotho and the various metals were analysed by multiple linear regression. BKMR with original componentwise variable selection was used to estimate the joint and independent effects of metal exposure on CKD risk[15]. We used 100 knots and 10,000 Markov chain Monte Carlo iterations to fit the BKMR model with a binomial distribution. The posterior inclusion probability (PIP) for each metal was computed as an indicator of its importance. We built a generalized additive model (GAM) with cubic regression splines and five knots to enhance the visualization and detailed investigation of the nonlinear relationships between metals and CKD risk. Stratified analyses were used to evaluate the potential effects of sex and age. Sensitivity analyses were used to assess the robustness of the results. To test whether the association between metal exposure and CKD risk was mediated by extreme values, we excluded participants with a history of diabetes, hypertension, those with both diseases, and individuals with outlier metal concentrations (defined as values exceeding three times the IQR of the original distribution). Mediation analysis helped clarify the impact of α-klotho on the metal exposure–CKD association. The mediation proportion refers to the ratio of α-klotho's mediated effect to the total

effect of metal exposure on CKD risk. To ensure the robustness of our results, all the analyses were conducted in the final group and extended cohort.

Two-sample Mendelian randomization (MR) analysis was conducted to validate the causal relationship between α-klotho levels and CKD risk. The GWAS summary statistics for 6,414,265 single nucleotide polymorphisms (SNPs) associated with α-klotho were obtained from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/) (GWASID: ebi-a-GCST90091247)[23]. SNPs associated with α-klotho at a genome-wide significance level ($P < 5 \times 10^{-8}$) were selected as instrumental variables. We applied linkage disequilibrium (LD) clumping to exclude SNPs in high LD ($r^2 < 0.001$, kb = 10000). Instrumental variables with an F statistic greater than 10 were retained to mitigate potential weak instrument bias. The GWAS summary statistics for 2,197,556 SNPs associated with CKD were obtained from the IEU OpenGWAS project (GWAS ID: ieu-a-1106), which included data from 33,152 mixed individuals[29]. The causal relationship between α-klotho and CKD was estimated using primary MR methods, including inverse-variance weighting with multiplicative random effects (IVW-MRE), MR-Egger regression (Egger), simple mode, weighted median, and weighted mode. To ensure robustness, Cochran's Q test was employed to assess heterogeneity among instrumental variable effects, and the MR-Egger intercept test was used to detect horizontal pleiotropy. Additionally, a leave-one-out sensitivity analysis was performed to evaluate the stability of the results and identify any influential SNPs that might impact the findings.

ML was performed via Python 2.7.11. All the statistical analyses were conducted using R (version 4.3.1; R Development Core Team, USA). The "stats" package was used for logistic regression analysis, the "bkmr" package was used for BKMR model construction and analysis, the "mediation" package was used for mediation analysis, and the "TwoSampleMR" package was used for two-sample MR analyses. A two-sided $P < 0.05$ was considered to indicate statistical significance.

Results and discussion

Priority screening of pollutants for CKD risk

Previous studies have indicated that multiple pollutants, such as heavy metals, phthalates, PAHs, PFASs, VOCs, pesticides, and phenols, can damage kidney function, causing albuminuria or a decrease in the eGFR[5,30]. However, few studies have simultaneously incorporated these pollutants into one model to predict CKD risk and analyse the contributions of various pollutants. Therefore, identifying the relative contributions of specific pollutants to CKD risk is critical for prioritizing targeted pollution control measures. We used the KNN imputation data (Table S1) to train the ML models. The XGB model achieved the highest predictive performance (AUC = 0.66), followed by GB (AUC = 0.64), NN (AUC = 0.63), and RF (AUC = 0.62) (Fig. S2). Consequently, SHAP was used to explain the XGB model for pollutant importance ranking. The SHAP results revealed that heavy metals were the most influential category, with Cd, Tl, Pb, and Hg ranking 1st, 2nd, 3rd, and 8th in all 51 kinds of pollutants (Fig. 2) in the training set and test set (Fig. S3). The concentrations of 51 pollutants in CKD and non-CKD

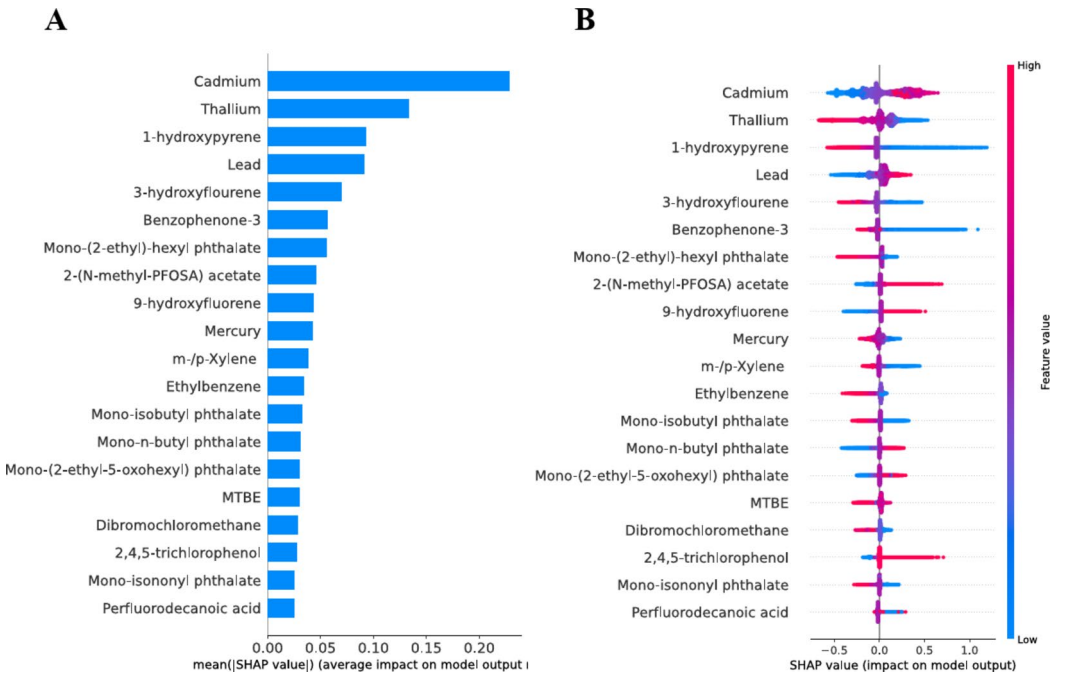

Fig. 2. Global model explanation by the SHAP method for XGB model. **(A)** SHAP summary bar plot. **(B)** SHAP summary dot plot. The probability of CKD increases with the SHAP value of a feature. A dot is made for SHAP value in the model for each single patient, so each patients has one dot on the line for each feature. The colours of the dots demonstrate the actual values of the features for each patient, as red means a higher feature value and blue mean a lower feature value. The dots are staked vertically to show density.

participants are summarized in Table S2. Furthermore, we investigated the associations of exposure to these four heavy metals with CKD risk in subsequent analyses.

Population characteristics

We built two cohorts after the screening of pollutants: the first is the final group, which comprised 2415 participants with data for all the covariates, and the second extended cohort comprised 3960 participants with data for all the covariates except smoking and drinking status. The average age of the 2415 participants was 56.7 ± 10.7 years. Statistically significant differences in age were observed between CKD patients and non-CKD patients. Patients with CKD presented lower family PIRs, higher BMIs, and higher prevalence rates of hypertension (62.4% vs. 40.0%) and diabetes (40.3% vs. 16.9%) history. Compared with that of non-CKD patients, a greater proportion of participants with CKD were male (53.6% vs. 48.6%), and a lower proportion had a college degree or higher (17.7% vs. 25.9%). The median urine levels of Cd, Hg, Pb, and Tl in all participants were relatively low, at 0.29, 0.42, 0.51, and 0.16 μg/g creatinine, respectively. In participants with CKD, urine Cd levels were significantly higher than those in the control group, whereas urine Hg and Tl levels were lower ($P < 0.05$). However, there were no statistically significant differences in the urinary Pb levels between the two groups ($P > 0.05$) (Table 1). The extended cohort presented demographic and clinical characteristics similar to those of the final group (Table S3).

Characteristic	Total ($n = 2415$)	Non-CKD ($n = 1997$)	CKD ($n = 418$)	*P* value[a]
Age (years)	56.70 ± 10.68	55.54 ± 10.29	62.23 ± 10.79	< 0.0001
Family PIR	2.92 ± 1.66	3.02 ± 1.66	2.47 ± 1.59	< 0.0001
Gender				0.1825
Male	1324 (54.82%)	1082 (54.18%)	242 (57.89%)	
Female	1091 (45.18%)	915 (45.82%)	176 (42.11%)	
Race				0.0163
Mexican American	324 (13.42%)	265 (13.27%)	59 (14.11%)	
Other Hispanic	254 (10.52%)	224 (11.22%)	30 (7.18%)	
Non-Hispanic White	1195 (49.48%)	985 (49.32%)	210 (50.24%)	
Non-Hispanic Black	438 (18.14%)	346 (17.33%)	92 (22.01%)	
Other Race	204 (8.45%)	177 (8.86%)	27 (6.46%)	
Education level				< 0.0001
Less Than 9th Grade	205 (8.49%)	148 (7.41%)	57 (13.64%)	
9–11th Grade	288 (11.93%)	234 (11.72%)	54 (12.92%)	
High School Grade	509 (21.08%)	408 (20.43%)	101 (24.16%)	
Some College or AA degree	698 (28.90%)	587 (29.39%)	111 (26.56%)	
College Graduate or above	715 (29.61%)	620 (31.05%)	95 (22.73%)	
Hypertension				< 0.0001
Yes	1060 (43.89%)	799 (40.01%)	261 (62.44%)	
No	1355 (56.11%)	1198 (59.99%)	157 (37.56%)	
Diabetes				< 0.0001
Yes	1984 (82.15%)	1703 (85.28%)	281 (67.22%)	
No	431 (17.85%)	294 (14.72%)	137 (32.78%)	
Smoking				0.0005
Yes	1321 (54.70%)	1060 (53.08%)	261 (62.44%)	
No	1094 (45.30%)	937 (46.92%)	157 (37.56%)	
Drinking frequency				0.1337
Low	1086 (44.97%)	915 (45.82%)	171 (40.91%)	
Moderate	557 (23.06%)	459 (22.98%)	98 (23.44%)	
High	772 (31.97%)	623 (31.20%)	149 (35.65%)	
BMI	28.28 ± 7.62	28.05 ± 7.35	29.40 ± 8.76	< 0.0001
α-Klotho	785.60 ± 323.10	798.80 ± 323.10	727.65 ± 310.18	< 0.0001
Cd[b]	0.29 ± 0.30	0.28 ± 0.30	0.32 ± 0.35	0.0021
Hg[b]	0.42 ± 0.58	0.43 ± 0.61	0.33 ± 0.49	< 0.0001
Pb[b]	0.51 ± 0.44	0.51 ± 0.44	0.52 ± 0.46	0.6977
Tl[b]	0.16 ± 0.11	0.16 ± 0.11	0.14 ± 0.10	< 0.0001

Table 1. Study population characteristics ($n = 2415$). [a]Wilcoxon rank-sum test for continuous variables and Pearson's chi-squared test or Fisher's exact test for categorical variables. [b]The concentration unit is expressed as Mg/g creatinine.

Among the 418 CKD patients, the majority exhibited preserved kidney function with an eGFR greater than 60 mL/min/1.73 m^2 but presented with albuminuria ($n = 230$), accounting for 9.52% of the total study population and 55.02% of the CKD patients. This was followed by patients with reduced kidney function (eGFR < 60 mL/min/1.73 m^2) but without albuminuria ($n = 144$), representing 5.96% of the total study population and 34.45% of all CKD cases. The smallest subgroup consisted of patients with both reduced eGFR (< 60 mL/min/1.73 m^2) and albuminuria ($n = 44$), comprising 1.82% of all study participants and 10.53% of the CKD patients. These findings indicated that only a small proportion of CKD patients have severely impaired kidney function, while the majority retain relatively preserved kidney function (Table S10).

Associations between urinary heavy metal concentrations and CKD risk

In the unadjusted logistic regression, higher Cd levels were significantly linked to increased CKD risk, whereas higher levels of Tl were associated with decreased CKD risk (Table 2). After adjusting for confounding factors, the associations between CKD risk and Tl and Pb concentrations were significant, with ORs of 0.6090 (95% CI: 0.4841, 0.7641) and 0.7919 (95% CI: 0.6535, 0.9570), whereas the associations with Cd and Hg were nonsignificant, with ORs of 1.1800 (95% CI: 0.9791, 1.4229) and 1.0080 (95% CI: 0.8883, 1.1433), respectively. When the concentrations were converted into categorical data, the associations between CKD risk and Cd and Hg disappeared, whereas the associations with Tl remained significant. The same associations were discovered in the extended cohort (Table S4).

Metal mixture and CKD: joint and independent effects

In the mixed-exposure model BKMR, the PIP is used to determine the relative importance of each metal for CKD risk. The PIP values indicated that Tl and Pb contributed significantly to CKD risk, with values of 1.0000 and 0.6080 (Table S5), respectively. These findings indicate that those metals play a dominant role in the mixed model, but their direction of action is to reduce the risk of developing CKD. The original componentwise variable selection BKMR also revealed a negative joint effect between low concentrations of heavy metal mixture exposure and CKD risk (Fig. 3A). We also investigated the independent effects of each metal on CKD risk. When other metals were fixed at specific thresholds (25th, 50th, and 75th percentiles), a significant negative correlation was observed between Tl and Pb concentrations and CKD risk (Fig. 3B). Univariate exposure–response plots indicated that Tl and Pb were significantly negatively correlated with CKD risk and presented more credible confidence intervals (CIs) than the other metals did (Fig. 3C). No interactions were detected between the four heavy metals (Fig. 3D). GAMs also revealed significant nonlinear negative relationships of Tl and Pb exposure concentrations with CKD risk ($P < 0.001$), whereas the negative relationship of Hg exposure concentrations and the positive relationship of Cd exposure concentrations with CKD risk were nonsignificant ($P > 0.05$). The

Metals	Metal concentrations (μg/g creatinine)	Controls/ Cases	Crude model[a] OR (95% CI)	Adjusted model[b] OR (95% CI)
Cd	Continuous	1997/418	**1.3811**(1.1929, 1.6005) ***	1.1800(0.9791,1.4229)
	T1 (< 0.2070)	666/113	1.0000 (reference)	1.0000 (reference)
	T2 (0.2070–0.4011)	665/147	**1.4022**(1.0683, 1.8450) *	1.0301(0.7629,1.3924)
	T3 (> 0.4011)	666/158	**1.5523**(1.1779, 2.0516) **	1.0683(0.7671,1.4900)
	P_{trend}[c]		0.0024	0.73913
Hg	Continuous	1997/418	0.9019(0.8047, 1.0100)	1.0080(0.8883,1.1433)
	T1 (< 0.4000)	666/182	1.0000 (reference)	1.0000 (reference)
	T2 (0.4000–0.6667)	665/127	**0.7477**(0.5781, 0.9650) *	0.8669(0.6574,1.1414)
	T3 (> 0.6667)	666/109	**0.6688**(0.5080, 0.8775) **	0.8659(0.6401,1.1690)
	P_{trend}		0.0029	0.30483
Pb	Continuous	1997/418	0.9209(0.7765, 1.0900)	**0.7919**(0.6535,0.9570) *
	T1 (< 0.2799)	664/148	1.0000 (reference)	1.0000 (reference)
	T2 (0.2799–0.6497)	666/129	0.8834(0.6761, 1.1531)	0.7824(0.5869,1.0418)
	T3 (> 0.6497)	667/141	0.9474(0.7243, 1.2388)	0.7601(0.5638,1.0236)
	P_{trend}		0.6961	0.07576
Tl	Continuous	1997/418	**0.5409**(0.4384, 0.6657) ***	**0.6090**(0.4841,0.7641) ***
	T1 (< 0.1288)	666/181	1.0000 (reference)	1.0000 (reference)
	T2 (0.1288–0.2007)	665/139	0.8257(0.6415, 1.0613)	0.8945(0.6804,1.1745)
	T3 (> 0.2007)	666/98	**0.5864**(0.4416, 0.7751) ***	**0.6796**(0.4982,0.9235) *
	P_{trend}		0.0002	0.01431

Table 2. Associations between urinary metal concentrations and CKD risk estimated using logistic regression models (n = 2415). [a]Crude model: unadjusted. [b]Adjusted model: adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency. [c]P_{trend} values were obtained from conditional logistic regression models by modelling the median value of each urinary metal tertile (natural log-transformed) as a continuous variable in the models. T1: first tertile, T2: second tertile, T3: third tertile. Bold indicates $P < 0.05$; ***$P < 0.00001$; **$P < 0.001$; *$P < 0.05$.

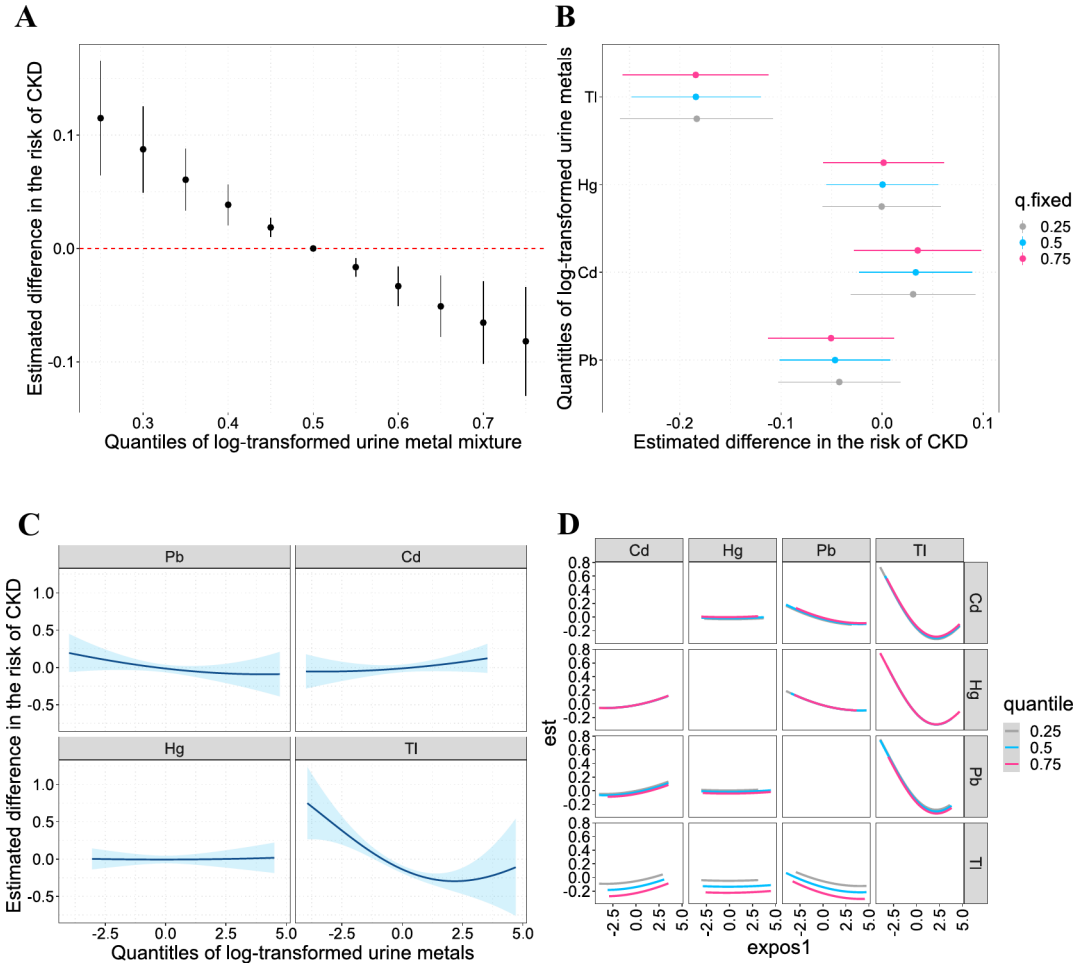

Fig. 3. Associations of the urinary metal mixture with CKD risk The associations were determined using BKMR with original component-wise variable selection and 10000 Markov chain Monte Carlo iteration (n = 2415). Models were adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency. Metals data were subjected to natural log-transformation for analyses. **(A)**, overall association between metal mixture (estimates and 95% CIs) and CKD risk. The figure plots the estimated difference in CKD risk when exposures are at a particular percentile (x axis) in comparison with when exposures are all at the 50th percentile; **(B)**, single-metal association (estimates and 95% CIs). The plot compares the risk of CKD when a single metal is at the 75th vs 25th percentile, while all the other metals are fixed at either the 25th, 50th, or 75th percentile; **(C)**, univariate exposure–response functions and 95% CIs for each metal with the other metals fixed at the medians; and**(D)**, bivariate exposure–response functions for single metal when other metal fixed at either the 25th, 50th, or 75th percentile and the CKD risk is fixed at the 50th.

SHAP-dependent plot revealed the same correlation between CKD risk and heavy metals except Pb. We believe that this might be due to differences in the participants included in the models (Fig. 4). The negative joint effect and nonlinear relationships still existed in the extended cohort (Fig. S4, S5).

Tl is a highly toxic substance that typically exists in low concentrations in the environment. Humans are primarily exposed to heavy metals through ingestion (water and food), with additional absorption via the skin and respiratory tract[31]. Animal experiments have shown that the stimulatory effect of Tl on the ATPase activity of chromaffin cells leads to increased release of catecholamines, thereby causing renal vascular constriction and significantly reducing the eGFR. Renal toxicity caused by Tl primarily involves interference with glutathione metabolism, disruption of mitochondrial function, generation of oxidative stress, and pathological changes in renal tubules[32]. Epidemiological research has revealed an association between low-level Tl exposure (13.4 μg/L to 60.1 μg/L) and a high risk for kidney damage in children[33]. The U.S. CDC and the German Federal Environment Agency consider urine Tl levels > 5 μg/L to be abnormal[34,35]. Previous studies also reported that the Tl concentration ranged from 0.27 to 0.525 μg/g creatinine in healthy and unexposed populations[36]. In our study, the average Tl concentration (0.156 μg/g creatinine) was much lower than the safety threshold. We observed a significant negative correlation between Tl exposure and CKD risk. The observed inverse association between Tl and CKD risk may reflect reverse causality, where impaired kidney function reduces urinary Tl excretion independently of exposure levels. Previous studies support the hypothesis of reverse causality since

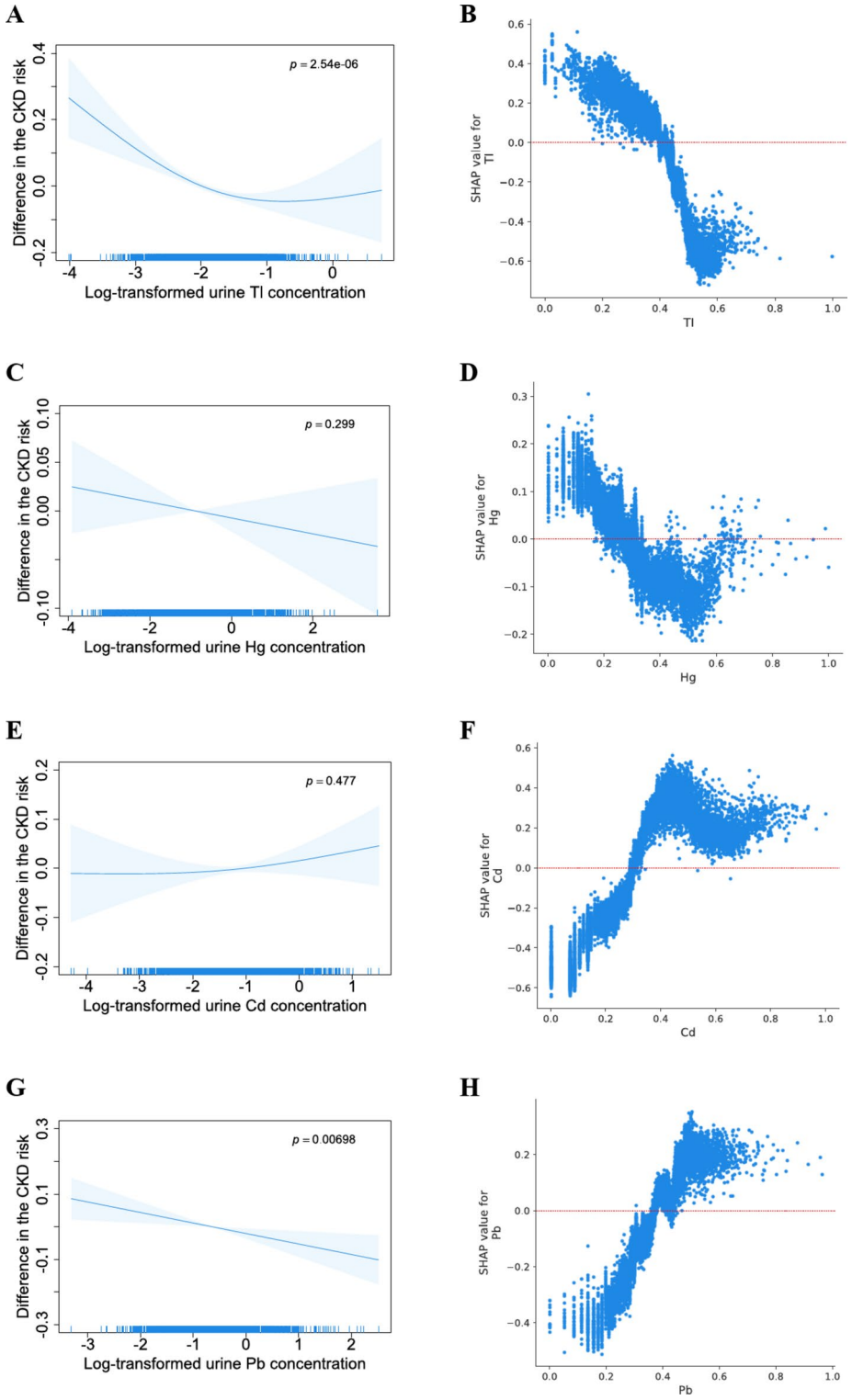

Fig. 4. Nonlinearity of the association between urinary heavy metal levels and CKD risk in GAMs and SHAP results. **(A, C, E, G)** show GAMs plots adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency. **(B, D, F, H)** display SHAP dependence plots. The SHAP values exceeding zero indicate a shift in model prediction toward the CKD class.

they reported similar relationships between exposure to metals and renal function[37,38]. Most metals are excreted mainly through the kidney, and impaired renal function logically reduces the elimination of metals. In addition to metals, poor renal function may reduce the excretion of triclosan and bisphenol A[38,39]. Hyperfiltration is a longitudinal and progressive process reported in diabetes patients, hypertension patients, obese patients and

Pd-exposed rodents, in which the initial increase in the glomerular filtration rate (GFR) subsequently decreases with disease progression. Nevertheless, further experimental studies are needed to explore the causality between them.

Pb increases intracellular lipid peroxidation in kidney cells, disrupting cell membrane integrity. Pb also elevates ROS levels in tubular and glomerular cells, causing oxidative stress, increasing cell apoptosis, and resulting in kidney damage[40]. A study in Sri Lanka revealed that high levels of Pb exposure were associated with high CKD risk[41]. The WHO emphasized that any level of Pb exposure may lead to potential adverse health effects, and the WHO clinical guidelines highlighted that even a blood level < 5 μg/dL may restrict foetal growth[42]. In healthy and unexposed populations, the urinary Pb concentrations range from 0.73 to 1.87 μg/g creatinine[43]. In comparison, the average Pb concentration in our study (0.516 μg/g creatinine) was relatively low. We observed a negative correlation between urinary Pb concentrations and CKD risk, which is consistent with findings from a cross-sectional study in Mexico[44]. Some studies have shown that Pb exposure might cause a mild hyperfiltration state in the kidneys, increasing the excretion of metals[38,45]. Therefore, the reasons for the negative correlation between the Pb concentration and CKD risk may be as follows: (1) there is reverse causation between Pb exposure and CKD risk, where impaired kidney function reduces Pb excretion[37,38,46], and (2) low-level Pb exposure induces a hyperfiltration state in the kidney, initially increasing the GFR.

Cd is a highly toxic heavy metal with a long biological half-life and strong bioaccumulation[47]. Nonoccupational exposure to Cd primarily occurs through food intake and smoking among the population[13]. Cd poisoning gained notoriety through itai-itai disease, a result of Jinzu River contamination in Japan attributed to mining operations. The urinary Cd half-life ranges from 15 to 30 years and serves as a biomarker for Cd exposure and absorption. In contrast, blood Cd levels only indicate recent exposure levels[47]. Cd primarily accumulates in the proximal tubules. Long-term Cd intake commonly leads to kidney damage, with proteinuria being one of the earliest signs[14]. Animal studies have suggested that Cd exposure can cause glomerulonephritis and tubular damage[47]. Epidemiological research has shown that low-level Cd exposure (0.64 μg/g creatinine) is associated with a decreased eGFR[12]. In our study, the average urine Cd concentration (0.31 μg/g creatinine) was lower than the threshold concentration (5.24 μg/g creatinine) proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[48], and it still demonstrated a significant positive correlation with CKD risk. These findings highlighted that Cd could be nephrotoxic even at low concentrations.

Hg is a ubiquitous environmental pollutant with nephrotoxic properties[49]. Human exposure to Hg occurs primarily through dietary intake, particularly in fish rich in methylmercury[50]. Human Hg exposure is assessed through urinary inorganic Hg, which is influenced by renal reabsorption and metabolic processes[50]. The kidneys serve as the primary organ of Hg accumulation and toxicity, with the proximal tubules being the most sensitive region[49]. In the clinic, proteinuria is a typical manifestation of Hg toxicity[51]. Epidemiological studies vary in their findings on the association between Hg exposure and CKD risk. For example, one cross-sectional study revealed a positive dose–response relationship between Hg and urinary β2-microglobulin (β2 M)[52], the biomarker of kidney disease, whereas other studies suggested an inverse or no association[53]. In our study, there was no significant association between Hg and CKD risk, either as a single metal exposure or in mixed metal exposure. A possible reason was that the Hg concentration (0.39 μg/g creatinine) was considerably below the WHO-recommended renal tubular damage threshold (50 μg/g creatinine)[48]. However, a previous study revealed that the level of Hg in the urine was significantly and positively associated with the eGFR. The heterogeneity may be due to differences in the confounding factors and the baseline characteristics of the study subjects. In conclusion, while Cd was significantly positively associated with CKD risk, Tl and Pb were negatively associated, potentially because of reverse causality or hyperfiltration effects. The relationship between urinary Hg concentrations and CKD risk was not significant in our study, likely because of low exposure levels. These findings highlight the complexity of the effects of metal exposure on CKD risk and the need for further research to elucidate the underlying mechanisms involved.

Although no significant interactions among heavy metals were found in our study, numerous other studies have demonstrated that Cd, Pb, and Hg exhibit complex interactions at low concentrations, which undeniably affects the physiological functions of organisms. At nanomolar concentrations related to the level of biological pollution in volcanic area residents, mixtures containing metals such as Hg, copper (Cu), and zinc (Zn) notably promoted the proliferation of undifferentiated thyroid cells. The effect was more pronounced with these mixtures, suggesting a possible synergistic effect among these metals on cell proliferation[54]. However, the role of Pd in this regard for this cell type has been less explored. With respect to the impact on overall physiological functions, especially with respect to children's health, studies have shown that even at relatively low exposure levels, Cd, Pb, and Hg can influence children's kidney and dopamine systems through complex interactions. They might affect physiological functions by influencing metal accumulation, excretion, and metabolism in the body[45]. Moreover, in studies on environmental exposure and health risks, at low concentrations of mixed exposure, these heavy metals may jointly affect biomarker levels through unclear mechanisms, such as those related to lipid metabolism and kidney function markers, indicating that their interactions start to impact the body's normal metabolic and physiological states at low concentrations[55,56]. However, these effects are rather concealed and need further detailed detection and long-term observation.

At toxic concentrations, the interactions among Cd, Pb, and Hg are manifested mainly as synergistic or antagonistic effects, causing significant harm to organisms. In cytotoxicity studies, when cells are jointly exposed to metals such as Cd, Pb, and Hg, the toxic effects on liver cells (e.g., HL7702 cells) exceed the sum of individual metal toxicities, resulting in synergistic toxicity and worsening liver damage[57]. In kidney cells, joint exposure to Cd and Pb can also affect renal function biomarkers through synergistic effects. For example, joint exposure to Cd–Pb has a synergistic effect on the eGFR-EPI, intensifying the adverse impact on renal function[58]. In addition, in studies on Hg accumulation in animals, a synergistic effect between Pb and Hg was found. Pretreatment with Pb can increase Hg deposition in the kidney, aggravating kidney damage, possibly because Pb interferes with

the metabolic or excretory pathways of Hg[59]. Antagonistic effects also exist. For example, some heavy metal mixtures with Mn show lower toxicity than the sum of individual metals do, indicating an antagonistic effect[57]. Overall, Cd, Pb, and Hg have complex interactions at both low and toxic concentrations, which should be taken into account in environmental health risk assessment and preventive strategy formulation.

Subgroup and sensitivity analyses

Stratified analysis indicated that there were no differences in the associations between metal exposure and CKD risk across different age or sex groups (Figs. S6-S9). In the younger subgroup of the extended cohort, no significant negative trend was observed. However, after adjusting for smoking and drinking status in the younger subgroup of the final group, the negative trend was significant. These findings indicate that there may be interactions among smoking, drinking and age. The single-metal associations and univariate exposure–response plots revealed no significant differences in the stratified analysis. In the sensitivity analysis, the association remained robust when participants with a history of diabetes or hypertension were excluded and metal concentration outliers were removed (Figs. S10, S11, S13). Furthermore, after excluding participants with a history of diabetes and hypertension, the association became nonsignificant in the extended cohort but significant in the final group (Fig. S12). We found that smoking and drinking status can obscure or confound the associations between heavy metals and CKD risk. Therefore, adjusting for smoking and drinking status was necessary in our study.

Associations among α-klotho levels, urinary metal concentrations, and CKD risk

After adjusting for confounding factors, multiple linear regression revealed a significant association of Hg→α-klotho, with a β value of 0.0295 (95% CI: 0.0159, 0.0431) (Table 3). In our study, we found that the α-klotho concentration in non-CKD patients (798.80 ± 323.10) was significantly greater than that in CKD patients (727.65 ± 310.18) (Table 1). Further logistic regression analysis revealed that α-klotho was a significant protective factor against the development of CKD, with an OR of 0.9244 (95% CI: 0.8923, 0.9577) (Table 3). Regression analysis revealed a significant positive relationship between Hg and α-klotho and a negative relationship between α-klotho and CKD risk. However, it remains unclear whether α-klotho plays a bridging role between exposure to metals and CKD risk. Therefore, we further analysed the mediating effect of α-klotho. After adjusting for confounding factors, α-klotho exerted a significant negative mediating effect on the Hg–CKD risk association, with a mediation proportion of 34.55%. The results of the mediation analysis revealed that both the total effect and direct effect of Hg on CKD risk were nonsignificant, which was consistent with the results of the logistic and nonlinear regressions. We assumed that low levels of Hg resulted in a weak direct effect on CKD risk, which was insufficient to achieve statistical significance. Since the total effect contains both direct and indirect effects, the nonsignificant direct effect may diminish the indirect effect to make the total effect statistically nonsignificant. In the extended cohort, this mediating effect still existed for the Hg–CKD risk association (Fig. 5), which confirmed the stability of the mediating role of α-klotho in the association between low-level metal exposure and CKD risk

The kidneys are vital metabolic organs rich in mitochondria that serve as a primary source of ROS and are susceptible to oxidative stress. The pathogenesis of CKD includes maladaptive repair of tubular epithelial cells, an excessive immune inflammatory response, capillary rarefaction, oxidative stress, etc. As a membrane-bound protein, klotho, including α-klotho, β-klotho, and γ-klotho, is considered a potential biomarker associated with CKD risk. α-Klotho is widely expressed in the human body, especially in the kidney[60]. The functions of α-klotho can be summarized as follows: (1) increased antioxidant capacity: α-klotho can regulate the expression and activity of intracellular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase-4 (GPX-4) and upregulate p53, glutathione, and SOD2 xNA levels, helping clear ROS and maintain redox balance[61]; (2) the inhibition of Toll-like receptor 4 (TLR4) signalling and the nuclear transcription factor NF-κB: α-klotho suppresses TLR4 signal transduction and inhibits the activation of NF-κB, a critical regulator of inflammation and oxidative stress, to alleviate inflammatory responses and oxidative stress[62]; (3) the induction of autophagy: α-klotho promotes autophagy, reducing the deposition of collagen I in the kidney and thus improving or inhibiting renal fibrosis[63]; and (4) the regulation of Ca homeostasis: α-klotho increases plasma 1,25-dihydroxyvitamin D_3concentrations, upregulates Na–Ca ion exchange, and/or regulates the expression of transient receptor potential cation channel subfamily V member 5 in distal tubular cells, maintaining Ca homeostasis in the kidney[64]. In our study, α-klotho was a protective factor against the development of CKD, which aligns with conclusions drawn from other studies. Above the nephrotoxicity threshold, metals typically induce damage through similar mechanisms, such as binding with crucial enzymes

Metals	Crude[a]β (95%CI)	*P* value	Adjusted[b]β (95%CI)	*P* value	Adjusted[c]β (95%CI)	*P* value
Cd	−0.0163(−0.0340,0.0014)	0.072	−0.0114(−0.0258,0.0030)	0.1206	−0.0133(−0.0334,0.0067)	0.191215
Hg	**0.0302**(0.0165,0.0438)	<0.0001	**0.0273**(0.0166,0.0380)	<0.0001	**0.0295**(0.0159,0.0431)	<0.0001
Pb	−0.0137(−0.0342,0.0068)	0.1894	0.0053(−0.0102,0.0207)	0.5029	0.0076(−0.0131,0.0283)	0.472889
Tl	**0.0332**(0.0098,0.0566)	0.0846	**0.0242**(0.0053,0.0432)	0.0123	0.0222(−0.0024,0.0468)	0.077606
Disease	**Crude[a]OR (95%CI)**	***P* value**	**Adjusted[b]OR (95%CI)**	***P* value**	**Adjusted[b]OR (95%CI)**	***P* value**
CKD	**0.9080**(0.8777,0.9393)	<0.0001	**0.9993**(0.9990,0.9996)	<0.0001	**0.9244**(0.8923,0.9577)	<0.0001

Table 3. Associations among α-Klotho concentrations, metal concentrations, and CKD risk. [a]Crude model: unadjusted. [b]Adjusted model 1: adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, and BMI. [c]Adjusted model 2: adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency.

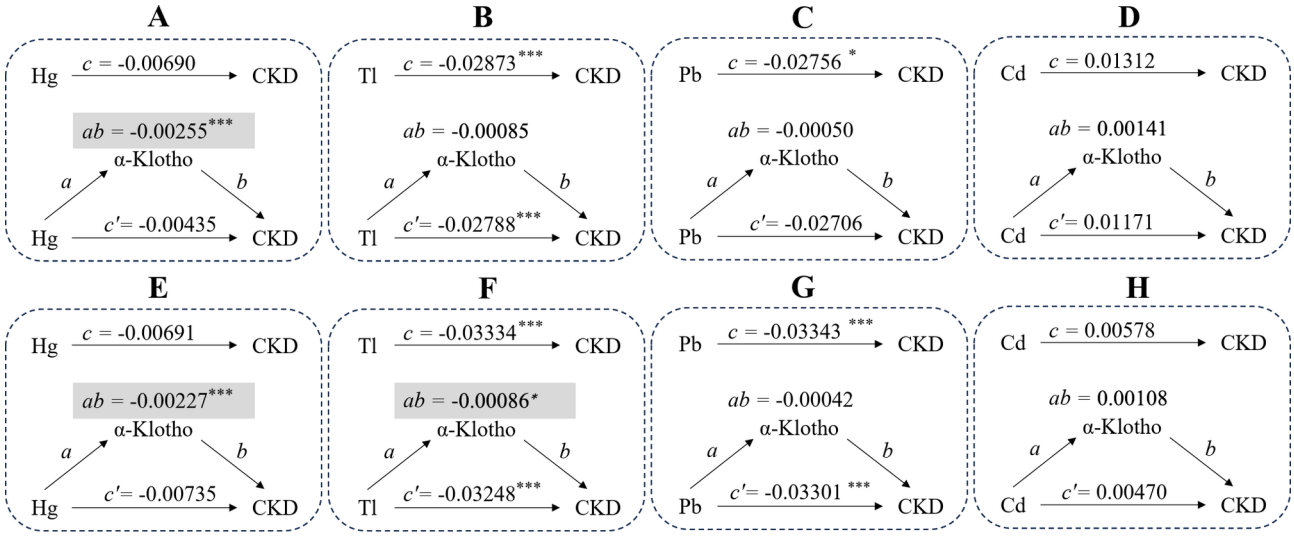

Fig. 5. Mediation model of α-Klotho in the correlation between each heavy metal and CKD risk. **A, B C, D** were the mediation models based on the final group and adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, BMI, smoking and drinking frequency. **E, F, G, H** were the mediation models based on the extended cohort and adjusted for age, sex, race, education level, family PIR, history of diabetes, history of hypertension, and BMI. Path **a** is a regression analysis between exposure and mediator. Path **b** is a regression analysis between mediator and outcome. Path **c** is a regression analysis between exposure and outcome. Path **c'** is a regression analysis between exposure and outcome. The indirect effect (ab) estimate is the amount of mediator contribution to the relationship between exposure and outcome. *P-value< 0.05. ** P-value < 0.001. *** P-value < 0.0001.

or macromolecules or substituting other elements in biochemical reactions. The mechanism of metal-induced damage involves (1) inhibiting the activity of SOD and CAT, significantly reducing the total antioxidant capacity of tissues. An experiment conducted on metal-exposed rats revealed that metal exposure can increase lipid peroxidation and oxidative stress (OS), as measured by serum malondialdehyde (MDA) and nitric oxide (NO), and decrease the total antioxidant capacity (TAC), as measured by glutathione (GSH), CAT and SOD[65]. Animal experiments have confirmed that the overexpression of klotho in long-lived mice increases resistance to oxidative stress, which includes UV radiation, heat, and heavy metals[66]. (2) Metals substitute for potassium (K) in biochemical reactions: A diuretic experiment in rats revealed that, owing to its similar physicochemical properties to those of K and its tenfold higher affinity for enzymes containing thiol groups, metals can affect Na+/K+-ATPase activity in renal cells[67], causing abnormal cellular energy metabolism, apoptosis, a reduced GFR and urine output, increased proteinuria, and resulting in kidney dysfunction. Hg also causes lipid peroxidation and oxidative stress in the kidneys through similar mechanisms, leading to an imbalance in metal homeostasis. However, few toxicological studies have explored the mechanism by which low-dose heavy metal exposure affects α-klotho levels. Research by Calabrese revealed that dose–response hormetic effects exist during low-dose pollutant exposure[68], which refers to stimulatory or beneficial effects at low doses but inhibitory or harmful effects at high doses. Multiple in vitro experiments have demonstrated that low concentrations of heavy metals such as arsenic (As), Cd, Pb, and Hg have hormetic effects on normal human mammary epithelial cells (MCF-12 A), human embryonic lung fibroblasts (HELF), immortalized keratinocyte cell lines (RHEK-1, HaCaT, and NM1), and primary keratinocytes (NHEK)[69–71]. These effects include promoting cell proliferation, inducing ROS generation without causing cytotoxicity, enhancing the activity of antioxidant enzymes such as glutathione peroxidase (GSH-Px) and SOD, strengthening antioxidant defence mechanisms, and increasing the levels of metallothionein and heat shock proteins (HSPs), including HSP27 and HSP70. In addition to these hormetic effects observed in vitro, a study on low-concentration heavy metal exposure in volcanic regions and the high incidence of thyroid cancer demonstrated that metal-induced proliferation was due to the activation of the ERK1/2 pathway[54]. In conclusion, above the nephrotoxicity threshold, Hg can increase ROS production or disrupt kidney function, leading to decreased levels of α-klotho in the body. However, below the nephrotoxicity threshold, low concentrations of Hg may increase klotho levels in the body because of dose–response hormetic effects or through reverse causality between metal exposure and CKD risk. On the basis of the hypothesis of hyperfiltration and dose–response hormetic effects, a reduced level of environmental heavy metals may increase the eGFR and stimulate α-klotho production, thereby lowering CKD risk. On the basis of the reverse causality hypothesis, poor renal function reduces the eGFR, which in turn decreases α-klotho production and reduces the excretion of heavy metals in the urine. Both explanations logically account for the relationships among them, but further experiments are needed to elucidate this potential mechanism.

We used the IEU OpenGWAS project and employed the MR method to validate the causal relationship of metals → α-klotho → CKD. However, owing to the lack of heavy metal GWAS data, we only verified the causal relationship of α-klotho → CKD in our study. After excluding SNPs with potential pleiotropic effects, 4

SNPs were retained. All selected SNPs had F statistics > 10, indicating strong instrument strength. Horizontal pleiotropy was assessed using the MR-Egger intercept, and no significant pleiotropy was observed ($P = 0.4548$). Detailed information on the instrumental variables is provided in Tables S6 and S8. MR studies indicated that higher α-klotho levels were associated with a reduced CKD risk, with an OR of 0.9842 (0.9715, 0.9970) (Table S7). This finding provides additional evidence for a causal role of higher α-klotho levels in reducing CKD risk. Sensitivity analyses confirmed the robustness and credibility of this causal relationship (Table S8).

Previous studies based on the GWAS database explored the causal relationship between circulating α-klotho levels and CKD, but no significant causal association was found[23]. This result differs from our study, and a possible reason could be differences in the choice of outcome variables. The CHST9 gene encodes carbohydrate sulfotransferase 9[72], an enzyme that can promote the recognition and degradation of the α-klotho protein through sulfation modifications. The ABO gene encodes α 1–3-N-acetylgalactosamine transferase and α 1–3-galactose transferase[73], which may affect the metabolism and clearance of α-klotho through glycosylation modification. The Klotho gene encodes the α-klotho protein, which serves as a membrane coreceptor for fibroblast growth factors (FGFs) and is involved in regulating kidney function[23]. The FGFR1 gene encodes components of the FGF receptor[23]. They act on fibroblasts in the kidney, helping maintain normal structure and repairing injury. However, when these signalling pathways are excessively activated, they may promote kidney fibrosis, ultimately leading to kidney damage. The causal relationship of metals → α-klotho still requires further research for confirmation.

Conclusion

In the present study, we conducted a unique priority screening of pollutants, ranked their contributions to CKD risk and revealed that, among various pollutants, heavy metals had the greatest effect on CKD risk. Moreover, our study revealed a negative joint effect of mixed metal exposure at low concentrations on CKD risk. Furthermore, we present supporting data on the mediating role of α-klotho in the association between Hg exposure and CKD risk. This discovery helps to elucidate the potential mechanisms underlying the association between mixed metal exposure and CKD risk.

Limitations

This study had several limitations. First, owing to its cross-sectional design, the measurement indicators and outcomes used in this study represented short-term results, potentially capturing only recent exposures of the participants. Second, despite controlling for several potential confounding factors in the multivariate models, unmeasured factors such as comorbidities of CKD might introduce residual confounding. In addition to metal exposure, other concurrent risk factors may exist, and their interactions could increase CKD risk. Third, our results do not establish a causal relationship between heavy metal exposure and CKD risk. Further studies are needed to explore the causal impact of heavy metals on CKD risk. Finally, our study focused on a homogeneous population with similar racial and geographic backgrounds; hence, caution should be exercised when generalizing our findings to other racial groups or locations. Further efforts are needed to validate our findings through multicentre investigations and investigate potential mechanisms through laboratory studies.

Data availability

The datasets supporting the conclusions of this article are available in NHANES repository, [https://www.cdc.gov/nchs/nhanes/index.htm].

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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