GMR
The Original

CAUSAL RELATIONSHIP BETWEEN SERUM CYSTATIN C AND HYPERTENSION IN MIDDLE-AGED AND ELDERLY PEOPLE BASED ON NESTED CASE-CONTROL AND MENDELIAN RANDOMIZATION STUDY: A NATIONWIDE COHORT STUDY

Haibo Shi[1], Zihang Dong[2], Xinyu Guo[3], Yangzong Ciren[4]*

[1,2,3,4]Xizang university, NO.10 Zang da Eest road, Lhasa, Xizang,P.R.China
Email: 2668601499@qq.com[1], 2529396643@qq.com[2], 2924165992@qq.com[3]
*Corresponding authors: Yangzong Ciren, Email: 2367451817@qq.com

ABSTRACT
Objective: Objective: Investigate dose-response and bidirectional causality between Cystatin C and hypertension in a national cohort.
Method: Utilizing data from the China Health and Retirement Longitudinal Survey (CHARLS) spanning 2011 to 2015, a nested case-control sample comprising 2,355 individuals was constructed. Logistic regression and restricted cubic splines were employed to analyze the dose-response relationship. A bidirectional two-sample Mendelian randomization was conducted to determine bidirectional causality.
Result: During the 4-year follow-up, 471 (20%) patients developed hypertension. For each 1 mg/L Cystatin C, the risk increased by 130% (OR=2.35, 95%CI 1.38-4.01), and there was a linear dose-response relationship, with Q4 being 59% higher than Q1, and the effect was stronger in <60 years old. MR: Cystatin C→ hypertension IVW OR=1.13 (p=0.005), adjusted OR=1.02 (p=0.89); Hypertension →Cystatin C IVW OR=1.08 (p<0.001), with no heterogeneity or pleiotropy, and the results were robust.
Conclusion: In the middle-aged and elderly population in China, elevated hypertension is a definitive cause of increased Cystatin C levels, whereas elevated Cystatin C does not exert a significant causal effect on hypertension. Cystatin C should be utilized as a real-time biomarker for assessing blood pressure-related renal filtration stress and can aid in the early evaluation, prevention, and monitoring of hypertension in middle-aged individuals.

KEYWORD: Causal link; CHARLS; Dose-response relationship; Machine learning; Middle-aged and elderly people

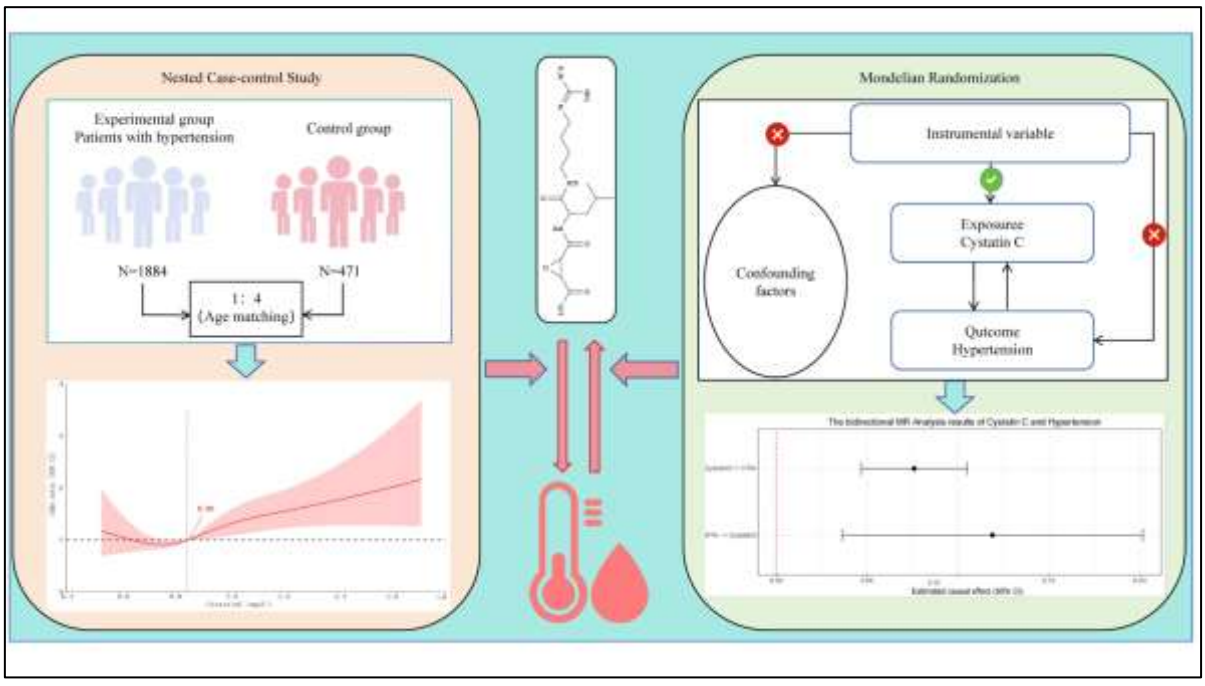

Figure. Central Illustration.

List of abbreviations

Full name	Abbreviation
Hypertension	HTN

Serum Creatinine	SCr
Estimated Glomerular Filtration Rate	eGFR
Body mass index	BMI
Systolic Blood Pressure	SBP
Diastolic Blood Pressure	DBP
Mendelian randomization	MR

BACKGROUND

Hypertension is a disease that is underdiagnosed and treated in real life [1][4]. It continues to be the most prevalent risk factor for cardiovascular diseases and mortality [5][6]. The 2023 World Report on Blood Pressure underscores the statistics related to hypertension prevalence, revealing that nearly 1.3 billion adults were affected by this condition as of 2019. Furthermore, the Global Burden of Disease Study conducted by the World Health Organization has recognized hypertension as a significant global risk factor for both the incidence and mortality associated with cardiovascular diseases [7]. Current international guidelines and literature consistently affirm that pharmacological therapy is the primary and essential treatment method for hypertension. Although lifestyle modifications are crucial, they cannot substitute for drug therapy [8]. While it is established that pharmacological treatment remains the foundation for the prevention and management of hypertension [8], the early identification of high-risk populations and precise risk stratification have emerged as critical factors for enhancing intervention efficacy. However, traditional indicators, such as creatinine levels, are susceptible to interference from variables including age, gender, and muscle mass. Consequently, there remains a clinical need for more stable and sensitive biomarkers.Serum Cystatin C is continuously synthesized by all nucleated cells and remains unaffected by factors such as muscle mass, gender, or diet [9][10]. It has been demonstrated to sensitively indicate changes in glomerular filtration rate and exhibits potential applications in vascular biology as well as in predicting adverse cardiovascular events [11].Although the value of serum Cystatin C (Cystatin C) in the assessment of renal function is widely recognized, its causal role in the pathogenesis of hypertension is still controversial. Prior cross-sectional studies have identified a significant association between Cystatin C levels and elevated blood pressure [12-13]. Nevertheless, these studies have not effectively differentiated between causal relationships and secondary phenomena. For example, increased glomerular pressure resulting from hypertension may subsequently elevate Cystatin C levels [14]. Additionally, Cystatin C may directly contribute to the pathological processes of hypertension by promoting vascular smooth muscle proliferation or exacerbating oxidative stress. The uncertainty surrounding this bidirectional relationship, particularly in the absence of extensive longitudinal population studies, hampers the clinical application of Cystatin C as a predictive marker.

In recent years, the emergence of the Mendelian Randomization (MR) method has introduced a novel approach to addressing this complex issue. By utilizing genetic variations as instrumental variables, MR can effectively mitigate the influence of confounding factors that often plague traditional observational studies, thereby enabling the assessment of causal relationships between exposure factors, such as Cystatin C, and outcomes, such as hypertension. However, the majority of existing genetic studies predominantly focus on European populations , which may not adequately reflect the significant differences in genetic backgrounds, lifestyles, and hypertension phenotypes present among East Asian populations, particularly among the middle-aged and elderly in China [15]. For example, the prevalence of salt-sensitive hypertension is notably higher in the Chinese population [16], while the potential regulatory role of Cystatin C in sodium metabolism remains poorly understood. Consequently, causal verification conducted with representative cohorts in China not only holds population-specific significance but also offers race-differentiated evidence that is crucial for the precise prevention and management of hypertension. Furthermore, the threshold for the dose-response relationship between Cystatin C and hypertension remains to be elucidated. Most existing studies treat Cystatin C as a continuous variable or employ simple stratification methods, such as quartiles [17]. However, clinical practice necessitates the establishment of clear cut-off values to facilitate risk stratification. Additionally, subgroup variations, including age and gender, may influence the predictive efficacy of Cystatin C. Therefore, this study enrolled middle-aged and elderly Chinese individuals as subjects. Using nationwide cohort data, we established a dose–response relationship between cystatin C and hypertension, determined its predictive threshold, and explored subgroup differences such as age. This study aimed to investigate the causal relationship between serum cystatin C levels and hypertension risk, so as to provide new biological evidence for risk identification prior to pharmaceutical intervention.

METHODS

1.Study population

This study established a prospective cohort utilizing the 2011 baseline data from the China Health and Retirement Longitudinal Survey (CHARLS) and employed a nested case-control design to evaluate the relationship between serum Cystatin C levels and hypertension risk. CHARLS is a multi-stage stratified probability sampling cohort that encompasses 28 provinces (municipalities) and 150 counties (districts) throughout the country (http://charls.pku.edu.cn). The investigation periods spanned from 2011 to 2012, 2013 to 2014, 2015 to 2016, 2017 to 2018, and 2018 to 2022, with approval granted by the Ethics Committee of Peking University. Given the completeness of blood biomarker data from the 2011 and 2015 rounds, this study exclusively utilized data from these two periods. The cohort construction process involved the following steps: among the 17,708 respondents in 2011, individuals with malignant tumors, those under 45 years of age, participants with baseline hypertension,

and those with Cystatin C deficiency were sequentially excluded. Ultimately, 6,057 individuals were included in the follow-up.From 2011 to 2015, new hypertensive events were identified through a combination of physical examinations and questionnaires, which included either a doctor's diagnosis or the use of antihypertensive medications, resulting in the confirmation of 493 cases. Following this, incidence-density sampling was employed for age matching at a ratio of 1:4 (±1 year) within the same follow-up population. Controls were required to complete the investigation concurrently, and no hypertension was detected during the follow-up period. Ultimately, 471 cases and 1,884 controls were included, leading to the formation of a nested case-control sample comprising 2,355 individuals for subsequent causal inference analysis (Figure 1).

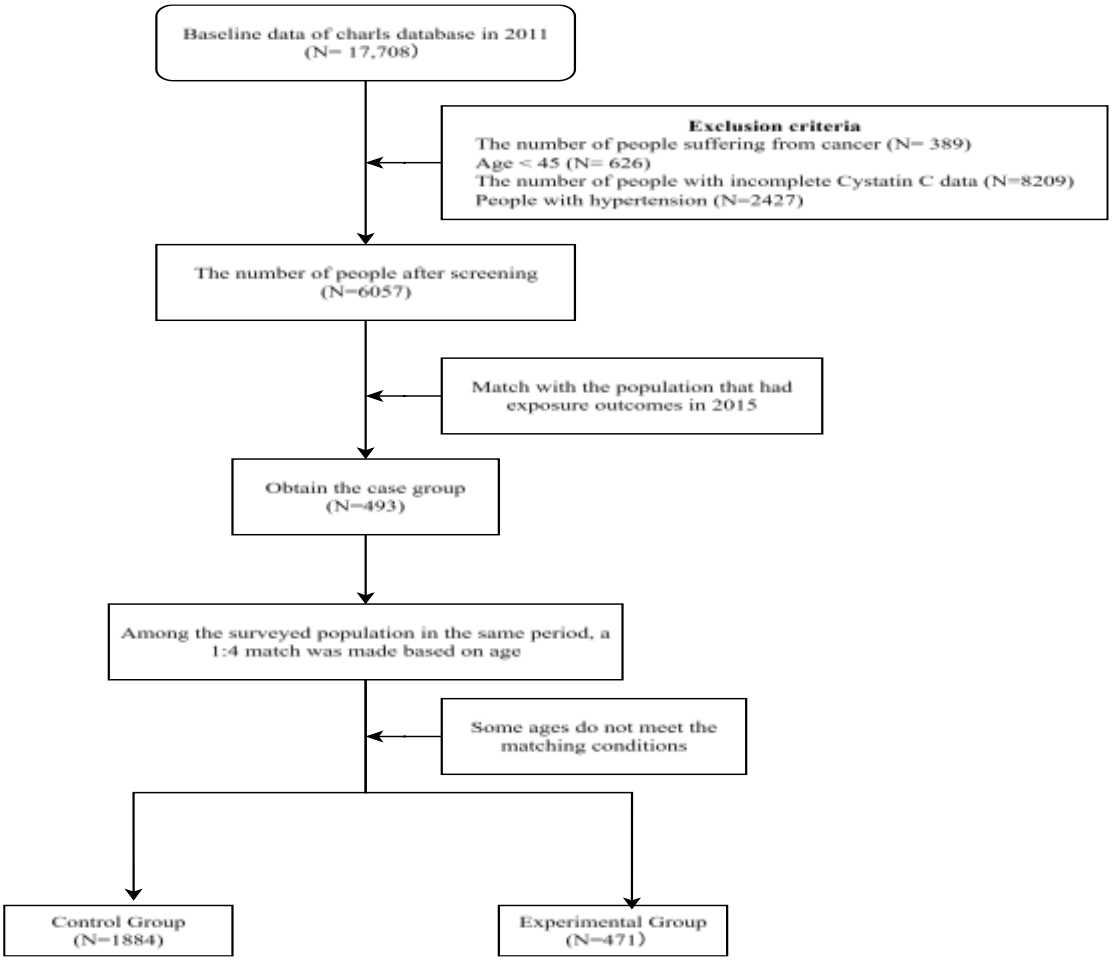

Figure 1. Flowchart of participant inclusion.

2.Exposure and covariates

The exposure factor in this study is serum Cystatin C. Data collection for serum Cystatin C was conducted by the Chinese Center for Disease Control and Prevention, which obtained three tubes of fasting venous blood from respondents according to a standardized protocol and promptly sent them for analysis. The particle-enhanced turbidimetric method (CV<5%) was employed to measure serum Cystatin C levels. Creatinine (mg/deciliter) was assessed using the rate blanking compensation Jaffe method. The estimated glomerular filtration rate (eGFR) for creatinine was calculated using the 2021 Chronic Kidney Disease Epidemiology Collaborative Equation. Baseline data from 2011 and follow-up data from 2015 were collected by well-trained interviewers utilizing standardized questionnaires that addressed sociodemographic factors, behavioral patterns, anthropometric measurements, and health status. Sociodemographic variables included age, gender, educational attainment (high school and below, high school and above), and marital status (married, unmarried). Behavioral factors encompassed smoking (never, once, currently) and drinking (never, once, currently).The average of two measurements for height and weight is calculated. Body Mass Index (BMI) is determined using the formula: BMI = weight (kg) / height² (m²). According to the Chinese standard, overweight and obesity are defined as BMI values of ≥24 kg/m² and ≥28 kg/m², respectively. Blood pressure is recorded as the average of three sitting measurements. Hypertension is classified as a systolic blood pressure (SBP) of ≥140 mmHg, a diastolic blood pressure (DBP) of ≥90 mmHg, a prior diagnosis by a physician, or current use of antihypertensive medications. Comorbidities and medication information are documented concurrently. The diagnosis of diabetes follows the criteria established by the American Diabetes Association: fasting blood glucose levels of ≥7.0 mmol/L, a previous diagnosis, or current use of hypoglycemic agents.

Female（SCr （mg/dL）≤ 0.7）：$eGFR = 142 \times （SCr/0.7）^{-0.241} \times 0.9938^{age} \times 1.012$

Female（SCr （mg/dL）> 0.7）：$eGFR = 142 \times （SCr/0.7）^{-1.200} \times 0.9938^{age} \times 1.012$

Male（SCr （mg/dL）≤ 0.9） : $eGFR = 142 \times （SCr/0.7）^{-0.302} \times 0.9938^{age}$

Male（SCr （mg/dL）> 0.9） : $eGFR = 142 \times （SCr/0.9）^{-1.200} \times 0.9938^{age}$

3.The definition of hypertension

This study defined new-onset hypertension during the follow-up period as the primary outcome event. Utilizing the standardized questionnaire modules from the International Aging Survey (HRS, ELSA, SHARE), diagnostic information was gathered through the question: "Have you ever been told by a doctor that you have hypertension?" Individuals without a prior history of hypertension, those first diagnosed during the follow-up period, or those currently taking antihypertensive medications were classified as having new-onset hypertension. Rigorous quality control and logical verification were employed throughout the process to ensure the accuracy and reliability of the outcome determination.

4. Statistical Analysis

4.1Analysis of the Relationship between Cystatin C and hypertension

Statistical analysis progresses from descriptive to causal inference and from observational data to genetic insights. Descriptive statistics were conducted for all variables: continuous variables are presented as mean ± standard deviation or median (interquartile range), while categorical variables are reported as the number of cases (percentage). Differences between groups were assessed using t-tests, Mann-Whitney U tests, or χ^2/Fisher's exact tests, contingent upon the data distribution. To address missing values, zero-order interpolation was utilized to replace absent classification data, while all count data served as substitutes. For the core exposure indicator, Cystatin C, we developed four stepwise adjusted logistic regression models within a nested case-control framework. Model 1 was a basic model that did not adjust for any factors. Model 2 incorporated gender and age. Model 3 further included marital status, smoking, alcohol consumption, urban versus rural residence, and education. Model 4 ultimately accounted for comorbidities such as diabetes, dyslipidemia, kidney disease, history of stroke, and estimated glomerular filtration rate. This model assessed the impact of a 1 unit increase in Cystatin C, calculated directly using the percentile method for the 25th, 50th, and 75th percentiles. The first quartile (Q1) was defined as a Cystatin C value lower than 25% of the overall distribution. A Cystatin C value lower than 50% of the overall distribution represented the second quartile (Q2), which is the median. The third quartile (Q3) was identified as the range where the Cystatin C value is lower than 75% of the overall distribution, while a Cystatin C value exceeding 75% of the overall distribution constituted the fourth quartile (Q4).The potential dose-response relationship between the opposite quartile grouping (Q1-Q4) and the incidence of new-onset hypertension over four years was evaluated using a restricted cubic spline to assess the nonlinear hypothesis. Subgroup analyses were stratified by age (≤60/>60 years), gender, smoking status (yes, no), alcohol consumption (yes, no), and body mass index (BMI) categorized as ≥ 24 kg/m² for overweight and < 24 kg/m² for non-overweight individuals. Interaction effects were examined using the product term, with Pinteraction < 0.05 indicating significant effect modification.All analyses were performed using R software, employing two-sided tests, with a P value of <0.05 deemed statistically significant.

4.2Previous causal analysis of Cystatin C and hypertension

In the genetic inference section, we employed two-sample bidirectional Mendelian randomization. For the forward direction, we utilized 188 independent SNPs associated with Cystatin C as instruments, while in the reverse direction, we used 198 independent SNPs related to hypertension. The primary estimates were calculated using random effects inverse variance weighting (IVW), with additional robustness assessments conducted through weighted median, MR-Egger, and weighted mode analyses. Heterogeneity was evaluated using the Cochran Q test, and pleiotropy was addressed through the MR-Egger intercept and the three-stage MR-PRESSO method (Global/Outlier/Distortion).

5.RESULTS

5.1 Baseline Characteristics of the Nested Case–Control Study

Nested sampling employs incidence-density sampling with a 1:4 age matching ratio. Due to a lack of sufficient controls within age groups, a total of 471 cases were successfully matched with 1,884 controls, resulting in a matching rate of 95.5%. The nested case-control sample comprised 2,355 participants from CHARLS, including 471 cases (20.0%) of newly diagnosed hypertension and 1,884 controls (80.0%). Table 1 indicates that waist circumference, weight, median Cystatin C levels, and the prevalence of dyslipidemia and diabetes, as well as place of residence, were significantly higher in the case group compared to the control group (P<0.01). In contrast, the matching variables—age, gender, BMI, smoking and drinking status, and educational level—were evenly distributed between the two groups (P>0.05), indicating effective matching.

Table 1. Baseline characteristics of the case group and the control group in nested case-control studies

Variables	Total	Control	Experimental	*P*
Participants, n	2,355	1,884	471	
Age(Mean ± SD)	65.00 ± 8.51	65.00 ± 8.51	65.00 ± 8.52	1.000
Bmi(Mean ± SD)	23.91 ± 17.23	23.76 ± 18.86	24.48 ± 7.81	0.421
Mheight(M)(Mean ± SD)	1.57 ± 0.10	1.57 ± 0.10	1.57 ± 0.10	0.589
Mwaist(Cm)(Mean ± SD)	83.81 ± 13.36	83.32 ± 12.97	85.77 ± 14.65	<.001
Mweight(Kg)(Mean ± SD)	57.71 ± 11.43	57.09 ± 11.21	60.21 ± 11.94	<.001
Cystatin C(mg/L) (Q_1, Q_3)	0.85 (0.74, 0.97)	0.85 (0.74, 0.96)	0.87 (0.75, 0.99)	0.002
Ragender, n(%)				0.606
Male	1,165 (49.47)	927 (49.20)	238 (50.53)	
Female	1,190 (50.53)	957 (50.80)	233 (49.47)	
Dyslipidemia, n(%)				<.001
No	1,962 (85.49)	1,626 (88.32)	336 (74.01)	
Yes	333 (14.51)	215 (11.68)	118 (25.99)	
Diabetes, n(%)				<.001
No	2,150 (92.12)	1,748 (93.38)	402 (87.01)	
Yes	184 (7.88)	124 (6.62)	60 (12.99)	
Stroke, n(%)				0.121
No	2,273 (96.85)	1,825 (97.13)	448 (95.73)	
Yes	74 (3.15)	54 (2.87)	20 (4.27)	
Drinking now, n(%)				0.897
No	1,516 (64.37)	1,214 (64.44)	302 (64.12)	
Yes	839 (35.63)	670 (35.56)	169 (35.88)	
Smoking status, n(%)				0.536
No	1230 (52.23)	978 (51.91)	252 (53.50)	
Yes	1125 (47.77)	906 (48.09)	219 (46.50)	
Current smoking, n(%)				0.314
No	1,619 (68.78)	1,286 (68.30)	333 (70.70)	
Yes	735 (31.22)	597 (31.70)	138 (29.30)	
Drinking alcohol, n(%)				0.749
No	1,245 (52.89)	999 (53.05)	246 (52.23)	
Yes	1109 (47.11)	884 (46.95)	225 (47.77)	
Place of residence, n(%)				0.043
Rural areas	803 (34.10)	661 (35.08)	142 (30.15)	
City	1,552 (65.90)	1,223 (64.92)	329 (69.85)	
Educational attainment, n(%)				0.169
Below high school	2,158 (91.63)	1,719 (91.24)	439 (93.21)	
High school and above	197 (8.37)	165 (8.76)	32 (6.79)	

Data are expressed as mean ± standard deviation or number of cases (%), where SD is the standard deviation and BMI is the body mass index.

5.2 Dose-response Relationship between Cystatin C Levels and Hypertension Risk

During the four-year follow-up period, a total of 471 new cases of hypertension were identified, representing 20.0% of the cohort. For each 1-unit (mg/L) increase in Cystatin C, the risk of developing hypertension increased by approximately 130%. This risk continued to rise across three subsequent stepwise adjusted models (Model 1 OR=2.30, 95% CI 1.43-3.70; Model 4 OR=2.49, 95% CI 1.35-4.58; P<0.002). The restricted cubic splines for the four models confirmed that the nonlinear assumption was invalid and demonstrated a consistently positive correlation (Figure 2) . Using Q1 (≤0.70 mg/L) as the reference group, Q4 (≥1.00 mg/L) exhibited an odds ratio of 1.61 (95% CI 1.61-2.25, P<0.001) in Model 4, with a trend test yielding P<0.001 (Table 2) , further substantiating the dose-response relationship (Table 2) . Notably, in the age stratification analysis, individuals younger than 60 years showed an odds ratio of 13.11 (95% CI 3.31-51.93, P<0.001), while those aged 60 years and older had an odds ratio of 1.73 (95% CI 0.98-3.05, P=0.061). The interaction term yielded P=0.005, indicating that age significantly modified the association between Cystatin C and hypertension, with a stronger effect observed in the population under 60 years of age. In contrast, the interaction P values for gender, smoking status (past/present), alcohol consumption (past/present) , and BMI stratification were all greater than 0.05, indicating no significant differences in effect (Table 3) .

Table 2. Relationship between baseline Cystatin C and the risk of Hypertension

		Model 1	Model 2	Model 3	Model 4

	No.of stroke/Total	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Cystatin C(per unit)	2355	2.30(1.43~3.70）	<0.001	2.68(1.59~4.52)	<0.001	2.77 (1.65 ~ 4.68)	<0.001	2.49(1.35~4.58)	<.0003
Quartile 1(**<=0.70**)	606/2355	Ref		Ref		Ref		Ref	
Quartile 2(**0.70-0.90**)	587/2355	0.93(0.69~1.25)	0.93	0.97(0.72–1.30)	0.817	0.96(0.71-1.30)	0.800	0.93 (0.69 ~ 1.27)	0.660
Quartile 3(**0.90-1.00**)	572/2355	0.97(0.72~1.30)	0.837	1.04(0.77–1.42)	0.788	1.05(0.77-1.43)	0.752	1.09 (0.81 ~ 1.52)	0.517
Quartile 4(**≥1.00**)	590/2355	1.50 (1.14 ~ 1.98)	0.004	1.71(1.24–2.37)	<.001	1.71(1.23-2.36)	0.001	1.61 (1.16 ~ 2.25)	<0.001

OR: Ratio ratio; CI: Confidence interval.

Model 1: Unadjusted;Model 2: Adjust: Gender, age; Model 3: Adjust: Marriage, whether one has smoked, whether one has drunk alcohol, gender, living in rural or urban areas, education, age; Model 4: Adjust: dia Diabetes, dyslipidemia, stroke, marital status, Kidney disease,whether one has smoked, whether one has drunk, gender, living in rural or urban areas, education, age, eGFR

Model 1: Unadjusted;Model 2: Adjust: Gender, age; Model 3: Adjust: Marriage, whether one has smoked, whether one has drunk alcohol, gender, living in rural or urban areas, education, age; Model 4: Adjust: dia Diabetes, dyslipidemia, stroke, marital status, Kidney disease,whether one has smoked, whether one has drunk, gender, living in rural or urban areas, education, age, eGFR

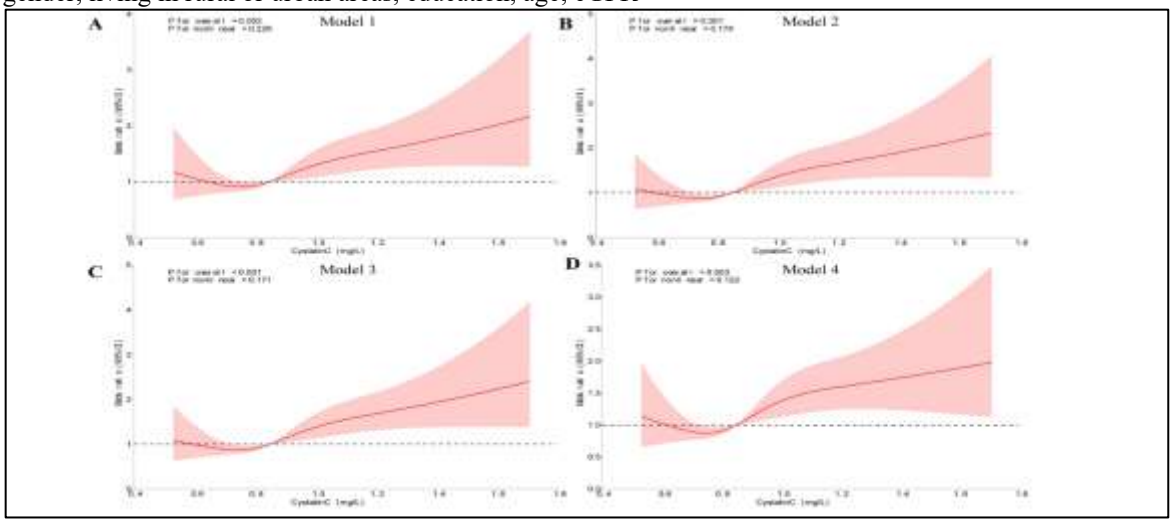

Figure 2. The association between Cystatin C and the risk of hypertension was analyzed by the restricted three-spline method.

Table 3. Subgroup analysis of the correlation between Cystatin C and hypertension risk.

Variables	n (%)	OR (95%CI)	P	P for interaction
All patients	2,355 (100.00)	2.30 (1.43 ~ 3.70)	<.001	
Drinking now				0.793
No	1,516 (64.37)	2.22 (1.27 ~ 3.86)	0.005	
Yes	839 (35.63)	2.55 (1.04 ~ 6.27)	0.041	
Smoking status				0.468
No	1,230 (52.23)	2.74 (1.45 ~ 5.18)	0.002	
Yes	1,125 (47.77)	1.91 (0.91 ~ 4.01)	0.085	

Current smoking				0.88
No	1,618 (68.76)	2.42 (1.37 ~ 4.27)	0.002	
Yes	735 (31.24)	2.23 (0.93 ~ 5.38)	0.074	
Drinking alcohol				0.28
No	1,245 (52.89)	2.90 (1.52 ~ 5.52)	0.001	
Yes	1,109 (47.11)	1.70 (0.82 ~ 3.52)	0.153	
Gender				0.256
Male	1,165 (49.47)	3.01 (1.57 ~ 5.78)	<.001	
Female	1,190 (50.53)	1.71 (0.83 ~ 3.55)	0.147	
Age				0.005
<60	655 (27.81)	13.11 (3.31 ~ 51.93)	<.001	
≥60	1,700 (72.19)	1.73 (0.98 ~ 3.05)	0.061	
BMI				0.955
<24	1,474 (62.59)	2.52 (1.39 ~ 4.56)	0.002	
≥24	881 (37.41)	2.44 (1.09 ~ 5.47)	0.03	
OR: Odds Ratio, CI: Confidence Interval				

5.3 Bidirectional two-sample Mendelian randomization

In the study examining Cystatin C as an exposure and hypertension as an outcome, a total of 188 single nucleotide polymorphisms (SNPs) were employed to investigate the causal relationship between Cystatin C and hypertension. Heterogeneity in Mendelian randomization (MR) analysis was assessed using Cochran's Q ($p < 0.001$), prompting the use of a random-effects model. The inverse variance weighted (IVW) model indicated that a genetically predicted increase of 1 standard deviation in Cystatin C was associated with a lifetime elevated risk of stroke [OR=1.127 (95% CI 1.04-1.22, P=0.005)]. MR-Egger analysis suggested slight directional pleiotropy (intercept = 0.000815, p = 0.012); however, the estimated direction was consistent with the IVW results, and no inversion occurred. No outliers were identified using MR-PRESSO. While IVW indicated a positive association between Cystatin C and hypertension, the causal relationship remains unclear. In the reverse MR analysis, where hypertension served as the exposure and Cystatin C as the outcome, a total of 198 SNPs were utilized to explore the causal relationship between hypertension and Cystatin C. Heterogeneity in MR analysis was estimated by Cochran's Q ($P < 0.001$), with IVW as the primary estimation method: OR=1.079 (95% CI 1.05-1.11, $P < 0.001$). No potential pleiotropic effects were detected (intercept = 0.000453, p = 0.18). Outliers were examined using MR-PRESSO, and none were found. This analysis indicates a positive association between hypertension and Cystatin C, suggesting that hypertension causally contributes to the increase in Cystatin C levels(Table 4).

Table 4. Results of Bidirectional two-sample Mendelian randomization

outcome	exposure	method	OR	pval
Hypertension	Cystatin C	MR Egger	1.009	0.887
		Weighted median	0.979	0.318
		Inverse variance weighted	1.127	0.005
		Weighted mode	0.974	0.223
		MR-PRESSO	1.127	0.005
		Cochran Q(Heterogeneity)		< 0.001
		MR-Egger(Pleiotropy)		0.012
Cystatin C	Hypertension	MR Egger	1.019	P = 0.67
		Weighted median	1.051	<0.001
		Inverse variance weighted	1.079	<0.001
		Weighted mode	1.049	0.033
		MR-PRESSO	1.079	<0.001
		Cochran Q(Heterogeneity)		$< 1\times10^{-6}$
		MR-Egger(Pleiotropy)		0.18

The bidirectional MR Forest map reveals significant causal effects in both directions: elevated levels of the Cystatin C gene increase the risk of hypertension (β=0.10, 95% CI excludes 0), while elevated hypertension levels, as predicted by genes, also result in increased Cystatin C levels (β=0.07, 95% CI excludes 0). This finding suggests the presence of a potential positive feedback loop (Figure 3).

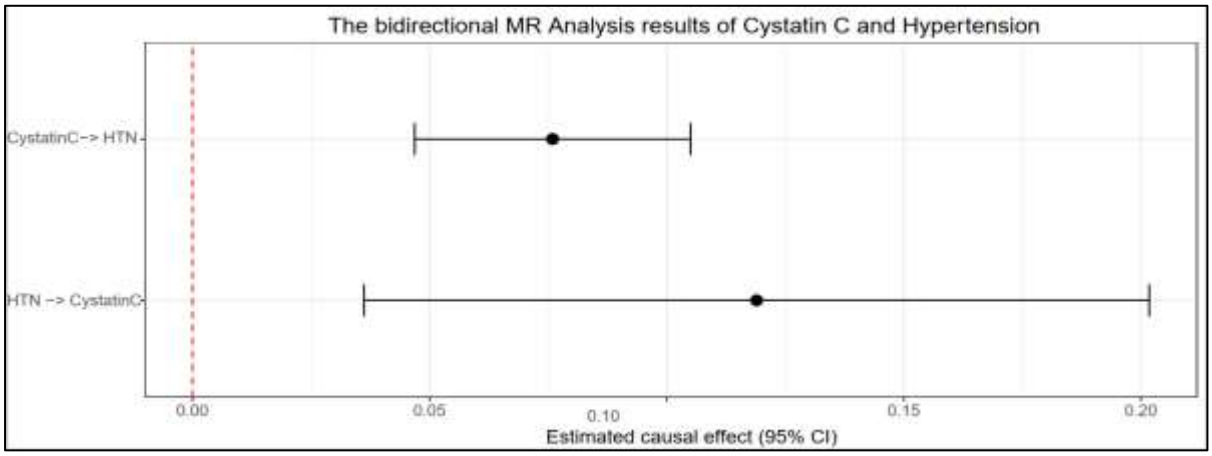

Figure 3. Bidirectional two-sample Mendelian randomization forest map.

DISCUSSION

This study, for the first time, integrates a nested case-control design with bidirectional two-sample Mendelian randomization to systematically elucidate the causal relationship between Cystatin C and hypertension in a middle-aged and elderly population in China. Epidemiologically, Cystatin C exhibits a linear dose-response relationship with the incidence of hypertension over a four-year period. Genetically, only hypertension is associated with an increase in Cystatin C, demonstrating robust causality. The reverse pathway showed no effect after adjusting for polymorphisms, confirming a unidirectional relationship for the first time in the Chinese population. Consequently, Cystatin C is not suitable as a predictor of hypertension; rather, it should be redefined as a real-time biomarker of blood pressure-related renal filtration stress. Mechanistically, elevated blood pressure increases glomerular pressure, heightens mechanical stress on podocytes, and alters the permeability of the filtration barrier.Renal tubular epithelial cells enhance the secretion of Cystatin C to locally mitigate proteolysis. Prolonged hypertension results in glomerular sclerosis and a slight reduction in glomerular filtration rate (GFR), which leads to a sustained increase in serum Cystatin C levels. This creates a positive feedback loop characterized by hypertension, renal stress, and elevated Cystatin C. Antihypertensive treatment can disrupt this cycle, offering a measurable and actionable early endpoint for blood pressure reduction and renal protection. At both clinical and public health levels, it is advisable to include Cystatin C levels ≥ 1.00 mg/L in the primary hypertension screening protocol. This inclusion serves as a dynamic monitoring indicator to enhance the effectiveness of blood pressure management and renal safeguarding, ultimately facilitating precise and cost-effective primary prevention of hypertension in the middle-aged population.

CONCLUSIONS

This study utilized a prospective nested case-control design from the China Health and Retirement Longitudinal Study (CHARLS) in conjunction with bidirectional two-sample Mendelian randomization analysis to systematically elucidate the causal relationship between Cystatin C and hypertension. The findings indicated that hypertension is a causative factor for elevated Cystatin C levels, whereas Cystatin C itself exerts minimal causal influence on hypertension. This unidirectional causal model reinforces the characterization of Cystatin C as a passive biomarker indicative of renal filtration stress related to blood pressure. These results provide new evidence supporting the clinical notion that "lowering blood pressure protects the kidney" and underscore the necessity for monitoring and intervening in early alterations of renal filtration function in future hypertension management.

Ethics approval and consent to participate

This study utilized the CHARLS database. The research involving the human body has been approved by the Institutional Review Board (IRB) of Peking University and was strictly carried out in accordance with local laws, regulations and relevant institutional rules. All participants were required to sign a written informed consent form by their legal guardians or close relatives to ensure that the research process met ethical requirements. with approval numbers IRB00001052-11015 andIRB00001052-11014.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.The survey using data sets can be tracked (CHARLS) health and pension in China, get online (http://charls.pku.edu.cn//).

Competing interests: The author of this article has no conflict of interest

Funding: No funding was granted for this article

Authors' contributions
The data in this article was collated and analyzed by HS,ZD and XG together,HS and ZD contributed equally to this work as co-first authors. The subsequent writing of the paper was completed by HS. The guidance work for CY 's replication of papers.

Acknowledgements
We express our gratitude to all the staff of the China Health and Retirement Longitudinal Survey (CHARLS)and Xizang Autonomous Region - Research on the Therapeutic Effect and Mechanism of Sanguisorba officinalis on Polycythemia at High Altitude (LSKJ202305).

REFERENCES
[1] Kearney PM, Whelton M, Reynolds K, et al. Global burden of hypertension: analysis of worldwide data. Lancet 2005; 365: 217-23.
[2] Egan BM, Zhao Y, Axon RN. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. JAMA 2010; 303: 2043-50.
[3] Gu Q, Burt VL, Dillon CF, et al. Trends in antihypertensive medication use and blood pressure control among United States adults with hypertension: the National Health And Nutrition Examination Survey, 2001 to 2010. Circulation 2012; 126: 2105-14.
[4] Onat A. Blood pressure in our community and hypertension. In: Onat A, editor. TEKHARF 2017, Leading the approach to chronic diseases in medicine. İstanbul, Logos; 2017. p.105-19.
[5] Francula-Zaninovic, S.; Nola, I.A. Management of measurable variable cardiovascular disease'risk factors. Curr. Cardiol. Rev. 2018, 14, 153–163. [CrossRef] [PubMed]
[6] Kuneinen, S.M, Kautiainen, H, Ekblad, M.O.et al. Multifactorial prevention program for cardiovascular disease in primary care: Hypertension status and effect on mortality. J. Hum. Hypertens. 2024, 38, 322–328.
[7] Kjeldsen, S.E. Hypertension and cardiovascular risk: General aspects. Pharmacol. Res. 2018, 129, 95–99. [CrossRef]
[8] Heidari B, Avenatti E, Nasir K. Pharmacotherapy for Essential Hypertension: A Brief Review. Methodist Debakey Cardiovasc J. 2022 Dec 6;18(5):5-16. PMID: 36561082; PMCID: PMC9733188.
[9] Duan A, Huang Z, Zhao Z, et al. The potential of Cystatin C as a predictive biomarker in pulmonary hypertension. BMC Pulm Med. 2023 Aug 26;23(1):311. PMID: 37633906; PMCID: PMC10463899.
[10] Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and Cystatin C-based equations to estimate GFR without race. N Engl J Med. 2021;385:1737–1749.
[11] Omaygenç MO, Özcan ÖU, Çakal B, et al. Cystatin C and uncontrolled hypertension. Anatol J Cardiol. 2020 Nov;24(5):309-315. doi: 10.14744/AnatolJCardiol.2020.78974. PMID: 33122483; PMCID: PMC7724385.
[12] Song X, Xiong L, Guo T, Chen X, Zhang P, Zhang X, Liang Z. Cystatin C Is a Predictor for Long-Term, All-Cause, and Cardiovascular Mortality in US Adults With Metabolic Syndrome. J Clin Endocrinol Metab. 2024 Oct 15;109(11):2905-2919. PMID: 38597157; PMCID: PMC11479697.
[13] Gu G, Yu N, Zhou Y, Cui W. Elevation of preoperative Cystatin C as an early predictor of contrast-induced nephropathy in patients receiving percutaneous coronary intervention. Singapore Med J. 2022 Aug;63(8):450-455. Epub 2021 Jan 21. PMID: 33472331; PMCID: PMC9584075.
[14] Elendu C, Amaechi DC, Elendu TC, Amaechi EC, Elendu ID. Dependable approaches to hypertension management: A review. Medicine (Baltimore). 2024 Jun 14;103(24):e38560. PMID: 38875433; PMCID: PMC11175961.
[15] Yang X, Liu L, Xiong X, Zhang Y, Liu Y, Li H, Yao K, Wang J. Effects of Bushen-Jiangya granules on blood pressure and pharmacogenomic evaluation in low-to-medium-risk hypertensive patients: study protocol for a randomized double-blind controlled trial. Trials. 2022 Jan 15;23(1):37. PMID: 35033168; PMCID: PMC8760657.
[16] Rahman ARA, Magno JDA, Cai J, Han M, Lee HY, Nair T, Narayan O, Panyapat J, Van Minh H, Khurana R. Management of Hypertension in the Asia-Pacific Region: A Structured Review. Am J Cardiovasc Drugs. 2024 Mar;24(2):141-170. Epub 2024 Feb 8. PMID: 38332411; PMCID: PMC10973088.
[17] Chen D, Sun W, Li J, Wei B, Liu W, Wang X, Song F, Chen L, Yang J, Yu L. Serum Cystatin C and Coronavirus Disease 2019: A Potential Inflammatory Biomarker in Predicting Critical Illness and Mortality for Adult Patients. Mediators Inflamm. 2020 Oct 8;2020:3764515. PMID: 33061826; PMCID: PMC7545455.