Cardiovascular Topics

Association between serum α-klotho level and the prevalence of heart failure in the general population

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Abstract

Background: Heart failure is a major cause of global morbidity and mortality. Studies in laboratory animals have shown the direct protective effects of α-klotho on the cardiovascular system although it has limited expression in the heart. The association between α-klotho and cardiovascular disease is still controversial in different clinical studies. We designed a cross-sectional study in order to investigate the association between serum α-klotho level and the prevalence of heart failure in the American general population.

Methods: The data were obtained from the National Health and Nutrition Examination Survey (NHANES), which included 11 271 participants aged 40–80 years. Serum α-klotho level was examined by enzyme-linked immunosorbent assay and divided into four quartiles for further analysis. Heart failure status was obtained from self-reported questionnaires. To estimate the association between α-klotho level and prevalence of heart failure, multivariate logistic regression analyses were conducted. Interaction and stratified analyses were performed to evaluate the potential modifiers.

Results: After adjusting for multiple covariates, a per-standard deviation increase in serum α-klotho level was associated with a decrease in prevalence of heart failure [odds ratio (OR): 0.76, 95% confidence interval (CI): 0.68–0.85). The ORs for participants in quartiles 2 to 4 were 0.77 (95% CI: 0.58–1.01), 0.70 (95% CI: 0.52–0.93) and 0.71 (95% CI: 0.53–0.95), respectively, compared with those in quartile 1. Stratified analysis revealed significant gender and racial differences.

Conclusion: We revealed an independent association between serum α-klotho level and the prevalence of heart failure in the American general population. The association was not always consistent and varied according to gender and race.

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Heart failure is a chronic and progressive condition that affects millions of people worldwide. It occurs when the heart is unable to pump enough blood to meet the body's needs, leading to a variety of symptoms and complications. The prevalence of heart failure has been steadily increasing over the years due to various factors such as aging populations, improved survival rates of heart attack patients and the rising prevalence of risk factors such as obesity, diabetes and hypertension.

Globally, heart failure affects around 64 million people, and its prevalence is expected to rise due to the aging population and the increasing burden of cardiovascular risk factors in developing countries.[1] It is a significant cause of morbidity and mortality, with a five-year mortality rate of around 50% for heart failure patients.[2] The current situation reflecting heart failure syndrome is rather complex, and there is an insufficient understanding of how it manifests and presents in different ways.

The α-klotho gene, named after the purported Greek goddess, is widely recognised as an anti-aging gene. Since its discovery by Kuro *et al*. more than two decades ago, numerous studies have consistently demonstrated the protective effects of α-klotho on various organs and tissues.[3,4]

α-klotho is primarily expressed in the kidney distal convoluted tubules and brain choroid plexus. In addition to its local actions, α-klotho can also exert its effects systemically through the blood circulation.

Circulating α-klotho acts as a hormone, potentially regulating the function of cells or tissues that do not express α-klotho. For instance, α-klotho exerts a regulatory influence on several crucial mechanisms implicated in the pathogenesis of cardiovascular diseases, despite its limited expression in cardiac tissue. It effectively mitigates oxidative stress and inflammation, safeguards against endothelial injury and vascular calcification, and impedes cardiovascular remodelling.[5,6]

Notably, varying perspectives persist regarding the correlation between α-klotho and cardiovascular diseases.[7-9] One significant factor contributing to this situation is the limited understanding of the molecular mechanisms and underlying impact factors that govern the actions of α-klotho at distant sites.

Numerous clinical studies have shown that α-klotho exhibits protective properties on the cardiovascular system in patients with end-stage renal disease.[4,10] It is tempting to draw the conclusion that renal damage, which leads to a decrease in α-klotho expression, may be responsible for this protective effect, considering that the kidney is the primary site of α-klotho expression.

However, certain studies have indicated that the correlation between α-klotho and cardiovascular diseases may not consistently align when individuals with renal diseases are excluded.[11-13] Therefore, it is imperative to establish conclusive evidence regarding this association among individuals from the general population who are at a moderate or high risk of developing cardiovascular disease.

The National Health and Nutrition Examination Survey (NHANES) is a national survey on nutrition and health information of the general population in the United States. NHANES measured concentrations of serum α-klotho in blood samples during the following cycles: 2007–2008, 2009–2010, 2013–2014 and 2015–2016. We designed a cross-sectional study by combining the four NHANES datasheets and investigated the association between serum α-klotho level and prevalence of heart failure in the general population.

Methods

The NHANES is a representative survey designed to understand the nutritional status and health in the United States. NHANES employs a complex, multistage, probability sampling design. The participants are representative of the civilian, non-institutionalised population. More details of NHANES can be found on the official website (https://www.cdc.gov/nchs/nhanes/).

The data to be analysed came from NHANES 2007–2008, 2009–2010, 2013–2014 and 2015–2016 cycles. We excluded participants with missing α-klotho data and those with missing data regarding the presence of heart failure. Our analysis finally included a total of 11 271 subjects. The study flow chart is available in Fig. 1.

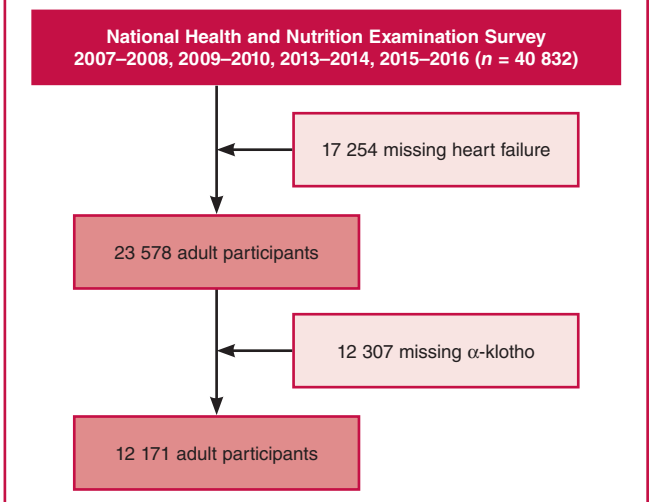

Fig. 1. Flow chart of the study.

All procedures in the NHANES survey cycles used in this study were approved by the National Center for Health Statistics Research Ethics Review Board. Written informed consent was obtained from all participants.

Demographic and lifestyle characteristics were collected via standard questionnaires. Race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic and other races. Educational level was categorised into below high school, high school and above high school.

Smoking status was described as never smoked, former smoker and current smoker. Non-smokers were defined as individuals smoking less than 100 cigarettes in their lifetime. Former smokers were defined as individuals smoking at least 100 cigarettes but who were not currently smoking. Current smokers were defined as individuals who currently smoked cigarettes every day or on some days.

Drinking habit was grouped into heavy drinker, moderate drinker and abstainer. Women who had two or more alcoholic drinks a day or men who had three or more were defined as heavy drinkers. Women who had up to two alcoholic drinks a day or men who had up to three were defined as moderate drinkers. Abstainers were defined as those who did not drink alcohol.

Income was evaluated by the poverty-to-income ratio (PIR) and categorised as low (≤ 1.3), middle (1.3–3.5) and high (> 3.5) income level.

Heart failure status was obtained from self-reported questionnaires: 'Has a doctor or other health professional ever told you that you had congestive heart failure?' Co-morbidities (cardiovascular diseases and diabetes mellitus) that had been diagnosed by a physician were obtained from the self-reported questionnaires. For these questionnaires, any answer other than 'no' was assumed to be 'yes'.

Physical examinations, such as body weight, height and blood pressure were performed according to a standard protocol. Body mass index was calculated as weight in kilograms divided by height in metres squared (kg/m^2).

Hypertension was defined as self-reported hypertension or self-reported prescription for anti-hypertensive medication. Participants with systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg were also considered hypertensive.

The NHANES provides a method file for each variable in the laboratory data. Additional information about the NHANES laboratory method document can be accessed on the official website (https://www.cdc.gov/nchs/nhanes/). To obtain the laboratory data, NHANES organisers followed established venipuncture protocols and procedures to collect either 3 ml or 5 ml of K3 EDTA anticoagulant whole blood from participants aged 18 years or older.

α-klotho concentration was analysed by a commercially available enzyme-linked immunosorbent assay (ELISA) kit produced by IBL International, Japan. The Northwest Lipid Metabolism and Diabetes Research Laboratories, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, performed analyses and ensured laboratory quality.

Other serum specimens were processed, stored and shipped to the Collaborative Laboratory Services, Ottumwa, Iowa for analysis. Enzymatic methods were employed using reagents to determine the concentration of creatinine, cholesterol, and triglycerides.

Statistical analysis

Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as means with standard deviation (SD). We calculated the differences among different α-klotho quartiles using the chi-squared or Fisher's exact test (categorical data) and the Kruskal–Wallis rank sum test (continuous data).

A multivariate logistic regression model was used to evaluate the association between α-klotho level and prevalence of heart failure. The results are expressed as odds ratios (ORs) and 95% confidence intervals (CIs). Interaction and stratified analyses were performed to evaluate potential effect modifiers. All analyses were performed with package R version 3.4.3 (http://www.R-project.org) and EmpowStats (http://www.empowerstats.com), with a p-value < 0.05 was considered statistically significant.

Results

Based on the inclusion and exclusion criteria, a total of 11 271 participants were enrolled. The mean age was 57.75 ± 10.89 years. Men accounted for 48.3% and women for 51.7% of participants. The mean concentration of α-klotho was 847.09 ± 307.55 pg/ml. Population characteristics of the participants stratified by α-klotho quartiles are shown in Table 1.

The prevalence of heart failure in this study was 4.13%, which was, respectively, 6.03, 3.91, 3.33 and 3.23% in α-klotho quartiles 1–4 (p < 0.05) (Table 1). Compared with the highest α-klotho quartile, participants in the lowest α-klotho quartile were mostly male, elderly, current smokers, heavy drinkers, had a higher serum level of creatinine and triglycerides, a lower educational level and higher prevalence of concomitant diseases (coronary heart diseases, diabetes mellitus and obesity).

Table 1. Baseline characteristics of the participants

		Serum α-klotho (pg/ml)				
Variables	*Overall*	*Q1*	*Q2*	*Q3*	*Q4*	p-*value*
Participants (*n*)	11271	2818	2816	2819	2818	
Serum α-klotho (pg/ml)	847.1 ± 307.6	538.4 ± 84.1	721.1 ± 41.3	881.6 ± 54.5	1247.1 ± 307	< 0.001
Heart failure prevalence, *n* (%)	465 (4.1)	170 (6.0)	110 (3.9)	94 (3.3)	91 (3.2)	< 0.001
Gender, *n* (%)						< 0.001
Male	5444 (48.3)	1462 (51.9)	1441 (51.2)	1325 (47.)	1216 (43.2)	
Female	5827 (51.7)	1356 (48.1)	1375 (48.8)	1494 (53.0)	1602 (56.9)	
Age group, *n* (%)						< 0.001
40–59 years	6102 (54.1)	1366 (48.5)	1486 (52.8)	1593 (56.5)	1657 (58.8)	
60–80 years	5169 (45.9)	1452 (51.5)	1330 (47.2)	1226 (43.5)	1161 (41.2)	
Race, *n* (%)						< 0.001
Non-Hispanic white	4991 (44.3)	1310 (46.5)	1320 (46.9)	1286 (45.6)	1075 (38.2)	
Non-Hispanic black	2080 (18.5)	523 (18.6)	443 (15.7)	450 (16.0)	664 (23.6)	
Mexican American	1933 (17.2)	483 (17.1)	487 (17.3)	477 (16.9)	486 (17.3)	
Other Hispanic	1295 (11.5)	293 (10.4)	306 (10.9)	324 (11.5)	372 (13.2)	
Other race	972 (8.6)	209 (7.4)	260 (9.2)	282 (10.0)	221 (7.8)	
Education level, *n* (%)						0.006
Below high school	3228 (28.6)	831 (29.5)	794 (28.2)	794 (28.2)	809 (28.7)	
High school	2522 (22.4)	687 (24.4)	607 (21.6)	643 (22.8)	585 (20.8)	
Above high school	5513 (48.9)	1296 (46.0)	1414 (50.2)	1379 (48.9)	1424 (50.5)	
Poverty-to-income ratio, *n* (%)						0.181
Low	3169 (28.1)	811 (28.8)	786 (27.9)	767 (27.2)	805 (28.6)	
Middle	3742 (33.2)	943 (33.5)	905 (32.1)	957 (34.0)	937 (33.3)	
High	3415 (30.3)	815 (28.9)	896 (31.8)	880 (31.2)	824 (29.2)	
Smoking status, *n* (%)						< 0.001
Never	5754 (51.1)	1269 (45.0)	1417 (50.3)	1482 (52.6)	1586 (56.3)	
Former	3284 (29.1)	908 (32.2)	825 (29.3)	806 (28.6)	745 (26.4)	
Current	2227 (19.8)	638 (22.6)	573 (20.4)	530 (18.8)	486 (17.3)	
Drinking behaviour, *n* (%)						< 0.001
Moderate	3642 (32.3)	863 (30.6)	924 (32.8)	950 (33.7)	905 (32.1)	
Heavy	3002 (26.6)	834 (29.6)	786 (27.9)	732 (26.0)	650 (23.1)	
Abstainer	4627 (41.1)	1121 (39.8)	1106 (39.3)	1137 (40.3)	1263 (44.8)	
Body mass index (kg/m^2), *n* (%)						0.003
18.5–24.9	2509 (22.3)	573 (20.3)	615 (21.8)	635 (22.5)	686 (24.3)	
25–29.9	3852 (34.2)	994 (35.3)	958 (34.0)	980 (34.8)	920 (32.7)	
≥ 30	4651 (41.3)	1197 (42.5)	1161 (41.2)	1139 (40.4)	1154 (41.0)	
Hypertension, *n* (%)	6031 (53.5)	1544 (54.8)	1510 (53.6)	1487 (52.8)	1490 (52.9)	0.394
Coronary heart diseases, *n* (%)	592 (5.25)	198 (7.03)	139 (4.94)	135 (4.79)	120 (4.26)	< 0.001
Diabetes mellitus, *n* (%)	2021 (17.9)	560 (19.9)	467 (16.6)	466 (16.5)	528 (18.7)	0.034
Total cholesterol (mmol/l)	5.2 ± 1.1	5.2 ± 1.2	5.2 ± 1.1	5.2 ± 1.1	5.1 ± 1.1	0.827
Triglycerides (mmol/l)	1.9 ± 1.6	2.0 ± 2.1	1.9 ± 1.4	1.9 ± 1.4	1.8 ± 1.5	< 0.001
Serum creatinine (mmol/l)	79.6 ± 44.2	88.4 ± 70.7	79.6 ± 44.2	79.6 ± 35.4	79.6 ± 26.5	< 0.001

Values are mean ± standard deviation or *n* (%). Q, quartile.

Table 2. Association between serum α-klotho level and prevalence of heart failure in different models

Serum α-klotho (pg/ml)	*Crude model*		*Model I*[a]		*Model II*[b]	
	OR (95% CI)	*p-value*	*OR (95% CI)*	p-*value*	*OR (95% CI)*	p-*value*
Per-SD increase	0.76 (0.68, 0.85)	< 0.001	0.80 (0.71, 0.89)	< 0.001	0.85 (0.75, 0.95)	0.005
Quartiles						
Q1	Reference		Reference		Reference	
Q2	0.63 (0.50, 0.81)	< 0.001	0.67 (0.52, 0.86)	0.002	0.77 (0.58, 1.01)	0.06
Q3	0.54 (0.42, 0.70)	< 0.001	0.61 (0.47, 0.79)	< 0.001	0.70 (0.52, 0.93)	0.01
Q4	0.52 (0.40, 0.67)	< 0.001	0.58 (0.45, 0.76)	< 0.001	0.71 (0.53, 0.95)	0.02
p-value for trend		< 0.001		< 0.004		0.038

[a]Model I adjusted for age, gender and race.
[b]Model II adjusted for age, gender, race, poverty-to-income ratio, educational level, smoking status, drinking behaviour, hypertension, coronary heart diseases, diabetes mellitus, body mass index, and cholesterol, triglyceride and creatinine levels. SD, standard deviation; CI, confidence interval; OR, odds ratio.

Table 2 shows the association between α-klotho and the prevalence of heart failure. In the crude model, a per-standard deviation (per-SD) increase in α-klotho level was associated with a decrease in the prevalence of heart failure (OR: 0.76, 95% CI: 0.68–0.85).

In a minimally adjusted model for age, gender and race, the OR for heart failure was 0.80 (95% CI: 0.71–0.89). After progressive adjustment for various cardiovascular risk factors, the negative association was maintained in the fully adjusted model (OR: 0.85, 95% CI: 0.75–0.95).

When α-klotho was converted into a categorical variable, the ORs for prevalence of heart failure decreased across α-klotho quartiles. In the all-adjusted model, the ORs for participants in α-klotho quartiles 2–4 were 0.77 (95% CI: 0.58–1.01), 0.70 (95% CI: 0.52–0.93) and 0.71 (95% CI: 0.53–0.95), respectively, compared with those in quartile 1. The trend remained among all models and the tests for trend were significant, as described in Table 2 (*p*-value for trend = 0.038).

We performed stratified analyses to assess the possible effect modifiers of α-klotho level on prevalence of heart failure in different subgroups. The results showed gender (*p*-value interaction = 0.01) and race (*p*-value interaction = 0.03) yielded a significant interaction with the association between α-klotho level and heart failure (Fig. 2). The correlation of α-klotho level with prevalence of heart failure remained significantly negative in males (OR: 0.67, CI: 0.57–0.78) and in non-Hispanic blacks (OR: 0.64, CI: 0.53–0.79), but was positive in other Hispanics (OR: 1.10, CI: 0.88–1.39).

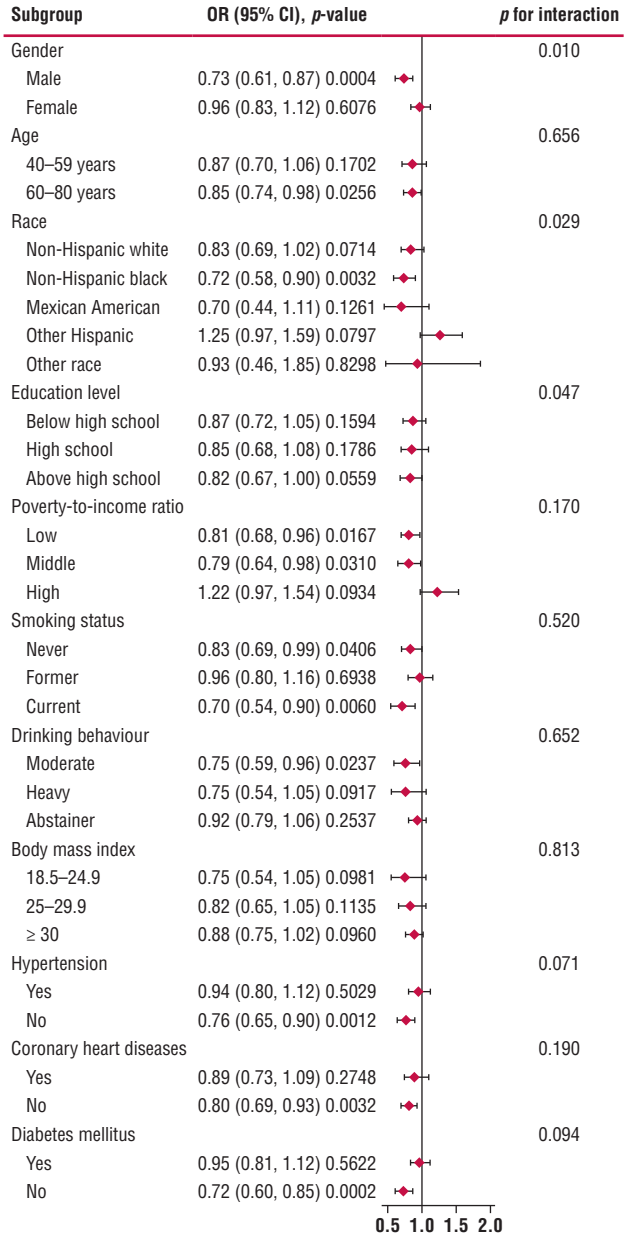

Fig. 2. Stratified analyses of the association between serum α-klotho level and prevalence of heart failure. BMI, body mass index; PIR, poverty-to-income ratio; SD, standard deviation; OR, odds ratio; CI, confidence interval.

Discussion

An association between α-klotho and cardiovascular events was first reported in patients with end-stage renal disease.[10] Kidney damage most likely contributed to an absolute reduction of α-klotho production and so α-klotho had a negative relationship with adverse cardiovascular outcomes in this particular population. Nevertheless, no significant change was expected in serum α-klotho levels in the general population without renal disease.[14]

Meanwhile some evidence suggested that the association between α-klotho and cardiovascular events was not always similar, although these studies did not negate any protective effect of α-klotho on the cardiovascular system.[12,13] These various results stimulate interest in discovering the unknown mechanism of α-klotho in heart failure and how its effect impacts on the patient's outcome.

NHANES offered the advantage of a large sample size and a standardised design that matches that of population-based studies. As far as we know, this was the first study to address the association between α-klotho and prevalence of heart failure in the general population. We discovered that lower α-klotho level was independently linked to higher prevalence of heart failure. Controlling for confounding factors, including demographic

and lifestyle characteristics, serum lipid levels, serum creatinine concentration and concomitant diseases did not considerably change the results. Likewise, a community-based study also found that adults with higher α-klotho levels were less likely to develop cardiovascular diseases.[15] The Prevention of Events with Angiotensin-Converting Enzyme (PEACE) trial showed patients with stable ischaemic heart disease who had lower α-klotho concentrations were at higher risk of cardiovascular mortality or hospitalisation for heart failure.[16]

Most of the cell and animal experimental studies have validated the direct protective effects of α-klotho on the cardiovascular system. Kawarazaki *et al*. and Chen *et al*. revealed that klotho-deficient mice exhibited salt-sensitive hypertension, cardiovascular remodelling and impaired heart function.[17,18] Xie *et al*. showed that α-klotho over-expression attenuated stress-induced cardiac hypertrophy and dysfunction.[19] Chen *et al*. found that exogenous α-klotho supplementation delayed cardiac aging in klotho-deficient mice and old mice.[18]

However, some studies present different perspectives. Svetlana *et al*. claimed there is no evidence that klotho plays a critical role in pressure overload-induced hypertrophy of the murine heart.[7] Shibata *et al*. and Poelzl *et al*. suggested that α-klotho levels did not correspond to cardiac function or severity of heart failure.[13,20] Manabu *et al*. drew the opposite conclusion and proposed that α-klotho level had a compensatory increase in heart failure patients.[21] Moreover, Poelzl *et al*. found that soluble α-klotho expression was increased in diseased heart specimens where α-klotho is barely expressed, and suggested a possible paracrine effect.[20] These discrepancies in the various studies are because the mechanisms by which α-klotho exerts its protective effect still remain unclear and poorly understood.[9]

Circulating α-klotho may include three components: long-form α-klotho, short-form α-klotho and secreted α-klotho. Because the sequences between different α-klotho forms are highly conserved, they are difficult to distinguish from each other. Compared to the full-length trans-membrane klotho in kidney tissues, the binding sites and receptors of circulating α-klotho have not been determined in the cardiovascular system. In addition, we do not know under what circumstances paracrine effects occur and how paracrine effects play a protective role in the cardiovascular system. Discovering these answers will improve our understanding of the complex association between α-klotho and risk of heart failure.

Moreover, to examine whether the outcomes were consistent, sensitivity analyses were performed with stratification to clarify potential confounding factors. Most of the investigated stratified subgroup analysis showed that α-klotho was independently associated with heart failure risk.

Gender- and race-stratified models were also analysed. Our study showed that lower α-klotho level was significantly associated with a higher prevalence of heart failure in males. Gender variations have been found in other population studies as well. Hideaki *et al*. indicated α-klotho was positively associated with aortic valve calcification only in men, independent of the confounding variables.[22] Dhayat *et al*. showed the association between α-klotho and parathyroid hormone level was positive for men but negative for women in the general population with mainly preserved kidney function.[23]

There were also differences found based on ethnicity. For non-Hispanic blacks, lower levels of α-klotho were independently associated with an increased prevalence of heart failure. For other Hispanics, the trend towards decreased prevalence was opposite to that of all other ethnic groups, although the difference was not significant.

Other studies about the association between α-klotho and blood pressure have also shown racial disparity. Drew *et al*. indicated that an elevated klotho level was associated with a higher baseline diastolic but not systolic blood pressure in a large community-dwelling and multiracial cohort study.[24] However, Liang *et al*. revealed that there was no correlation between serum α-klotho level and blood pressure in the general Chinese population.[25]

It appears that the function of α-klotho is regulated by stable factors within different individuals, which may be genetic or environmental. An important example is the genetic polymorphism of α-klotho. Gao *et al*. found that the genotype distribution of G-395A was significantly different in female hypertensive patients but not in males.[26] Friedman *et al*. showed the klotho variants (rs577912) would be more closely linked with mortality from haemodialysis in whites than in blacks.[27]

The klotho variants modulate klotho expression or influence its function, which may lead to gender and racial disparities at the onset of disease and affect the severity of disease phenotypes.[27-30] In addition, genetic or environmental factors contribute to epigenetic changes, which may also play an important role in disease development and assist in explaining gender and racial differences.[31,32]

Accumulated evidence suggests the potential for α-klotho administration as a promising therapeutic strategy to protect against age-related cardiovascular diseases.[4,9,33] Identifying which individuals are suitable for α-klotho interventions will be one of the key challenges. Therefore, our results may help understand individual therapeutic responsiveness to α-klotho application and determine suitable therapeutic windows in the future.

Study limitations

This study has several limitations that should be considered. First, despite being more inclusive than registries, self-reported information relies on patients knowing their diagnoses and could be subject to recall bias. Researchers often combine self-reported data with other sources of information, such as medical records or clinical examinations, to obtain a more comprehensive and accurate understanding of the prevalence of heart failure. Additionally, efforts are made to ensure the anonymity and confidentiality of participants to encourage honest and unbiased reporting.

Second, the cross-sectional design is unable to establish causality. Third, the study population was from the USA, therefore, the results should be validated in other population groups.

Conclusion

We revealed an independent association between serum α-klotho and prevalence of heart failure in the general population, which may vary based on gender and race. Further research is needed to clarify the causal relationship and underlying mechanisms in the wider context, such as genetic and environmental background factors.

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