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ORIGINAL RESEARCH

Fibroblast Growth Factor 23 as a Prognostic Biomarker in Post-Myocardial Infarction Outcomes: Influence of Renal Function and Its Modulation by Klotho

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BACKGROUND: Elevation of FGF23 (fibroblast growth factor 23) and decreased Klotho levels have been associated with various cardiovascular and renal diseases. However, the combined study of the FGF23–Klotho axis in ischemic heart disease remains elusive.

METHODS: We analyzed the associations between circulating FGF23 and Klotho levels with cardiac and renal parameters, as well as mortality outcomes following myocardial infarction (MI). Cardiac tissue from patients with ischemic heart disease and a post-MI mouse model were analyzed to assess myocardial FGF23 expression. Proteomic analysis was performed to examine myocardial pathways activated by FGF23 and regulated by Klotho.

RESULTS: We observed an inverse correlation between circulating levels of FGF23 and Klotho in patients after MI. Elevated plasma FGF23 levels were particularly associated with ST-segment–elevation MI and cardiac dysfunction, including reduced left ventricular ejection fraction, prolonged corrected interval, and higher Killip–Kimball classification of cardiac risk, identifying FGF23 as a potential prognostic marker of overall and cardiac-related mortality. In contrast, systemic Klotho levels were reduced in patients with ST-segment–elevation MI but did not correlate with mortality. In cardiac tissue, ischemic injury significantly upregulated FGF23 expression. Although Klotho prevented FGF23-induced proteomic alterations, it did not inhibit cardiac FGF23 overexpression in the post-MI model.

CONCLUSIONS: FGF23 represents a promising biomarker for mortality and cardiac dysfunction in patients with MI. Although Klotho does not appear to be a reliable predictor of mortality after MI, its cardioprotective properties suggest a role in modulating FGF23-driven metabolic, structural, and proliferative processes in the myocardium.

Key Words: FGF23 ■ ischemic heart disease ■ Klotho ■ mortality ■ myocardial infarction

Ischemic heart disease (IHD) is a leading cause of morbidity and mortality worldwide and arises from reduced coronary blood flow, which results in myocardial oxygen deprivation. The primary clinical manifestation of IHD is myocardial infarction (MI),[1,2] which is categorized as ST-segment–elevation MI

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RESEARCH PERSPECTIVE

What is New?

- Following myocardial infarction, elevated circulating levels of FGF23 (fibroblast growth factor 23), but not reduced levels of Klotho, are strongly associated with adverse cardiac outcomes and increased cardiovascular mortality, particularly in patients with ST-segment–elevation myocardial infarction, highlighting FGF23 as a relevant biomarker linked to cardiac risk after myocardial infarction.
- Supplementation with recombinant Klotho appears to exert cardioprotective effects by attenuating FGF23-dependent signaling pathways in cardiac tissue.

What Question Should Be Addressed Next?

- This study raises the question of whether targeting the FGF23–Klotho axis may improve post-myocardial infarction cardiac prognosis, and future research should investigate the therapeutic potential and mechanisms of Klotho supplementation in ischemic heart disease.

Nonstandard Abbreviations and Acronyms

FGF23	fibroblast growth factor 23
FGFR1	fibroblast growth factor receptor 1
GRACE	Global Registry of Acute Coronary Events
IHD	ischemic heart disease
PMI	post-myocardial infarction
QTc	corrected QT interval
TGF-β	transforming growth factor β

(STEMI) or non–ST-segment–elevation MI (NSTEMI) on the basis of ECG findings.[3] Following an acute ischemic event, the myocardium undergoes a complex remodeling process. Initially, compensatory hypertrophy occurs in noninfarcted regions of the heart to maintain cardiac output. However, over time, this adaptative mechanism can progress to left ventricular dilatation, impairing ventricular function and increasing the risk of heart failure (HF), ultimately leading to cardiovascular death in many cases. The prognosis is particularly poor in patients with concomitant renal dysfunction, because reduced renal function is a strong predictor of adverse outcomes, including higher rates of in-hospital mortality and long-term mortality from any cause.[4] Alterations in mineral metabolism, including increased circulating levels of FGF23 (fibroblast growth factor 23) and decreased levels of Klotho, an antiaging factor, are established biomarkers of progressive renal disease.[5,6] The FGF23–Klotho axis plays a critical role in phosphate homeostasis. FGF23, a phosphaturic hormone, primarily acts on the kidneys, inhibiting sodium–phosphate cotransporters in the proximal tubule to increase urinary phosphate excretion.[7] This action requires Klotho, a necessary cofactor for FGF23 to bind to and activate FGFR1 (FGF23 receptor 1) in the renal tubules.[8] In addition to its established renal role, the FGF23–Klotho axis has recently emerged as a significant factor in cardiac tissue.[9,10] Although the precise role of circulating and cardiac FGF23 is still under investigation, it has been proposed to contribute to the complex interplay between cardiomyocytes, fibroblasts, endothelial cells, and macrophages through autocrine or paracrine signaling.[11] Similarly, soluble systemic Klotho has been implicated in cardiovascular diseases, with lower levels associated with the presence and severity of HF.[12] Experimental studies, including our own, have demonstrated the cardioprotective effects of Klotho therapy in various cardiovascular and renal pathologies. These benefits include anti-inflammatory effects, the mitigation of necrosis and maladaptive cardiac and electrical remodeling, the prevention of intracellular Ca^{2+} mishandling, and the reduction of arrhythmic events in cardiomyocytes.[13–17] These findings suggest that exogenous Klotho supplementation could be a promising therapeutic strategy for cardiorenal disease. Despite growing evidence supporting the roles of FGF23 and Klotho in cardiovascular pathology, their specific interplay remains unclear. There is a need for additional predictive biomarkers to improve early risk stratification following MI and to identify novel therapeutic strategies. This study investigated the potential role of the FGF23–Klotho axis in IHD by evaluating its function as a potential predictive biomarker for adverse outcomes after MI as a therapeutic target.

METHODS

Data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure, upon reasonable request to the corresponding author. Ethical approval was obtained from the Clinical Trials and Medicines Ethics Committee of the University Hospital 12 de Octubre (16/250). All procedures were conducted in accordance with the Declaration of Helsinki and approved through the Biobanks and Biomodels Platform of the Carlos III Health Institute. Signed informed consent was obtained from all patients. The study involving experimental animals was approved by the Bioethics Committee of the

Autonomous University of Madrid and the Directorate General of Agriculture and Environment, Community of Madrid (PROEX 186.5/20), according to the Guide for the Care and Use of Laboratory Animals and Ethical Care and Welfare Guidelines (2013/175) of the European Union (2010/63/EU).

Study Population

This observational study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[18] Eligible patients were recruited from 2 independent cohorts. We performed a retrospective analysis of 2 cohorts of patients. The first cohort included 148 patients (aged >18 years) diagnosed with MI. The plasma samples collected at hospital admission were provided by the biobank of the University Hospital Puerta del Hierro Majadahonda (Madrid, Spain). The second cohort comprised 10 patients with IHD and 7 healthy controls. In this cohort, cardiac tissue samples were obtained from the biobank of the Principado de Asturias (Oviedo, Spain).

Experimental Post-Myocardial Infarction Model

All experimental procedures involving animals were conducted in accordance with the Animal Research: Reporting of In Vivo Experiments guidelines to ensure transparency and reproducibility in animal research.[19] C57BL/6J adult mice underwent permanent ligation of the left anterior descending coronary artery. The effectiveness of the MI murine model was confirmed by quantification of infarct size 24 hours after permanent left anterior descending coronary artery ligation using Evans Blue and 2,3,5-triphenyltetrazolium chloride staining. Sham-operated and MI mice were randomly sex-matched and assigned to 2 groups. Each group received daily intraperitoneal injections of either 0.9% sodium chloride (vehicle) or recombinant murine Klotho (10 μg/kg per day) (1819-KL-050; R&D Systems, Minneapolis, MN). Sham or post-MI (PMI) surgery mice were blindly randomized using simple randomization to receive either vehicle solution (0.9% sodium chloride) or murine recombinant Klotho (10 μg/kg per day). The animals were distributed into the following 4 experimental groups: sham mice treated with vehicle (Sham) or recombinant Klotho (Sham+Klotho) and PMI mice treated with vehicle (PMI) or recombinant Klotho (PMI+Klotho). All the animals were euthanized 15 days postsurgery, and heart tissue samples were collected for molecular analysis. A total number of 65 mice were used to perform all experimental procedures; 7 animals died before the predefined end point, and 2 were excluded according to the exclusion criteria. Twenty-four hours after surgery, an ECG was recorded for the inclusion/exclusion criteria. Thus, animals were included for subsequent analyses only if they exhibited a clear deep and wide negative Q wave on the 24-hour ECG, indicative of a transmural infarct and significant ischemic necrosis. This variable was used as the primary outcome. Animals that did not show this electrocardiographic pattern were excluded. Following this criterion, 2 mice were excluded.

Analysis of Systemic FGF23, Klotho, and Phosphorus Levels

Plasma samples from patients with MI were analyzed for systemic levels of FGF23, Klotho, and phosphates via the following commercial ELISA kits: microVue human FGF23 C-Term (Quidel, San Clemente, CA), human soluble α Klotho (IBL-America, Minneapolis, MN), and a colorimetric phosphorus assay kit (Abcam, Cambridge, United Kingdom).

Real-Time Quantitative Polymerase Chain Reaction, Western Blotting, and Immunohistochemistry

For molecular analyses (protein and RNA extraction) of murine FGF23, the entire left ventricles were processed, excluding the infarcted area. Paraffin-embedded and frozen tissues from human left ventricles were used for immunohistochemistry and protein/RNA extraction. Real-time quantitative polymerase chain reaction was performed on a LightCycler 480 II instrument (Roche, Basel, Switzerland) in 96-well plates with FastStart Essential DNA Green Master Mix (Roche). Western blotting analysis was used to detect FGF23 protein expression. Bands were visualized via chemiluminescence with an enhanced chemiluminescence substrate (Thermo Fisher Scientific, San Jose, CA) and detected with an ImageQuant LAS4000 imaging system (GE Healthcare, Piscataway, NJ) via ImageQuant TL 8.1 software. Cardiac tissue samples were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, embedded in paraffin, and processed for FGF23 immunohistochemical staining. Sections were examined under blinded conditions using an optical microscope (BX41; Olympus, Tokyo, Japan). For murine immunohistochemistry, myocardial sections were consistently taken from the left ventricle approximately 2 to 3 mm below the left atrium, using the ligation site as reference.

Proteomic Analysis

A total of 9 healthy Wistar rats (200 g) were used to perform this proteomic analysis. Rat hearts were retrogradely perfused via the ascending aorta with

vehicle (0.9% saline solution), FGF23 (100 ng/mL), or Klotho (100 ng/mL), which was administered 20 minutes before the administration of FGF23. Perfusion was conducted via a Langendorff system at 37 °C. Recombinant murine FGF23 and Klotho were purchased from R&D Systems. Frozen heart samples were lysed. Protein extracts were digested via the filter-aided sample preparation method.[20] High-resolution analysis of the tandem mass tag-labeled peptides was conducted on an Ultimate 3000 high-performance liquid chromatography system (Thermo Fisher Scientific) coupled to a Tribrid Orbitrap mass spectrometer (Orbitrap Fusion; Thermo Fisher Scientific). For peptide identification, the tandem mass spectrometry spectra were searched against the UniProt rat protein database (UniProt_Nov 2017; 29 971 sequences) via the Sequest algorithm[21] implemented in Proteome Discoverer 2.1 (Thermo Fisher Scientific,[22] concatenated with decoy sequences generated via DecoyPyrat).[23] The iSanXoT program[24,25] was used to quantify the tandem mass tag reporter ion intensities of the fragmentation spectra. Quantitative information was integrated from the spectrum to the peptide and protein levels via the weighted spectrum, peptide, and protein statistical model[26] and the generic integration algorithm.[27] Quantitative protein values are expressed via the standardized variable Zq (ie, normalized log2 ratios expressed in units of standard deviation according to the estimated variances). Twenty-one proteins of interest were analyzed via online DAVID (Database for Annotation, Visualization, and Integrated Discovery) Bioinformatics Resources software (v2023q4), and their functions were assessed according to Gene Ontology terms.[28,29] Cord graphs were plotted via a free online platform for data analysis and visualization, representing biological process terms according to Gene Ontology (https://www.bioinformatics.com.cn/en).

Statistical Analysis

Parametric comparisons of means were evaluated via Student *t* test or 1-way ANOVA, followed by the Newman–Keuls test post hoc. The Spearman coefficient was used to assess correlations. Binary logistic regression analysis was used to develop mortality prediction models, and their performance was evaluated via receiver operating characteristic analysis to calculate the area under the curve. Direct comparisons between areas under the curve were performed using the paired method described by Hanley and McNeil for correlated receiver operating characteristic curves.[30] Optimal cutoff points were identified via the Youden index. Multivariate linear regression analysis was performed to evaluate the relationship between FGF23 levels and the Killip–Kimball classification adjusted by age, sex, estimated glomerular filtration rate (eGFR), or C-reactive protein. Data analysis was performed via GraphPad Prism version 10 software (GraphPad Software, La Jolla, CA) or SPSS Statistics v22 (IBM, Armonk, NY). The data are presented as the means±SDs, and statistical significance was indicated as specific *P* values in each figure.

A more extensive section of material and methods is provided in Data S1.

RESULTS

Elevated Systemic FGF23 and Lower Klotho Levels Are Linked to Increased Myocardial Damage in Patients With MI

The demographic characteristics of the 148 patients with MI included in the study are presented in Table S1. No differences were observed in FGF23 levels by the presence or absence of major comorbidities/risk factors such as diabetes, hyperlipidemia, hypertension, previous IHD, or smoking, as well as by sex (data not shown). FGF23 levels were stratified by quartiles of systemic FGF23 levels (reference units per milliliter) in Table S2. Although sex distribution and body mass index were similar across quartiles, significant differences were observed in age and cardiac mortality. Patients in the highest FGF23 quartile (Q4) were older and exhibited a greater incidence of cardiac death. Medical history, including hypertension, dyslipidemia, diabetes, current smoking, or previous IHD, was similar across quartiles. However, there was a significant difference in the percentage of patients with STEMI across FGF23 quartile levels, with a lower percentage of patients with STEMI in Q1. With respect to laboratory findings, significant differences were found in C-reactive protein, with higher FGF23 levels associated with increased inflammation. No significant differences were found in N-terminal pro-brain natriuretic peptide, high-sensitivity troponin I (TnI), phosphate, or Klotho levels between quartiles. A significant inverse correlation was identified between systemic FGF23 and Klotho levels (r=−0.166; P=0.043). Compared with patients with NSTEMI, patients with STEMI presented significantly higher systemic FGF23 levels (Figure 1A) and lower Klotho levels (Figure 1B), suggesting an association between elevated FGF23, reduced Klotho, and greater myocardial ischemic injury characteristic of STEMI. To further investigate this relationship, patients were stratified by renal function: eGFR ≥90 mL/min per 1.73 m^2 (preserved) and eGFR <90 mL/min per 1.73 m^2 (impaired). Among patients with preserved renal function, those with STEMI had significantly higher FGF23 levels and lower Klotho levels (Figure 1C and 1D). Compared with patients with NSTEMI, patients with STEMI presented elevated FGF23 and reduced Klotho levels in impaired renal

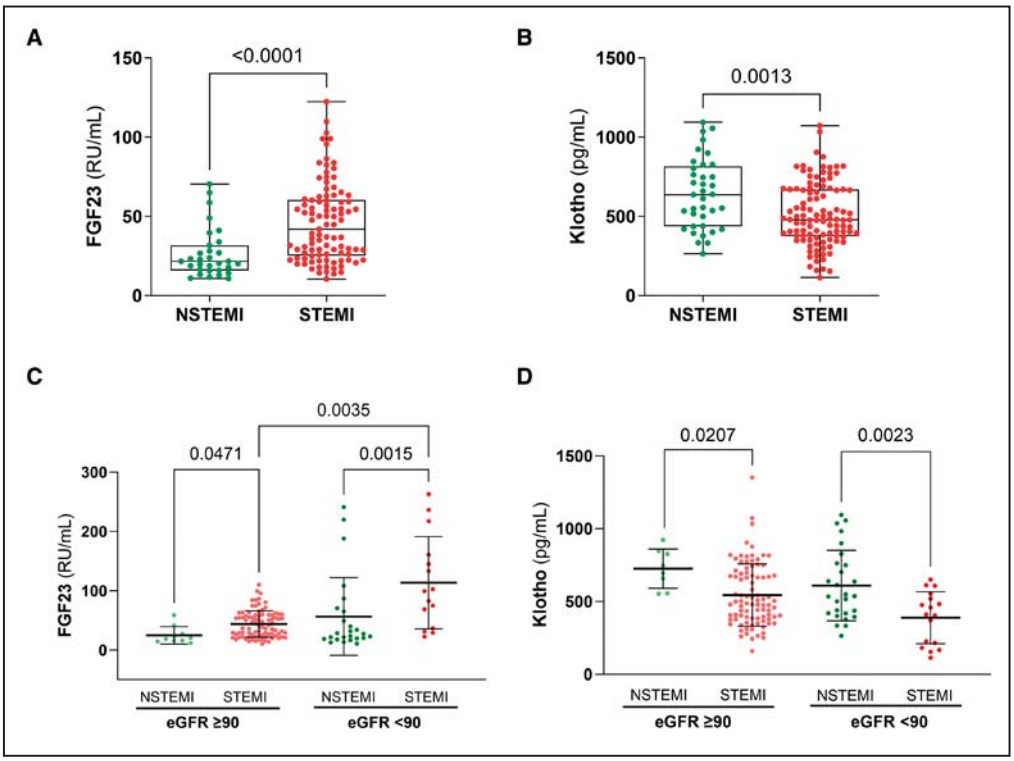

Figure 1. FGF23 and Klotho levels and their relationship with renal function in patients with ischemic heart disease.

Plasma levels of (**A**) FGF23 and (**B**) Klotho in patients with NSTEMI (N=31 and N=37, respectively) and patients with STEMI (N=97 and N=107, respectively). Plasma levels of (**C**) FGF23 and (**D**) Klotho in patients with NSTEMI and STEMI classified by renal function (eGFR ≥90 mL/min per 1.73 m^2 or <90 mL/min per 1.73 m^2). Beeswarm plots show the median, the interquartile range (25th–75th percentiles), and the minimum and maximum values. Scatter plots show the mean±SD. eGFR indicates estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; NSTEMI, non–ST-segment–elevation myocardial infarction; and STEMI, ST-segment–elevation myocardial infarction.

function (Figure 1C and 1D). Notably, patients with STEMI with impaired renal function had significantly higher FGF23 levels than did those with preserved renal function (Figure 1C), and there was a trend toward lower Klotho levels (Figure 1D). Overall, these findings suggest that elevated systemic FGF23 and lower Klotho levels are associated with more severe myocardial ischemic damage in patients with STEMI regardless of renal function, although these changes are exacerbated by renal impairment.

Elevated Systemic FGF23 Plasma Levels in Patients With MI Are Associated With Long-QT Syndrome, Reduced Left Ventricular Ejection Fraction, and Increased Killip–Kimball Classification

Patients were first stratified by the corrected QT (QTc) interval at hospital discharge; a prolonged QTc interval ≥450 ms is linked to an increased risk of ventricular arrhythmias and sudden cardiac death.[31] Patients with a prolonged QTc ≥450 ms had significantly higher systemic FGF23 levels than did those with a normal QTc interval (<450 ms; Figure 2A). Patients with a reduced left ventricular ejection fraction (LVEF) (<50%), indicative of impaired cardiac function,[31] presented higher, although not statistically significant, FGF23 levels than those with a preserved LVEF (≥50%; Figure 2C). These associations remained significant in patients with normal renal function (eGFR ≥90 mL/min per 1.73 m^2; Figure 2B and 2C), whereas no significant differences were observed in patients with eGFRs <90 mL/min per 1.73 m^2. To further evaluate cardiac risk, the Killip–Kimball scale was used to stratify patients into 4 classes on the basis of cardiac complications[32]: low-risk (Killip I) and high-risk (Killip II–IV). Systemic FGF23 levels were significantly elevated in high-risk patients compared with low-risk patients (Figure 2E). These associations remained significant in patients with eGFR <90 mL/min per 1.73 m^2 (Figure 2F). Furthermore, among high-risk patients, those with impaired renal function presented the highest FGF23 levels (Figure 2F). Multivariate linear regression analysis confirmed an association between

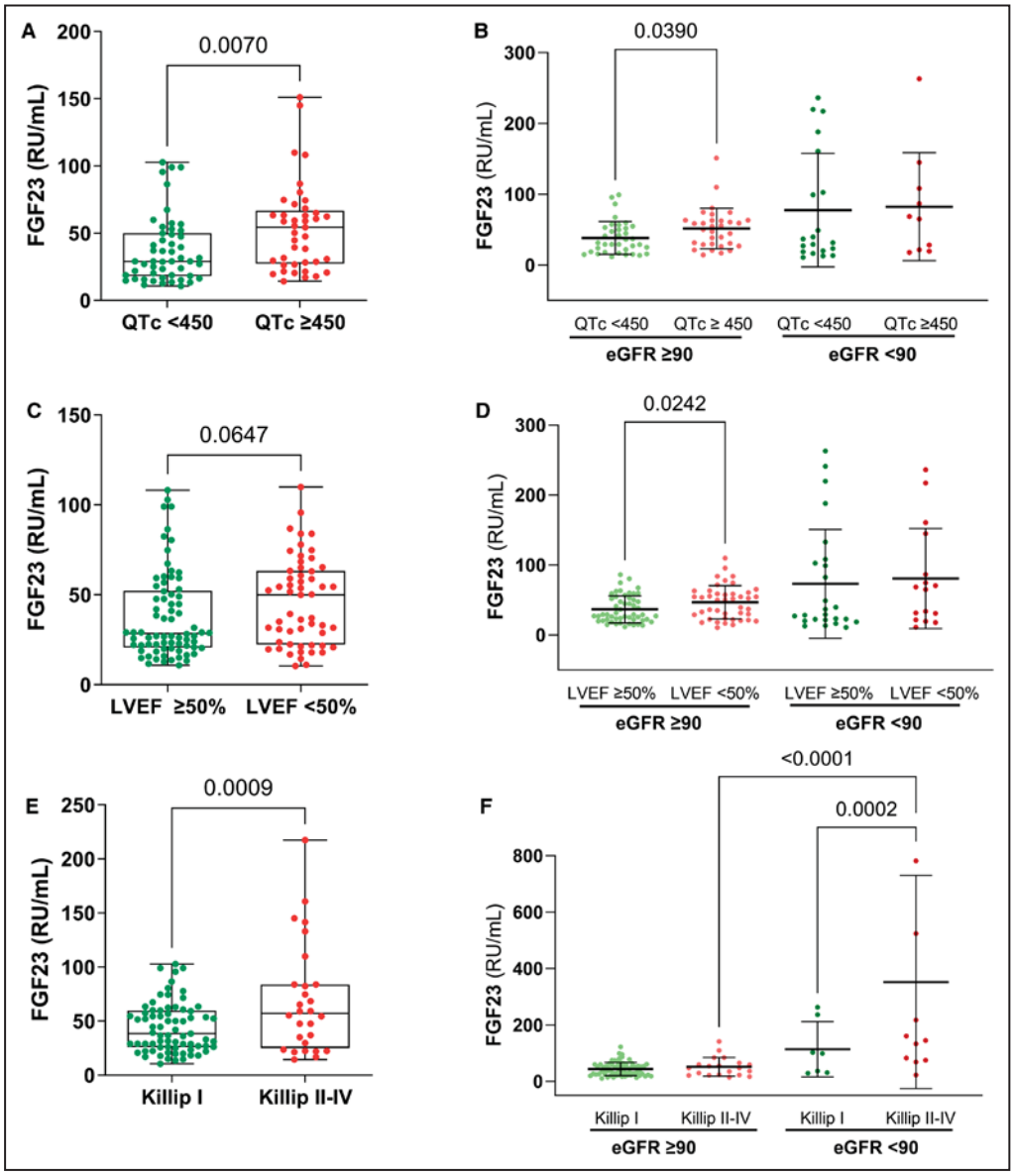

Figure 2. FGF23 levels and their relationship with cardiac function, rhythm, and risk in patients with ischemic heart disease.
Systemic levels of FGF23 in patients after myocardial infarction based on QTc values at discharge (<450 ms or ≥450 ms) (N=50 and N=41, respectively) (**A**), according to cardiac function (LVEF ≥50% or <50%) (N=74 and N=55, respectively) (**C**), according to the Killip–Kimball classification in low-risk (Killip I) and high-risk (Killip II–IV) patients (**E**), and after stratification by renal function (eGFR ≥90 mL/min per 1.73 m^2 or <90 mL/min per 1.73 m^2) (**B**, **D**, **F**). Beeswarm plots show the median, the interquartile range (25th–75th percentiles), and the minimum and maximum values. Scatter plots show the mean±SD. eGFR indicates estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; LVEF, left ventricular ejection fraction; and QTc, corrected QT.

plasma FGF23 levels and the Killip–Kimball classification, independent of age, sex, eGFR, or C-reactive protein (Table 1). These findings suggest that elevated systemic FGF23 levels are associated with worse prognosis and cardiac conditions, highlighting the significant impact of renal impairment on FGF23 levels and underscoring the prognostic value of FGF23 as a biomarker of adverse cardiac outcomes after MI.

Systemic FGF23, but Not Klotho, Demonstrates Predictive Value for Mortality in Patients With MI at Hospital Admission

The predictive value of FGF23 and Klotho for all-cause mortality and cardiac-specific mortality was assessed in patients with MI. Compared with survivors,

Table 1. Multivariate Linear Regression Analysis of the Contribution of FGF23 Plasma Levels to Killip–Kimball Classification

	Killip–Kimball			
	β	Upper limit	Lower limit	*P* value
Not adjusted	0.003	0.004	0.002	<0.001
Adjusted by age and sex	0.002	0.003	0.001	<0.001
Adjusted by eGFR	0.002	0.003	0.001	0.002
Adjusted by CRP	0.0025	0.004	0.001	0.007

CRP indicates C-reactive protein; eGFR, estimated glomerular filtration rate; and FGF23, fibroblast growth factor 23.

nonsurvivors presented significantly higher systemic FGF23 levels (Figure 3A), whereas no significant differences in systemic Klotho levels were observed between these groups (Figure 3B). Similarly, cardiac death was associated with elevated FGF23 levels in patients after MI (Figure 3D), whereas Klotho plasma levels remained unchanged (Figure 3E). To assess the ability of these biomarkers to be associated with mortality, we analyzed the area under the curve of the receiver operating characteristic curves (Figure 3C and 3F; Table 2). The results revealed that systemic FGF23, but not Klotho, effectively differentiated between patients with MI who died and those who survived, irrespective of the cause of death (*P*=0.0014 for all-cause mortality and *P*=0.0073 for cardiac-specific mortality; Table 2). Furthermore, the Youden index identified cutoff values for systemic FGF23 of 64.2 RU/mL for all-cause mortality and 65.2 RU/mL for cardiac-specific mortality, which yielded a positive predictive value of 0.73 and a negative predictive value of 0.88, indicating a low probability of false negatives. The area under the curve comparisons between FGF23 and Klotho for both all-cause and cardiac mortality did not reach statistical significance. These findings suggest that FGF23, unlike Klotho, may serve as a valuable prognostic biomarker for mortality risk assessment in patients with MI.

Cardiac FGF23 Is Overexpressed in the Ischemic Myocardium and Remains Elevated in the Presence of Klotho

To investigate the impact of ischemic cardiomyopathy on cardiac FGF23 levels, we examined cardiac tissue from patients with IHD. Compared with healthy donors, patients with IHD presented significantly elevated levels of cardiac FGF23 at both the mRNA (Figure 4A) and protein (Figure 4B) levels. Immunohistochemical analysis confirmed strong cardiac FGF23 overexpression (brown staining) in both transverse (Figure 4C, panel b)

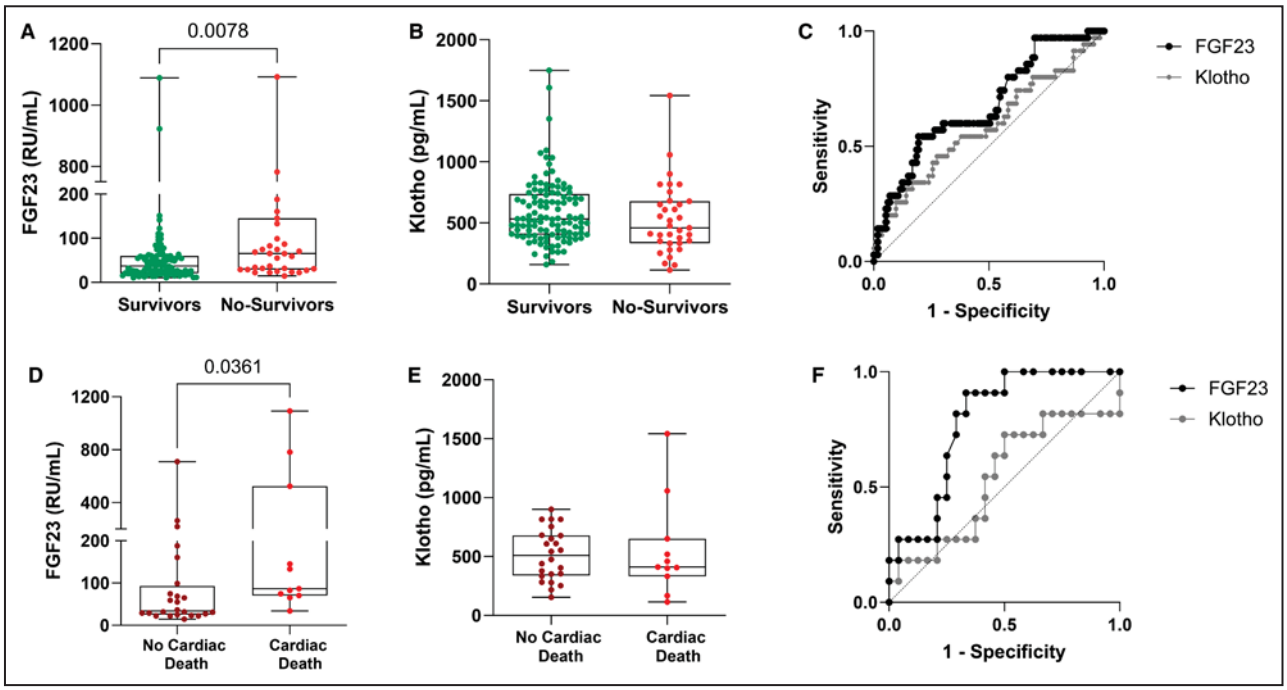

Figure 3. Systemic FGF23, but not Klotho, predicts global and cardiac mortality in patients after myocardial infarction. Systemic levels of FGF23 (**A**) and Klotho (**B**) in surviving (N=113) and nonsurviving (N=35) patients. **C**, Receiver operating characteristic curves of FGF23 and Klotho levels and their capacity to predict overall mortality. Systemic levels of FGF23 (**D**) and Klotho (**E**) in patients with noncardiac death (n=19) or cardiac death (n=8). **F**, Receiver operating characteristic curves of FGF23 and Klotho levels and their capacity to predict cardiac mortality. Beeswarm plots show the median, the interquartile range (25th–75th percentiles), and the minimum and maximum values. FGF23 indicates fibroblast growth factor 23.

Table 2. AUC of FGF23 and Klotho for Prediction of Global and Cardiac Mortality

	Mortality		Cardiac death	
	AUC (95% CI)	*P* value	AUC (95% CI)	*P* value
FGF23 (RU/mL)	0.6793 (0.5774–0.7811)	0.0014	0.786 (0.6361–0.9359)	0.0073
Klotho (pg/mL)	0.5855 (0.4704–0.7005)	0.1272	0.5379 (0.3196–0.7562)	0.7223

AUC indicates area under the curve; and FGF23, fibroblast growth factor 23.

and longitudinal (Figure 4C, panel d) myocardial tissue sections from patients with IHD compared with healthy donor tissue (Figure 4C, panels a and c).

To validate these findings, we used a PMI mouse model. A significant positive correlation was observed between circulating FGF23 and troponin T (TnT) levels 24 hours after left anterior descending coronary artery ligation (r=0.6035, P=0.0493), indicating that FGF23 release is associated with the extent of acute myocardial injury during the early phase. In contrast, no correlation was detected between TnT and Klotho levels following experimental MI induction. PMI mice presented significant increases in cardiac FGF23 at both the mRNA (Figure 5A) and protein (Figure 5B) levels compared with sham-operated mice. Immunohistochemical analysis corroborated these results, revealing elevated FGF23 expression in transverse and longitudinal cardiac sections from PMI mice (Figure 5C, panels b and e). Because Klotho may have therapeutic potential in IHD,[17] we studied whether its application could restore FGF23 levels after MI. PMI mice were treated with recombinant murine Klotho (10 μg/kg per day) for 15 days, beginning immediately after infarction induction. PMI mice showed a survival rate of 62.5%, whereas Klotho-treated PMI animals reached 75.5% (Figure S1). However, Klotho treatment failed to prevent the increase in cardiac FGF23 levels (Figure 5A and 5B; Figure 5C, panels c and f), suggesting that ischemic damage triggers the overexpression of myocardial FGF23 independently of Klotho.

FGF23 Modulates Metabolic, Structural, and Proliferative Cardiac Processes That Are Blocked by Klotho

To uncover the specific mechanisms/pathways involved in the cardiac effects of FGF23, proteomic analysis of rat hearts perfused with FGF23 was performed, which revealed that FGF23 induced significant alterations in 21 proteins compared with those in control hearts (Figure 6A); 11 were downregulated, and 10 were upregulated (Figure 6B). The functional annotation of the differentially expressed proteins revealed their involvement in 15 gene ontology terms (Table 3; Figure 6C), with the majority integrated into metabolic processes such as the regulation of oxaloacetate, aspartate, and the tricarboxylic acid cycle; energetic processes such as mitochondrial matrix function and response to muscle activity; proliferative processes such as the cellular response to TFG-β (transforming growth factor β) and the regulation of endothelial cell proliferation; and structural processes such as modifications in T-tubules and binding receptor responses. Notably, the administration of Klotho before FGF23 perfusion completely abolished these proteomic changes. This was evident in the proteomic profile, which closely resembled that of control hearts (Figure 6A through 6D). These results strongly corroborate the observation of a different proteomic profile in FGF23-treated hearts, which is effectively normalized by the presence of Klotho.

DISCUSSION

This study offers novel insights into the interplay between the mineral metabolism biomarker FGF23 in IHD. A summary of the results of this study is shown in Figure 7. Our findings demonstrate that elevated circulating FGF23, but not decreased systemic Klotho, has predictive value for all-cause and cardiac-related mortality in patients with IHD at hospital admission following MI. Furthermore, ischemic myocardial injury was shown to significantly upregulate cardiac FGF23 expression, independent of the presence of Klotho. Importantly, elevated circulating FGF23 induces substantial changes in the cardiac proteome that are completely prevented by Klotho administration. Our findings suggest that circulating FGF23, rather than its levels within the heart muscle itself, may be a detrimental factor for cardiac damage following an ischemic event, making it a valuable biomarker to risk stratification for post-MI mortality risk.

In recent years, FGF23 has emerged as a predictor of both mortality and cardiovascular events. Previous studies in patients with MI have established FGF23 as a reliable predictor of long-term adverse cardiovascular outcomes, including HF.[33] Moreover, FGF23 is independently associated with mortality at 5 years.[34] Consistent with these findings, our cohort analysis demonstrated that FGF23 measured at hospital admission effectively discriminates short-term all-cause and cardiac mortality in patients with MI.

Interestingly, other studies have demonstrated that the predictive value of FGF23 is stronger in patients with MI who develop HF, even surpassing the GRACE (Global Registry of Acute Coronary Events)

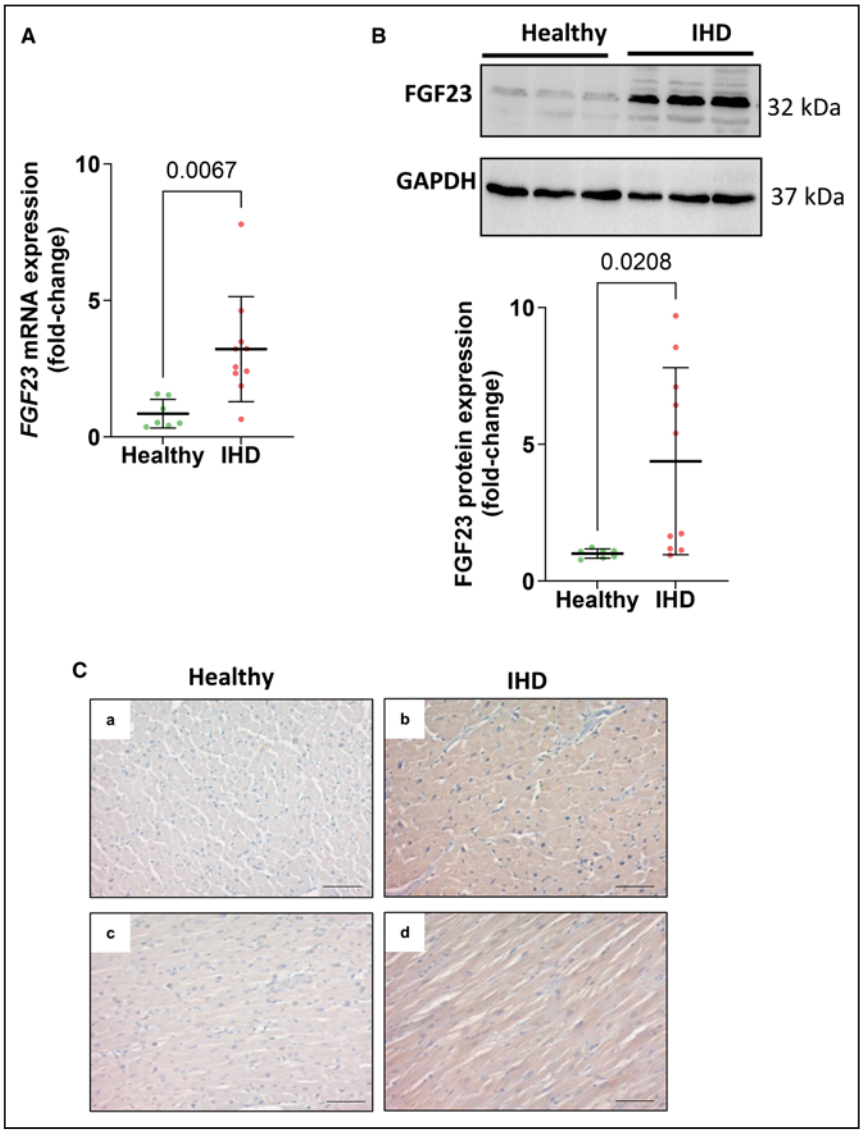

Figure 4. Cardiac FGF23 is upregulated in patients with IHD.
A through **C**, Cardiac samples from healthy donors (N=7) and patients with IHD (N=10) were obtained. (**A**) Cardiac gene expression of FGF23. (**B**) Representative Western blots and quantification of the cardiac protein expression of FGF23 and GAPDH. (**C**) Immunohistochemical staining of intracardiac FGF23 in transverse and longitudinal sections at ×20 magnification and 50 μm scale (N=3 per group). Scatter plots show the mean±SD. FGF23 indicates fibroblast growth factor 23; and IHD, ischemic heart disease.

score.[35] In the present study, patients with the highest FGF23 levels also had higher Killip–Kimball classification scores and cardiac-specific deaths. In support of these findings, several studies have demonstrated the prognostic usefulness of FGF23 in predicting cardiac mortality in patients with diabetes and MI,[36] patients undergoing coronary angiography,[37] or patients with stable IHD.[38] Furthermore, elevated FGF23 is considered a biomarker of inflammation and detrimental cardiac remodeling in patients with HF and a reduced LVEF.[39] Collectively, our data, along with findings from other researchers,[40–42] support FGF23 as a valuable biomarker of both short- and long-term IHD, with increased levels associated with increased cardiac death rates and poorer MI outcomes. Our findings suggest that elevated circulating levels of FGF23, particularly in patients with STEMI, are associated with worse cardiac outcomes and increased cardiovascular mortality. This raises the possibility that FGF23 testing could be incorporated into early triage protocols to identify high-risk patients who may benefit from closer monitoring or more aggressive therapeutic strategies. However, before FGF23 measurement can be implemented in routine clinical practice, prospective studies

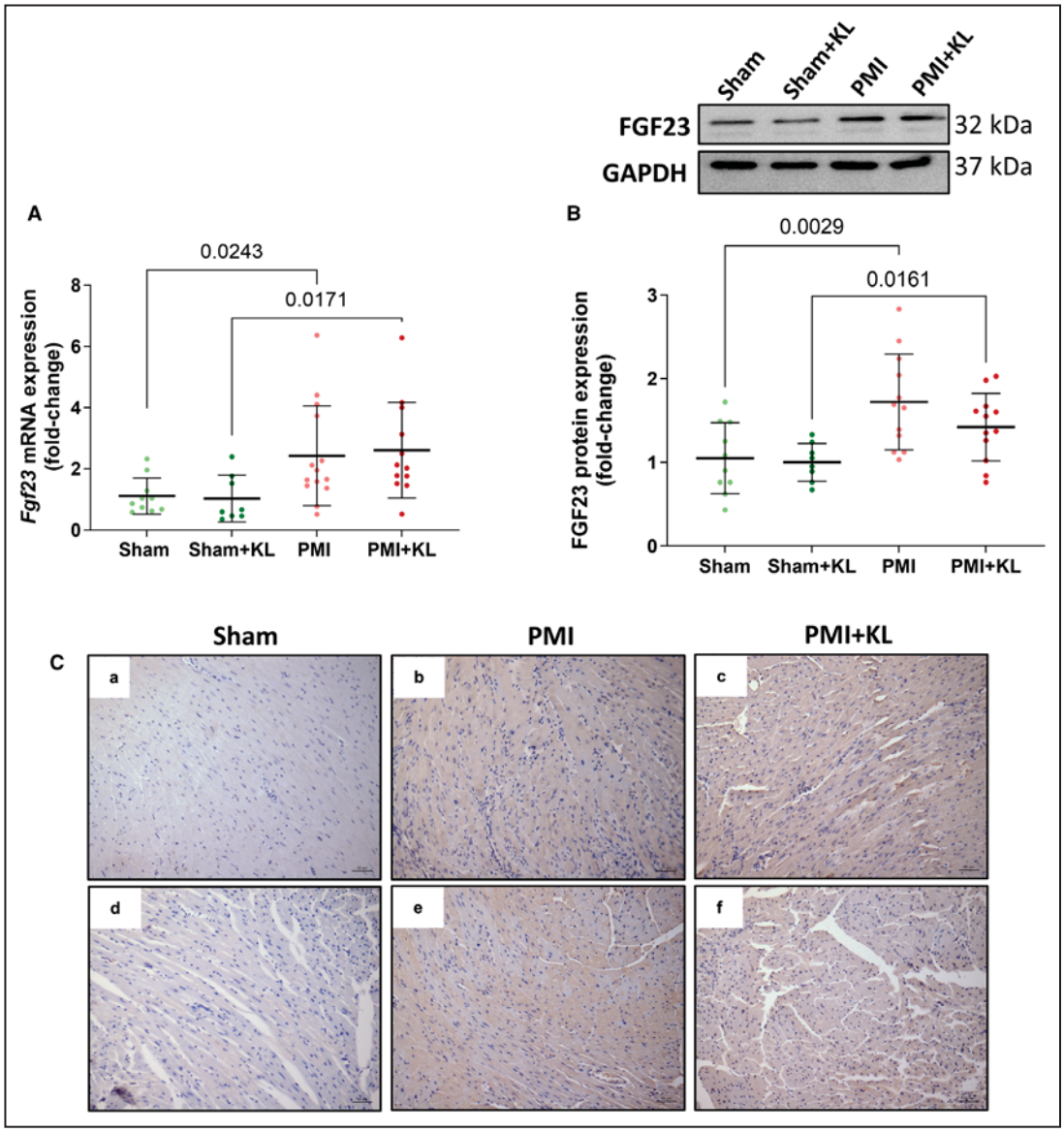

Figure 5. Cardiac FGF23 is upregulated in mice after myocardial infarction, and this elevation is not prevented by Klotho treatment.

A through **C**, Cardiac murine samples from the Sham (N=10), Sham+KL (N=8), PMI (N=14), and PMI+KL (N=12) groups. **A**, Cardiac gene expression of FGF23. **B**, Representative Western blots and quantification of the cardiac protein expression of FGF23 and GAPDH. **C**, Immunohistochemical staining of intracardiac FGF23 in transverse and longitudinal sections at ×20 magnification and 50 μm scale (N=3 per group). Scatter plots show the mean±SD. FGF23 indicates fibroblast growth factor 23; KL, Klotho; and PMI, post-myocardial infarction.

are needed to validate its predictive value, determine optimal timing for assessment, and evaluate its cost-effectiveness and integration with existing risk models. Finally, it is important to note that incorporating genetic risk variants could enhance the prediction accuracy of specific biomarkers.[43] In this context, a recent preliminary genetic analysis has identified a polygenic score for FGF23 that is associated with a reduced risk of heart failure but an increased risk of all-cause mortality and ischemic stroke.[44,45] Therefore, integrating network-based genetic analysis with systemic FGF23 levels may provide valuable insights for predicting mortality in the context of IHD.

Our results show that systemic levels of FGF23 are greater in patients with STEMI than in patients with NSTEMI, particularly when renal function is impaired. ST-segment elevation on an ECG is associated with MI severity, with patients with STEMI exhibiting a 40% higher 30-day mortality rate than those with NSTEMI.[46] Only a few prior studies have explored the relationship between FGF23 levels and STEMI/NSTEMI stratification, with some even reporting higher FGF23 levels in

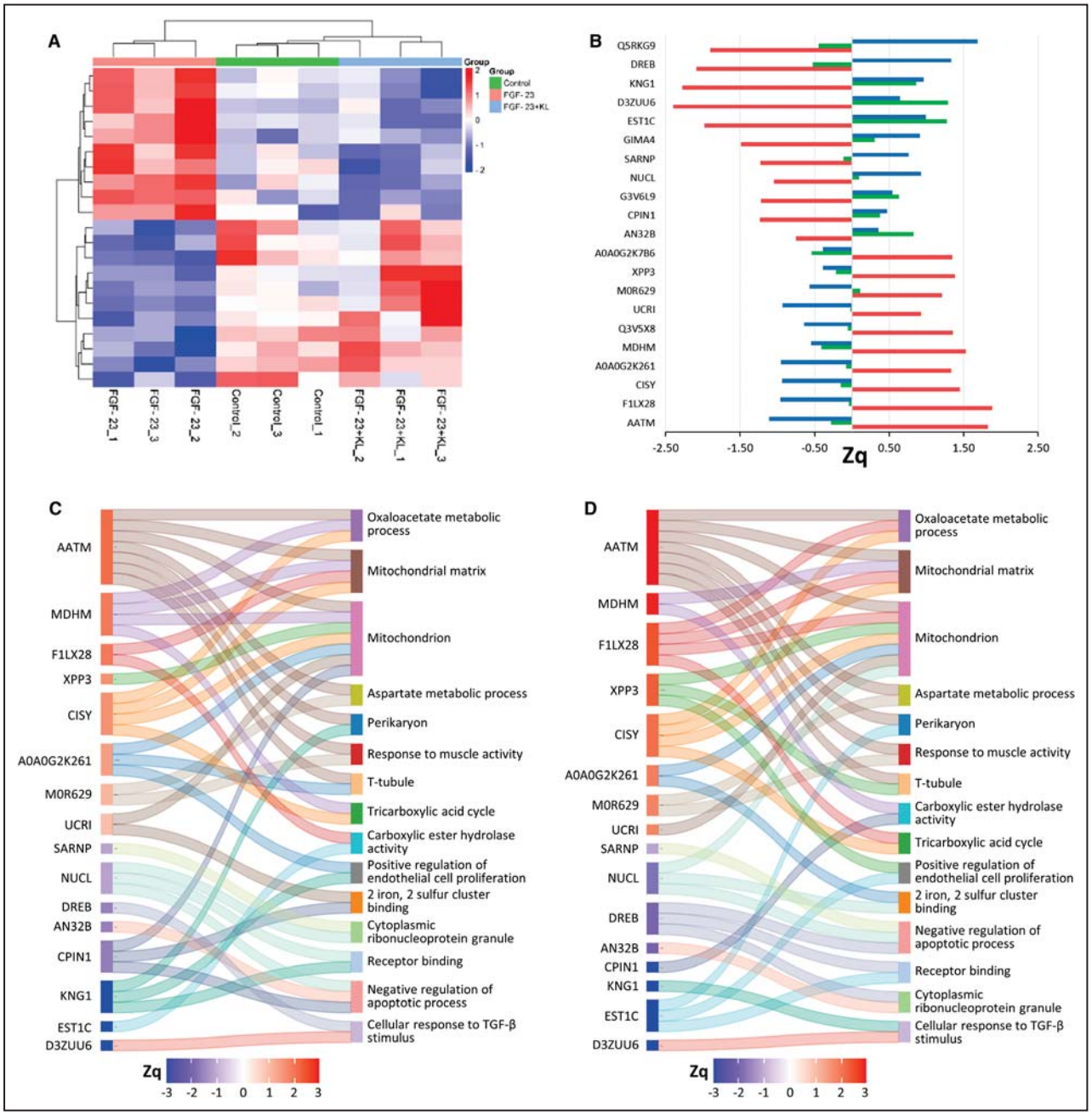

Figure 6. Proteomic changes in the heart exposed to FGF23 that are prevented by Klotho.
A, Hierarchical clustering heatmap and dendrogram of hearts exposed to vehicle (control), FGF23, and FGF23+KL. **B**, 21 proteins significantly altered (diminished or increased) in hearts from the FGF23 (red) and FGF23+KL (green) groups compared with those from the control group (blue). **C** and **D**, Sankey diagram showing the GO biological processes of the 21 proteins of interest. The diagrams show the clustered genes and their assigned GO terms, which are connected by ribbons. **C**, Sankey plot comparing FGF23 hearts with control hearts. **D**, Sankey plot comparing FGF23+KL hearts with FGF23 hearts. FGF23 indicates fibroblast growth factor 23; FGF23+KL, FGF23 pretreated with Klotho; GO, Gene Ontology; KL, Klotho; and TGF-β, transforming growth factor β.

patients with NSTEMI than in patients with STEMI.[47] This discrepancy may be due to the more varied subclinical causes and preexisting conditions often observed in patients with NSTEMI, leading to more complex and heterogeneous disease processes, including cardiometabolic backgrounds. Because STEMI typically results in more severe transmural ischemia and a larger infarct area, the association observed in our study suggests a significant contribution of FGF23 to the classification and prognosis of the MI type.

In addition to their prognostic value for mortality, elevated systemic FGF23 levels are associated with key markers of cardiac dysfunction. LVEF, measured by echocardiography, remains the gold standard for

Table 3. Functional Annotation of Differentially Expressed Proteins According to Gene Ontology Terms

Function	*P* value	FC	Proteins
Oxaloacetate metabolic process	3.84E-05	298.32	CISY, MDHM, AATM
Mitochondrial matrix	0.0012	17.70	CISY, MDHM, F1LX28, AATM
Mitochondrion	0.0015	4.99	CPIN1, CISY, UCRI, XPP3, MDHM, AATM, A0A0G2K261
Positive regulation of endothelial cell proliferation	0.0024	38.61	A0A0G2K7B6, KNG1
Aspartate metabolic process	0.0069	273.46	M0R629, AATM
Perikaryon	0.0174	14.04	KNG1, AATM
2 iron, 2 sulfur cluster binding	0.0299	62.81	CPIN1, UCRI
Tricarboxylic acid cycle	0.0306	60.77	CISY, MDHM
Response to muscle activity	0.0398	46.55	M0R629, AATM
Carboxylic ester hydrolase activity	0.0559	33.18	EST1C, F1LX28
Receptor binding	0.0614	7.03	KNG1, NUCL
T-tubule	0.0639	28.90	A0A0G2K7B6, AATM
Cytoplasmic ribonucleoprotein granule	0.0775	23.68	SARNP, NUCL
Negative regulation of apoptotic process	0.0965	5.36	CPIN1, AN32B, NUCL
Cellular response to transforming growth factor β stimulus	0.0980	18.38	D3ZUU6, DREB

FC indicates fold change.

diagnosing HF.[48] In our study, patients with an LVEF <50% had significantly higher systemic levels of FGF23, suggesting a potential link between elevated FGF23 and cardiac dysfunction, particularly when renal function was preserved. These findings align with previous research demonstrating increased FGF23 levels within the first week following MI in patients experiencing greater LVEF reduction at 6 months.[49] Moreover, elevated plasma FGF23 levels in patients with HF are associated with HF severity and all-cause mortality independent of the eGFR.[50,51]

A prolonged QTc interval is another relevant clinical parameter related to cardiac rhythm, because it is associated with the onset of fatal ventricular arrhythmias and sudden cardiac death, even after MI,[31,52] and is correlated with in-hospital mortality in patients with STEMI.[53] We observed higher FGF23 levels in patients with a pathological QTc ≥450 ms. Although previous

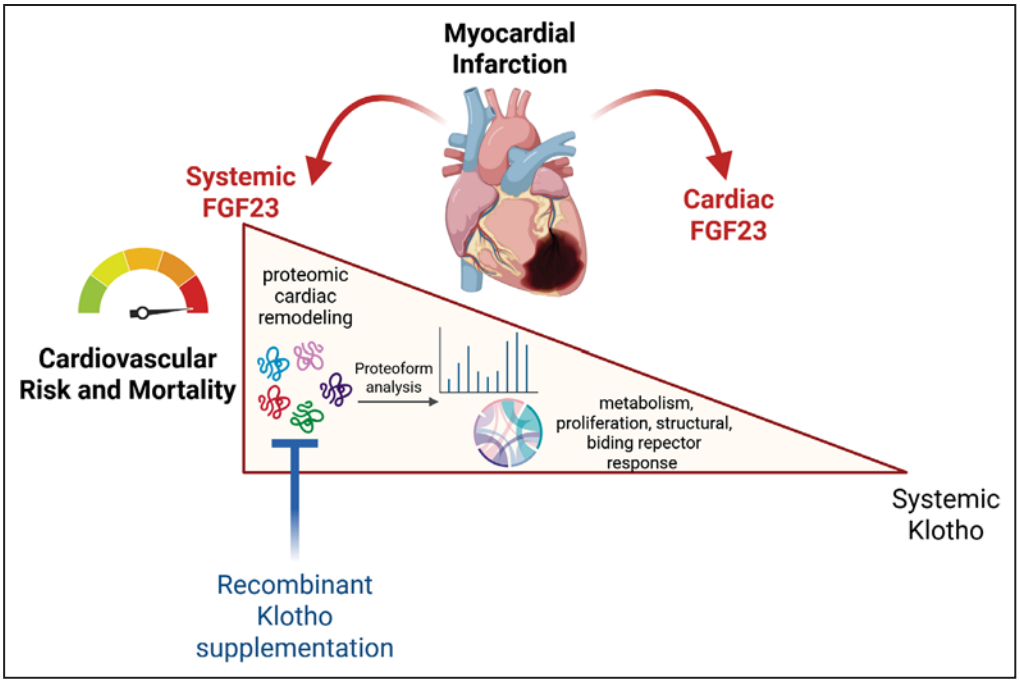

Figure 7. Schematic drawing with the summary of the results of this study.
FGF23 indicates fibroblast growth factor 23.

studies noted an association between elevated FGF23 levels and prolonged QTc in chronic kidney disease,[15,54] this relationship has not been explored in the context of IHD. When interpreting these results, it is important to consider the potential impact of renal impairment, a common condition associated with elevated FGF23 levels. In our cohort, we observed that FGF23 levels were not significantly affected by prolonged QTc or reduced LVEF in the presence of impaired renal function, because this condition inherently predisposes patients to elevated FGF23 levels. However, renal impairment was found to further increase systemic FGF23 levels in patients with STEMI and those with higher Killip–Kimball scores. These results indicate that the severity of HF and 30-day mortality after MI are associated with FGF23 independently of renal function.

We also aimed to elucidate the relationship between FGF23 and Klotho levels. Prior research on stable coronary artery disease has shown conflicting results on their association, with a significant negative correlation[55] or no association.[56] Here, we observed a weak negative correlation between FGF23 and Klotho levels (r=−0.166; P=0.043). Our findings show that Klotho levels are reduced after MI in patients with STEMI, regardless of renal function. Recent data have demonstrated that low serum Klotho levels may be associated with increased all-cause mortality in the general adult American population.[57] However, in our study, Klotho levels did not significantly predict all-cause or cardiac-specific mortality, and it should not be considered a valuable predictor biomarker in the clinical context of IHD. One possible explanation for this incongruity is that the predictive value of Klotho for mortality in the general population may be linked to individual cardiovascular risk factors, including elevated body mass index, smoking, alcohol consumption, and lipid parameters, especially when Klotho is combined with advanced age.[58] In contrast, in patients with cardiovascular disease, reduced Klotho levels are significantly associated with an increased incidence of MI and congestive HF.[59] However, its association with cardiovascular mortality remains unclear. It is possible that the decrease in Klotho levels could be a consequence of cardiovascular disease, particularly when accompanied by declining renal function. This perspective may shift our focus away from using Klotho more as a potential therapeutic strategy than as a predictive biomarker.

In addition, experimental research has identified Klotho as a promising therapeutic agent capable of mitigating cardiac damage associated with acute or chronic kidney disease.[13,15,60] Several studies in PMI models have demonstrated that Klotho treatment improves cardiac function parameters, attenuates ventricular remodeling, and reduces myocardial apoptosis.[16,61] Moreover, our group recently demonstrated that administering Klotho immediately after experimental MI significantly reduces infarct size, tissue hypertrophy, and fibrosis while improving contractile function and decreasing arrhythmogenic and apoptotic events.[17] On the basis of this evidence, our study aimed to evaluate Klotho as a potential treatment for IHD, particularly in relation to the cardiac actions of FGF23. We observed that patients with IHD exhibited cardiac overexpression of FGF23. Due to the low number of heart samples from the IHD condition tested, we replicated experimental ischemia in the PMI murine model exhibiting similar cardiac overexpression of FGF23 following the ischemic event. Although these results should be validated in a larger and external cohort of patients with IHD, they align with previous reports in experimental models of MI, which demonstrated FGF23 overexpression in the left ventricle 4 weeks postintervention[62] and within 4 to 7 days in the infarcted area.[63] Similarly, human studies have reported the upregulation of FGF23 expression in the hearts of patients with coronary artery disease.[64] Interestingly, here we observed that myocardial FGF23 expression analyzed by Western blot displayed a bimodal distribution, with a subset of samples showing levels slightly higher than those found in healthy individuals and another with markedly elevated expression. Stratification by histopathological features indicated that elevated FGF23 was predominantly associated with acute ischemic lesions, whereas baseline-like levels corresponded to chronic ischemic remodeling. These observations suggest a closer link between myocardial FGF23 accumulation and acute injury/inflammation rather than chronic fibrotic injury. Given the confirmed increased cardiac overexpression of FGF23 in both clinical and experimental IHD, and considering the established cardioprotective properties of Klotho, we investigated the impact of recombinant Klotho treatment on cardiac FGF23 expression after MI. Klotho did not prevent the cardiac overexpression of FGF23 in IHD conditions, suggesting that this cardiac FGF23 may constitute a specific response related to intrinsic ischemic damage.

To further elucidate the cardiac consequences of elevated systemic FGF23 levels, we used a proteomic approach using an ex vivo model, in which healthy rat hearts were exposed to pathological concentrations of FGF23. Our analysis revealed that systemic FGF23 significantly altered the myocardial proteome profile, affecting pathways involved in metabolism, mitochondrial function, proliferation or muscle activity response in the myocardium, which are all crucial in ischemic and HF conditions. Importantly, these proteomic alterations induced by FGF23 were not observed in the presence of Klotho, reinforcing the notion that Klotho may counteract the deleterious cardiac effects of systemic FGF23 levels, because intrinsic cardiac FGF23 overexpression is likely a compensatory cardiac response to ischemic

events independent of Klotho. However, directly inhibiting elevated cardiac FGF23 might be detrimental, because FGF23, a growth factor, may play a critical role in transient compensatory mechanisms after cardiac ischemia. Specifically, FGF23 could contribute to enhanced contractility and hypertrophy in noninfarcted myocardial regions as an adaptative response to the loss of contractile tissue within the infarcted area, particularly during the early stage of ischemic damage.[63] Therefore, rather than directly blocking FGF23, modulating its systemic effects with agents such as Klotho might be a more promising therapeutic strategy. Despite the cardioprotective properties of Klotho, further research is essential to explore its therapeutic potential in ischemic injury, especially independent of its regulatory effects on cardiac FGF23 expression. The proteomic alterations observed in this study will allow for the molecular characterization of several processes involved in the development of IHD after MI and their associations with FGF23 and Klotho, paving the way for personalized therapies.

CONCLUSIONS

This study underscores the intricate relationship between FGF23 and Klotho in the context of IHD. Our results indicate that elevated systemic FGF23, but not Klotho, serves as a novel independent biomarker with predictive value for all-cause and cardiac-specific mortality, establishing its significance as a valuable biomarker for short-term clinical outcomes in patients with MI. Moreover, this research identified Klotho as a promising therapeutic candidate capable of mitigating the detrimental cardiac effects of FGF23. However, the specific cardioprotective mechanisms of Klotho within the heart still remain elusive and warrant further investigation in future studies.

LIMITATIONS AND FUTURE DIRECTIONS

A key limitation of our study is the absence of long-term follow-up samples for assessing FGF23 and Klotho levels beyond the acute phase of MI. Although we attempted to estimate clinical progression using the Killip–Kimball score and its relationship with FGF23 levels, it was not possible to analyze temporal dynamics of FGF23 and its potential prognostic value over time. However, to fully understand the prognostic value of these biomarkers related to mineral bone disorders in MI, future studies should incorporate serial measurements over time. In addition, Klotho treatment applied in this study was limited to purely experimental and exploratory approaches; therefore, its translation into clinical practice in the context of IHD still remains speculative.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Supplemental Methods S1
Tables S1–S2
Figure S1
ARRIVE_checklist
STROBE_checklist
United Gels

REFERENCES

1. Kaski JC, Crea F, Gersh BJ, Camici PG. Reappraisal of ischemic heart disease. *Circulation*. 2018;138:1463–1480. doi: 10.1161/CIRCULATIONAHA.118.031373
2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Task Force for the Universal Definition of Myocardial Infarction.

Third universal definition of myocardial infarction. *Nat Rev Cardiol*. 2012;9:620–633. doi: 10.1093/eurheartj/ehs184
3. Mitsis A, Gragnano F. Myocardial infarction with and without ST-segment elevation: a contemporary reappraisal of similarities and differences. *Curr Cardiol Rev*. 2021;17:e230421189013. doi: 10.2174/1573403X16999201210195702
4. Mc Causland FR, McGrath MM, Claggett BL, Barkoudah E, East C, Fernandez A, Jering KS, Lewis EF, McMurray JJV, Mody FV, et al. Acute changes in kidney function and outcomes following an acute myocardial infarction: insights from PARADISE-MI. *Eur J Heart Fail*. 2024;26:1984–1992. doi: 10.1002/ejhf.3386
5. Kuro-O M. The FGF23 and klotho system beyond mineral metabolism. *Clin Exp Nephrol*. 2017;21(Suppl 1):64–69. doi: 10.1007/s10157-016-1357-6
6. Navarro-García JA, González-Lafuente L, Fernández-Velasco M, Ruilope LM, Ruiz-Hurtado G. Fibroblast growth Factor-23-klotho Axis in cardiorenal syndrome: mediators and potential therapeutic targets. *Front Physiol*. 2021;12:775029. doi: 10.3389/fphys.2021.775029
7. Prié D. FGF23 and cardiovascular risk. *Ann Endocrinol (Paris)*. 2021;82:141–143. doi: 10.1016/j.ando.2020.03.007
8. Ho BB, Bergwitz C. FGF23 signalling and physiology. *J Mol Endocrinol*. 2021;66:R23–R32. doi: 10.1530/JME-20-0178
9. Vázquez-Sánchez S, Poveda J, Navarro-García JA, González-Lafuente L, Rodríguez-Sánchez E, Ruilope LM, Ruiz-Hurtado G. An overview of FGF-23 as a novel candidate biomarker of cardiovascular risk. *Front Physiol*. 2021;12:632260. doi: 10.3389/fphys.2021.632260
10. Richter B, Faul C. FGF23 actions on target tissues-with and without klotho. *Front Endocrinol (Lausanne)*. 2018;9:189. doi: 10.3389/fendo.2018.00189
11. Leifheit-Nestler M, Haffner D. Paracrine effects of FGF23 on the heart. *Front Endocrinol (Lausanne)*. 2018;9:278.
12. Navarro-González JF, Donate-Correa J, Muros de Fuentes M, Pérez-Hernández H, Martínez-Sanz R, Mora-Fernández C. Reduced klotho is associated with the presence and severity of coronary artery disease. *Heart*. 2014;100:34–40. doi: 10.1136/heartjnl-2013-304746
13. Junho CVC, González-Lafuente L, Neres-Santos RS, Navarro-García JA, Rodríguez-Sánchez E, Ruiz-Hurtado G, Carneiro-Ramos MS. Klotho relieves inflammation and exerts a cardioprotective effect during renal ischemia/reperfusion-induced cardiorenal syndrome. *Biomed Pharmacother*. 2022;153:113515. doi: 10.1016/j.biopha.2022.113515
14. Ding J, Tang Q, Luo B, Zhang L, Lin L, Han L, Hao M, Li M, Yu L. Klotho inhibits angiotensin II-induced cardiac hypertrophy, fibrosis, and dysfunction in mice through suppression of transforming growth factor-β1 signaling pathway. *Eur J Pharmacol*. 2019;859:172549. doi: 10.1016/j.ejphar.2019.172549
15. Navarro-García JA, Salguero-Bodes R, González-Lafuente L, Martín-Nunes L, Rodríguez-Sánchez E, Bada-Bosch T, Hernández E, Merida-Herrero E, Praga M, Solís J, et al. The anti-aging factor klotho protects against acquired long QT syndrome induced by uremia and promoted by fibroblast growth factor 23. *BMC Med*. 2022;20:14. doi: 10.1186/s12916-021-02209-9
16. Wang K, Li Z, Ding Y, Liu Z, Li Y, Liu X, Sun Y, Hong J, Zheng W, Qian L, et al. Klotho improves cardiac fibrosis, inflammatory cytokines, ferroptosis, and oxidative stress in mice with myocardial infarction. *J Physiol Biochem*. 2023;79:341–353. doi: 10.1007/s13105-023-00945-5
17. Vázquez-Sánchez S, Blasco A, Fernández-Corredoira P, Cantolla P, Mercado-García E, Rodríguez-Sánchez E, González-Lafuente L, Poveda J, González-Moreno D, Matutano A, et al. Recombinant klotho administration after myocardial infarction reduces ischaemic injury and arrhythmias by blocking intracellular calcium mishandling and CaMKII activation. *J Pathol*. 2025;265:342–356. doi: 10.1002/path.6388
18. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. STROBE Initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007;370:1453–1457.
19. du Percie Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *J Physiol*. 2020;598:3793–3801.doi: 10.1371/journal.pbio.3000410
20. Wiśniewski JR, Zougman A, Nagaraj N, Mann M. Universal sample preparation method for proteome analysis. *Nat Methods*. 2009;6:359–362. doi: 10.1038/nmeth.1322
21. Tabb DL. The SEQUEST family tree. *J Am Soc Mass Spectrom*. 2015;26:1814–1819. doi: 10.1007/s13361-015-1201-3
22. Orsburn BC. Proteome discoverer-a community enhanced data processing suite for protein informatics. *PRO*. 2021;9:15. doi: 10.3390/proteomes9010015
23. Wright JC, Choudhary JS. DecoyPyrat: fast non-redundant hybrid decoy sequence generation for large scale proteomics. *J Proteomics Bioinform*. 2016;9:176–180. doi: 10.4172/jpb.1000404
24. Rodríguez JM, Jorge I, Martinez-Val A, Barrero-Rodríguez R, Magni R, Núñez E, Laguillo A, Devesa CA, López JA, Camafeita E, et al. iSanXoT: a standalone application for the integrative analysis of mass spectrometry-based quantitative proteomics data. *Comput Struct Biotechnol J*. 2024;23:452–459. doi: 10.1016/j.csbj.2023.12.034
25. Trevisan-Herraz M, Bagwan N, Garcia-Marques F, Rodriguez JM, Jorge I, Ezkurdia I, Bonzon-Kulichenko E, Vázquez J. SanXoT: a modular and versatile package for the quantitative analysis of high-throughput proteomics experiments. *Bioinformatics*. 2019;35:1594–1596. doi: 10.1093/bioinformatics/bty815
26. Navarro P, Vázquez J. A refined method to calculate false discovery rates for peptide identification using decoy databases. *J Proteome Res*. 2009;8:1792–1796. doi: 10.1021/pr800362h
27. Garcia-Marques F, Trevisan-Herraz M, Martinez-Martinez S, Camafeita E, Jorge I, Lopez JA, Méndez-Barbero N, Méndez-Ferrer S, Del Pozo MA, Ibáñez B, et al. A novel systems-biology algorithm for the analysis of coordinated protein responses using quantitative proteomics. *Mol Cell Proteomics*. 2016;15:1740–1760. doi: 10.1074/mcp.M115.055905
28. Huang dW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4:44–57. doi: 10.1038/nprot.2008.211
29. Huang dW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37:1–13. doi: 10.1093/nar/gkn923
30. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology*. 1983;148:839–843. doi: 10.1148/radiology.148.3.6878708
31. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, Charron P, Corrado D, Dagres N, de Chillou C, et al. 2022 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J*. 2022;43:3997–4126. doi: 10.1093/eurheartj/ehac262
32. Garcia-Osuna A, Sans-Rosello J, Ferrero-Gregori A, Alquezar-Arbe A, Sionis A, Ordóñez-Llanos J. Risk assessment after ST-segment elevation myocardial infarction: can biomarkers improve the performance of clinical variables? *J Clin Med*. 2022;11:1266. doi: 10.3390/jcm11051266
33. Kallmeyer A, Pello A, Cánovas E, Aceña Á, González-Casaus ML, Tarín N, Cristóbal C, Gutiérrez-Landaluce C, Huelmos A, Rodríguez-Valer A, et al. Fibroblast growth factor 23 independently predicts adverse outcomes after an acute coronary syndrome. *ESC Heart Fail*. 2024;11:240–250. doi: 10.1002/ehf2.14568
34. Schmitz T, Wein B, Heier M, Peters A, Meisinger C, Linseisen J. Baseline fibroblast growth factor 23 is associated with long-term mortality in ST-elevation myocardial infarction-results from the augsburg myocardial infarction registry. *Front Cardiovasc Med*. 2023;10:1173281. doi: 10.3389/fcvm.2023.1173281
35. Cornelissen A, Florescu R, Kneizeh K, Cornelissen C, Liehn E, Brandenburg V, Schuh A. Fibroblast growth factor 23 and outcome prediction in patients with acute myocardial infarction. *J Clin Med*. 2022;11:601. doi: 10.3390/jcm11030601
36. Ferreira JP, Sharma A, Mehta C, Bakris G, Rossignol P, White WB, Zannad F. Multi-proteomic approach to predict specific cardiovascular events in patients with diabetes and myocardial infarction: findings from the EXAMINE trial. *Clin Res Cardiol*. 2021;110:1006–1019. doi: 10.1007/s00392-020-01729-3
37. Brandenburg VM, Kleber ME, Vervloet MG, Tomaschitz A, Pilz S, Stojakovic T, Delgado G, Grammer TB, Marx N, März W, et al. Fibroblast growth factor 23 (FGF23) and mortality: the Ludwigshafen risk and cardiovascular health study. *Atherosclerosis*. 2014;237:53–59. doi: 10.1016/j.atherosclerosis.2014.08.037
38. Udell JA, Morrow DA, Jarolim P, Sloan S, Hoffman EB, O'Donnell TF, Vora AN, Omland T, Solomon SD, Pfeffer MA, et al. Fibroblast growth factor-23, cardiovascular prognosis, and benefit of angiotensin-converting enzyme inhibition in stable ischemic heart disease. *J Am Coll Cardiol*. 2014;63:2421–2428. doi: 10.1016/j.jacc.2014.03.026
39. Girerd N, Cleland J, Anker SD, Byra W, Lam CSP, Lapolice D, Mehra MR, van Veldhuisen DJ, Bresso E, Lamiral Z, et al. Inflammation and remodeling pathways and risk of cardiovascular events in patients

with ischemic heart failure and reduced ejection fraction. *Sci Rep*. 2022;12:8574. doi: 10.1038/s41598-022-12385-0
40. Mirna M, Lauten A, Jirak P, Rezar R, Wernly B, Paar V, Felder TK, Hoppe UC, Motloch LJ, Jung C, et al. Serum levels of C-terminal FGF23 (cFGF23) are associated with 1-year-mortality in patients undergoing transcatheter aortic valve replacement (TAVR). *Eur J Intern Med*. 2021;85:98–107. doi: 10.1016/j.ejim.2020.09.022
41. Pramong N, Gojaseni P, Suttipongkeat S, Kiattisunthorn K, Chittinandana A. Diagnostic accuracy of fibroblast growth factor 23 for predicting acute kidney injury in patients with acute decompensated heart failure. *Nephrology (Carlton)*. 2021;26:126–133. doi: 10.1111/nep.13780
42. Palupi-Baroto R, Hermawan K, Murni IK, Nurlitasari T, Prihastuti Y, Sekali DRK, Ambarsari CG. High fibroblast growth factor 23 as a biomarker for severe cardiac impairment in children with chronic kidney disease: a single tertiary center study. *Int J Nephrol Renov Dis*. 2021;14:165–171. doi: 10.2147/IJNRD.S304143
43. Müller B, Wilcke A, Boulesteix AL, Brauer J, Passarge E, Boltze J, Kirsten H. Improved prediction of complex diseases by common genetic markers: state of the art and further perspectives. *Hum Genet*. 2016;135:259–272. doi: 10.1007/s00439-016-1636-z
44. Li J, Lai Y, Jiang C, Li M, Yang Z, Zhao M, Peng X, Li S, Li Q, Chen J, et al. Fibroblast growth factor 23 and outcomes of atrial fibrillation: from clinical association to genetic evidence. *Eur J Prev Cardiol*. 2025;zwaf001. doi: 10.1093/eurjpc/zwaf001
45. Navarro-García JA, Wehrens XHT, Ruiz-Hurtado G. Is fibroblast growth factor (FGF)23 a player or a trigger in atrial fibrillation? *Eur J Prev Cardiol*. 2025;zwaf053. doi: 10.1093/eurjpc/zwaf053
46. Martínez MJ, Rueda F, Labata C, Oliveras T, Montero S, Ferrer M, El Ouaddi N, Serra J, Lupón J, Bayés-Genís A, et al. Non-STEMI vs. STEMI cardiogenic shock: clinical profile and long-term outcomes. *J Clin Med*. 2022;11:3558. doi: 10.3390/jcm11123558
47. Hjort M, Eggers KM, Lindhagen L, Baron T, Erlinge D, Jernberg T, Marko-Varga G, Rezeli M, Spaak J, Lindahl B. Differences in biomarker concentrations and predictions of long-term outcome in patients with ST-elevation and non-ST-elevation myocardial infarction. *Clin Biochem*. 2021;98:17–23. doi: 10.1016/j.clinbiochem.2021.09.001
48. Lund LH, Pitt B, Metra M. Left ventricular ejection fraction as the primary heart failure phenotyping parameter. *Eur J Heart Fail*. 2022;24:1158–1161. doi: 10.1002/ejhf.2576
49. Takahashi H, Ozeki M, Fujisaka T, Morita H, Fujita SI, Takeda Y, Shibata K, Sohmiya K, Hoshiga M, Tamaki J, et al. Changes in serum fibroblast growth factor 23 in patients with acute myocardial infarction. *Circ J*. 2018;82:767–774. doi: 10.1253/circj.CJ-17-0826
50. von Jeinsen B, Sopova K, Palapies L, Leistner DM, Fichtlscherer S, Seeger FH, Honold J, Dimmeler S, Aßmus B, Zeiher AM, et al. Bone marrow and plasma FGF-23 in heart failure patients: novel insights into the heart-bone axis. *ESC Heart Fail*. 2019;6:536–544. doi: 10.1002/ehf2.12416
51. Vergaro G, Del Franco A, Aimo A, Gentile F, Castiglione V, Saponaro F, Masotti S, Prontera C, Fusari N, Emdin M, et al. Intact fibroblast growth factor 23 in heart failure with reduced and mildly reduced ejection fraction. *BMC Cardiovasc Disord*. 2023;23:433. doi: 10.1186/s12872-023-03441-2
52. Cupa J, Strebel I, Badertscher P, Abächerli R, Twerenbold R, Schumacher L, Boeddinghaus J, Nestelberger T, Maechler P, Kozhuharov N, et al. Diagnostic and prognostic value of QRS duration and QTc interval in patients with suspected myocardial infarction. *Cardiol J*. 2018;25:601–610. doi: 10.5603/CJ.a2018.0033
53. Wattoo MA, Tabassum M, Bhutta KR, Kaneez M, Zaidi SMJ, Ijaz H, Awan J, Irshad U, Azhar MJ, Rafi Z. Correlation of prolonged corrected QT interval with ventricular arrhythmias and in-hospital mortality among ST-elevation myocardial infarction patients: a mystique or lucidity? *Cureus*. 2020;12:e12356. doi: 10.7759/cureus.12356
54. Sikaneta T, Ho N, Bellasi A, Mahdavi S, Taskapan H, Svendrovski A, Makanjee B, Roberts J, Wu G, Nathoo B, et al. QTc interval prolongation is independently associated with FGF23 and predicts mortality in Predialysis chronic kidney disease. *Cardiorenal Med*. 2024;14:45–57. doi: 10.1159/000535133
55. Bergmark BA, Udell JA, Morrow DA, Jarolim P, Kuder JF, Solomon SD, Pfeffer MA, Braunwald E, Sabatine MS. Klotho, fibroblast growth factor-23, and the renin-angiotensin system—an analysis from the PEACE trial. *Eur J Heart Fail*. 2019;21:462–470. doi: 10.1002/ejhf.1424
56. Yang Z, Wang J, Ma J, Ren D, Li Z, Fang K, Shi Z. Fibroblast growth factor 23 during septic shock and myocardial injury in ICU patients. *Heliyon*. 2024;10:e27939. doi: 10.1016/j.heliyon.2024.e27939
57. Kresovich JK, Bulka CM. Low serum klotho associated with all-cause mortality among a nationally representative sample of American adults. *J Gerontol A Biol Sci Med Sci*. 2022;77:452–456. doi: 10.1093/gerona/glab308
58. Lee J, Kim D, Lee HJ, Choi JY, Min JY, Min KB. Association between serum klotho levels and cardiovascular disease risk factors in older adults. *BMC Cardiovasc Disord*. 2022;22:442. doi: 10.1186/s12872-022-02885-2
59. Xu JP, Zeng RX, He MH, Lin SS, Guo LH, Zhang MZ. Associations between serum soluble α-klotho and the prevalence of specific cardiovascular disease. *Front Cardiovasc Med*. 2022;9:899307. doi: 10.3389/fcvm.2022.899307
60. González-Lafuente L, Navarro-García JA, Rodríguez-Sánchez E, Aceves-Ripoll J, Poveda J, Vázquez-Sánchez S, Mercado-García E, Fernández-Velasco M, Kuro-O M, Liaño F, et al. Interplay between mineral bone disorder and cardiac damage in acute kidney injury: from Ca. *Transl Res*. 2022;243:60–77. doi: 10.1016/j.trsl.2022.01.002
61. Wang K, Li Z, Li Y, Liu X, Sun Y, Hong J, Ding Y, Zheng W, Qian L, Xu D. Cardioprotection of klotho against myocardial infarction-induced heart failure through inducing autophagy. *Mech Ageing Dev*. 2022;207:111714. doi: 10.1016/j.mad.2022.111714
62. Andrukhova O, Slavic S, Odörfer KI, Erben RG. Experimental myocardial infarction upregulates circulating fibroblast growth Factor-23. *J Bone Miner Res*. 2015;30:1831–1839. doi: 10.1002/jbmr.2527
63. Schumacher D, Alampour-Rajabi S, Ponomariov V, Curaj A, Wu Z, Staudt M, Rusu M, Jankowski V, Marx N, Jankowski J. Cardiac FGF23: new insights into the role and function of FGF23 after acute myocardial infarction. *Cardiovasc Pathol*. 2019;40:47–54. doi: 10.1016/j.carpath.2019.02.001
64. Corsetti G, Pasini E, Scarabelli TM, Romano C, Agrawal PR, Chen-Scarabelli C, Knight R, Saravolatz L, Narula J, Ferrari-Vivaldi M, et al. Decreased expression of klotho in cardiac atria biopsy samples from patients at higher risk of atherosclerotic cardiovascular disease. *J Geriatr Cardiol*. 2016;13:701–711. doi: 10.11909/j.issn.1671-5411.2016.08.009