Mendelian Randomization and the association of Fibroblast Growth Factor-23 with Heart Failure with Preserved Ejection Fraction

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Abstract

Purpose of review—Observational data provide compelling evidence for elevated fibroblast growth factor-23 (FGF23) as a risk factor for heart failure (HF), particularly heart failure with preserved ejection fraction (HFpEF). Given the limitations of observational studies, uncertainties persist regarding the causal role of FGF23 in the pathogenesis of HF and HFpEF. Recently, Mendelian randomization (MR) studies have been performed to examine causal associations between FGF23 and HF phenotypes.

Recent findings—The current review describes the methodological basis of the MR techniques used to examine the causal role of FGF23 on HF phenotypes, highlighting the importance of large-scale multi-omics data. The findings from most of the MR studies indicate an absence of evidence of a causal effect of FGF23 on the risk of HF in general population settings. However, analysis using individual-level data showed a strong association between genetically-predicted FGF23 and HFpEF in individuals with a genetic predisposition to low eGFR.

Summary—Evidence from MR analysis suggests a causal role of FGF23 in the pathogenesis of HFpEF in low eGFR settings – a finding supported by experimental, clinical, and epidemiological data. While future MR studies of FGF23 and HFpEF could provide further evidence, randomized trials of FGF23-lowering agents could provide the most definitive answers on the association in chronic kidney disease (CKD) populations.

Keywords

mendelian randomization; fibroblast growth factor-23; heart failure with preserved ejection fraction; causality; chronic kidney disease

Introduction

Consistent associations across multiple cohort studies provide compelling evidence of the role of mineral metabolism markers, including phosphate, parathyroid hormone, and

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Conflicts of Interest

There are no conflicts of interest.

fibroblast growth factor-23 (FGF23) in the pathophysiology of cardiovascular disease[1–5]. Over the last decade, observational studies have implicated elevated levels of circulating FGF23 as a risk factor for heart failure (HF) in patients with chronic kidney disease (CKD) and individuals in the general population [6–10]. Independent associations were reported between serum FGF23 concentration and multiple HF-related phenotypes, including HF with preserved ejection fraction (HFpEF) and left ventricular hypertrophy (LVH) [11–13]. Observational epidemiological studies suffer from inherent biases such as confounding and reverse causation, limiting their ability to robustly identify causal associations. In addition, the lack of specificity of associations and an unclear dose-response relationship raised the usual questions on whether the observed correlations signify cause and effect.[14]

Recently, several research teams have examined the potential causal role of FGF23 on HF and HF subtypes using mendelian randomization (MR) experiments, designed to mitigate some of the pitfalls of observational studies[15–18]. This review discusses the MR studies that utilize large-scale multi-omics data to bring clarity to the debate regarding the causal nature of these associations. The review also highlights other experimental, animal model and mechanistic data that clarify the potential role of FGF23 on the pathophysiology of HF and HFpEF.

Mendelian randomization methods for the FGF23-heart failure association

MR techniques leverage genetic variants associated with circulating biomarker levels as instruments to investigate the causal effect of the candidate biomarker on the risk of disease [19–22]. Akin to a randomized trial, because of the random assortment and segregation of alleles at meiosis, genetic variants associated with a risk factor through genome-wide association studies (GWAS) can be used as instruments to investigate the relationship between the risk factor and disease in an un-confounded manner (Figure 1). Since genetic variants are assigned at conception and largely stable over the course of an individual's life, MR studies also have the correct temporal ordering to overcome the possibility of reverse causation.

GWAS can identify genetic variants correlated with interindividual differences in protein levels that may indirectly or directly influence the protein-coding gene [23, 24]. A GWAS of 16,624 individuals of European ancestry in the CHARGE consortium identified five FGF23-associated genetic loci, some of which were located near genes involved in vitamin D metabolism and renal phosphate transport [25]. A second FGF23 GWAS from the SCALLOP consortium using a proteomic assay (multiplex immunoassay) on 19,195 individuals replicated most of the signals from the prior meta-GWAS and additionally identified one genome-wide-significant pQTL (protein quantitative trait locus) near the *FGF23* gene itself [26]. Mendelian randomization studies investigating the FGF23-HF association have primarily used a two-sample MR design [27] were the single nucleotide polymorphism (SNP)-exposure (FGF23) and the SNP-outcome (HF) data come from two different studies with similar ancestry or population structure.

MR studies of serum biomarkers often utilize data from GWAS to identify independent genetic variants which together predict a non-negligible proportion of the variance of

the circulating biomarker levels [28, 29]. These genetic variants must meet the three assumptions of instrumental variables to be considered valid instruments [30]. For the FGF23-HF association, these are (1) the genetic markers are strongly associated with circulating FGF23 levels (relevance assumption); (2) the variants are independent of any confounders of the association between circulating FGF23 and HF (independence assumption); and (3) the variants affect HF only through their effect on FGF23 (no horizontal pleiotropy) (Figure 2). Even if only one of the genetic variants is not a valid instrumental variable, the causal estimate based on all the variants from a conventional Mendelian randomization analysis will be biased, with inflated false positive error rates. [31, 32] For example, a genetic variant in the *ABO* gene identified as an independent signal in the GWAS of FGF23 has known pleiotropic effects and was not included in the primary analysis of the MR studies included in the current review.

When all variants satisfy the assumptions, a Wald ratio estimate is obtained by dividing the beta coefficient of the variant-HF association by the beta coefficient of the variant-FGF23 association. An overall inverse-variance weighted (IVW) estimate is obtained by combining the variants' ratio estimates using standard meta-analysis [33]. Alternative MR approaches allow consideration or inclusion of pleiotropic variants and are included as sensitivity analyses in MR efforts. The weighted median estimate is more robust to the presence of pleiotropic effects, allowing up to 50% of variants to violate MR assumptions[34]. MR-Egger regression, in contrast to conventional MR, allows for pleiotropy and fits the weighted linear regression model with an intercept term that represents the average pleiotropic effect [33]. Assuming that the distribution of pleiotropic effects is independent from the genetic associations with exposure, (*i.e.* Instrument Strength Independent of Direct Effect assumption), the MR-Egger approach provides a consistent and valid estimate of the causal effect.[35] In MR studies of FGF23 and HF, additional sensitivity analyses have been performed, including leave-one-out analyses, where variants are removed one at a time to investigate their impact on the primary analysis. Other alternatives include using only cis-PQTLs or utilizing a larger set of genetic instruments meeting a lower threshold for significance (*e.g.* $p < 1 \times 10^{-6}$) and combining their effects in a genetic risk score to increase the proportion of variance of FGF23 explained by the instruments [16].

Current evidence from Mendelian randomization studies of FGF23 and overall heart failure

Four recent studies have examined the causal nature of the association between FGF23 and overall HF using MR techniques [15–18]. Liang et al performed a two-sample MR using genome-wide association data of individuals of European ancestry from large consortia [18, 36]. Genetic instruments were defined as independent variants ($r^2 < 0.01$) that were genome-wide significant signals ($p < 5 \times 10^{-8}$)[25] for log-FGF23 in models adjusted for age, sex, and 10 principal components of ancestry and did not have known pleiotropic effects. These variants were rs17216707 upstream of *CYP24A1*, rs11741640 within *RGS14*, rs17479566 near *LINC01506*, rs9925837 near *LINC01229*. The pleiotropic rs2769071 variant in *ABO* gene was excluded. There was no weak instrument bias as the F-statistics for these variants ranged from 34.0 to 117.5 [18]. A meta-analysis of 26 GWAS from the

HERMES consortium, including 47,309 cases of HF and 930,014 controls [37], was used to capture the association of the genetic instruments with overall HF. Cases of HF were all patients with a clinical diagnosis of HF regardless of left ventricular ejection fraction (LVEF) while controls were individuals without HF. In the primary analysis using an IVW approach, no evidence of a causal effect of genetically-predicted FGF23 on HF was detected (OR: 0.99, 95%CI: 0.75–1.31) (Table 1). A similar finding was obtained using the weighted median approach (OR: 1.01, 95%CI: 0.73–1.38) and there was no evidence of heterogeneity between the SNPs (p-value for Cochran's Q = 0.24) or directional pleiotropy (p-value for MR-Egger intercept = 0.20) [18].

A subsequent MR study used the same FGF23 GWAS to identify genetic instruments[25], alongside individual-level data from BioVU, the Vanderbilt University Medical Center DNA biobank linked to de-identified electronic medical records to identify heart failure cases (n=18,415), with differentiation for preserved (HFpEF) and reduced ejection fraction (HFrEF) and controls (n=13,903), who had no HF-related ICD codes or text mentions in their record, and never had an LVEF 50%.[15, 38]. After obtaining beta coefficients for the association between the FGF23 genetic instruments and overall HF in BioVU, IVW MR analysis showed no evidence of a causal effect of genetically-predicted FGF23 on HF (OR: 1.32, 95%CI: 0.95–1.84) [15] (Table 1). MR analyses were then repeated within strata of estimated glomerular filtration (eGFR) polygenic risk score (PRS) based on eGFR GWAS data from the CKDGen consortium.[15] (Figure 3) In the low eGFR PRS stratum, genetically-predicted FGF23 was associated with 3-fold higher odds of overall HF (OR: 3.09, 95%CI: 1.38–6.91) [15] (Table 1). This suggests that individuals with a genetic liability for low eGFR or CKD may have greater susceptibility to the adverse effects of FGF23 on the risk of heart failure. No evidence of a causal effect of genetically-predicted FGF23 was observed in the high eGFR PRS stratum suggesting perhaps that genetic liability to elevated FGF23 may be benign in settings of preserved kidney function [15].

More recently, Donovan and collaborators used a different approach to instrument selection, involving building a genetic risk score for FGF23,[39] based on a GWAS of FGF23 in 19,195 individuals of European ancestry from the Systematic and Combined Analysis of Olink Proteins (SCALLOP) consortium, a collaboration of 14 cohorts with proteomic assays for 90 cardiovascular proteins. Genetic instruments included in the GRS were 34 independent ($r^2<0.1$) variants associated with FGF23 at $p<5\times10^{-6}$, explaining up to 6.4% of FGF23 variance. The GRS was validated in the ORIGIN consortium and included two *cis*-variants (variants within 100 kb of the *FGF23* transcription start site, less likely to have unidentified pleiotropic effects, rs6489536 and rs7955866). Outcome data were from the UK Biobank (n=337,448) with 10,177 cases of HF hospitalizations and the HERMES consortium.[37] Both sets of analyses found no evidence of a causal association between genetically-predicted FGF23 and HF, OR = 1.01 (95%CI: 0.94–1.10) in the UK Biobank and 1.00 (95%CI: 0.95–1.05) in the HERMES consortium (Table 1). As these cohorts were both general population cohorts, these data re-emphasize the absence of a causal effect of FGF23 on HF risk in such settings.

Henry et al used the *cis*-variants in the FGF23 meta-GWAS in the SCALLOP consortium to perform a 2-sample *cis*-MR analysis of the FGF23-HF association. In the analysis using the

only genome-wide significant *cis*-variant (rs6489536) the effect estimate was not different from the null (OR: 0.79, 95%CI: 0.62–1.02) (Table 1). When including a second cis-variant with a less stringent FGF23 GWAS p-value (p=2.7×10⁻⁷) there was unexpectedly evidence of a protective effect of FGF23 on HF risk (OR: 0.80, 95%CI: 0.71–0.89). Since *cis*-MR studies use variants that are near or within the cognate gene, if their effects are indeed causal and replicated, the findings are often used to inform drug target discovery and validation. However, both *cis*-variants explained only 0.4% of the variance of FGF23 and the directionality of the MR estimates obtained using these cis-variants was inconsistent with the bulk of the data from other MR studies using instruments explaining a greater proportion of the variance of FGF23. [15, 25, 39] Given that previous studies have established the significant role of trans-acting variants in the regulation of gene expression and consequently the circulating levels of the encoded proteins, it is difficult to predict how omitting trans-regulatory variants in the cis-MR analysis may have impacted the MR estimate. Importantly, analyses within subgroups of eGFR PRS and according to HF-subtype suggest that there is significant heterogeneity of effects. As such, MR analyses that do not account for these differences in associations may produce estimates that obfuscate specific pathophysiological pathways.

Current evidence from mendelian randomization studies of FGF23 and HFpEF

To our knowledge, only one MR study has examined the association of FGF23 with HF subtypes defined by LVEF cut-points. Using individual-level data in BioVU, the algorithm used to ascertain HF allowed for differentiation of cases by LVEF determined using transthoracic echocardiographic measurements [15] [38]. A case-control design was used to estimate the effects of the FGF23 genetic instruments (4 SNPs) on HFpEF (all LVEF measures ≥ 50%) and HFrEF (any left ventricular ejection fraction ≤ 40%). In the primary IVW analysis, genetically-predicted FGF23 was significantly associated with HFpEF (OR: 1.47, 95%CI: 1.01–2.14) but not HFrEF (OR: 0.86, 95%CI: 0.38–1.96) [15]. The findings from the eGFR PRS-stratified analysis were particularly striking for HFpEF. In the low eGFR PRS stratum, a one-unit higher genetically predicted logFGF23 was associated with an over 7-fold higher odds of HFpEF (OR:7.20, 95%CI: 2.80–18.5) while the association was not different from the null (OR:1.03, 95%CI: 0.67–1.57) in the high eGFR PRS stratum [15]. There was no evidence of a significant FGF23-HF association for HFrEF in either PRS strata.

Potential causal role of FGF23 on the risk of HFpEF

The contemporary debate on the potential causal role of FGF23 in the pathogenesis of HFpEF must be weighed against the totality of the evidence from observational studies, animal models, and MR experiments leveraging proteogenomic data.

Initial epidemiologic evidence described an association between elevated FGF23 levels and incident HF in general population [6, 8–10] and CKD settings [9] and subsequent data emphasized the association with HFpEF [10] which is thought to be mediated in part via the direct effect of FGF23 on cardiomyocyte hypertrophy [11–13]. Data from observational

studies have highlighted the prospective independent association between elevated FGF23 at baseline and incident left ventricular hypertrophy (LVH) that was preponderantly concentric [11–13] – a typical feature of HFpEF.

In animal models, rat ventricular cardiomyocytes treated with FGF23 experienced activation of pro-hypertrophic genes and underwent pathological hypertrophy that was mediated via FGF receptor (FGFR)-dependent activation of the calcineurin-NFAT signaling pathway [11]. Additional experimental data showed that FGF23 induced pathological LVH [40] via activation of FGFR4, [41, 42] and blockade of FGFR4 attenuated this response [43] further emphasizing a direct likely causal effect of FGF23 in the development of LVH and the pathogenesis of HFpEF. Other mechanisms have been suggested to further explain the role of FGF23 on the heart. FGF23 potentially exacerbates diastolic dysfunction by promoting myocardial fibrosis via upregulation of TGF-β and β-catenin [44]. Elevated FGF23 may lead to LVH in a calcium-dependent pathway similar to angiotensin II and this pathway can be inhibited with angiotensin receptor blockers [45] and/or by directly regulating sodium chloride cotransporter expression in the distal convoluted tubule via a signaling mechanism involving the FGFR-klotho complex [46].

Further evidence of a potential causal role of FGF23 on HFpEF in CKD is provided by the striking results of the eGFR PRS-stratified MR analysis of the FGF23-HFpEF association in the BioVU biobank [15] highlighting the importance of large-scale EMR-linked genomics data for investigating causal effects. The observed 3-fold higher odds of overall HF compared to the more than 7-fold higher odds of HFpEF per log-unit higher genetically-predicted FGF23 among patients with a genetic liability for lower eGFR, implies that the association with overall HF was likely driven by the more specific association with HFpEF, which is supported by the biological and experimental data linking elevated FGF23 to concentric LVH that underlies HFpEF [1]. Secreted by osteocytes in the incipient stages of kidney dysfunction, FGF23 is well known for its role in suppressing type 2a sodium sodium-phosphate cotransporters to facilitate renal phosphate excretion. As circulating FGF23 levels rise sharply from 2 to 5-fold above normal in early CKD to reach 100 to 1000-fold in advanced kidney failure [47, 48], this potentially explains the stronger effect of FGF23 observed in low eGFR settings in the stratified MR analysis in the BioVU biobank. [15]

A recent study demonstrated a significant longitudinal association between reduction in serum FGF23 after kidney transplantation and improvements in cardiovascular functional capacity (VO2 max), suggesting a potential surrogate endpoint for randomized trials of FGF23-lowering interventions in advanced CKD [49, 50]. While there was no significant improvement in LV mass index with decreasing FGF23 in the study, the accompanying editorial underscored the fact that VO2 max is a measure of cardiopulmonary fitness for which diastolic dysfunction (which is a feature of HFpEF) is one of the components [49, 50]. Targeting improvements in diastolic dysfunction and consequently VO2 max in randomized trials for FGF23-lowering agents would provide compelling evidence that could influence clinical practice.

Conclusion

The aggregate evidence from epidemiologic, experimental, and genetic data suggests that while the association of FGF23 with overall HF in the general population may be driven in part by residual confounding, there appears to be a causal role for FGF23 in the pathogenesis of HFpEF in CKD patients that is likely mediated via the effect of pronounced chronically-elevated levels of FGF23 on concentric LVH and enhanced by the biological synergism between elevated FGF23 and CKD.

Taken together, these data suggest it may be time to consider designing randomized trials to test the safety and efficacy of FGF23 lowering agents in CKD patients. Meanwhile additional mendelian randomization studies could provide more critical evidence. Nonlinear MR for HFpEF using larger datasets with individual-level data could demonstrate dose-response revealing any threshold effects and multivariable mendelian randomization of several mineral metabolism markers with shared genetic architecture with HFpEF as the outcome could provide critical evidence further deconstructing the mechanisms by which FGF23 is implicated in the pathogenesis of HFpEF.

Summary

- Elevated FGF23 has been associated with heart failure in observational and experimental studies, yet it is unknown whether FGF23 levels themselves represent causal processes for complications, how kidney function impacts these processes, and if it may be a promising interventional target.

- Mendelian randomization represents an inexpensive alternative or adjunct to randomized controlled trials and incorporates genetic information into traditional epidemiological methods to address the causality of a risk factor for important clinical outcomes.

- Several Mendelian randomization studies of FGF23 and heart failure have been conducted and the consensus is that while the association of FGF23 with overall heart failure may be driven by residual confounding, there appears to be a causal role for FGF23 in the pathogenesis of HFpEF in CKD patients.

- Results from the MR analyses have the potential to suggest new therapies for mineral metabolism disturbances and to identify pathways/targets amenable to pharmaceutical or biological intervention.

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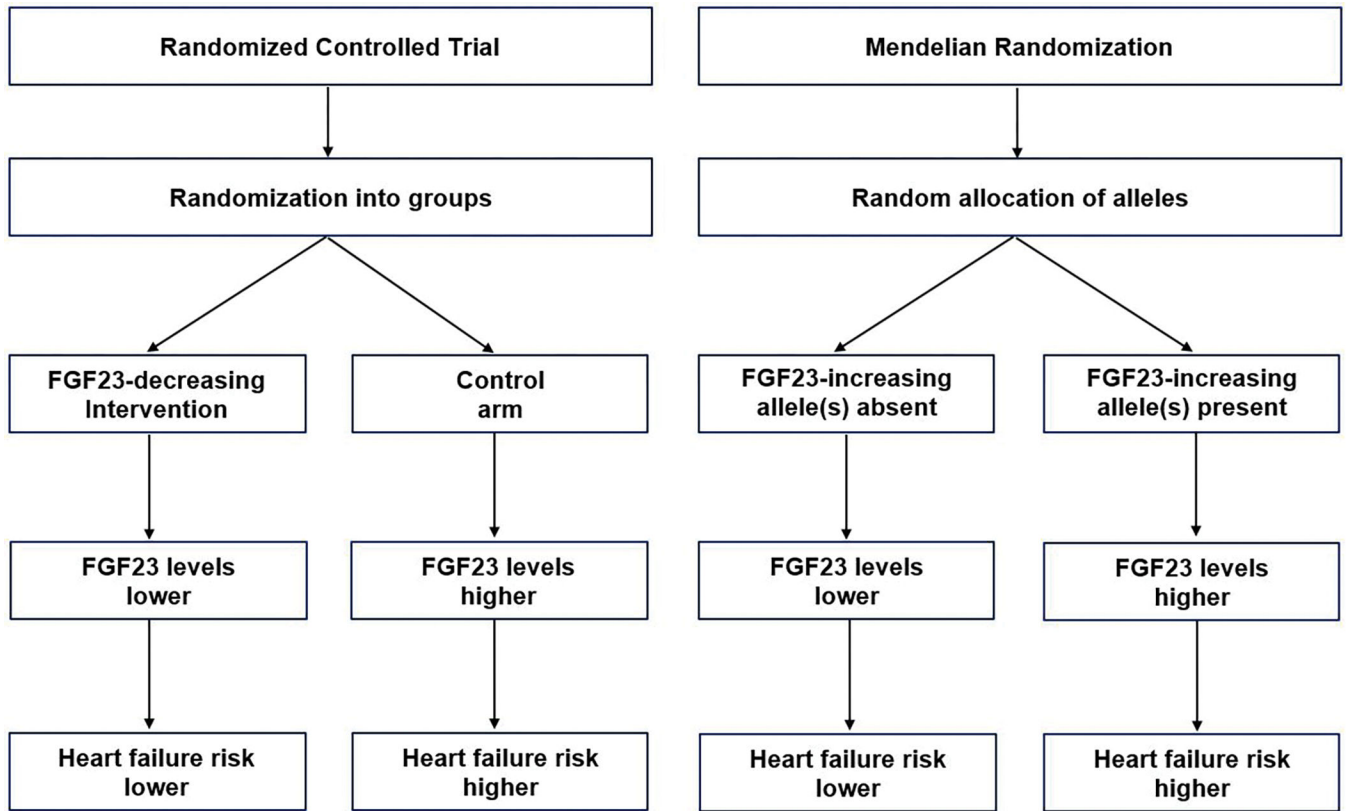
Figure 1.
Random allocation of FGF23 alleles in Mendelian randomization versus randomized control trials. Legend: This figure demonstrates the analogy between Mendelian Randomization (MR) designs and randomized controlled trial (RCT) designs. In MR designs, genetic variants are randomly allocated at conception, while in RCT designs, participants are randomly allocated to intervention or control groups.
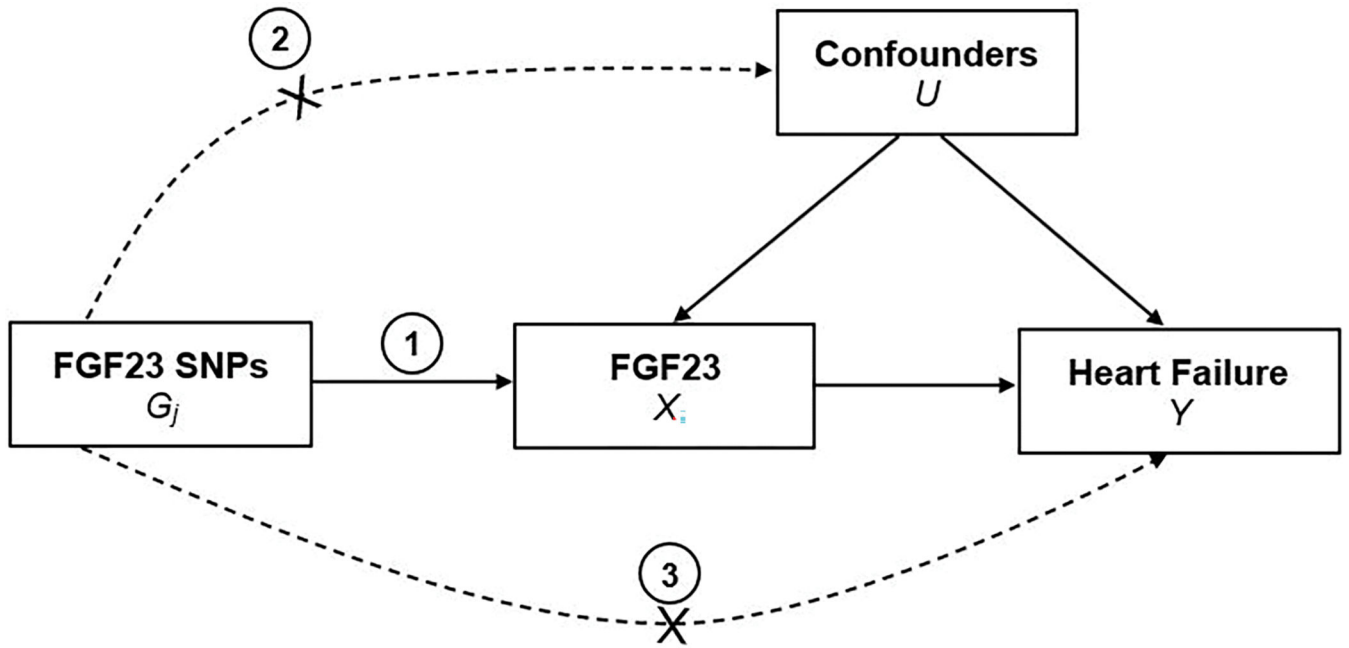
Figure 2.
Instrumental variable assumptions for the Mendelian randomization studies of FGF23 and heart failure. Legend: The three assumptions are: (1) the genetic variant must be robustly associated with the exposure (FGF23); (2) the genetic variant should not be associated with confounders of the exposure-outcome (heart failure) association; and (3) the genetic variant must influence the outcome through the exposure only and not through any direct or alternative pathways. The dashed lines represent pathways that violate the assumptions.
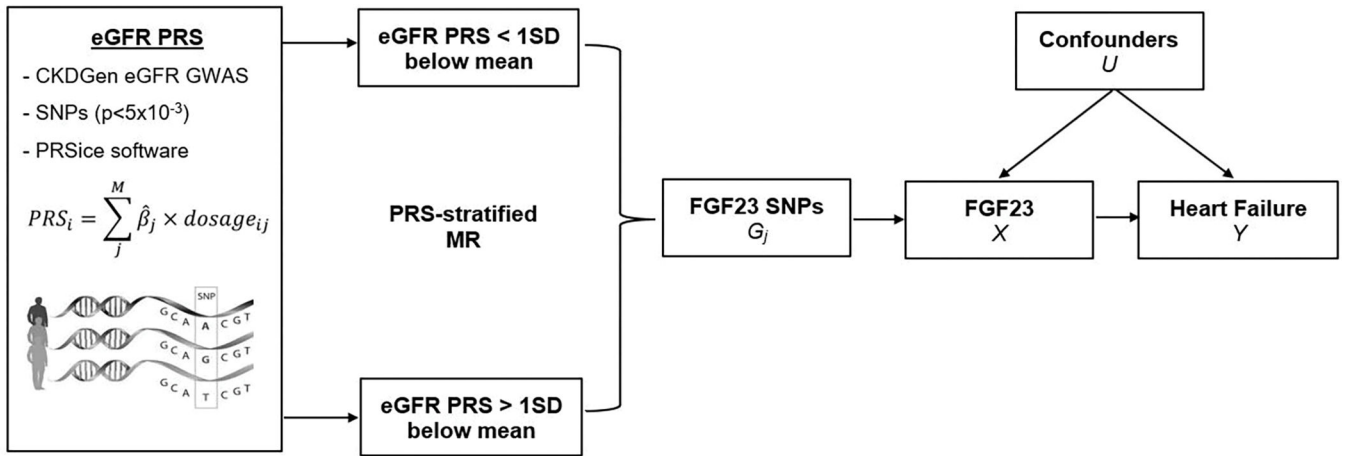
Figure 3.
Polygenic risk score-stratified analysis for Mendelian randomization study of FGF23
and heart failure. Figure: An eGFR polygenic risk score was developed using summary
statistics from the Chronic Kidney Disease Genetics Consortium (CKDGen) genome-wide
association study of eGFR in >1 million individuals and stratified MR analyses of FGF23
and heart failure were performed across eGFR polygenic risk score strata.

Table 1.

Methods and summary estimates from mendelian randomization studies of FGF23 and heart failure phenotypes

Study (First Author, Journal, Publication date)	FGF23 GWAS	FGF23 Instruments	Source of Outcome data (n = number of HF cases)	MR IVW OR (95%CI)
Liang Y et al. *Frontiers in Genetics* 07/23/2021	CHARGE Consortium, FGF23 GWAS meta-analysis, n =16,624	4 SNPs with p<5×10⁻⁸ (rs17216707 near *CYP24A1*, rs11741640 near *RGS14*, rs17479566 near *LINC01229*, rs9925837 near *LINC01506*	HERMES (n = 47,309)	0.99 (0.75, 1.31)
			Overall HF	
			HERMES (n = 47,309)	1.13 (0.89, 1.42)
			BioVU, Overall (n = 18,415)	1.32 (0.95, 1.84)
			BioVU, low eGFR PRS[a] (n=3086)	3.09 (1.38, 6.91)
			BioVU, low eGFR PRS[a] (n=15,329)	1.11 (0.77, 1.54)
Akwo E et al *CJASN* 08/01/2022	CHARGE Consortium, FGF23 GWAS meta-analysis, n=16,624	4 SNPs with p<5×10⁻⁸ (rs17216707 near *CYP24A1*, rs11741640 near *RGS14*, rs17479566 near *LINC01229*, rs9925837 near *LINC01506*	**HFpEF**	
			BioVU, Overall (n = 13,141)	1.47 (1.01, 2.14
			BioVU, low eGFR PRS[a] (n=2223)	7.20 (2.80, 18.5)
			BioVU, low eGFR PRS[a] (n=10,918)	1.03 (0.67, 1.57)
			HFrEF	
			BioVU, Overall (n = 3394)	0.86 (0.38, 1.96)
			BioVU, low eGFR PRS[a] (n=554)	0.24 (0.03, 1.72)
			BioVU, low eGFR PRS[a] (n=2840)	1.12 (0.45, 2.77)
Donovan et al *CJASN* 01/01/2023	SCALLOP Consortium, FGF23 GWAS meta-analysis, n = 19,195	Genetic score of 34 SNPs with p<5×10⁻⁶	HERMES (n = 47,309)	1.00 (0.94, 1.05)
			UK Biobank (n=10,177)	1.01 (0.94, 1.10)
Henry et al *Circulation* 04/19/2022	SCALLOP Consortium, FGF23 GWAS meta-analysis, n = 19,195	1 Cis-variant (rs6489536) near the *FGF23* gene with p < 5×10⁻⁸	HERMES (n = 47,309)	0.79 (0.62, 1.02)

Study (First Author, Journal, Publication date)	FGF23 GWAS	FGF23 Instruments	Source of Outcome data (n = number of HF cases)	MR IVW OR (95%CI)
		2 Cis-variants (rs6489536 and rs7955866) near the *FGF23* gene with $p < 1\times10^{-6}$	HERMES (n = 47,309)	0.80 (0.71–0.89)

BioVU, Vanderbilt University Medical Center DNA Biobank; CHARGE, Cohorts for Heart and Aging Research in Genetic Epidemiology; eGFR, estimated Glomerular Filtration Rate; FGF23, Fibroblast Growth Factor-23; GWAS, Genome-Wide Association Study; HERMES, Heart Failure Molecular Epidemiology Therapeutic Targets; HF, heart failure; HFpEF heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IVW, Inverse Variance Weighted; MR, mendelian randomization; OR, Odds Ratio; PRS, Polygenic Risk Score; SNP, single nucleotide polymorphism