Causal Association and Mechanism Linking Basal Metabolic Rate and Atrial Fibrillation with Plasma Protein Levels:

A Mendelian Randomization Analysis

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Abstract

Background: Atrial fibrillation is commonly diagnosed worldwide; however, whether and how the basal metabolic rate affects atrial fibrillation remains unclear. The aim of this study is to evaluate their causal relationship and the potential mechanism using serum proteomic profiling.

Methods: Bidirectional two-sample Mendelian randomization studies on summary statistics of genome-wide association studies were conducted, along with an analysis of thirteen potential biomarkers of incident atrial fibrillation. Multivariable Mendelian randomization is also conducted by controlling for other risk factors, including heigh, fat free mass, coronary heart disease, heart failure, obesity, hypertension, thyrotoxicosis, and diabetes.

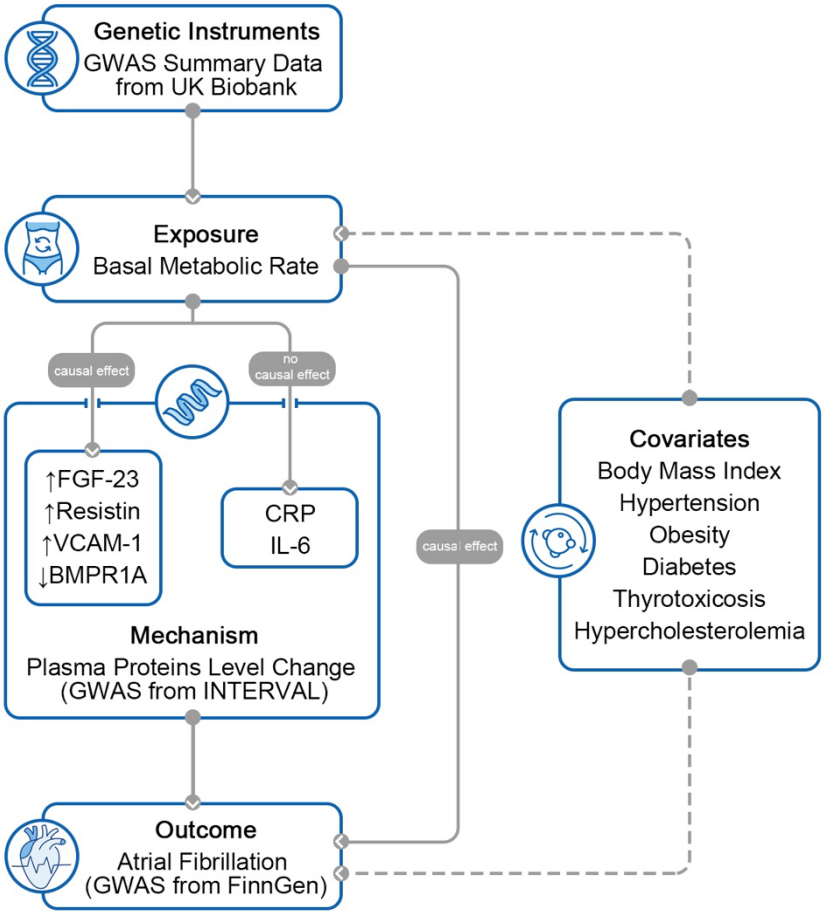
Results: Basal metabolic rate increases incident atrial fibrillation ($\beta = 0.77$, 95% CI 0.64-0.90, $p < 0.0001$). This effect persisted after adjusting for body composition or metabolic diseases using multivariable Mendelian randomization. Higher basal metabolic rate has a positive causal effect on resistin ($\beta = 0.24$, 95% CI 0.64-0.41, $p < 0.01$), growth differentiation factor 15 ($\beta = 0.62$, 95% CI 0.15-1.08, $p = 0.01$), and a negative causal association on bone morphogenetic protein receptor type-1A ($\beta = -0.30$, 95% CI -0.94-0.11, $p = 0.002$). However, it did not have a significant causal effect on inflammatory cytokines (C-reactive protein and interleukin-6).

Conclusions: A higher basal metabolic rate is a causal risk factor for atrial fibrillation. Furthermore, it is causally associated with several atrial remodelling-associated proteins but not inflammatory cytokines, indicating that atrial remodelling influenced by the basal metabolic rate may not involve systemic inflammation. Our findings suggest that behaviours and lifestyles of reducing or maintaining BMR prevent AF incidence.

Key Words:

Basal metabolic rate; Atrial fibrillation; Mendelian randomization; Plasma proteins; Biomarker

Graphical Abstract



Key Question: Is a higher basal metabolic rate causally related to atrial fibrillation and if so, what is the mechanism?

Key Findings: In this Mendelian randomisation study, basal metabolic rate had a significantly positive causal association with the incidence of atrial fibrillation and several atrial remodelling-associated proteins but not on C-reactive protein or interleukin-6 (inflammatory cytokines).

Take-home message: A higher basal metabolic rate causes atrial fibrillation incidence, and atrial remodelling influenced by the basal metabolic rate may not involve systemic

Introduction

Atrial fibrillation (AF) is the most commonly diagnosed cardiac arrhythmia, affecting approximately 30 million individuals worldwide.[1] AF contributes to several cardiovascular, such as ischemic stroke and heart failure, and non-cardiovascular outcomes, such as cognitive impairment, which increase socioeconomic burden and mortality risk. Risk factors for AF include advanced age, obesity, hypertension, diabetes, obstructive sleep apnea, and other cardiovascular diseases.[2] Recent studies have identified independent genetic variants at more than 100 loci that are significantly involved in AF.[3,4]

Metabolic rate is associated with key physiological and life history traits, including survival, growth, immunity, predation, and reproductive output.[5] Basal metabolic rate (BMR) is the daily energy required to preserve vital function integrity and accounts for 35–70% of the total energy requirement. An increased BMR is reportedly associated with a greater mortality risk. [6,7] Additionally, BMR is positively associated with blood pressure after adjusting for Body Mass Index (BMI),[8,9] and cancer and diabetes are associated with a higher BMR at baseline.[10]

A recent cohort study showed that body composition parameters, including metabolic rate, are associated with AF occurrence and type.[11] Although these observational data indicate an association between the two, the actual effect of BMR on AF remains unclear. Moreover, little is known regarding the biological pathways linking BMR and AF. Genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with BMR, AF, and plasma proteins. Therefore, we used a two-sample Mendelian randomization (MR) approach that infers the causality among correlated traits with genetic instrumental variants, which are less vulnerable to confounding and reverse causation bias. To

investigate the potential causal mechanism, we also conducted serum proteomic profiling using MR, which may clarify the pathophysiological mechanisms linking BMR and AF.

Our findings can shed light on many clinic implications; for example, high-volume endurance exercise by elite athletes increases their AF risk, which is five times higher than that in non-athletes.[2] Conversely, athletes have significantly higher resting metabolic rates than non-athletes.[12]

Methods

Study Design and Data Sources

This study investigated the causal relationship between BMR and AF using a bidirectional two-sample MR analysis. To ensure the selection of a similar study population while minimizing sample overlap, we used summary statistical data from two large-scale GWAS analyses: the Neale lab study from the UK Biobank (UKBB) and FinnGen biobank for BMR and AF, respectively. Ethical approval was obtained in the original studies. We then performed a two-sample MR analysis between BMR and several plasma proteins, which were significantly associated with the risk of incident AF and identified as potential biomarkers of incident AF previously.[13–16] GWAS summary data of plasma proteins were obtained from a plasma proteome study of the INTERVAL study.[17] Figure 1 gives an overview of the study. All GWAS data used were de-identified summary data that were publicly available. Information regarding the data sources and sample sizes used in this study is summarized in Additional file1: Table S1.

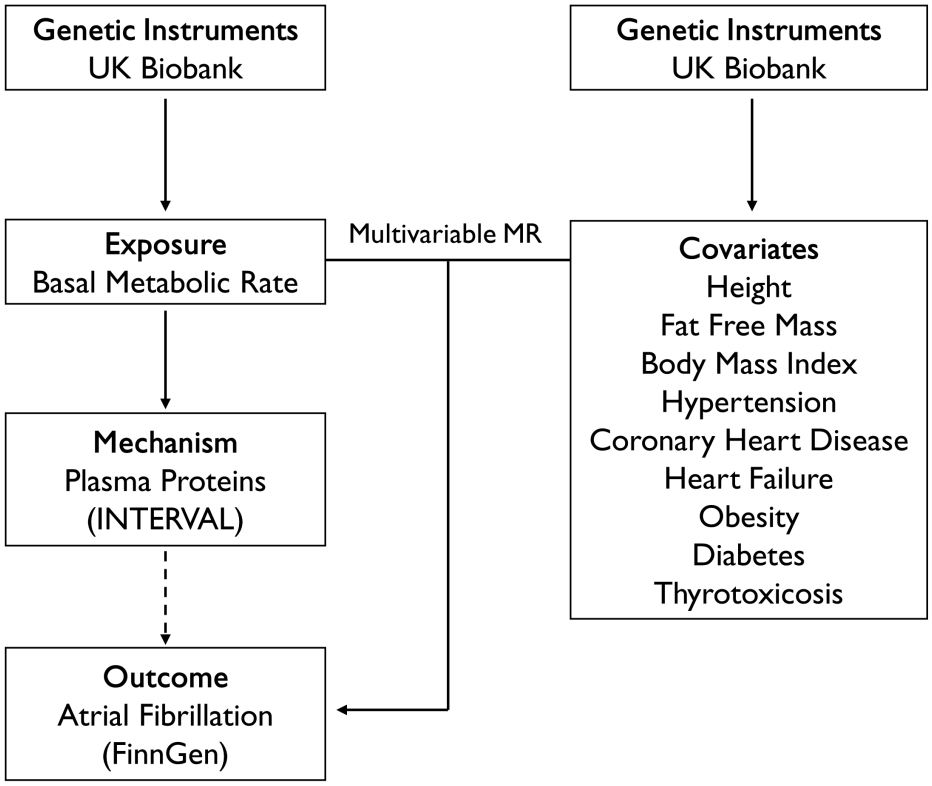


Figure 1. Mendelian Randomization Analysis Overview. The left flow chart illustrates our main two-sample mendelian randomization analysis. The right part shows the covariates we used in the multivariable MR. Line from proteins to AF indicates the significant relationship in previous studies.

Statistical Analyses

As an instrumental variable (IV) method, MR must satisfy three main identification assumptions. First, genetic variants should be associated with exposure (BMR). If the association is weak, the estimate is biased and leads to a greater type 1 error in a finite sample.[18] To avoid this, we used a two-sample analysis strategy rather than the traditional individual IV analysis.[19] F-statistics were calculated to assess the strength of each selected IV.[20] The second assumption requires there are no unmeasured confounders of the associations between genetic

variants and outcomes. The last one is the exclusion restriction: IV affects the outcome only through exposure.

Assuming all the above IV assumptions are satisfied, our favoured estimate is the efficient inverse-variance weighted (IVW) estimate, which is asymptotically equal to the two-stage least squares estimator using individual data.[21] However, IVW is likely to be biased, commonly due to horizontal pleiotropy.[22] To address this issue, we also conduct MR-Egger regression, which can be consistent even under a weaker Strength Independent of Direct Effect assumption.[23] Recently, a unified framework is developed to resolve weak IVs, horizontal pleiotropy, and selection bias by adjusting the profile likelihood.[24] We report the proposed MR-RAPS estimator.

Although uncommon, IV may correlate unobserved confounder, possibly because of population stratification. Therefore, we applied a new and robust method. The contamination mixture (Conmix) allows invalidity of some genetic variants.[25] Based on the profile-likelihood approach, MR-Conmix efficiently computes causal effects with hundreds of SNPs.

Many other risk factors are closely related to BMR and AF. Therefore, we additionally conducted the multivariable MR (MVMR). This approach allows us to estimate the direct causal effect of BMR.[27,28] All GWAS summary data of covariates were obtained from the Neale lab. The data processing was similar to that of the exposure. We used multiple models, including MVMR-IVW, MVMR-Egger, and MVMR-Lasso, for a robustness check.

Statistical analysis was performed using the TwoSampleMR, MRInstruments, and MendelianRandomization R packages, version 3.6.1 (RStudio, Boston, MA, USA).

Exposure: BMR-associated Genetic IVs

IVs for BMR were obtained from the UKBB GWAS phenotypes released by the Neale lab (phenotype code: 23105). BMR was measured with a body composition analyser using data derived by dual-energy X-ray absorptiometry using bioelectrical impedance analysis at an Assessment Centre of the UK Biobank. Because BMR is a continuous variable, we used a revised version with inverse-rank normalized values.[29] Single- or both-sex GWAS summary data were extracted for analysis. For each BMR trait, SNPs were included if they obtained a significant genome-wide association level ($p \leq 5 \times 10^{-8}$) or had a minor allele frequency over 0.01. We only contained independent SNPs (linkage disequilibrium distance threshold, 10,000 kb; coefficient of determination $R^2 < 0.001$).

Selection bias indicates the overestimation of the relationship between exposure and outcome, which may lead the MR estimate towards a null effect.[30] To avoid this, we follow the literature to conduct a robust analysis. [31] First, we identify IVs for BMR only in the single-sex set rather than in the whole sample. Then, we use identified IVs to conduct the original MR.

In the GWAS results released by the Neale laboratory, age, sex, and principal components 1-20 were included as covariates in their model. In our sex-specific analyses, age and principal components were included as covariates in the model. Detailed SNPs for the IVs of BMR and F-statistics are presented in Additional file1: Table S2-S4.

Outcome: AF Outcome Data

We extracted GWAS summary data associated with AF from the FinnGen Study Release 6 (phenotype code: I9 AF). The International Classification of Diseases diagnosis codes were

used to define AF in the FinnGen study. GWAS were adjusted for age, sex, ten principal components, and the genotyping batch.

For each BMR-instrumental SNP, corresponding SNP information, including SNP position, reference SNP identification number, effect allele frequency, effect size, standard error, and p-values, was extracted from the AF GWAS.

Plasma Proteins Data

Sun et al. performed genome-wide testing of the human plasma proteome in healthy blood donors from the INTERVAL study, including 2,994 plasma proteins in 3,301 individuals of European descent.[17] Based on previous studies,[13–16] 11 plasma proteins, identified as potential biomarkers of incident AF. Their hazard ratio were summarized in Additional file1: Table S5. Protein levels were adjusted using linear regression for age, sex, and the first three principal components.

Sensitivity Analysis

We conducted three broad classes of sensitivity analyses. First, we conducted multiple tests for measurement errors, heterogeneity, and pleiotropy. (1) We checked I^2 statistics; if SNP-exposure association is measured without error, I^2 will tend towards one.[32] (2) To test directional pleiotropy, we subsequently assessed the estimate, confidence interval (CI), and p-value for the intercept in the MR-Egger.[23] (3) Cochran's and Rucker's Q tests along with MR-PRESSO for the main regressions were performed; MR-PRESSO can correct horizontal

pleiotropy by removing outliers.[33] (4) In addition, we used a funnel plot to visually check for directional pleiotropy.[34] Plot asymmetry is a sign of directional pleiotropy.[35]

Second, we tested whether our results were sensitive to the data and model specifications. (1) We used MR-LASSO to re-estimate the main results and (2) checked whether the results were driven by specific SNPs. Because we have more than 500 SNPs, we used the bootstrap technique that randomly excludes 30% of the SNPs and re-performs MR-Egger 1000 times.[36]

Third, we conducted bidirectional MR to determine whether AF results in a higher BMR. Detecting the effect of AF on BMR may indicate that some SNPs violated the identification assumptions.

Results

Two-sample MR: Higher BMR Causes AF

We identified 458 SNPs for BMR-associated IVs (235 and 227 SNPs in females and males, respectively). The F statistics for IVs ranged from 29.74 to 678.91, indicating that these analyses were minimally affected by weak instrument bias (Additional file1: Tables S2-S4). BMR showed a statistically significant causal effect on AF (β = 0.77 , 95% CI 0.64 − 0.90), indicating that a higher BMR causes AF (Figure 2). The direction of this causal effect remained consistent across all the methods, confirming the robustness of the MR results. By consistently using female or male BMR summary GWAS to identify IVs, the estimates confirmed a significant causal relationship between BMR and AF (β = 0.83, 95% CI 0.67−1.00 and β = 0.84, 95% CI 0.67 − 1.01 respectively). We found little evidence ($I^2 > 0.9$) of attenuation bias due to measurement error (Additional file1: Table S6). The MR-Egger intercept test provided

little evidence ($p > 0.1$) of directional pleiotropy (Additional file1: Table S7). Additionally, the funnel plot indicated balanced pleiotropy of individual SNP, with a symmetrical variant effect distribution (Additional file1: Figure S1). Results are consistent under Bootstrap (Additional file1: Figure S2) . The Cochran's and Rucker's Q values are reported in Additional file1: Table S8. Due to the large number of SNPs, evidence of heterogeneity was observed. The estimates from the MR-Presso (β = 0.77, 95% CI 0.65−0.90) conducted to correct horizontal pleiotropy were consistent with the results of the other methods (Additional file1: Table S9).

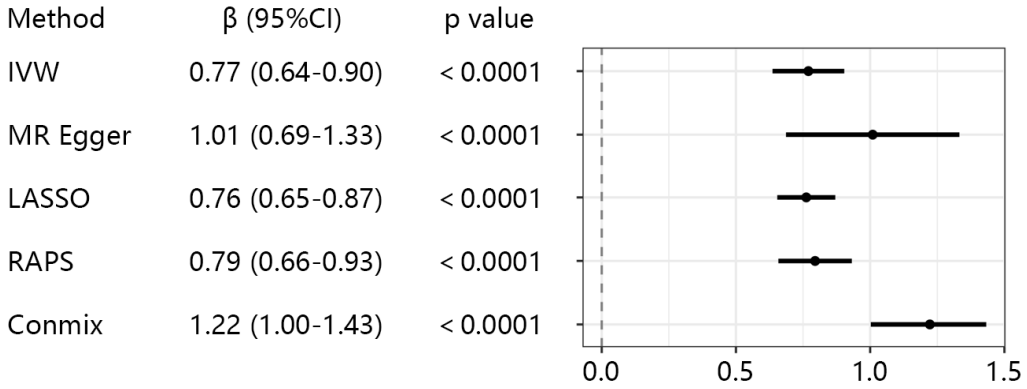


Figure 2. Univariable Causal Association Between Basal Metabolic Rate and Atrial Fibrillation. IV, instrumental variable; SNP, single nucleotide polymorphism; IVW, Inverse variance weighted; MR Egger, Mendelian randomization Egger; LASSO, least absolute shrinkage and selection operator; *RAPS,* robust adjusted profile score; *Conmix*, contamination mixture method. Exposure IVs were obtained from significant SNPs for BMR in both sexes, females and males, respectively.

Bidirectional MR was used to assess the causality and direction of the association between BMR and AF. Conversely, we estimated the causal effect of AF on BMR using significant SNP as AF genetic IVs. The MR results provided little evidence that AF affected the BMR ($p = 0.35$) (Additional file1: Table S10).

MVMR: BMR and AF

MVMR analyses were used to estimate the direct effect of BMR on AF, conditioned on measured potential confounders, including height, fat free mass and major cardiac and metabolic diseases (Table 1 and Additional file1: Table S11). The IVW MVMR results showed that this causal effect of BMR on AF was unaffected after controlling for height and fat free mass (β = 2.624, 95% CI 0.527 – 4.721), as well as major cardiac and metabolic diseases (β = 0.752, 95% CI 0.608−0.895). This estimated result was consistent across all methods. Little directional pleiotropy was observed in the MR-Egger intercept test.

Two-sample MR: BMR and Plasma Protein Level

The estimated causal effects between BMR and proteins level are shown in Table 2. The results showed that higher BMR had a positive causal effect on resistin (IVW β = 0.24, 95% CI 0.06 − 0.41, p < 0.01) and a negative effect on bone morphogenetic protein receptor type-1A (BMPR1A) (IVW β = −0.30, 95% CI [−0.49,−0.11], p = 0.002), which was consistent across all methods and are robust after controlling the false discovery rate of 5%. Fibroblast growth factor 23 (FGF-23) (IVW β = 0.15, 95% CI −0.03−0.33, p = 0.097) shows a marginal relationship. Considering the pleiotropic effects, a higher BMR also has a positive causal effect on growth differentiation factor 15 (MR-Egger β = 0.62, 95% CI 0.15 − 1.08, p = 0.01) and vascular cell adhesion molecule 1 (VCAM-1) (MR-Egger β = 0.44, 95% CI −0.02 − 0.90, p = 0.06). These estimated effects were consistent across other MR methods. In contrast, a consistently negative causal effect was also observed for BMR on neural cell adhesion molecule 1 (IVW β = −0.21, 95% CI [−0.41,−0.01], p = 0.037). However, no significant causal

effect was found between BMR and inflammatory cytokines, such as C-reactive protein (CRP) and interleukin-6 (IL-6).

Discussion

In this study, we examined the causal effect of BMR on AF using MR. Our univariable two-sample MR analysis suggested that higher BMR is a causal risk factor for AF incidence. This estimated effect is consistent with estimates from other MR analyses, indicating that horizontal pleiotropy had minimal association with this result. The direction of this relationship was confirmed using bidirectional MR analysis.

Previous studies showed that height and fat free mass are likely a positive causal risk factor for atrial fibrillation.[37,38] Our MVMR results corroborated the association between higher BMR and the risk of AF incidence independently of height, fat free mass, and several potential confounders considered in the present study. Studies showed that resting energy expenditure was found to be significantly higher in obese individuals[39] and individuals with type 2 diabetes mellitus have higher BMRs than the non-diabetic control group.[40] Therefore, we adjusted for common metabolic diseases, such as obesity, diabetes, thyrotoxicosis, hypertension in the MVMR. We also included cardiac event such as heart failure and coronary heart disease. Our results also confirmed this estimation. Furthermore, we found that coronary heart disease and hypertension are strongly associated with AF incidence.

Our findings correlated with those of previous population-based cohort studies, which suggested that patients with AF had significantly higher values of bioelectrical impedance analysis parameters, including BMR.[11] However, this correlation provided by observational studies might suffer from confounding or reverse causation bias. The MR study design

identifies causation. Moreover, the bidirectional MR analysis made any impact of reverse causation unlikely.

Potential Mechanism by Protein Analysis

We reviewed four prospective proteomics studies that reported the simultaneous screening of several proteins as potential AF biomarkers.[13–16] Eleven proteins were selected for screening the potential biological links between BMR and AF. N-terminal pro-brain natriuretic peptide (NT-proBNP) is reportedly a predictor of AF incidence.[13,14,41,42] Due to the absence of GWAS summary statistical data of NT-proBNP in the plasma proteome study by Sun et al.,[17] we used BNP32 instead, which is the C-terminal fragment of NT-proBNP. However, we did not find a statistically significant causal effect between BMR and BNP32, suggesting that NT-proBNP might not be a biological link between BMR and AF.

A cohort study showed that elevated FGF-23 levels are associated with higher risks of AF and reduced left ventricular (LV) function independent of kidney disease, which also affects all-cause and cardiovascular mortality.[43,44] Several prospective studies have validated the role of FGF-23 as a biomarker of prevalent AF.[44–45] In vitro and in vivo studies have implicated FGF-23 in promoting myocardial remodelling and cardiac hypertrophy[46] and fibrosis,[47] which can alter atrial electrophysiology and enhance atrial arrhythmogenesis, leading to AF.[48] Moreover, FGF-23 is associated with endothelial dysfunction.[49]

Resistin is associated with increased insulin resistance, and has pro-inflammatory and pro-hypertrophic effects.[50] Increased plasma resistin concentrations are significantly related to AF incidence.[51–53] Resistin directly affects the heart and its electrical properties. Mouse studies

have demonstrated that resistin can depress cardiac contractility, promote cardiac hypertrophy,[54] and promote endothelial cell activation and smooth muscle cell proliferation.[55]

VCAM-1 is a transmembrane type 1 protein, and increased levels of its soluble form (sVCAM-1) are associated with various cardiac diseases, such as ischemic cardiomyopathy, acute myocarditis, and AF.[56] A cohort study with a 20-year follow-up suggested that sVCAM1 was significantly associated with AF incidence.[15] One hypothesis of VCAM-1 in AF pathophysiology is that circulating VCAM-1 partially reflects local endocardial activation that influences atrial remodelling. Reportedly, sVCAM-1 levels significantly correlate with ventricular remodelling in patients with ST elevation myocardial infarction.[57]

Bone morphogenetic protein ligands and receptors, including BMPR1A, are essential for embryonic cardiac and vascular development. Ko et al. showed that decreased BMPR1A expression was significantly associated with AF incidence.[14] Furthermore, BMPR1A signalling for bone morphogenetic proteins is required for proper atrioventricular junction development.[58] BMP7-based small peptides, which function as BMPR1A agonists, inhibit pathological LV remodelling induced by pressure overload.[59]

The generation and persistence of AF depend on the trigger and substrate, including electrical and structural atrial remodelling. In this study, several potential AF biomarkers which are causally affected by higher BMR influence structural atrial remodelling. Furthermore, inflammation can alter the atrial electrophysiology and structure to increase vulnerability to AF. However, we found that two important inflammatory cytokines, CRP and IL-6, had no causal relation with BMR in this MR analysis. Thus, it was assumed that BMR was associated with atrial remodelling that leads to AF and systemic inflammation was not participate in this pathway (Figure 3).

Recent studies on the basal metabolism focused more on diet and exercise program. Although there was no agreement in the literature regarding the effect of exercise on BMR, the resistance exercise appeared to increase BMR, while aerobic exercise did not have similar effect.[60,61] And daily carbohydrate restriction, such as the ketogenic diet and western diet, is accompanied by a decline in the reduction of BMR.[60,62] Thus, changes in behaviour and lifestyle, such as dietary intake and regular physical activity, might prevent the incidence of AF by reducing or maintaining BMR.

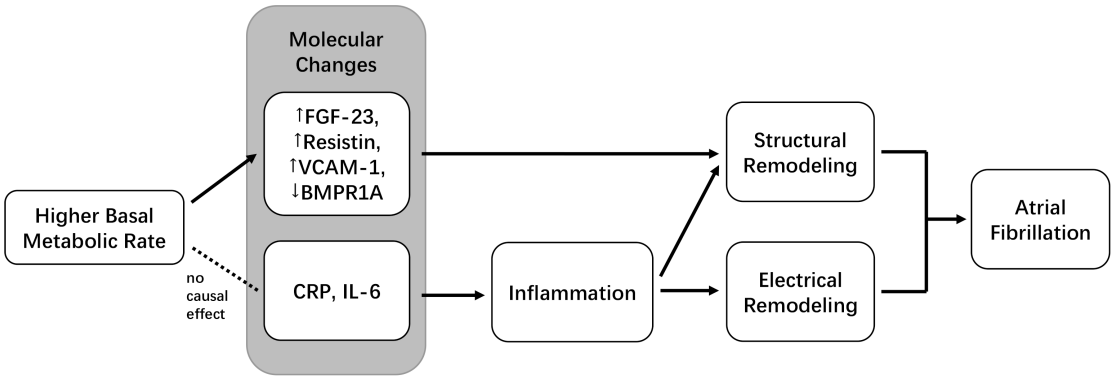


Figure 3. Overview of Potential Mechanism from Basal Metabolic Rate to Incident Atrial Fibrillation in Plasma Protein Level. FGF-23, fibroblast growth factor 23; VCAM-1, vascular cell adhesion molecule 1; BMPR1A, bone morphogenetic protein receptor type-1A; CRP, C-reactive protein; IL-6 interleukin-6.

Strengths and Limitations

The strengths of this study include the identification and estimation of the causal effect of BMR on AF, usage of the latest MR estimators, combination of multiple data sources, and most importantly, application of human plasma proteome GWAS studies. We not only established the causal relationship but also attempted to explain the potential mechanism.

However, our study had a few limitations. First, almost all study participants were of European ancestry; therefore, our results may be inapplicable in other ethnic groups. Second, the association between BMR and protein levels was only determined under the MR assumption, and sample of the plasma proteome GWAS study was insufficient to avoid a weak instrument bias. Finally, the association between increasing BMR and AF assumes a linear relationship; thus, this study could not determine the optimal BMR for AF occurrence.

Conclusion

In conclusion, this two-sample MR study showed that a higher BMR is a causal risk factor for AF incidence, regardless of height, BMI or cardiac and metabolic diseases. Furthermore, a higher BMR has a causal association with several atrial remodelling-associated proteins but not inflammatory cytokines, indicating that atrial remodelling influenced by the basal metabolic rate may not involve systemic inflammation. Our findings suggest that behaviours and lifestyle of reducing or maintaining BMR prevent AF incidence. Further work is needed to assess the overall impact of the BMR, dietary intake, and physical activity on AF.

List of abbreviations:

BMR = basal metabolic rate

AF = atrial fibrillation

GWAS = genome-wide association study

MR = Mendelian randomization

BMPR1A = bone morphogenetic protein receptor type-1A

FGF-23 = fibroblast growth factor 23

VCAM-1 = vascular cell adhesion molecule 1

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Table 1 The Multivariable Mendelian Randomization results of Basal Metabolic Rate on Atrial Fibrillation

		Model 1	Model 2
N (SNPs)		662	396
Exposure	BMR	2.624** (1.070)	0.752*** (0.073)
Covariate	Height	0.009 (0.088)	
	Fat Free Mass	-1.787 (1.128)	
	Hypertension		19.297*** (6.107)
	Coronary Heart Disease		6.322*** (1.619)
	Heart Failure		7.599 (5.992)
	Obesity		-8.573 (9.734)
	Diabetes		2.677 (6.600)
	Thyrotoxicosis		-6.106 (17.511)
RSE		1.259	1.296

Note: All models were estimated with inverse variance weighted multivariable Mendelian randomization analyses. Data were represented as estimated β(SE). SNP, single nucleotide polymorphism; BMR, basal metabolic rate; AF, atrial fibrillation; IVW, Inverse variance weighted; RSE, residual standard error. ∗p<0.1; ∗∗p<0.05; ∗∗∗p<0.01

Table 2: Univariable causal effects of basal metabolic rate on potential plasma biomarkers to incident AF

Protein	Beta (95%CI)	P value
Growth/differentiation factor 15		
Inverse variance weighted	0.04 (-0.15, 0.23)	0.692
MR Egger	0.62 (0.15, 1.08)	0.010
LASSO	0.00 (-0.18, 0.18)	0.974
RAPS	0.04 (-0.15, 0.24)	0.668
Conmix	-0.02 (-0.34, 0.33)	0.845
Resistin		
Inverse variance weighted	0.24 (0.06, 0.41)	0.008
MR Egger	0.50 (0.06, 0.93)	0.027
LASSO	0.25 (0.08, 0.42)	0.005
RAPS	0.29 (0.10, 0.47)	0.002
Conmix	0.71 (0.39, 0.94)	0.000
Vascular cell adhesion protein 1		
Inverse variance weighted	0.09 (-0.09, 0.28)	0.330
MR Egger	0.44 (-0.02, 0.90)	0.064
LASSO	0.14 (-0.03, 0.32)	0.114
RAPS	0.14 (-0.04, 0.33)	0.130
Conmix	0.23 (-0.01, 0.55)	0.065
Brain natriuretic peptide 32		
Inverse variance weighted	-0.13 (-0.32, 0.06)	0.171
MR Egger	-0.28 (-0.75, 0.18)	0.227
LASSO	-0.05 (-0.23, 0.12)	0.565
RAPS	-0.07 (-0.26, 0.12)	0.468
Conmix	-0.06 (-0.34, 0.26)	0.835
Fibroblast growth factor 23		
Inverse variance weighted	0.15 (-0.03, 0.33)	0.097
MR Egger	0.27 (-0.17, 0.70)	0.235
LASSO	0.16 (-0.01, 0.34)	0.066
RAPS	0.18 (0.00, 0.36)	0.054
Conmix	0.26 (0.00, 0.66)	0.054
Interleukin-6		
Inverse variance weighted	-0.04 (-0.22, 0.14)	0.687
MR Egger	-0.28 (-0.73, 0.16)	0.216
LASSO	-0.06 (-0.23, 0.12)	0.533
RAPS	-0.06 (-0.25, 0.12)	0.492
Conmix	-0.23 (-0.53, 0.21)	0.188
Fatty acid-binding protein, adipocyte		
Inverse variance weighted	0.05 (-0.13, 0.22)	0.599
MR Egger	0.00 (-0.43, 0.44)	0.989
LASSO	0.06 (-0.12, 0.23)	0.518
RAPS	0.07 (-0.11, 0.25)	0.459
Conmix	0.20 (-0.08, 0.46)	0.191

C-reactive protein		
Inverse variance weighted	-0.01 (-0.20, 0.18)	0.950
MR Egger	-0.03 (-0.50, 0.44)	0.906
LASSO	0.03 (-0.15, 0.21)	0.735
RAPS	0.02 (-0.17, 0.22)	0.806
Conmix	0.29 (-0.04, 0.56)	0.075
Neural cell adhesion molecule 1, 120 kDa isoform		
Inverse variance weighted	-0.21 (-0.41, -0.01)	0.037
MR Egger	-0.33 (-0.82, 0.16)	0.186
LASSO	-0.27 (-0.45, -0.09)	0.003
RAPS	-0.23 (-0.44, -0.03)	0.025
Conmix	-0.37 (-0.68, -0.11)	0.008
Angiopoietin-2		
Inverse variance weighted	0.11 (-0.06, 0.29)	0.206
MR Egger	0.04 (-0.39, 0.48)	0.850
LASSO	0.12 (-0.05, 0.30)	0.166
RAPS	0.14 (-0.05, 0.32)	0.144
Conmix	0.12 (-0.13, 0.46)	0.290
Bone morphogenetic protein receptor type-1A		
Inverse variance weighted	-0.30 (-0.49, -0.11)	0.002
MR Egger	-0.39 (-0.85, 0.08)	0.102
LASSO	-0.27 (-0.44, -0.09)	0.003
RAPS	-0.27 (-0.46, -0.08)	0.006
Conmix	-0.07 (-0.39, 0.19)	0.594

Note: MR Egger, Mendelian randomization Egger; LASSO, least absolute shrinkage and selection operator; *RAPS,* robust adjusted profile score; *Conmix*: contamination mixture method.