Assessment of causality between modifiable factors and heart failure: A Mendelian randomization analysis

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WW, WJ and TH designed the research. WW and TH had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. WW, WJ, ZZ, MG, RY, ZL, and TH wrote the paper and performed the data analysis. All authors contributed to the statistical analysis, critically reviewed the manuscript during the writing process, and approved the final version to be published. WW, WJ and TH are the guarantors for the study.

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ABSTRACT

Background and Objectives: Observational studies have associated lifestyle, dietary, adiposity, biochemical and clinical measures with heart failure. Whether the associations are causal remains unclear. We aimed to determine the causal associations between modifiable risk factors and incidence or mortality of heart failure. **Methods and Study Design:** Using single-nucleotide polymorphism (SNP) as genetic instruments, we conducted a two-sample Mendelian randomization (MR) analysis to estimate the causal effects of 27 modifiable risk factors on incident heart failure (2526 cases; 20926 participants) and mortality of heart failure (1798 deaths; 2828 patients). **Results:** None of 27 modifiable risk factors were significantly associated with incidence or mortality of heart failure after the Bonferroni correction (p<0.0019). However, there was suggestive evidence for genetically predicted educational attainment (odds ratio [OR] per educational year increase: 0.57, 95% CI 0.33-0.99, p=0.049), circulating mono-unsaturated fatty acid concentrations (OR per 1-SD increase [ORSD] : 1.50, 1.10-2.04, p=0.011), C-reactive protein (CRP) (1.53, 1.04-2.25, p=0.031), high-density lipoprotein (HDL) (0.84, 0.72-0.99, p=0.036), triglycerides (1.24, 1.00-1.52, p=0.045), and systolic blood pressure (SBP) (1.06, 1.01-1.11, p=0.017) with incident heart failure. **Conclusions:** Our findings provide supporting evidence for prioritizing certain modifiable risk factors such as education, lipids, and blood pressure for primary prevention of heart failure, suggesting important clues for further mechanism research.

Key Words: heart failure, Mendelian randomization, modifiable factors, incidence, mortality

INTRODUCTION

Heart failure is a syndrome driven by abnormalities of cardiac structure and function, involving increased intracardiac pressure or decreased cardiac output during stress or rest.[1] Due to population aging and growth, heart failure has been the fastest-growing cardiovascular event in the world and thought to affect more than 26 million people, imposing a heavy burden on health-care systems.[2-4] In spite of advances in therapeutic alternatives, mortality in heart failure remains high, 5-year survival ranging from 20-50% after first diagnosis.[5,6] Therefore, understanding the role of modifiable risk factors in development of heart failure is of great significance for its prevention and management.

A scientific statement from the American Heart Association suggests that elevated blood pressure, diabetes mellitus, body mass index (BMI), and hyperlipidemia are associated with

an increased risk of heart failure.[7] Inconclusive evidence from conventional observational studies have reported that moderate alcohol intake, coffee consumption, adiponectin, physical activity, and a healthy diet are associated with lower risk,[8-14] whereas smoking status, heart rate, sleep apnea, uric acid, C-reactive protein (CRP), depression and atrial fibrillation are associated with higher risk of heart failure.[15-19] Available evidence is largely inadequate as conventional observational studies are susceptible to reverse causation and residual confounding, and evidence from randomized controlled trials (RCT) is inclined to be insufficient and inconclusive.[20-25] Therefore, which specific factors affect heart failure risk and the strength of these effects remain unknown.

Mendelian randomization (MR) approach has been widely used to uncover unbiased causality among exposure and outcome where genetic variants are used as instruments for risk factors.[26] As genetic variants are fixed at conception and segregate randomly from parents to offspring, results from MR analyses are unlikely to be influenced by confounding and reverse causation bias. MR approach therefore circumvents the above-mentioned limitations of conventional observational studies and has been increasingly used to explore the potential causal effects of risk factors on disease. However, few MR studies were conducted to investigate the etiology of heart failure and only focused on the metabolic factors such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, BMI, blood pressure and uric acid.[27-32]

Given the unclear causal relevance of previously reported observational associations of modifiable risk factors with heart failure and limited exploration in MR analyses to date, we performed a two-sample MR analysis to evaluate the causal association of potentially modifiable risk factors with heart failure.

MATERIALS AND METHODS

Heart failure outcome genetic data

Summarized statistics of the association between genetic variants and incident heart failure were assessed from Cohorts for Heart and Aging Research in Genomic Epidemiology-Heart Failure Working Group (CHARGE-HF) of adults of European ancestry, including 20926 participants who had not been diagnosed with heart failure at baseline, of whom 2526 cases occurred during an average of 11.5 years of follow-up.[33] In addition, genotyping data for mortality of heart failure were identified from a genome-wide association study (GWAS) involving 2828 new-onset heart failure patients of European ancestry and 20-30% had a

myocardial infarction history, of which 1798 died during an average of 3.5 years follow-up time.[34]

Modifiable risk factors

We focused on potentially modifiable risk factors, divided into obesity-related, biochemical and clinical measures, lifestyle and dietary factors groups, which were identified from 2019 guideline issued by American College of Cardiology/American Heart Association (ACC/AHA) on primary prevention of cardiovascular disease.[35] In addition, we also identified other risk factors for heart failure from literature review of published epidemiological studies using PubMed (up to Jan 31, 2020). To be more specific, 3 obesity-related traits (BMI, body fat, waist : hip ratio adjusted by BMI [WHRadjBMI]),[35,36] 9 lifestyle and dietary factors (education, smoking, alcohol intake, coffee consumption, circulating iron and zinc, circulating 25-hydroxyvitamin D, circulating mono-unsaturated and omega-3 fatty acids),[15,19,35,37-40] 11 biochemical measures (fasting glucose, fasting insulin, 2h glucose, glycated hemoglobin [HbA1c], adiponectin, CRP, HDL, LDL, total cholesterol, triglycerides, uric acid),[11,35,41,42] 4 clinical measures (systolic blood pressure [SBP], diastolic blood pressure [DBP], pulse pressure, heart rate),[9,35] were considered as potentially risk factors and were brought into the two-sample MR analysis.

Genetic instruments selection

Single-nucleotide polymorphism (SNP) used as genetic instruments were selected from the largest genome-wide association studies (GWAS) (Table 1). For each SNP, only those below threshold of genome-wide significance ($p \leq 5\times10^{-8}$) and independent from each other for each trait - that is, linkage disequilibrium (LD) threshold of r^2 less than 0.2 were considered as potential instruments.[43] When there was LD between genetic variants, the one with the lowest *p* value for the associated risk factor was retained. The estimates of association between SNPs and risk factors were derived from sex-combined results of European ancestry or multi-ancestries are presented in eTable 1.

Statistical analysis

The MR is based on the assumptions that genetic instruments (1) are only associated with risk factors, (2) not associated with any confounders, and (3) influence heart failure only through risk factors (Figure 1). Another assumption is the associations of risk factors with heart failure are linear, without statistical interaction.[44]

For traits with 2 or more SNPs as genetic instruments, we estimated the causal effect by the inverse-variance weighted (IVW) method to combine the ratio estimates of each variant.[45] To evaluate the robustness of causal inference, we also conducted the weighted median, simple median, and MR-Egger regression approach for sensitivity analysis. Potential pleiotropy was assessed by the intercept obtained by MR-Egger approach.[46] For those traits with one SNP as instrument, the Wald ratio was used to estimate the causal effect by dividing the estimate for the association between the variant and risk factors by the association between the variant and incidence or mortality of heart failure.[45]

Results are reported as ORs with their 95% confidence intervals per unit genetically predicted increase in each risk factor. We included 27 risk factors in our analysis. Considering multiple testing, a Bonferroni correction significance threshold was set as 0.0019 (0.05/27). Also, we established a threshold for suggestive evidence when p values are between 0.0019 and 0.05. All of the above analysis were performed using R version 3.6.2.

We did power calculations for risk factors with significant or suggestive associations in IVW analysis using an online calculation tool (https://shiny.cnsgenomics.com/mRnd/) (eTable 3).

RESULTS

Lifestyle and dietary factors

Genetically predicted higher education showed a suggestive association with lower incident heart failure risk (OR per educational year increase: 0.57, 95% CI 0.33-0.99, *p*=0.049) (Figure 2), but not with mortality of heart failure (0.97, 0.51-1.86, *p*=0.927). The associations observed in sensitivity analysis using simple-median and weighted-median method were consistent. The MR-Egger method indicated no directional pleiotropy (*p*=0.82) (eTable 2). In addition, genetically predicted smoking, alcohol and coffee consumption were not associated with incident or mortality of heart failure (Figure 2).

For dietary factors, we observed a suggestive association of genetically determined higher circulating monounsaturated fatty acid levels with increased risk of heart failure (ORSD=1.50, 1.10-2.04, *p*=0.011), but not with mortality of heart failure (0.94, 0.64-1.39, *p*=0.769) (Figure 2). Simple median and weighted median methods supported the association with incidence risk, but with less precision (eTable 2). No significant results were observed for genetically predicted circulating iron, zinc, 25-hydroxyvitamin D and omega-3 fatty acid (Figure 2).

Obesity-related traits

There was little evidence that genetically predicted BMI was associated with risk of heart failure (ORSD=1.10, 0.99-1.46, p=0.065), with a stronger association for simple median method (1.85, 1.24-2.75, p=0.002) (Figure 2 and eTable 2). However, MR-Egger regression analysis showed evidence of pleiotropy for the association (p=0.008) (eTable 2). Genetically predicted BMI was not associated with mortality of heart failure. Likewise, there was no evidence of causal association of body fat percentage and WHRadjBMI with risk of heart failure (Figure 2 and eTable 2).

Biochemical and clinical measures

We observed suggestive associations of genetically determined higher HDL (ORSD=0.84, 0.72-0.99, p=0.036) with lower risk of heart failure, whereas an incident higher risk for genetically predicted higher triglycerides (1.24, 1.00-1.52, p=0.045), CRP (1.53, 1.04-2.25, p=0.031), and SBP (1.06, 1.01-1.11, p=0.017) (Figure 2). Except for simple-median method showing a stronger estimate of HDL (0.65, 0.49-0.87, p=0.004), none of the other factors showed associations in sensitivity analysis using simple median, weighted median or MR-Egger method (eTable 2). The MR-Egger method showed directional pleiotropy in the analysis of association between HDL and heart failure (p=0.027), but not in other biochemical and clinical measures associations (eTable 2). We didn't find associations between genetically predicted LDL, total cholesterol, fasting glucose, fasting insulin, 2h glucose, HbA1C, uric acid, adiponectin, DBP, pulse pressure and heart rate with risk of heart failure (Figure 2).

DISCUSSION

In the present study, we for the first time systematically investigated causal relationships of modifiable risk factors with heart failure using a MR approach. We found suggestive associations of genetically predicted education, circulating mono-unsaturated fatty acid, CRP, HDL, triglycerides, and SBP with incident heart failure ($0.0019<p<0.05$), whereas none of the modifiable risk factors were significantly associated with incidence rate or mortality of heart failure after Bonferroni correction ($p<0.0019$).

As far as we know, no studies have used MR framework to reveal the causal association between level of education and heart failure. Consistent with conventional observational studies,[37] educational attainment was found to have causal protective effect on incident heart failure in our analysis. One possible reason is that people with low level of education might have a poorer lifestyle and cardiometabolic profile.[47,48] Health care inequality and occupational status might also explain the higher risk of heart failure.[49] Moreover, we

provided evidence for the first time that circulating mono-unsaturated fatty acid is causally associated with increased incident heart failure using MR approach, which was suggested by previous conventional observational studies.[50,51] The potential mechanisms might be explained by increased blood pressure or heightened inflammation,[52,53] which were also identified as risk factors by our analysis. In our results, the positive associations of hypertension, hyperlipidemia and high CRP concentrations with incident heart failure were in accordance with several RCTs.[54-56] We didn't find associations between genetically predicted glycemic index and risk of heart failure, indicating metabolic disorders might not affect heart failure via glucose metabolism. However, we cannot exclude small effect estimates on account of limited power of our analyses. Corroborating previous MR,[31] we found no association between uric acid and heart failure, suggesting the association observed in observational studies could be affected by reverse causation or confounders.[11]

We didn't find any significant causal association for mortality of heart failure. Our findings are consistent with RCTs in which targeting traditional risk factors (e.g. hypertension, hyperlipidemia) didn't have a beneficial effect on heart failure patients,[57-59] although it is generally believed that the pathophysiological mechanism leading to incident heart failure is similar to the progression of heart failure. Therefore, it is suggested that decreasing the incidence rate is the primary goal for reducing the burden of heart failure.

Strengths of our study include the use of the MR framework in assessing the etiology of disease to avoid reverse causation and confounding, the derivation of data from large GWAS of risk factors, and the restriction to data from predominantly European ancestry cohorts to reduce the confounding caused by population stratification. More importantly, by leveraging large scale GWAS summary statistics, we systematically explored multiple risk factors for heart failure which have not been investigated in previous MR studies. Even when compared to those risk factors examined in previous MR studies, our analysis provides several advantages. For example, though genetically predicted BMI was previously identified as risk factor of incident heart failure, we constructed genetic instruments for BMI using 77 SNPs (versus 1 or 32 SNPs of previous studies),[30,60] which might allow us to detect false positives with greater instrument strength. However, our analysis also has some limitations. First, we derived genetic variants from large GWAS data, which were significantly associated with corresponding risk factors, but our findings may still be subject to weak instrument bias. However, the majority of risk factors have an F-statistic of >10, and thus likelihood of instrument bias is greatly reduced. Second, it is difficult to exclude the possibility of pleiotropy from causal effects in view of the imprecision of the MR-Egger method in all MR

analysis. Finally, the power of some of our analysis is limited, so that we cannot rule out false negative results. Large-scale MR studies should be done to identify additional associations.

In summary, our analysis provides suggestive evidence that several modifiable risk factors play an important role in development of heart failure, underscoring the importance of interventions for primary prevention and management of heart failure. First, given the causal estimates of our analysis, one-year increase of education attained is equivalent to a 43% decrease in incident heart failure, suggesting that promoting education is of promising practical significance for the prevention of heart failure. Preventive cardiovascular services should be extended to underprivileged communities for the sake of equitable provision of health care. What's more, our results indicated that lipid traits, fatty acids and inflammation may be causally related to heart failure risk, and thus early detection and treatment of people with metabolic dysregulation has great importance in the prevention of heart failure and will provide effective targets to improve the prognosis of patients.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors declare: no support from companies for the submitted work; no relationships with companies that might have an interest in the submitted work in the previous three years; no spouses, partners, or children have no financial relationships that may be relevant to the submitted work; no non-financial interests that may be relevant to the submitted work. The study was supported by grants from the Peking University Start-up Grant (BMU2018YJ002), High-performance Computing Platform of Peking University. The funding organization had no role in the preparation of the manuscript.

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Table 1. Description of modifiable risk factors

	Consortium or study	Maximum sample size	Population	Reference	Number of SNPs identified in GWAS	Number of SNPs included in MR (incidence/mortality)[†]	R^2(%) explained by the SNPs
Obesity-related traits							
BMI, kg/m^2	GIANT	322,154	European	Locke et al, 2015	77	77	2.4
Body fat, %	56 studies	100,716	Trans-ethnic	Lu Y et al, 2016	12	12	0.6
WHRadjBMI	GIANT	224,459	European	Shungin et al, 2015	39	39	1.2
Lifestyle and dietary factors							
Education, year	SSGAC	405,072	European	Okbay et al, 2016	162	57	0.5
Smoking, cigarettes per day	TGC	74,053	European	Furberg et al, 2010	3	3	0.5
Alcohol intake, drinks per week	GSCAN	945,988	European	Liu et al, 2019	94	47	0.2
Coffee, cups consumed per day	28 studies	87,998	European	Marilyn et al, 2015	4	4	1.3
Circulating iron, μmol/L	ATR	2,299	European	Beben et al, 2011	2	1	1.2
Circulating zinc, μmol/L	QIMR	2,603	European	Evans et al, 2013	3	2	4.6
Circulating 25-hydroxyvitamin D, nmol/L	SUNLIGHT	79,366	European	Xia et al, 2018	6	6	2.6
Circulating MUFA, %[‡]	14 studies	13,535	European	Kettunen J et al,2016	7	3	1.8
Circulating omega-3 fatty acid, %[‡]	14 studies	13,544	European	Kettunen J et al,2016	5	3	1.8
Biochemical measures							
Fasting glucose, mmol/L	MAGIC	133,010	European	Robert et al, 2013	36	36	4.8
Fasting insulin, pmol/L	MAGIC	133,010	European	Robert et al, 2013	19	19	1.2
2h glucose, mmol/L	MAGIC	133,010	European	Robert et al, 2013	9	9	0.3
HbA1C, %	MAGIC	46,368	European	Soranzo et al, 2010	11	11/10	1.8
Adiponectin, μg/mL	16 studies	45,891	Trans-ethnic	Dastani et al, 2012	18	17	1.9
CRP, mg/L	CHARGE	204,402	European	Symen et al, 2018	40	35	0.8
HDL, mg/dL	GLGC	188,578	European	Willer et al, 2013	71	70	13.7
LDL, mg/dL	GLGC	188,578	European	Willer et al, 2013	57	56/55	14.6
TC, mg/dL	GLGC	188,578	European	Willer et al, 2013	74	73/74	15.0
TG, mg/dL	GLGC	188,578	European	Willer et al, 2013	40	40/39	11.7
Uric acid, mg/dL	GUGC	110,347	European	Köttgen et al, 2013	30	30	7.0
Clinical measures							
SBP, mmHg	GERA, UKB, ICBP	321,262	Trans-ethnic	Hoffmann et al, 2017	103	42	0.5
DBP, mmHg	GERA, UKB, ICBP	321,262	Trans-ethnic	Hoffmann et al, 2017	118	52/51	0.6
Pulse pressure, mmHg	GERA, UKB, ICBP	321,262	Trans-ethnic	Hoffmann et al, 2017	157	73/72	0.9
Heart rate, bpm	UKB	265,046	European	Eppinga et al, 2016	66	30	0.9

GWAS: Genome-wide association study; MR: Mendelian Randomization; BMI: body mass index; WHRadjBMI: waist-hip ratio adjusted by BMI; HbA1C: glycated hemoglobin; MUFA: mono-unsaturated fatty acid; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reative protein; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglycerides; SNP: single nucleotide polymorphism; CHARGE: Cohorts for Heart and Aging Research in Genomic Epidemiology; MAGIC: Meta-Analyses of Glucose and Insulin-Related Traits Consortium; ICBP: International Consortium for Blood Pressure; GIANT: Genetic Investigation of Anthropometric Traits; GLGC: Global Lipids Genetics Consortium; TGC: Tobacco and Genetics Consortium; ATR: Australian Twin Registry; QIMR: Queensland Institute of Medical Research; GUGC: Global Urate Genetics Consortium; GERA: Genetic Epidemiology Research on Adult Health and

Aging; ICBP: International Consortium for Blood Pressure; SSGAC: Social Science Genetic Association Consortium; GSCAN: GWAS and Sequencing Consortium of Alcohol and Nicotine use; UKB: UK Biobank.
[†]Number of SNPs excluded for each exposure because of missing in heart failure summary statistics data, in linkage disequilibrium with another SNP or derived from sex-specific results.
[‡]Restricted analyses that excluded SNPs associated with other classes of fatty acids.

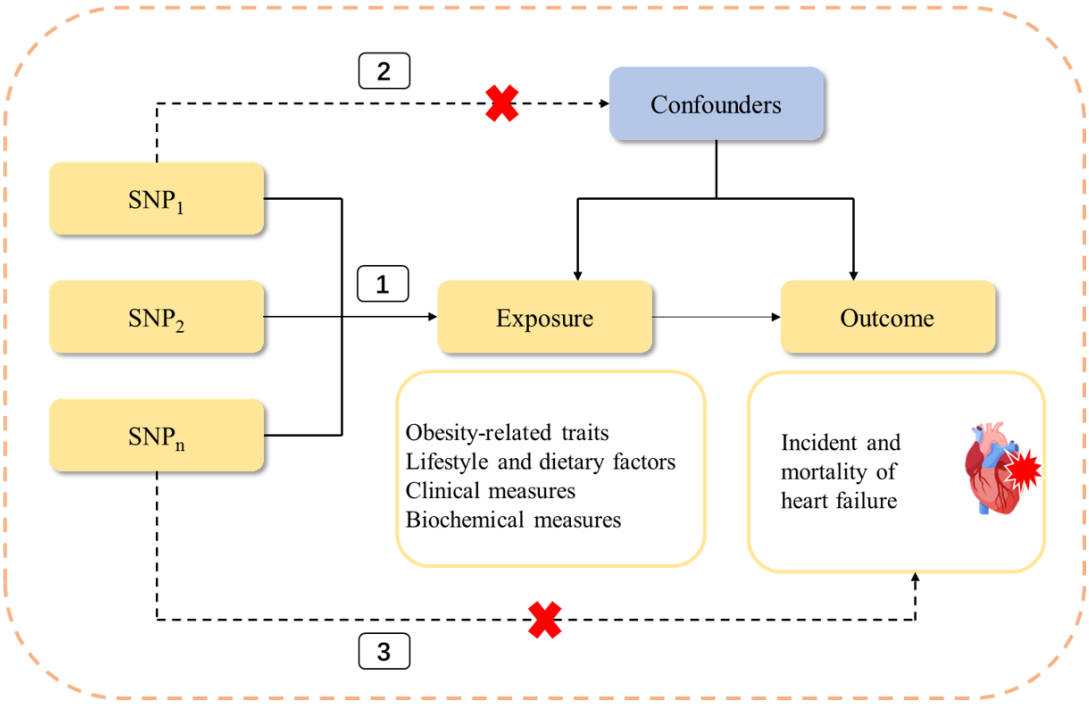

Figure 1. Principles of Mendelian randomization analysis and assumptions that need to be met to obtain unbiased causal effect estimates. Dashed lines represent potential pleiotropic or direct causal effects that could violate Mendelian randomization assumptions. Three assumptions of MR are as follows: (1) genetic variants used as instrumental variables are associated with the exposure, (2) genetic variants are not associated with any confounders and (3) genetic variants influence outcome only through exposure. SNP=single nucleotide polymorphisms.

Risk factors	No. of SNPs	OR (95CI%)	P-value	No. of SNPs	OR (95CI%)	P-value
Obesity-related traits						
BMI	77	1.10 (0.99,1.46)	0.065	77	1.02(0.81,1.29)	0.859
Body fat	12	1.46 (0.75,2.82)	0.265	12	1.26(0.66,2.41)	0.476
WHRadjBMI	39	0.90 (0.62,1.31)	0.588	39	1.33(0.85,2.07)	0.211
Lifestyle and dietary factors						
Education	57	0.57 (0.33,0.99)	0.049	57	0.97(0.51,1.86)	0.927
Smoking	3	1.04 (0.99,1.10)	0.104	3	1.04(0.98,1.11)	0.217
Alcohol intake	47	0.76 (0.32,1.84)	0.547	47	1.38(0.51,3.75)	0.523
Coffee	4	1.12 (0.67,1.87)	0.673	4	1.19(0.79,1.80)	0.402
Circulating iron	1	1.04 (0.72,1.52)	0.840	1	0.89(0.56,1.40)	0.618
Circulating zinc	2	0.94 (0.72,1.25)	0.687	2	1.00(0.78,1.27)	0.983
Circulating 25-hydroxyvitamin D	6	1.54 (0.82,2.89)	0.184	6	1.20(0.43,3.38)	0.727
Circulating MUFA	3	1.50 (1.10,2.04)	0.011	3	0.94(0.64,1.39)	0.769
Circulating omega-3 fatty acid	3	1.33 (0.91,1.94)	0.143	3	0.92(0.64,1.33)	0.668
Biochemical measures						
Fasting glucose	36	1.27 (0.83,1.94)	0.271	36	0.90(0.55,1.47)	0.676
Fasting insulin	19	0.55 (0.22,1.36)	0.196	19	0.53(0.19,1.50)	0.230
2h glucose	9	1.01 (0.72,1.41)	0.946	9	1.23(0.91,1.65)	0.177
HbA1c	11	0.79 (0.45,1.40)	0.429	10	1.00(0.47,2.15)	0.999
Adiponectin	17	0.74 (0.45,1.21)	0.230	17	0.85(0.47,1.52)	0.577
C-reactive protein	35	1.53 (1.04,2.25)	0.031	35	1.25(0.79,1.97)	0.337
High-density lipoprotein	70	0.84 (0.72,0.99)	0.036	70	0.89(0.73,1.07)	0.215
Low-density lipoprotein	56	1.19 (1.00,1.41)	0.051	55	1.14(0.95,1.38)	0.162
Total cholesterol	73	1.13 (0.95,1.34)	0.170	74	1.18(0.98,1.41)	0.082
Triglycerides	40	1.24 (1.00,1.52)	0.045	39	1.23(0.98,1.55)	0.072
Uric acid	30	1.06 (0.89,1.26)	0.527	30	1.15(0.97,1.35)	0.101
Clinical measures						
SBP	42	1.06 (1.01,1.11)	0.017	42	0.96(0.91,1.01)	0.136
DBP	52	1.01 (0.98,1.03)	0.537	51	1.02(0.99,1.05)	0.139
Pulse pressure	73	1.00 (1.00,1.00)	0.634	72	1.00(1.00,1.00)	0.831
Heart rate	30	1.03 (0.99,1.07)	0.198	30	1.00(0.95,1.05)	0.948

Figure 2. Odds ratio for associations between genetically predicted obesity-related traits, lifestyle and dietary factors, biochemical and clinical measures and incident and mortality of heart failure. The estimates are scaled to represent the association of 1-cigarette a day of smoking, 1-year increase of education attained, 1-cup a day of coffee consumption, 1-drink a week of alcohol consumption, 1-unit increase in natural log transformed 25-hydroxyvitamin D and C-reactive protein, 5-bpm increase in resting heart rate, 1-SD for the other continuous risk factors and 1-unit higher log odds of binary risk factors. BMI: body mass index; WHRadjBMI: waist-hip ratio adjusted by BMI; HbA1C: glycated hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure; MUFA: mono-unsaturated fatty acid.