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Causal Pathways Between Breast Cancer and Cardiovascular Disease Through Mediator Factors: A Two-Step Mendelian Randomization Analysis

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Background: The causal relationship of breast cancer (BC) with cardiovascular disease (CVD) and the underlying mediating pathways remains elusive. Our study endeavors to investigate the causal association between BC and CVD, with a focus on identifying potential metabolic mediators and elucidating their mediation effects in this causality.

Methods: In this study, we conducted two-sample Mendelian randomization (MR) to estimate the causal effect of BC (overall BC, ER + BC, ER- BC) from the Breast Cancer Association Consortium (BCAC) on CVD including coronary heart disease (CHD), hypertensive heart disease (HHD), ischaemic heart disease (IHD), and heart failure (HF) from the FinnGen consortium. Then, we used two-step MR to evaluate 18 metabolic mediators of the association and calculate the mediated proportions.

Results: Genetically predicted ER+ BC was causally associated with an increased risk of CVD including CHD (OR = 1.034, 95% CI: 1.004–1.065, p = 0.026), HHD (OR = 1.061, 95% CI: 1.002–1.124, p = 0.041), IHD (OR = 1.034, 95% CI: 1.007–1.062, p=0.013), and HF (OR = 1.055, 95% CI: 1.013–1.099, p = 0.010), while no causality was observed for overall BC and ER- BC. Furthermore, high-density lipoprotein cholesterol (HDL-C) was identified as a mediator of the association between ER+BC and CVD, including CHD (with 15.2% proportion)) and IHD (with 15.5% proportion), respectively.

Conclusion: This study elucidates the potential causal impact of ER+ BC on subsequent risk of CVD, including CHD, HHD, IHD, and HF. We also outline the metabolic mediator HDL-C as a priority target for preventive measures to reduce excessive risk of CVD among patients diagnosed with ER+BC.

Keywords: breast cancer, cardiovascular disease, ER status, Mendelian randomization, metabolic mediators, HDL-C

Introduction

Breast cancer (BC) is the most frequently diagnosed malignant cancer as well as the leading cause of cancer-related mortality in women globally.[1] According to the American Cancer Society, BC stood out as the leading contributor to new cancer cases among females in the United States, comprising a staggering 32% of the total incidences. Besides, it occupied the second-most significant position in terms of mortality rates, accounting for 15% of the female deaths recorded in 2024.[2] BC can be classified into two main subtypes: ER+ and ER-, differentiated by the presence or absence of estrogen receptor (ER) expression on cancer cells. Notably, approximately 70% of BC patients exhibit ER expression, thereby rendering it a pivotal therapeutic target for precision-based treatments.[3]

Over the last two decades, with advancements in early detection, precision therapy, and supportive care, there has been a consequent increase in survival following BC diagnosis. However, non-cancer-related mortality, including cardiovascular disease(CVD), cerebrovascular disease, chronic obstructive pulmonary disease (COPD), and

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Alzheimer's disease, has been increasing in BC survivors.[4] Among them, CVD stands as the primary cause of mortality among women in the United States, posing a significant impediment to achieving optimal outcomes for BC survivors.[5,6]

Epidemiological evidence concerning the correlation between BC and CVD remains inconclusive. A population-based cohort of 6641 BC patients who survived at least 10 years demonstrated that an increased risk of newly diagnosed diseases of the circulatory system was observed compared with the general population.[7] Several cohort studies have examined the CVD risks among BC survivors, which showed that women with a history of BC are more predisposed to developing and dying of CVD compared to women without a history of BC.[8–10] However, the latest published meta-analysis indicated that BC is associated with a higher risk of atrial fibrillation, and heart failure when compared to the general population, but not with myocardial infarction, coronary artery disease, or ischaemic stroke,[11] contradicting some of the previous studies.

Considering the diverse CVD risk among various primary cancers, the malignant tumor itself or the cardiotoxic effects of certain cancer therapies are likely to be pivotal factors contributing to the risk of CVD.[12] As mentioned earlier, BC can be classified into ER+, and ER- cancers based on the presence of estrogen receptors and these subtypes may have different etiologies. Therefore, without further stratification, some previous measurements of the impact of BC on CVD may have been mixed and less accurate. Furthermore, due to the presence of pre-existing CVD in some BC patients before diagnosis, many cohort studies have focused on mortality rather than incidence as the outcome measure. Consequently, it remains blurry to what extent BC is directly associated with the incidence of CVD, and to what potentially modifiable risk factors mediate this association. Advancement in early detection and diagnosis of BC has resulted in a growing cohort of survivors, who are increasingly susceptible to long-term cardiac complications. As the population ages, more women may suffer from BC, CVD, or both, and thus knowledge of this topic is therefore warranted.[13]

To fill the knowledge gap, we conducted Mendelian randomization (MR) analyses leveraging genetic variants as instrumental variables (IVs) to deduce causal associations between pertinent traits. As genetic variation is randomly allocated at conception, MR analyses are akin to randomized controlled trials. In addition, multivariable Mendelian randomization (MVMR) is employed as an extended methodology, to disentangle the independent impacts of correlated exposures on an outcome by incorporating genetic variants specific to each exposure within a consolidated analytical framework.[14] Furthermore, a two-step MR study can be utilized to delineate the pathways through which an exposure affects an outcome, thereby enhancing causal inference in mediating effects. This methodology addresses several limitations encountered in traditional methods, including the issue of reverse causation and the potential for unmeasured confounding.[15]

In this study, we conducted two-step MR analyses to elucidate the causal association between BC (overall BC, ER+ BC, ER- BC) and CVD, specifically CHD, HHD, IHD, and HF, with particular focus on identifying metabolic mediators and quantifying their mediation effects on this association pathway.

Methods

Study Design

This MR study consisted of two stages and an overview of the study design is presented in Figure 1. In stage 1, a two-sample MR was utilized to evaluate the causal association between BC (overall BC, ER+, and ER− subtypes) and CVD (CHD, HHD, IHD, and HF) based on data from genome-wide association studies (GWASs) summary statistics. In stage 2, we initially screened 18 potential candidates as mediators in the pathway between BC and CVD, and then implemented a two-step MR to calculate the mediation effect of each selected mediator on the causal relationship between BC and CVD.[16,17] The MR analysis was conducted in accordance with the STROBE-MR guidelines.[18]

Data Source

GWASs summary level data, primarily originating from individuals of European ancestry, was utilized to obtain data resources pertaining to exposures, mediators, and outcomes. Ethical approval for the original studies was obtained, ensuring compliance with ethical standards. Detailed information was summarized in Table 1.

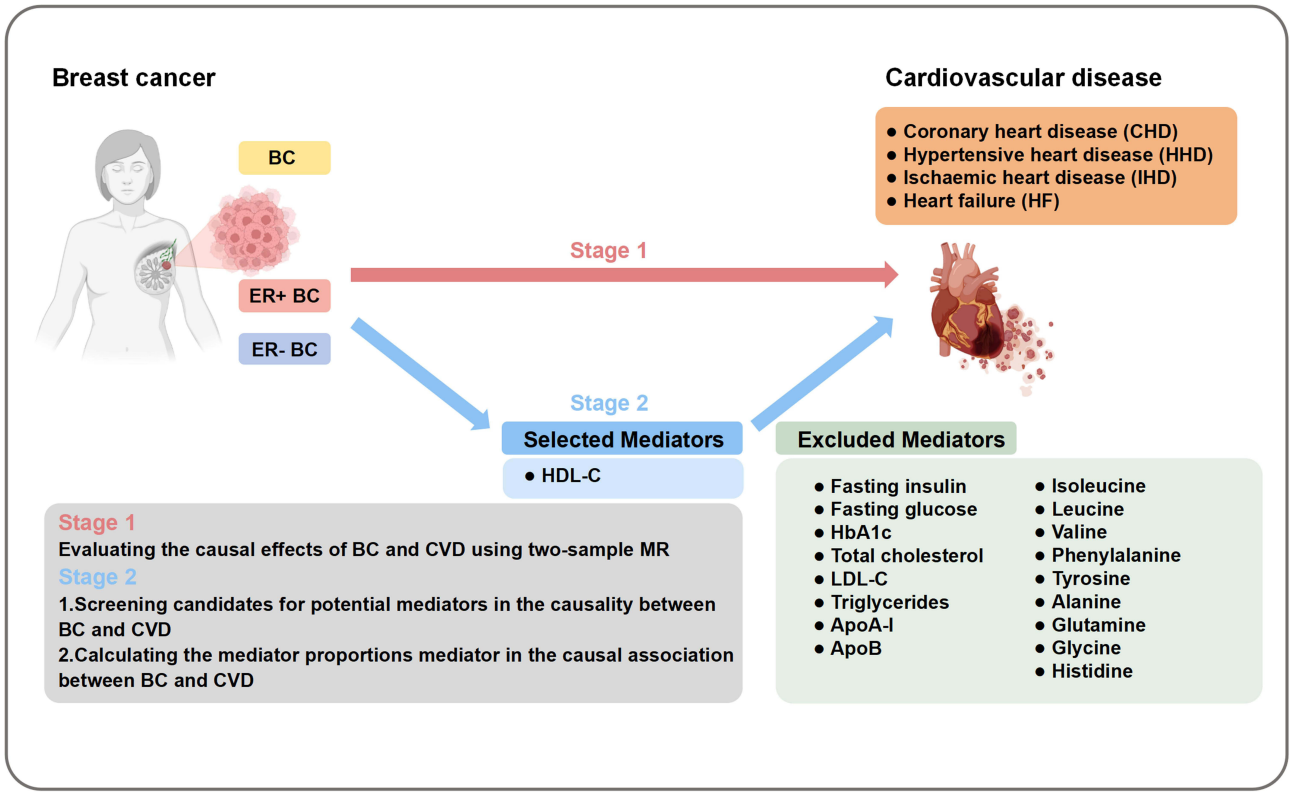

Figure 1 Overview of the study design CHD, coronary heart disease; HHD, hypertensive heart disease; IHD, ischaemic heart disease; HF, heart failure; ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Exposures

Genetic IVs for the exposures in individuals of European descent were retrieved from the GWAS summary data (https://gwas.mrcieu.ac.uk/) and obtained from the Breast Cancer Association Consortium (BCAC), including overall BC (14,910 cases and 17,588 controls), ER+ BC (4,226 cases and 17,588 controls), and ER- BC (4,480 cases and 17,588 controls).[19] Single nucleotide polymorphisms (SNPs) with genome-wide significance ($p < 5 \times 10^{-6}$) were derived from the corresponding GWASs and then clumped to a linkage disequilibrium threshold of $r^2<0.001$ within a 5000 kb window to select independent genetic variants. We further eliminated the potential pleiotropic confounders using Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/). Finally, to ensure that genetic variants solely influence the outcome via the risk factors, we excluded those SNPs that are directly related to CVD (CHD, HHD, IHD, and HF) by applying a threshold at genome-wide significance ($p > 5 \times 10^{-5}$).[20] After removing the SNPs that were palindromic with intermediate allele frequencies, weak IVs, and IVs that explained more of the variance in the outcome than in the exposure, there were 34, 22.17 SNPs with regards to overall BC, ER+ BC, ER− BC, respectively, which were extracted for further MR. Detailed information about all SNPs is shown in Supplementary Table S1.

Mediators

BC and CVD exhibit a profound correlation with the intricate metabolism of insulin, glucose, lipids, fatty acids, and amino acids. Therefore, our research focused on candidate mediators that encompass these metabolic characteristics, subsequently classifying them into three distinct clusters,[1] insulin and glycaemic traits including fasting glucose, fasting insulin, and glycated hemoglobin (HbA1c)[2,21] lipid traits including triglycerides, total cholesterol,[22] high-density lipoprotein cholesterol (HDL-C),[23] low-density lipoprotein cholesterol (LDL-C),[24] apolipoprotein A-I (ApoA-I) and apolipoprotein B (ApoB)[3,25] amino acids traits including isoleucine, phenylalanine, alanine, glutamine,[26] leucine, valine, tyrosine, glycine, and histidine.[27]

Our criteria for identifying the potential mediators between ER+BC and CVD (CHD, HHD, IHD, and HF) included: (1) ER+BC and the mediator are causally related, and the effect of ER+BC on the mediator should be unidirectional;

Table 1 GWAS Data Sources of the MR Study

Trait	Consortium	Ancestry	Sample size	GWAS ID
Exposure				
BC	BCAC	European	32498	ieu-a-1131
ER+ BC	BCAC	European	21814	ieu-a-1134
ER- BC	BCAC	European	22068	ieu-a-1137
Mediator				
Fasting insulin	MAGIC	European	108557	ieu-b-116
Fasting glucose	MAGIC	European	133010	ieu-b-114
HbA1c	MAGIC	European	46368	ieu-b-104
Triglycerides	EBI	European	343992	ebi-a-GCST90018975
Total cholesterol	EBI	European	344278	ebi-a-GCST90018974
HDL-C	EBI	European	94595	ebi-a-GCST002223
LDL-C	EBI	European	9961	ebi-a-GCST005068
ApoA-I	EBI	European	398508	ebi-a-GCST90025955
ApoB	EBI	European	435744	ebi-a-GCST90025952
Isoleucine	EBI	European	291	ebi-a-GCST90026189
Phenylalanine	EBI	European	291	ebi-a-GCST90026249
Alanine	EBI	European	291	ebi-a-GCST90026114
Glutamine	EBI	European	291	ebi-a-GCST90026170
Leucine	UK Biobank	European	115074	met-d-Leu
Valine	UK Biobank	European	115048	met-d-Val
Tyrosine	UK Biobank	European	115075	met-d-Tyr
Glycine	UK Biobank	European	114972	met-d-Gly
Histidine	UK Biobank	European	114895	met-d-His
Outcome				
CHD	FinnGen	European	218792	finn-b-I9_CHD
HHD	FinnGen	European	218792	finn-b-I9_HYPTENSHD_EXNONE
IHD	FinnGen	European	218792	finn-b-I9_IHD
HF	FinnGen	European	218792	finn-b-I9_HEARTFAIL

Notes: ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CHD, coronary heart disease; HHD, hypertensive heart disease; IHD, ischaemic heart disease; HF, heart failure.

(2) the causality between the mediator and CVD is consistent, regardless of the adjustment for ER+BC; (3) The association of ER+ BC with the mediator and the association of the mediator with CVD should be in same directions. The detailed process for selecting mediators is presented in Figure 2.

Following a rigorous selection process, HDL-C emerged as a significant risk factor that met all established criteria and was consequently included in the mediation analyses, aimed at elucidating its mediating role in the causal relationship between ER+BC and CVD (CHD, IHD).

Outcomes

In this study, we selected four representative subtypes of CVD (CHD, HHD, IHD, and HF) that are widely recognized due to their high incidence and significant impact on public health. The FinnGen research project represents a collaborative public–private partnership that seamlessly integrates genotype information derived from Finnish biobanks with comprehensive digital health records maintained by the Finnish health registry. For the genetic instruments for CVD, we performed an in-depth analysis utilizing the data on CHD (21,012 cases and 197,780 controls), HHD (3,938 cases and 214,854 controls), IHD (31,640 cases and 187,152 controls), and HF (13,087 cases and 195,091 controls) from FinnGen consortium.

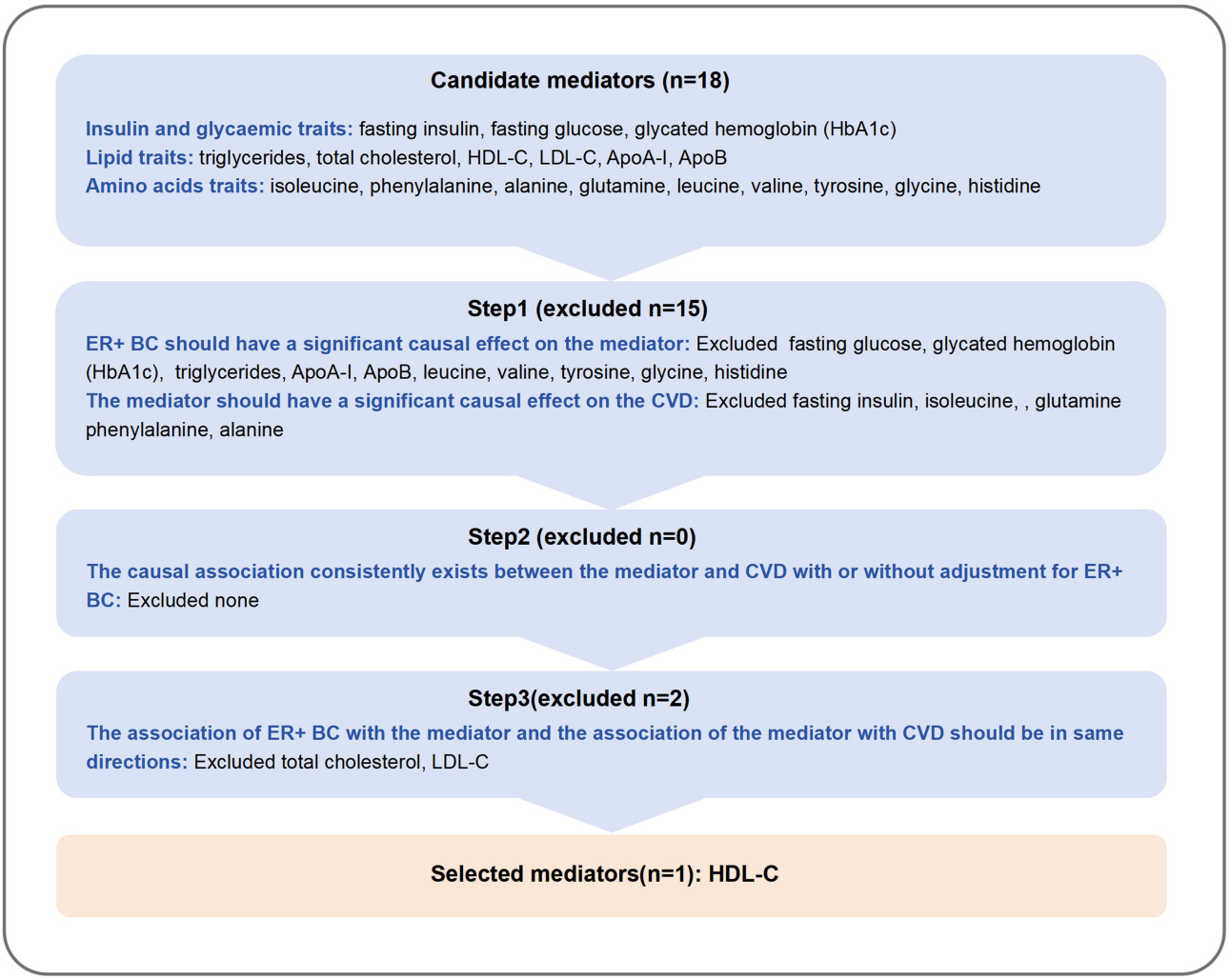

Figure 2 Mediator selection process ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Statistical Analysis

Two-Sample MR and MVMR Analyses

For two-sample MR analyses, the inverse variance weighted (IVW) approach served as the primary analytical strategy, with the MR-Egger, weighted median, simple mode, and weight mode methodologies acting as supplementary methods.[28] For the implementation of MVMR analysis, a comprehensive GWAS result is imperative to extract IVs information. As the primary analytical tool, we employed IVW, which leverages a random-effects meta-analysis framework to aggregate Wald Ratio estimates for individual SNPs, thereby generating a single causal estimate for each exposure variable. All MR analyses fulfilled three critical assumptions: (1) relevance assumption: SNPs chosen as IVs must exhibit a strong association with the exposure; (2) independence assumption: the genetic variants selected should not be correlated with potential confounders; (3) exclusion-restriction assumption: IVs influence the outcome solely through the exposure, not via alternative pathways.[29]

Mediation MR Analyses

To assess the intermediary impacts of risk factors on the association between ER+BC and CVD (CHD, HHD, IHD, and HF), we performed a two-step MR.[30] Firstly, we leveraged UVMR to quantify the causal effect of genetically predicted ER+BC on the mediator (β1). Secondly, we utilized MVMR to evaluate the causal impact of the mediator on the incidence of CVD. To

determine the fraction of the total effect of ER+BC on CVD (CHD, HHD, IHD, and HF) that is mediated through each intermediary factor, we calculated the proportion by dividing the mediation effect (β1 × β2) by the total effect (β). Standard errors for the mediation effects were obtained by the delta method.

Sensitivity Analyses

Comprehensive sensitivity analyses were conducted to estimate potential violations of the model assumptions in the MR analysis. To detect the presence of horizontal pleiotropy, we utilized the intercept test of the MR-Egger regression.[31] Additionally, the MR-PRESSO was employed to identify outliers among SNPs exhibiting pleiotropic effects, enabling the generation of estimates following the exclusion of such outliers.[32] Besides, Cochran's Q value served as a quantitative measure of heterogeneity among SNP estimates.[33] Moreover, a leave-one-out analysis was conducted to assess the potential contribution of any individual SNP to a significant effect. Finally, a funnel plot was implemented to assess the potential directional bias resulting from pleiotropy.[34]

With the aim of uncovering as many potential positive associations as possible, we did not apply multiple testing corrections in this exploratory study. All MR analyses were conducted with the R (version 4.2.3) using the package "TwoSampleMR", "MR-PRESSO" and "MendelianRandomization".

Results

Causal Effects of BC on CVD

The potential risk of overall BC on four kinds of cardiovascular diseases, including CHD, HHD, IHD, and HF, were evaluated as the first step of our study. Consequently, we have not obtained causal relationships with statistical significance (Figure 3).

To determine whether these negative results were related to the interference with the ER status of BC, we further performed subtypes analysis based on ER status (ER+ and ER−) and the results are summarized in Figure 4. Our analyses evaluated by the IVW method uncovered a positive correlation between genetically predicted ER+BC and a slightly elevated risk of CHD (OR = 1.034, 95% CI: 1.004–1.065, p = 0.026), HHD (OR = 1.061, 95% CI: 1.002–1.124, p = 0.041), IHD (OR = 1.034, 95% CI: 1.007–1.062, p=0.013), and HF (OR = 1.055, 95% CI: 1.013–1.099, p = 0.010), although MR-Egger and weighted median did not attain statistical significance. As for ER-BC, based on the IVW analysis, no remarkable association was observed between ER-BC and CVD (Supplementary Figure S1). The results of the two-sample MR are detailed in Supplementary Table S2-4.

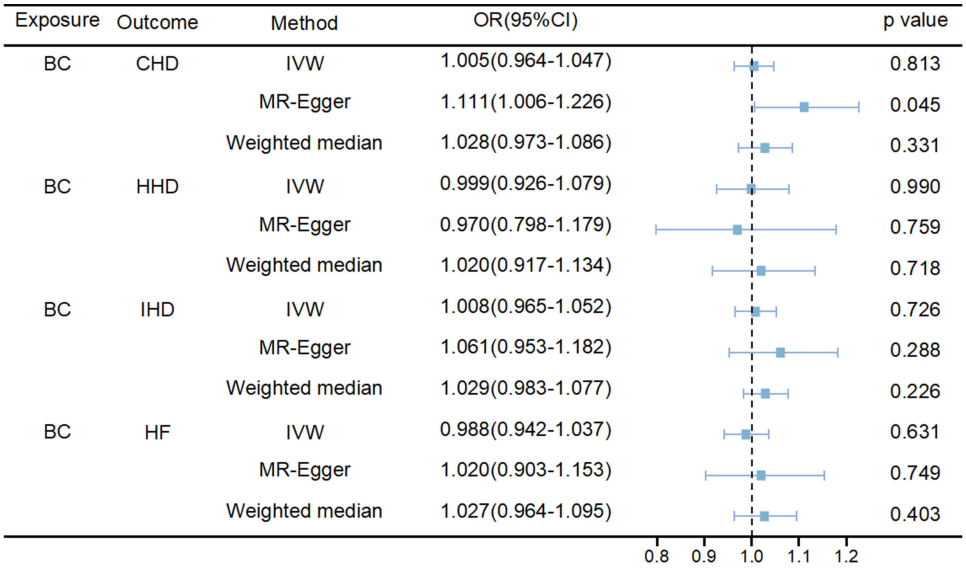

Figure 3 Forrest plot of the causal effects of overall BC on cardiovascular disease CHD, coronary heart disease; HHD, hypertensive heart disease; IHD, ischaemic heart disease; HF, heart failure; CI, confidence interval; OR, odds ratio.

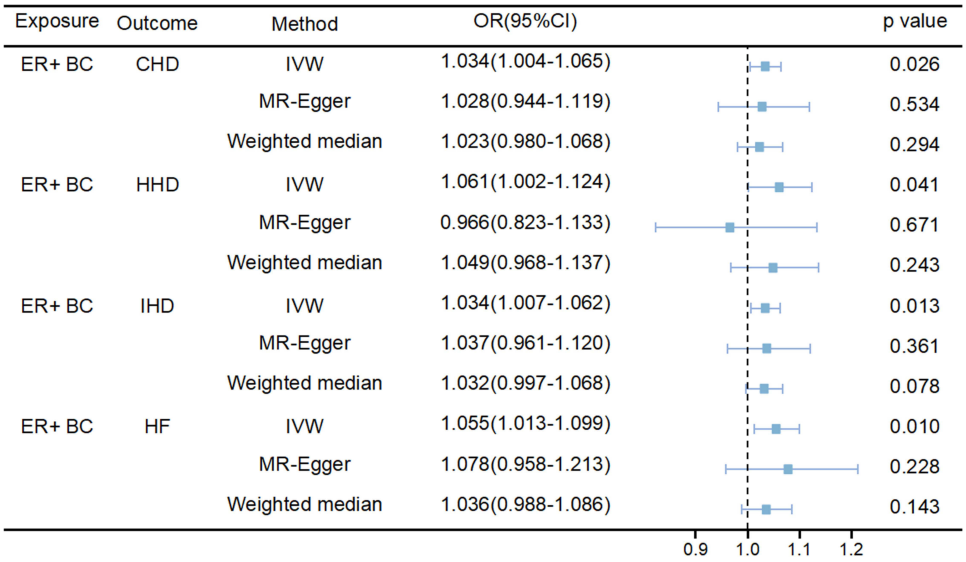

Figure 4 Forrest plot of the causal effects of ER+ BC on cardiovascular disease CHD, coronary heart disease; HHD, hypertensive heart disease; IHD, ischaemic heart disease; HF, heart failure; CI, confidence interval; OR, odds ratio.

Causal Effects of ER+ BC on Mediators

Our analyses revealed a positive association between a genetically predicted 1-SD increase in the risk of ER+ BC and a subsequent elevation in the risk of isoleucine by 2.6% (OR: 1.026, 95% CI: 1.004–1.050, p = 0.023), phenylalanine by 2.3% (OR: 1.023, 95% CI: 1.006–1.041, p = 0.009), alanine by 2.2% (OR: 1.022, 95% CI: 1.000–1.043, p = 0.047), and glutamine by 1.2% (OR: 1.012, 95% CI: 1.001–1.022, p = 0.028). Conversely, a genetically predicted 1-SD increment in the risk of ER+ BC was found to be associated with a 1.5% decrement in the risk of fasting insulin (OR: 0.985, 95% CI: 0.973–0.996, p = 0.011), a 0.7% decrement in the risk of total cholesterol (OR: 0.993, 95% CI: 0.987–0.999, p = 0.023), a 2.7% decrement in the risk of HDL-C (OR: 0.973, 95% CI: 0.958–0.988, p = 0.001), a 4.9% decrement in the risk of LDL-C (OR: 0.951, 95% CI: 0.911–0.993, p = 0.021). Besides, no significant association was found between ER+ BC and fasting glucose, HbA1c, triglycerides, ApoA-I, ApoB, leucine, valine, tyrosine, glycine, or histidine. The comprehensive results, along with their respective details, are visually depicted in Figure 5 and Supplementary Table S5, offering a concise representation of the significant causal linkages between ER+ BC and the aforementioned mediators.

Causal Effects of Mediators on CVD

Among the eight candidate mediators exhibiting a causal relationship with ER+ BC, a genetically predicted 1-SD increase in the risk of total cholesterol was significantly associated with a 63.5% increment in the risk of CHD (OR: 1.635, 95% CI: 1.440–1.856, p < 0.001), a 58.3% increment in the risk of IHD (OR: 1.583, 95% CI: 1.417–1.770, p < 0.001). A genetically predicted 1-SD increase in the risk of HDL-C was associated with a 17.1% decrement in the risk of CHD (OR: 0.829, 95% CI: 0.748–0.920, p < 0.001), a 16.9% decrement in the risk of IHD (OR: 0.831, 95% CI: 0.755–0.915, p < 0.001). A genetically predicted 1-SD increase in the risk of LDL-C was associated with a 47.0% increment in the risk of CHD (OR: 1.470, 95% CI: 1.223–1.768, p < 0.001), a 41.9% increment in the risk of IHD (OR: 1.419, 95% CI: 1.193–1.687, p < 0.001). However, no significant association between HDL and HHD or HF was observed. The forest plots in Figure 6 and Supplementary Table S6-13 depict the significant causal relationship between these mediators and CVD.

Mediation Effects of Mediators Between ER+ BC and CVD

Due to the inconsistency in the MR direction of total cholesterol and LDL-C, the final mediator that meets the screening criteria is HDL-C. In the MVMR, after accounting for ER+ BC, the causal effect of HDL-C was negatively associated with a 17.0% reduction in the risk of CHD (OR: 0.830, 95% CI: 0.742–0.929, p = 0.001), a 17.5% reduction in the risk of in IHD (OR: 0.825,

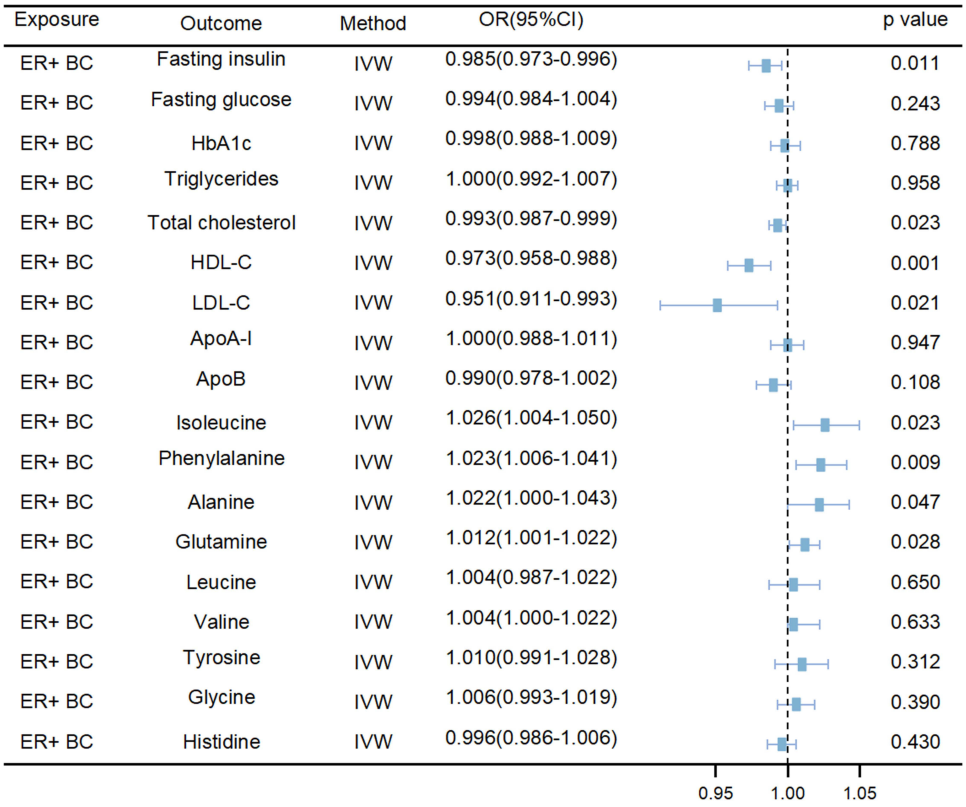

Figure 5 ApoA-I, Apolipoprotein A-I; ApoB, Apolipoprotein B; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CI, confidence interval; OR, odds ratio.

95% CI: 0.746–0.913, $p < 0.001$). The MVMR results are presented in Supplementary Table S14-16 The process of mediation MR analyses is exhibited in Figure 7, illustrating the stepwise tests on the direct and indirect effect. The proportion of the effect mediated by HDL-C was 15.2% (3.4%–27.0%) and 15.5% (3.9%–27.2%) on CHD and IHD, respectively. The relevant details of the mediation MR are presented in Table 2.

Sensitivity Analyses

Based on the presence of pleiotropy or not, we employed different methods. Specifically, in the absence of pleiotropy, we used the IVW method as the primary analytical approach. Conversely, when pleiotropy was present, the MR-Egger method was adopted as the primary analytical approach, with the addition of the MR-PRESSO outlier test to mitigate potential biases stemming from pleiotropic influences. The sensitivity analyses conducted within this study further reinforced the robustness and credibility of the causal estimates observed, and the outcomes are detailed in Supplementary Table S2-13 and Supplementary Figure S2-37.

Discussion

Mendelian randomization (MR) serves as a robust approach leveraging genetic variability to investigate the potential causal linkages between risk factors and health outcomes. In the current MR study, we found that genetically predicted ER+ BC was associated with a slightly increased risk of cardiovascular disease in the European population, including CHD, HHD, IHD, and HF. However, such causal associations have not been replicated in ER− BC. Additionally, our research has identified one mediator out of 18 metabolic traits, HDL-C, with a mediated proportion of 15.2% (3.4%–27.0%) and 15.5% (3.9%–27.2%) in the association between ER+ BC and CHD, IHD, respectively.

Due to the aging population and improved long-term survival rates for BC, an increasing number of older BC survivors are prone to developing chronic age-related conditions, such as CVD.[35] A retrospective cohort analysis

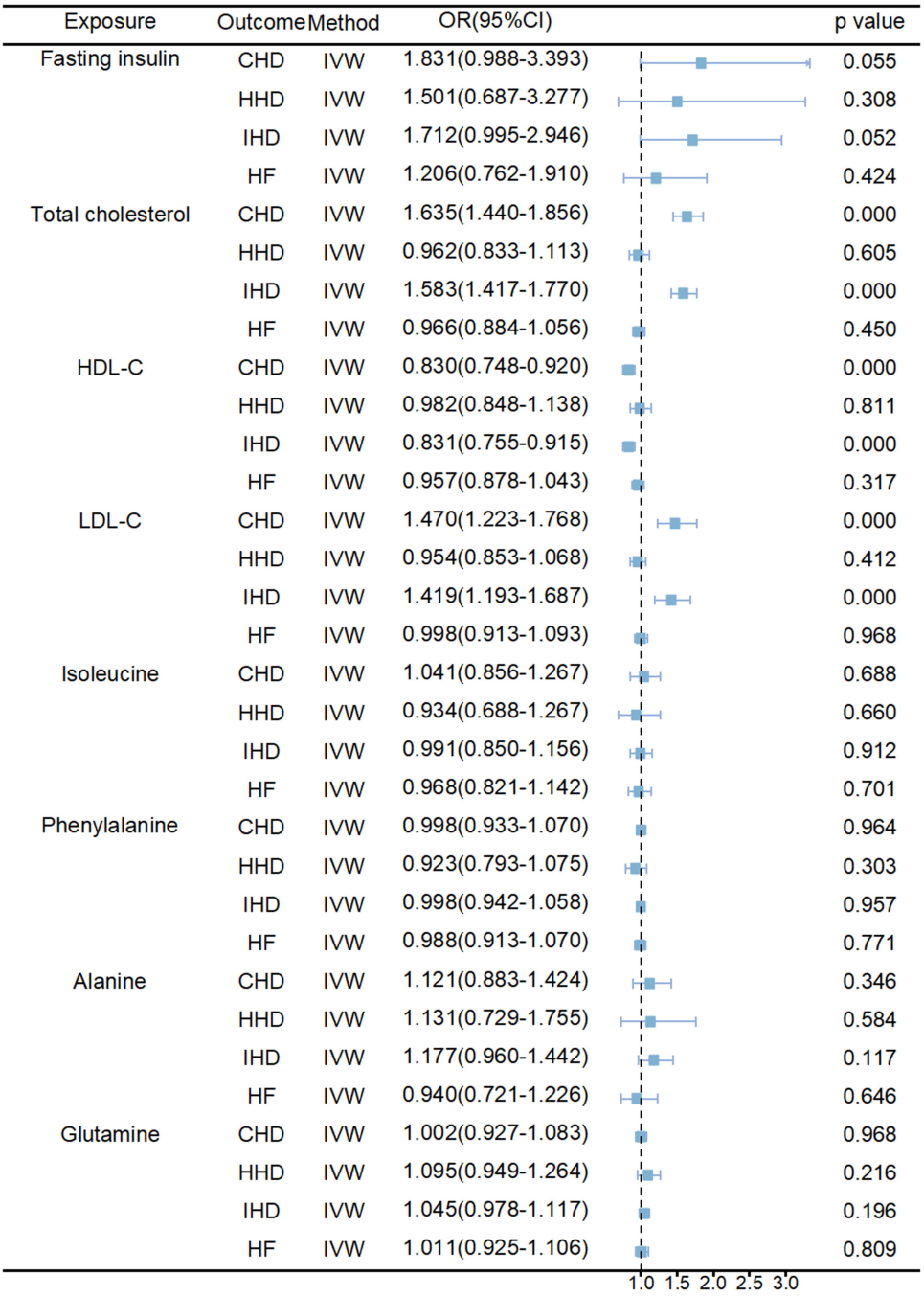

Figure 6 Forrest plot of the mediators on cardiovascular disease CHD, coronary heart disease; HHD, hypertensive heart disease; IHD, ischaemic heart disease; HF, heart failure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CI, confidence interval; OR, odds ratio.

utilizing the SEER-Medicare database highlighted CVD as the primary cause of mortality among older (>66 years) survivors of early-stage or localized BC, particularly those over 75 years old.[36] Another research determined that mortality stemming from CVD is significantly elevated in a population-based cohort of BC survivors compared to a matched cohort of women without BC (HR = 1.8, 95% CI = 1.5–2.1) and this increase in risk is manifest approximately 7 years post-diagnosis.[37] In a cohort study conducted by Ramin et al, involving 628 BC patients and 3140 age-matched cancer-free women, it was observed that after a 25-year follow-up period, BC survivors exhibited a greater incidence of cardiovascular-related deaths compared to their cancer-free counterparts, especially in older survivors and in women with ER+ BC.[38] However, most cohort studies above have focused on changes in CVD mortality after BC diagnosis rather

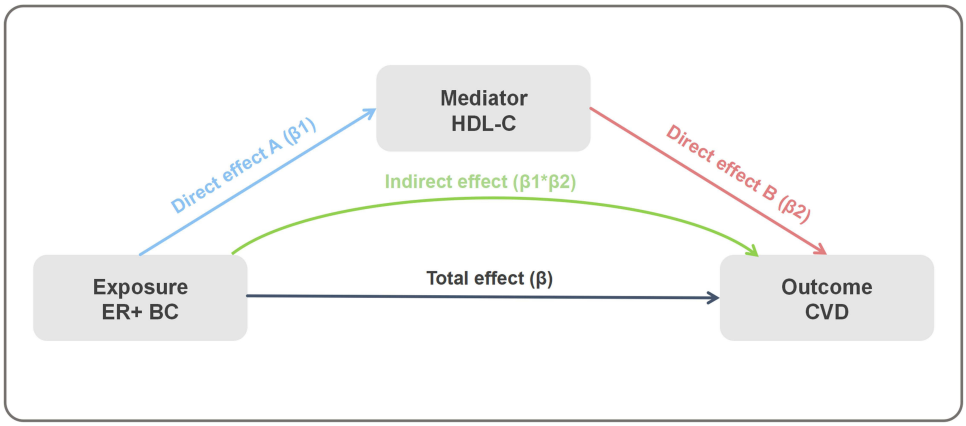

Figure 7 Two-step MR analysis framework The direct effect A (β1) signifies the impact of ER+ BC on the mediator, obtained via the IVW method within UVMR. The direct effect B (β2) signifies the impact of the mediator on CVD with adjustment for ER+ BC, obtained via the IVW method within MVMR. The total effect signifies the impact of ER+ BC on CVD, obtained via the IVW method within UVMR. Lastly, the indirect effect (β1×β2) signifies the impact of ER+ BC on CVD mediated through the mediator, calculated by delta method. CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol.

than on incidence, as BC and CVD share multiple risk factors. Thus, many women were already at risk of CVD at their BC diagnosis.[39,40] Therefore, our understanding of whether BC is associated with an elevated risk of CVD incidence is limited. Few studies have explored the association between BC and CVD incidence, prompting a shift in the focus from CVD mortality to incidence. Previous research reported that apart from traditional risk factors, higher BC stages, and grades at diagnosis are associated with an increased risk of atrial fibrillation (AF), indicating a systemic effect of advanced BC itself on the heart.[41] Another prospective cohort study found that women with BC compared with those without BC had a higher risk of developing heart failure, cardiomyopathy, arrhythmia, cardiac arrest, and venous thromboembolism.[42] Nonetheless, no MR study has verified such an association so far. To fill this gap, our Mendelian randomization analysis provides an opportunity to investigate the causal relationship between BC and the CVD incidence. This analysis is valuable in aiding the early detection and diagnosis of CVD risk factors and symptoms in BC patients.

In the pathway from ER+ BC to CHD and IHD, 15.2% and 15.5% of the mediation effect was explained by HDL-C, respectively. Based on the findings of two-step MR, genetically predicted ER+ BC is correlated with lower HDL-C levels, and lower HDL-C levels are associated with higher risk of developing CVD (CHD and IHD), which are both consistent with contemporary epidemiological insights.[43–45] Previous studies have shown abnormal lipoprotein profiles in breast cancer patients, with the levels of TG, LDL-C and VLDL-C increased, and the level of HDL-C decreased.[46–49] However, our MR study did not provide insights into the relationship between BC and other lipid traits such as TG, LDL-C, and VLDL-C. Consequently, further research is warranted to examine the alterations in other lipid profiles among BC patients. Regarding the association between HDL and other lipid traits with CVD, numerous conclusions have been drawn from previous studies.[44,45] In a prospective, observational study, 795 individuals had extensive cardiometabolic profiling, including emerging biomarkers. The low ApoE-HDL-C group had more severe stenosis (11% vs 2%, $p < 0.001$), with higher coronary artery calcium (CAC) as compared with high ApoE-HDL-C, suggesting that low ApoE-HDL-C may be key markers of CVD severity.[50] Similarly, in both the UVMR and MVMR methods we employed, HDL-C demonstrated a negative association with CVD (CHD and IHD). Subsequent research should further explore the relationship between

Table 2 The Mediation Effect of ER+ BC on CVD via HDL-C

Outcome	Total effect	Direct effect A	Direct effect B	Indirect effect	Mediated proportion (%)
	β(95% CI)	β1(95% CI)	β2(95% CI)	β1*β2 (95% CI)	(95% CI)
CHD	0.033(0.004 to 0.062)	−0.027(−0.043 to −0.012)	−0.186(−0.299 to −0.073)	0.005(0.001 to 0.009)	15.2(3.4 to 27.0)
IHD	0.034(0.007 to 0.060)	−0.027(−0.043 to −0.012)	−0.192(−0.293 to −0.091)	0.005(0.001 to 0.009)	15.5(3.8 to 27.2)

HDL-C and other CVD. Mechanistically, the abnormalities in blood lipids among BC survivors and their subsequent impact on CVD remain unclear. One possible explanation involves genes related to lipoprotein metabolism. Specifically, peroxisome proliferator-activated receptors (PPARs), as a group of nuclear hormone receptors, regulate the transcription of adipocyte genes in the body and participate in processes such as adipocyte differentiation, lipid and glucose metabolism. In breast cancer patients, the expression and activity of PPARs may be influenced by factors such as chemotherapy and endocrine therapy, thereby further affecting blood lipid levels and the risk of CVD.[51]

Over 80% of the observed effects remained unexplained despite the inclusion of mediators in the model. This can be attributed to several factors: (1) the IVs we chose did not have sufficient explanatory power for the mediator; (2) the potential existence of other unexamined mediators, given that our selection was confined to metabolic pathways; (3) the mediator from ER+ BC to CVD was inherently rare or had a weak mediating effect. Consequently, the interpretation of these findings should be undertaken with prudence, and further exploration of potential mediating factors, along with the utilization of complementary methodologies, is warranted to validate the conclusions.

Our study provided evidence for a causal link between genetically predicted ER+ BC and CVD (CHD, HHD, IHD, and HF), while no similar causal relationship was observed in ER− BC. Furthermore, we identified a potential mediator, HDL-C, and calculated the mediation effect. Initially, we considered BC at the overall level, but did not find statistically significant associations with CVD. Recognizing the influence of ER status on BC,[3] we further investigated the impact of BC on CVD based on estrogen receptor status separately. Consequently, we discovered an association between ER + BC and an increased risk of CVD including CHD, HHD, IHD, and HF. Then, we conducted a two-step MR analysis to explore the mediation effects of 18 metabolic candidates, and ultimately discovered HDL-C. The current findings possess profound implications for clinical practice and public health strategies. The screening and prevention of CVD among cancer survivors are frequently neglected due to the scarcity of evidence to guide these practices and the prevailing misperceptions surrounding the competing risks of cancer mortality. In our study, BC patients, especially those with ER+ status, demonstrated a higher risk of CVD (CHD, HHD, IHD, and HF), which suggested that this population may benefit from proactive screening and preventive interventions. Considering the mediation effect of HDL-C between ER+ BC and CVD, HDL-C can be clinically used for early screening and predicting the occurrence of CVD in BC survivors. Subsequent research should focus on expanding such causal relationship to other BC subtypes, a broader range of CVD subtypes, as well as more potential mediator pathways, and provide screening and prevention strategies tailored to this unique patient population.

Nevertheless, our study encountered certain limitations. Firstly, while we employed summary-level data and subgroup analyses stratified by ER status, we were constrained in stratifying BC analyses based on additional risk variables, such as age, body mass index (BMI), menopausal status, and tumor stage, which are pivotal factors influencing the incidence of CVD. Secondly, MR approach is inherently limited to drawing inferences regarding trait associations within the specific populations from which the genome-wide association studies (GWAS) are derived. Consequently, given the limitations of available online databases, we did not utilize sex-specific CVD data in our MR analysis, instead relying on mixed-sex cohorts. In the future, an increasing number of large-scale genomic studies may have the potential to change this situation and bring about new insights. We recognize the importance of sex-specific analysis and plan to conduct more tailored MR studies in our future work. Thirdly, our findings did not provide evidence of the specific mechanisms underlying the causal relationship between BC and CVD. Instead, we put forward a potential mediator that partially explains the underlying reasons for the causal effect and discussed the feasibility and plausibility. Finally, considering multiple factors such as evolutionary history, genetic diversity, environment, and natural selection, it is evident that there are differences in genetic variations among different racial populations. For instance, genetic variations related to thrombosis differ between European and East Asian populations.[52] Therefore, to ensure consistency in genetic background, this MR study was almost exclusively restricted to individuals of European ancestry, thus the generalization of our findings to other ethnic groups should be approached with caution.

Conclusion

In conclusion, this two-step MR study elaborates on the potential causal impact of ER+ BC on the risk of CVD and proposes one potential mediator, HDL-C. Our finding enhances our understanding of the possible mechanism for the

occurrence of CVD after BC diagnosis and informs early screening and intervention strategies to curb the BC-related CVD and corresponding disease burden.

Abbreviation

BCAC, Breast Cancer Association Consortium; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; ER, estrogen-receptor; GWAS, genome-wide association study; HF, heart failure; HHD, hypertensive heart disease; IHD, ischaemic heart disease; IVW, inverse variance weighted; MR, Mendelian randomization; MR-PRESSO, MR-pleiotropy residual sum and outlier; SNPs, single-nucleotide polymorphisms.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics Statement

The original genome-wide association studies (GWASs) had been approved by the corresponding ethical committee. The Ethics Committee of Taizhou People's Hospital, affiliated with Nanjing Medical University, approved this project in accordance with the Declaration of Helsinki (approval number: KY2023-044-01).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval for the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors assert that this research was conducted without any commercial or financial ties that might be perceived as a potential conflict of interest.

References

1. Giaquinto AN, Sung H, Miller KD, et al. Breast Cancer Statistics, 2022. *CA Cancer J Clin*. 2022;72(6):524–541. doi:10.3322/caac.21754
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin*. 2024;74(1):12–49. doi:10.3322/caac.21820
3. Nolan E, Lindeman GJ, Visvader JE. Deciphering breast cancer: from biology to the clinic. *Cell*. 2023;186(8):1708–1728. doi:10.1016/j.cell.2023.01.040
4. Afifi AM, Saad AM, Al-Husseini MJ, Elmehrath AO, Northfelt DW, Sonbol MB. Causes of death after breast cancer diagnosis: a US population-based analysis. *Cancer*. 2020;126(7):1559–1567. doi:10.1002/cncr.32648
5. Blaes AH, Konety SH. Cardiovascular Disease in Breast Cancer Survivors: an Important Topic in Breast Cancer Survivorship. *J Natl Cancer Inst*. 2021;113(2):105–106. doi:10.1093/jnci/djaa097
6. Sturgeon KM, Deng L, Bluethmann SM, et al. A population-based study of cardiovascular disease mortality risk in US cancer patients. *Eur Heart J*. 2019;40(48):3889–3897. doi:10.1093/eurheartj/ehz766
7. Koric A, Chang CP, Mark B, et al. Cardiovascular disease risk in long-term breast cancer survivors: a population-based cohort study. *Cancer*. 2022;128(14):2826–2835. doi:10.1002/cncr.34224

8. Möhl A, Behrens S, Flaßkamp F, et al. The impact of cardiovascular disease on all-cause and cancer mortality: results from a 16-year follow-up of a German breast cancer case-control study. *Breast Cancer Res*. 2023;25(1):89. doi:10.1186/s13058-023-01680-x
9. Mehta LS, Watson KE, Barac A, et al. Cardiovascular Disease and Breast Cancer: where These Entities Intersect: a Scientific Statement From the American Heart Association. *Circulation*. 2018;137(8):e30–e66. doi:10.1161/cir.0000000000000556
10. Abdel-Qadir H, Austin PC, Lee DS, et al. A Population-Based Study of Cardiovascular Mortality Following Early-Stage Breast Cancer. *JAMA Cardiol*. 2017;2(1):88–93. doi:10.1001/jamacardio.2016.3841
11. Galimzhanov A, Istanbuly S, Tun HN, et al. Cardiovascular outcomes in breast cancer survivors: a systematic review and meta-analysis. *Eur J Prev Cardiol*. 2023. doi:10.1093/eurjpc/zwad243
12. Lenneman CG, Sawyer DB. Cardio-Oncology: an Update on Cardiotoxicity of Cancer-Related Treatment. *Circ Res*. 2016;118(6):1008–1020. doi:10.1161/circresaha.115.303633
13. Miller KD, Nogueira L, Devasia T, et al. Cancer treatment and survivorship statistics, 2022. *CA Cancer J Clin*. 2022;72(5):409–436. doi:10.3322/caac.21731
14. Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *Int J Epidemiol*. 2019;48(3):713–727. doi:10.1093/ije/dyy262
15. Carter AR, Sanderson E, Hammerton G, et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur J Epidemiol*. 2021;36(5):465–478. doi:10.1007/s10654-021-00757-1
16. Gao Q, Tan JS, Fan L, Wang X, Hua L, Cai J. Causal associations between disorders of lipoprotein metabolism and ten cardiovascular diseases. *Front Cell Dev Biol*. 2022;10:1023006. doi:10.3389/fcell.2022.1023006
17. Quan L, Tan J, Hua L, You X. Genetic predisposition between coronavirus disease 2019 and rheumatic diseases: a 2-sample Mendelian randomization study. *Int J Rheum Dis*. 2023;26(4):710–717. doi:10.1111/1756-185x.14624
18. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: the STROBE-MR Statement. *JAMA*. 2021;326(16):1614–1621. doi:10.1001/jama.2021.18236
19. Michailidou K, Lindström S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature*. 2017;551(7678):92–94. doi:10.1038/nature24284
20. Tan H, Wang S, Huang F, Tong Z. Association between breast cancer and thyroid cancer risk: a two-sample Mendelian randomization study. *Front Endocrinol*. 2023;14:1138149. doi:10.3389/fendo.2023.1138149
21. Scott RA, Lagou V, Welch RP, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet*. 2012;44(9):991–1005. doi:10.1038/ng.2385
22. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet*. 2021;53(10):1415–1424. doi:10.1038/s41588-021-00931-x
23. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274–1283. doi:10.1038/ng.2797
24. Prins BP, Kuchenbaecker KB, Bao Y, et al. Genome-wide analysis of health-related biomarkers in the UK Household Longitudinal Study reveals novel associations. *Sci Rep*. 2017;7(1):11008. doi:10.1038/s41598-017-10812-1
25. Barton AR, Sherman MA, Mukamel RE, Loh PR. Whole-exome imputation within UK Biobank powers rare coding variant association and fine-mapping analyses. *Nat Genet*. 2021;53(8):1260–1269. doi:10.1038/s41588-021-00892-1
26. Panyard DJ, Kim KM, Darst BF, et al. Cerebrospinal fluid metabolomics identifies 19 brain-related phenotype associations. *Commun Biol*. 2021;4(1):63. doi:10.1038/s42003-020-01583-z
27. Julkunen H, Cichońska A, Slagboom PE, Würtz P. Metabolic biomarker profiling for identification of susceptibility to severe pneumonia and COVID-19 in the general population. *Elife*. 2021;10. doi:10.7554/eLife.63033
28. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol*. 2015;30(7):543–552. doi:10.1007/s10654-015-0011-z
29. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian Randomization as an Approach to Assess Causality Using Observational Data. *J Am Soc Nephrol*. 2016;27(11):3253–3265. doi:10.1681/asn.2016010098
30. Liu N, Tan JS, Liu L, et al. Roles of obesity in mediating the causal effect of attention-deficit/hyperactivity disorder on diabetes. *Epidemiol Psychiatr Sci*. 2023;32:e32. doi:10.1017/s2045796023000173
31. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol*. 2017;32(5):377–389. doi:10.1007/s10654-017-0255-x
32. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693–698. doi:10.1038/s41588-018-0099-7
33. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife*. 2018;7. doi:10.7554/eLife.34408
34. Millard LA, Davies NM, Timpson NJ, Tilling K, Flach PA, Davey Smith G. MR-PheWAS: hypothesis prioritization among potential causal effects of body mass index on many outcomes, using Mendelian randomization. *Sci Rep*. 2015;5:16645. doi:10.1038/srep16645
35. Colzani E, Liljegren A, Johansson AL, et al. Prognosis of patients with breast cancer: causes of death and effects of time since diagnosis, age, and tumor characteristics. *J Clin Oncol*. 2011;29(30):4014–4021. doi:10.1200/jco.2010.32.6462
36. Patnaik JL, Byers T, DiGuiseppi C, Dabelea D, Denberg TD. Cardiovascular disease competes with breast cancer as the leading cause of death for older females diagnosed with breast cancer: a retrospective cohort study. *Breast Cancer Res*. 2011;13(3):R64. doi:10.1186/bcr2901
37. Bradshaw PT, Stevens J, Khankari N, Teitelbaum SL, Neugut AI, Gammon MD. Cardiovascular Disease Mortality Among Breast Cancer Survivors. *Epidemiology*. 2016;27(1):6–13. doi:10.1097/ede.0000000000000394
38. Ramin C, Schaeffer ML, Zheng Z, et al. All-Cause and Cardiovascular Disease Mortality Among Breast Cancer Survivors in CLUE II, a Long-Standing Community-Based Cohort. *J Natl Cancer Inst*. 2021;113(2):137–145. doi:10.1093/jnci/djaa096
39. Bardia A, Arieas ET, Zhang Z, et al. Comparison of breast cancer recurrence risk and cardiovascular disease incidence risk among postmenopausal women with breast cancer. *Breast Cancer Res Treat*. 2012;131(3):907–914. doi:10.1007/s10549-011-1843-1
40. Barac A, Murtagh G, Carver JR, et al. Cardiovascular Health of Patients With Cancer and Cancer Survivors: a Roadmap to the Next Level. *J Am Coll Cardiol*. 2015;65(25):2739–2746. doi:10.1016/j.jacc.2015.04.059

41. Guha A, Fradley MG, Dent SF, et al. Incidence, risk factors, and mortality of atrial fibrillation in breast cancer: a SEER-Medicare analysis. *Eur Heart J*. 2022;43(4):300–312. doi:10.1093/eurheartj/ehab745
42. Greenlee H, Iribarren C, Rana JS, et al. Risk of Cardiovascular Disease in Women With and Without Breast Cancer: the Pathways Heart Study. *J Clin Oncol*. 2022;40(15):1647–1658. doi:10.1200/jco.21.01736
43. Lu CW, Lo YH, Chen CH, et al. VLDL and LDL, but not HDL, promote breast cancer cell proliferation, metastasis and angiogenesis. *Cancer Lett*. 2017;388:130–138. PubMed PMID: 27940127. doi:10.1016/j.canlet.2016.11.033
44. Georgoulis M, Chrysohoou C, Georgousopoulou E, et al. Long-term prognostic value of LDL-C, HDL-C, lp(a) and TG levels on cardiovascular disease incidence, by body weight status, dietary habits and lipid-lowering treatment: the ATTICA epidemiological cohort study (2002-2012). *Lipids Health Dis*. 2022;21(1):141. doi:10.1186/s12944-022-01747-2
45. Chen JX, Li Y, Zhang YB, et al. Nonlinear relationship between high-density lipoprotein cholesterol and cardiovascular disease: an observational and Mendelian randomization analysis. *Metabolism*. 2024;154:155817. doi:10.1016/j.metabol.2024.155817
46. Kökoğlu E, Karaarslan I, Karaarslan HM, Baloğlu H. Alterations of serum lipids and lipoproteins in breast cancer. *Cancer Lett*. 1994;82 (2):175–178. PubMed PMID: 8050088. doi:10.1016/0304-3835(94)90008-6
47. Kumar K, Sachdanandam P, Arivazhagan R. Studies on the changes in plasma lipids and lipoproteins in patients with benign and malignant breast cancer. *Biochem Int*. 1991;23(3):581–589.
48. Alexopoulos CG, Blatsios B, Avgerinos A. Serum lipids and lipoprotein disorders in cancer patients. *Cancer*. 1987;60(12):3065–3070. doi:10.1002/1097-0142(19871215)60:12<3065::aid-cncr2820601234>3.0.co;2-q
49. Chang SJ, Hou MF, Tsai SM, et al. The association between lipid profiles and breast cancer among Taiwanese women. *Clin Chem Lab Med*. 2007;45(9):1219–1223. doi:10.1515/cclm.2007.263
50. Sorokin AV, Patel N, Abdelrahman KM, et al. Complex association of apolipoprotein E-containing HDL with coronary artery disease burden in cardiovascular disease. *JCI Insight*. 2022;7(10). doi:10.1172/jci.insight.159577
51. Sharma M, Tuaine J, McLaren B, et al. Chemotherapy Agents Alter Plasma Lipids in Breast Cancer Patients and Show Differential Effects on Lipid Metabolism Genes in Liver Cells. *PLoS One*. 2016;11(1):e0148049. doi:10.1371/journal.pone.0148049
52. Tan JS, Yan W XX, Gao X Y, et al. Rare variants in MTHFR predispose to occurrence and recurrence of pulmonary embolism. *Int J Cardiol*. 2021;331:236–242. PubMed PMID: 33571559. doi:10.1016/j.ijcard.2021.01.073

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