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ORIGINAL ARTICLE

Ferritin and Serum Iron in a Causal Relationship with Estrogen Receptor-Negative Breast Cancer: a Two-Sample Mendelian Randomization Study

Yi Lu [1], Liumei Lin [1, 2], Jun Shen [1]

[1] *Department of Surgical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang Province, China*
[2] *Department of Thyroid and Breast Surgery, Nanping First Hospital, Fujian Medical University, Nanping, Fujian, China*

SUMMARY

Background: **The aim of this study was to explore the causal relationship between different serum iron statuses (ferritin, transferrin, transferrin saturation, and serum iron) and the occurrence of estrogen receptor (ER)-positive or ER-negative breast cancer.**
Methods: **The summary data on serum iron status exposure were gathered from the IEU OpenGWAS Project, the UK Biobank, and other databases. Concurrently, the summary data for ER^+ and ER^- breast cancer are sourced from the Breast Cancer Association Consortium (BCAC). By examining the causal link between iron status and breast cancer, we deployed five distinct Mendelian randomization (MR) algorithms, namely MR-Egger, inverse variance weighted (IVW), weighted median, simple mode, and MR-PRESSO. To assess heterogeneity and horizontal pleiotropy, Cochran's Q and MR-Egger algorithms were applied, respectively.**
Results: **Elevated ferritin levels are associated with an increased risk of ER-negative breast cancer (OR(IVW) = 1.042, 95% CI (1.005, 1.081), p = 0.025; OR (weighted median) = 1.050, 95% CI (1.001, 1.102), p = 0.046; and OR (MR-PRESSO) = 1.042, 95% CI (1.005, 1.081), p = 0.039). Conversely, an increase in the serum iron level is linked to a reduced risk of ER-negative breast cancer (OR (IVW) = 0.791, 95% CI (0.649, 0.962), p = 0.019; and OR (MR-PRESSO) = 0.791, 95% CI (0.649, 0.962), p = 0.028). However, there is no evidence of a causal relationship between transferrin, transferrin saturation, and ER-negative breast cancer. For ER-positive breast cancer, none of the four different iron statuses demonstrated a causal relationship.**
Conclusions: **Ferritin is positively correlated with ER-negative breast cancer, while serum iron is negatively associated with ER-negative breast cancer. However, there is no causal relationship between the four iron statuses and ER-positive breast cancer.**
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Correspondence:
Jun Shen
Department of Surgical Oncology
Sir Run Run Shaw Hospital
Zhejiang University School of Medicine
No. 3, Road of Qingchun East
Hangzhou 310016
Zhejiang Province
China
Email: shenjun0909@zju.edu.cn

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KEYWORDS

iron status, estrogen receptor, breast cancer, SNP, Mendelian randomization

INTRODUCTION

Breast cancer is a malignancy that is originating from the deterioration of glandular or ductal epithelial tissues in the breast, primarily affecting female patients and representing 99% of all breast cancers [1]. In 2020, around 2.26 million women worldwide received a diagnosis of breast cancer, resulting in a staggering 685,000

deaths, solidifying its status as one of the most prevalent and lethal malignancies [2]. Beyond threatening patients' health, breast cancer and its complications impose substantial economic burdens on families and society at large. Increasing clinical research underscores that advancements in screening methods, early diagnosis, and treatment significantly contribute to enhancing the survival rates of breast cancer patients [3]. Notably, studies, such as the one led by Taylor et al. [4], that use cohort research, have unveiled reduced mortality rates among female patients that were diagnosed early with invasive breast cancer and underwent surgical treatment, underscoring the pivotal importance of an early diagnosis. Despite these advances, the lack of effective biomarkers remains a significant impediment to early screening and diagnosis for breast cancer.

The concept of iron death, initially proposed by Dixon et al. [5], elucidates the iron-dependent regulation of cell death induced by lipid peroxidation. Its distinguishing features encompass the oxidation of multiple unsaturated fatty acid phospholipids, the involvement of redox-active iron, and the impairment of lipid peroxide repair mechanisms. Doll et al. [6], in mouse experiments, observed that triple-negative breast cancer exhibits a greater sensitivity to iron death compared to ER^+ breast cancer. Ma et al. [7] demonstrated that the treatment with lapatinib alone or in combination with celecoxib effectively addresses breast cancer by activating the iron death pathway. Yang et al. [8], in their investigation of triple-negative breast cancer, found that inhibiting GPX4 not only triggers iron death in tumor cells, but also enhances the anti-tumor immune response. Lin et al. [9] discovered in their research that dihydroisotanshinone IIA in Danshen can improve the survival rate of breast cancer patients by inducing apoptosis and iron death. In conclusion, there exists a discernible correlation between iron death and breast cancer, suggesting its potential utility as a biological marker for breast cancer treatment. However, the incomplete depth of research on iron death in breast cancer and the absence of consistent conclusions underscore the inconclusive role of iron status (typically assessed in clinical practice through measurements such as serum iron, transferrin saturation, ferritin, and transferrin) in the development of breast cancer.

MR stands as a statistical approach, utilizing genetic variation as an exposure tool to evaluate causal connections between exposure and outcomes [10]. In recent years, it has found widespread application in the realm of biomedical research [11,12]. Compared to conventional cohort studies or randomized controlled trials, MR boasts the following advantages: 1) maximizing the reduction of confounding factors; 2) genetic variations are randomly assigned during meiosis, circumventing the impact of subsequent lifestyle and environmental factors; and 3) the random allocation of alleles precedes the onset of the disease, thereby averting the repercussions of reverse causation. The present study intends to use a two-sample MR analysis to probe potential causal links between four iron status biomarkers and ER^+ and ER^- breast cancer. The goal is to offer fresh perspectives for the development of early diagnostic markers and drug targets for breast cancer.

MATERIALS AND METHODS

Study design

In this study, we investigate the causal relationships between four distinct iron status indicators (ferritin, transferrin, transferrin saturation, and serum iron) as exposure factors and ER^+ and ER^- breast cancer as outcome variables. The analysis uses a two-sample MR approach, with additional tests conducted for heterogeneity, horizontal pleiotropy, and stability. Detailed information regarding the genome-wide association study (GWAS) statistical data, utilized in this study, is provided in Table 1. Ethical and institutional review board approvals for each included GWAS have been obtained from local institutions, and this information is accessible in the original publications of each study. As a result, no further ethical approval was required for this study.

Data sources

The compiled data for the exposure levels of the four iron status indicators are sourced from the UK Biobank and the Iron Status Genetics Consortium database, as shown in Table 1. The data summarization for ferritin originates from an extensive study conducted by the National Health Service Blood and Transplant Tissue Centre in England, involving the recruitment of 50,000 adults, aged 18 and above, from mid-2012 to mid-2014 [13]. The relative abundance of ferritin undergoes initial natural logarithm transformation, followed by linear regression adjustments based on age, gender, duration between blood draw and processing, and the first three principal components derived from multidimensional ancestry. Summary data for transferrin and transferrin saturation are drawn from genetic association data on iron status biochemical markers in 11 European population studies [14]. Ferritin data is extracted from the UK Biobank database, encompassing 64,979 European adult individuals. The summarized outcome data (ER^+ breast cancer and ER^- breast cancer) are obtained from the Breast Cancer Association Consortium (BCAC), as is detailed in Table 1. The GWAS results for ER^+ and ER^- breast cancer emanate from a large-scale cancer genome-wide association study, encompassing 106,776 patients of European ancestry (61,282 breast cancer cases and 45,494 controls) [15]. The breast cancer GWAS utilized the 1,000 Genomes Project (Phase 3) reference panel during the attribution phase and adjusted for logistic regression analysis with principal components representing national and ancestry information. The aforementioned genetic data is available for download on the GWAS summary website (https://gwas.mrcieu.ac.uk/).

Table 1. The aggregation of genetic data for exposure and outcome.

GWAS ID	Trait	Year	PMID	Consortium	Sample size (n (case)/ n (control))	Number of SNPs	Population	Gender
ieu-a-1127	ER^+ breast cancer	2017	29059683	BCAC	175,475 (69,501/105,974)	10,680,257	european	females
ieu-a-1128	ER^- breast cancer	2017	29059683	BCAC	127,442 (21,468/105,974)	10,680,257	european	females
prot-a-1148	Ferritin	2018	29875488	EGA	3,301	10,534,735	european	males and females
ieu-a-1052	Transferrin	2014	25352340	GISC	23,986	2,104,242	european	males and females
ieu-a-1051	Transferrin saturation	2014	25352340	GISC	23,986	2,102,226	european	males and females
ukb-b-20447	Iron	2018	-	UKB (MRC-IEU)	64,979	9,851,867	european	males and females

BCAC - Breast Cancer Association Consortium, EGA - European Genotype Archive, GISC - Genetics of Iron Status Consortium, UKB - UK Biobank.

Instrumental variables

Based on the GWAS datasets obtained, single nucleotide polymorphisms (SNPs) were selected as instrumental variables (IVs) to evaluate the causal relationship between four different iron statuses and ER^+ and ER^- breast cancer. Firstly, SNPs phenotypically associated with iron statuses were extracted by using a genome-wide significance threshold of $p < 1 \times 10^{-5}$, as the stricter threshold of $p < 5 \times 10^{-8}$ yielded too few SNPs. Subsequently, to eliminate potential bias due to linkage disequilibrium, the PLINK clustering method with a threshold of $r^2 < 0.001$ and a distance of 10,000 kb was employed. To further mitigate the impact of weak IVs, the F-statistic ($F = \beta^2/SE^2$, where β represents the SNP's effect size on the exposure factor, and SE is the standard error of β) was calculated, and only SNPs with an F-statistic exceeding 10 were retained [16]. We concluded by extracting essential information, including SNP identifiers, effective alleles, effective allele frequencies, and associations between effective alleles and exposure phenotypes, along with effect sizes, standard errors, and p-values for ER^+ and ER^- breast cancer. Standardization was implemented to ensure an alignment between exposure and outcome effect values and their corresponding effective alleles.

Mendelian randomization analysis

Classical MR studies must adhere to three fundamental assumptions: 1) relevance assumption: the selected SNPs must demonstrate a significant association with the exposure factor; 2) independence assumption: it ensures that SNPs are not linked to confounding factors along the pathway between SNPs and exposure outcomes; and 3) exclusion restriction assumption: this assumption mandates that SNPs exclusively impact outcomes through their correlation with the exposure, excluding any influence through alternative pathways. Only when these assumptions are met, can MR studies effectively correct for unknown confounding factors (Figure 1).

This study used a two-sample MR approach, utilizing MR-Egger, inverse variance weighted (IVW), weighted median, simple mode, and MR-PRESSO methods to assess the causal relationship between iron status and breast cancer. Cochran's Q statistic and the MR-Egger algorithm were used for heterogeneity and horizontal pleiotropy testing, respectively, with a significance threshold of $p < 0.05$ indicating the presence of heterogeneity or horizontal pleiotropy. The inverse variance weighted (IVW) method determined MR results for each risk factor. In the absence of horizontal pleiotropy, IVW combines the exposure and outcome effect values for each SNP through meta-analysis, providing a relatively stable and accurate causal assessment. In case of significant heterogeneity, a multiplicative random-effects model was used.

The sensitivity analyses included the weighted median method [17] and MR-PRESSO [18]. The weighted median method provides stable results when more than 50% of SNPs are effective instrumental variables, reducing Type I errors in the presence of horizontal pleiotropy, and leading to more accurate causal effect estimates [17]. MR-PRESSO provides post-correction results after removing horizontal pleiotropy [19]. To better interpret the results, β and se values were converted to odds ratios (OR), and 95% confidence intervals (CI) were calculated. Lastly, a leave-one-out approach, systematically removing each SNP to calculate the combined effect of the remaining SNPs, was employed to verify the impact of each SNP on the overall causal estimate. All analyses were conducted by using the TwoSampleMR package (Version: 0.5.6) and MRPRESSO package (Version: 1.0) in R software (Version: 4.2.0).

Table 2. MR results for the relationship between the four iron statuses and ER$^+$/ER$^-$ breast cancer.

MR info								Cochran's Q			Horizontal pleiotropy		
Exposure	Outcome	SNP (n)	Method	Beta	se	OR (95% CI)	p	Q	Q _df	p	Intercept	se	p
Ferritin	ER$^+$ breast cancer	14	MR-Egger	0.008	0.026	1.008 (0.958 - 1.060)	0.768	25.754	12.000	0.079	-0.003	0.001	0.647
			IVW	-0.003	0.014	0.998 (0.972 - 1.024)	0.855	26.082	13.000	0.098	-	-	-
			Weighted median	0.022	0.017	1.020 (0.989 - 1.056)	0.190	-	-	-	-	-	-
			Simple mode	0.011	0.036	1.011 (0.942 - 1.085)	0.767	-	-	-	-	-	-
			MR-PRESSO	-0.003	0.014	0.998 (0.972 - 1.024)	0.857	-	-	-	-	-	-
	ER$^-$ breast cancer	19	MR-Egger	0.037	0.036	1.038 (0.967 - 1.114)	0.320	20.883	17.000	0.232	0.001	0.008	0.885
			IVW	0.042	0.019	1.042 (1.005 - 1.081)	0.025	53.224	18.000	0.284	-	-	-
			Weighted median	0.049	0.024	1.050 (1.001 - 1.102)	0.046	-	-	-	-	-	-
			Simple mode	0.031	0.042	1.032 (0.949 - 1.121)	0.471	-	-	-	-	-	-
			MR-PRESSO	0.042	0.019	1.042 (1.005 - 1.081)	0.038	-	-	-	-	-	-
Transferrin	ER$^+$ breast cancer	28	MR-Egger	-0.028	0.028	0.972 (0.921 - 1.026)	0.316	52.364	26.000	0.002	-0.002	0.004	0.668
			IVW	-0.037	0.020	0.964 (0.928 - 1.002)	0.062	52.744	27.000	0.002	-	-	-
			Weighted median	-0.038	0.018	0.962 (0.929 - 0.997)	0.034	-	-	-	-	-	-
			Simple mode	-0.071	0.049	0.932 (0.846 - 1.026)	0.163	-	-	-	-	-	-
			MR-PRESSO	-0.037	0.019	0.964 (0.928 - 1.001)	0.066	-	-	-	-	-	-
	ER$^-$ breast cancer	28	MR-Egger	-0.012	0.034	0.989 (0.926 - 1.056)	0.734	32.341	26.000	0.182	0.003	0.004	0.474
			IVW	0.005	0.024	1.005 (0.960 - 1.054)	0.820	32.997	27.000	0.197	-	-	-
			Weighted median	-0.008	0.029	0.992 (0.938 - 1.050)	0.785	-	-	-	-	-	-
			Simple mode	0.139	0.078	1.149 (0.986 - 1.339)	0.086	-	-	-	-	-	-
			MR-PRESSO	0.003	0.024	1.003 (0.956 - 1.051)	0.913	-	-	-	-	-	-
Transferrin saturation	ER$^+$ breast cancer	14	MR-Egger	0.015	0.041	1.015 (0.937 - 1.100)	0.718	25.626	26.000	0.012	0.001	0.005	0.821
			IVW	0.022	0.028	1.022 (0.967 - 1.080)	0.441	25.739	27.000	0.018	-	-	-
			Weighted median	0.015	0.025	1.016 (0.967 - 1.067)	0.540	-	-	-	-	-	-
			Simple mode	0.073	0.065	1.076 (0.947 - 1.222)	0.282	-	-	-	-	-	-
			MR-PRESSO	0.019	0.027	1.020 (0.967 - 1.075)	0.489	-	-	-	-	-	-
	ER$^-$ breast cancer	14	MR-Egger	-0.070	0.043	0.933 (0.857 - 1.015)	0.133	10.532	12.000	0.569	0.009	0.005	0.105
			IVW	-0.017	0.032	0.984 (0.925 - 1.046)	0.598	13.604	13.000	0.402	-	-	-
			Weighted median	-0.050	0.038	0.952 (0.884 - 1.024)	0.187	-	-	-	-	-	-
			Simple mode	0.196	0.101	1.217 (0.998 - 1.483)	0.074	-	-	-	-	-	-
			MR-PRESSO	-0.013	0.031	0.987 (0.930 - 1.048)	0.674	-	-	-	-	-	-

Table 2. MR results for the relationship between the four iron statuses and ER+/ER- breast cancer (continued).

MR info								Cochran's Q			Horizontal pleiotropy		
Exposure	Outcome	SNP (n)	Method	Beta	se	OR (95% CI)	p	Q	Q _df	p	Intercept	se	p
Iron	ER+ breast cancer	25	MR-Egger	-0.085	0.179	0.919 (0.647 - 1.304)	0.640	39.696	23.000	0.017	0.005	0.007	0.499
			IVW	0.024	0.082	1.025 (0.873 - 1.202)	0.766	40.512	24.000	0.019	-	-	-
			Weighted median	0.032	0.091	1.032 (0.864 - 1.233)	0.726	-	-	-	-	-	-
			Simple mode	-0.061	0.168	0.941 (0.677 - 1.308)	0.721	-	-	-	-	-	-
			MR-PRESSO	0.024	0.082	1.025 (0.873 - 1.202)	0.768	-	-	-	-	-	-
	ER- breast cancer	25	MR-Egger	-0.093	0.223	0.911 (0.589 - 1.409)	0.680	25.581	23.000	0.321	-0.006	0.009	0.481
			IVW	-0.235	0.100	0.791 (0.649 - 0.962)	0.019	26.152	24.000	0.346	-	-	-
			Weighted median	-0.188	0.147	0.828 (0.621 - 1.105)	0.200	-	-	-	-	-	-
			Simple mode	-0.054	0.314	0.947 (0.5123-1.751)	0.864	-	-	-	-	-	-
			MR-PRESSO	-0.235	0.100	0.791 (0.649 - 0.962)	0.028	-	-	-	-	-	-

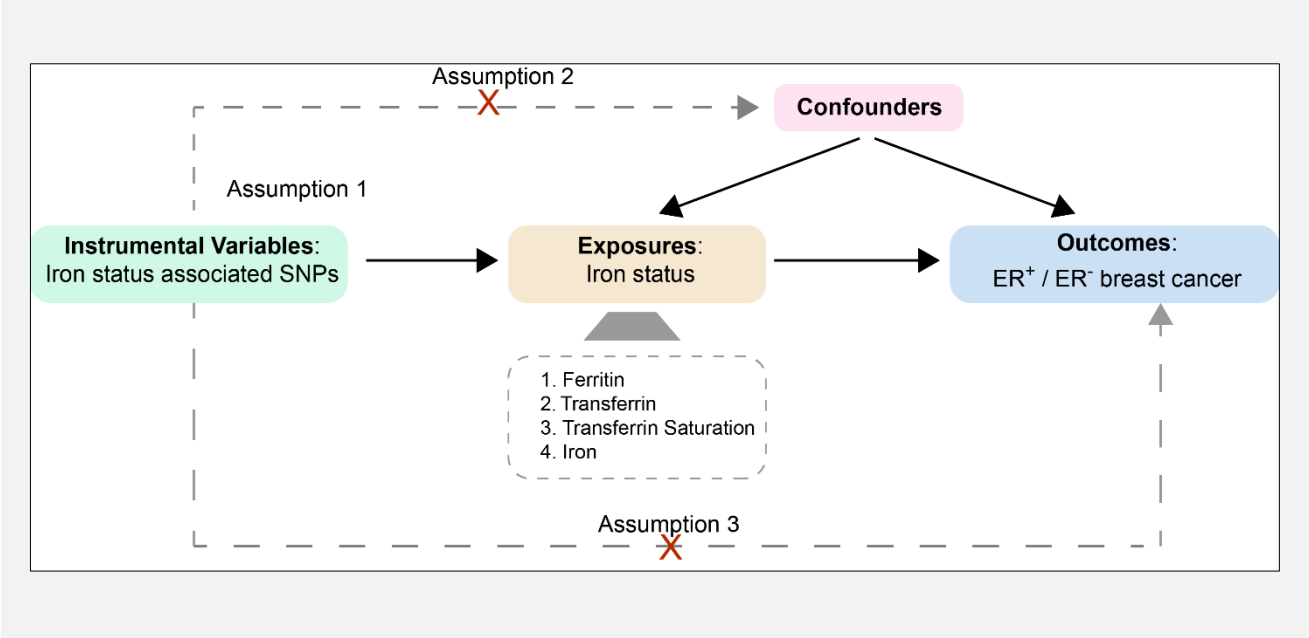

Figure 1. The three main assumptions of MR analysis on exposure and outcome.

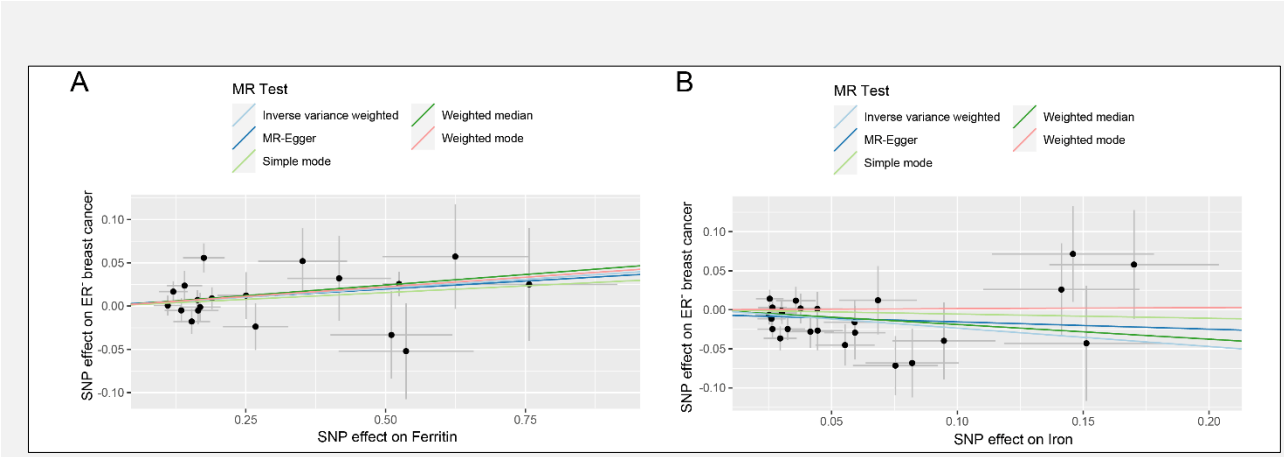

Figure 2. Scatterplots illustrating the influence of SNPs on both exposure and outcome.

2A - for ferritin levels and ER-negative breast cancer, 2B - for serum iron levels and ER-negative breast cancer.

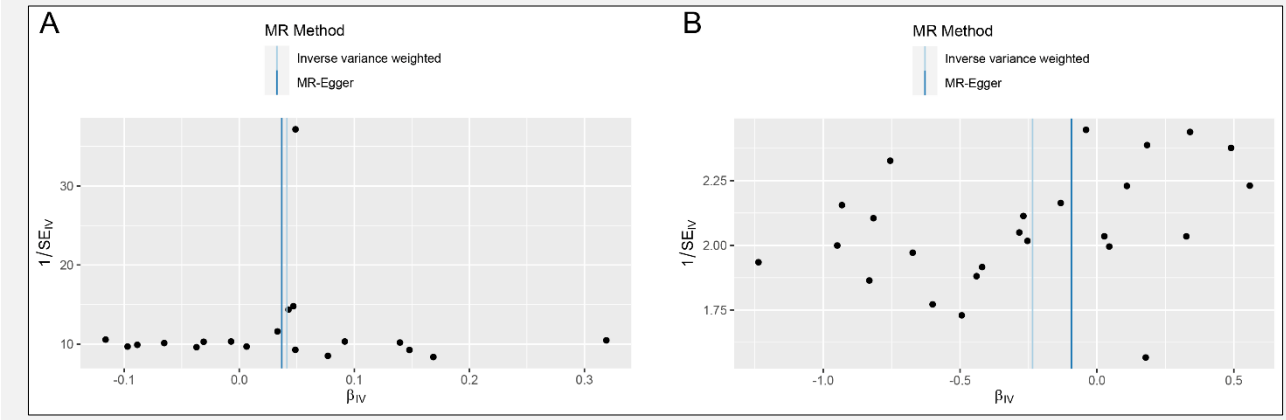

Figure 3. Funnels illustrating the association.

3A - for ferritin and ER-negative breast cancer, 3B - for serum iron and ER-negative breast cancer.

RESULTS

Mendelian randomization estimates

This study employed five MR methods to investigate the causal relationships between four iron status indicators (serum iron, transferrin saturation, ferritin, and transferrin) and ER^+ and ER^- breast cancer. The MR analysis revealed a correlation between ferritin and serum iron with ER^- breast cancer, but no association was found with ER^+ breast cancer. Transferrin and transferrin saturation showed no correlation with either ER^+ or ER^- breast cancer. The results of the inverse variance weighted (IVW) analysis indicated that an elevated expression of ferritin increases the risk of ER^- breast cancer (OR = 1.042, 95% CI (1.005, 1.081), p = 0.025), with no observed heterogeneity or horizontal pleiotropy. Therefore, ferritin is identified as a risk factor for ER^- breast cancer (see Table 2 and Figure 2A). Serum iron exhibited a negative correlation with the risk of ER^- breast cancer (OR = 0.791, 95% CI (0.649, 0.962), p =

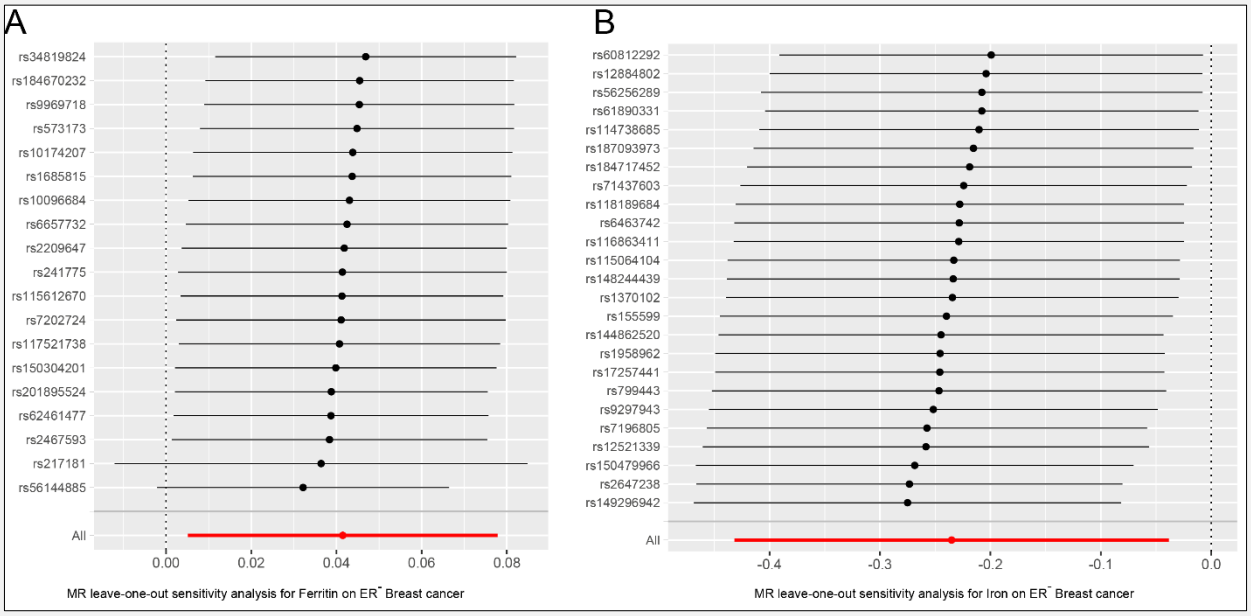

Figure 4. Results of the leave-one-out analysis.

4A - for ferritin and ER-negative breast cancer, 4B - for serum iron and ER-negative breast cancer.

0.019), and no heterogeneity or pleiotropy was observed. Hence, serum iron is recognized as a protective factor for ER- breast cancer (see Table 2 and Figure 2B).

Sensitivity analysis

In comparison to the IVW results, the weighted median method yielded a conclusion supporting only an increased expression of ferritin as a risk factor for ER- breast cancer (OR = 1.050, 95% CI (1.001, 1.102), p = 0.046). Upon the application of the MR-PRESSO method to exclude abnormal SNPs, the causal relationships between ER- breast cancer and ferritin (OR = 1.042, 95% CI (1.005, 1.081), p = 0.039), as well as serum iron (OR = 0.791, 95% CI (0.649, 0.962), p = 0.028), retained statistical significance, aligning with the IVW conclusions, as elaborated in Table 1. Funnel plot and leave-one-out analyses for the MR results of ferritin and serum iron with ER- breast cancer is presented in Figures 3 and 4. The funnel plot results indicate no evidence of an asymmetry in the causal relationships among these SNPs, and the leave-one-out results show no changes in the results after sequentially excluding each SNP. These analytical findings provide a certain degree of evidence for the stability of the results regarding the causal associations between ferritin and serum iron with ER- breast cancer.

DISCUSSION

This study used a two-sample MR approach to comprehensively investigate the causal relationships between four iron status biomarkers (ferritin, transferrin, transferrin saturation, and serum iron) and ER+ and ER- breast cancer. The IVW results suggested that an elevated level of ferritin expression may be a potential risk factor for ER- breast cancer, while an increased serum iron expression may be a potential protective factor for ER- breast cancer. However, no association was observed between transferrin and transferrin saturation with ER- breast cancer. Additionally, no significant correlations were found between the four iron status biomarkers and ER+ breast cancer. In the sensitivity analysis, the weighted median estimation (WME) results aligned closely with the IVW results.

Ferritin is a glycoprotein synthesized by the liver and is widely recognized as an acute-phase reactant protein. Its levels exhibit non-specific elevation in various inflammatory conditions, including malignancies, infections, and autoimmune diseases. Moore et al. [20], through an analysis of data from adult patients with ferritin levels exceeding 1,000 ug/L, found a more prevalent increase in the ferritin levels in patients with malignancies or infections. Another clinical study revealed a significantly higher ferritin content in breast cancer patients compared to a healthy control group (p = 0.083), confirming

a close association between ferritin and breast cancer [21]. The link between malignancies and elevated ferritin levels may be attributed to ferritin's involvement in pathways such as antioxidant damage, immune suppression, promotion of cell proliferation, and angiogenesis, thereby promoting the occurrence and development of malignancies [22]. The results of this study suggest that elevated ferritin levels are a potential risk factor for ER-negative breast cancer, which is consistent with findings from other clinical studies. High expression of ferritin in tumor patients is correlated with a shorter survival period, making it a biochemical indicator for assessing tumor prognosis [23]. Additionally, downregulating ferritin expression not only increases the sensitivity to chemotherapy drugs, but also inhibits the proliferation of tumor cells [24]. Collectively, these studies indicate that ferritin holds promise in the identification and treatment of anticancer therapies.

Iron serves as a crucial nutrient that facilitates cell growth and proliferation, playing a role in oxidation-reduction processes and the formation of free radicals, thereby fostering the onset and progression of tumors [25]. In their Cox proportional hazards regression analysis, Ferrucci et al. [26] found a positive correlation between elevated dietary iron content and the risk of breast cancer, though a linear trend was not discerned. In summary, an increased iron content exhibits a positive association with breast cancer risk. Nevertheless, other studies [5,27] indicate that an augmentation in intracellular Fe2+ levels can trigger the Fenton reaction, leading to the generation of hydroxyl radicals. These radicals, by oxidizing membrane-bound polyunsaturated fatty acids, induce lipid peroxidation. In instances where the antioxidant system is hindered, an excess of lipid peroxidation and reactive oxygen species remains uncleared, resulting in cellular membrane damage and the initiation of iron-dependent programmed cell death, recognized as iron death [28]. An activation of the iron death pathway proves effective in suppressing the proliferation of breast cancer tumor cells, enhancing resistance to chemotherapy, fortifying anti-tumor immunity, and curbing distant metastasis of tumor cells [29]. These collective findings suggest that an elevated iron content, through the activation of iron death, contributes to anti-breast cancer effects, indicating a negative correlation between heightened intracellular iron levels and the risk of breast cancer. This is consistent with the outcomes of this study.

In a previous study, Yuan et al. [30] used a two-sample MR approach to explore the causal relationships between four distinct iron status biomarkers (ferritin, transferrin, transferrin saturation, and serum iron) and 22 specific-site cancers, including breast cancer. The findings suggested that there was no discernible causal link between the four different iron statuses and breast cancer, as well as ER^+ and ER^- breast cancer. Using a similar MR methodology, this current study delved into the causal associations between the four iron status biomarkers and ER^+ and ER^- breast cancer. The majority of the results from this analysis affirmed the absence of a correlation between the four iron status biomarkers and ER^+ breast cancer. Furthermore, no causal relationship was identified between transferrin and transferrin saturation and ER^- breast cancer. However, a minority of the findings in this analysis contradicted the conclusions drawn by Yuan et al. [30], indicating a correlation between ferritin and serum iron with ER^- breast cancer. The variance in these results may be attributed to inconsistencies in the criteria for selecting genetic instrumental variables (IVs) for the four different iron statuses. While Yuan et al. chose only three IVs (rs1800 562, rs1799945, and rs855791) as instrumental variables, this study implemented a broader threshold for site selection ($p = 1 \times 10^{-5}$), leading to the inclusion of a more extensive set of IVs. Additionally, disparities in the GWAS datasets for ER^+ and ER^- breast cancer between the two analyses could contribute to the observed differences. In conclusion, further analyses, incorporating a larger patient cohort and utilizing the latest GWAS data, are imperative to unravel the underlying mechanisms between the four distinct iron statuses and breast cancer.

Two-sample MR serves as a robust approach for drawing causal inferences between exposures and outcomes, by using summarized statistical data. However, it carries inherent strengths and limitations, demanding careful consideration when interpreting study outcomes. This research boasts several strengths: 1) genetic exploration: the study delves into the causal relationships between four distinct iron status biomarkers (ferritin, transferrin, transferrin saturation, and serum iron) and ER^+ and ER^- breast cancer from a genetic standpoint; 2) confounding mitigation: MR methods effectively mitigate the impact of most confounding factors, diminishing the potential for confounding bias and delivering more stable estimates of causal effects; 3) data prowess: the utilization of the latest and most extensive exposure genome-wide association study (GWAS) data, coupled with stringent adherence to the three fundamental MR assumptions during instrumental variable SNP selection, ensures robustness; and 4) methodological variety: employing five distinct methods for MR analysis and scrutinizing results by using Cochran's Q statistic, MR-Egger regression, funnel plots, and leave-one-out analysis enhance the reliability of the findings. However, akin to other MR studies, this research grapples with certain limitations: 1) population homogeneity: inclusion of GWAS data solely from European populations limits the generalizability of the findings; 2) SNP selection threshold: the relatively lenient SNP threshold ($p = 1 \times 10\text{-}5$) for the GWAS of the four different iron statuses may result in a weaker association between selected instrumental variables and exposure; 3) handling outliers: despite employing diverse methods to eliminate outliers, complete negation of the impact of horizontal pleiotropy on results may not be achieved; 4) stratification omission: the study does not stratify ER^+ and ER^- breast cancer based on severity, gender, and age; 5)

population specificity: the reliance on GWAS data from European populations may reduce the universality of the research findings; and 6) algorithmic heterogeneity: heterogeneity exists in the MR-Egger and inverse variance weighted (IVW) algorithms in the correlation analysis of ER^+ breast cancer. In conclusion, future analyses should incorporate more diverse and up-to-date GWAS data, further elucidating the mechanistic intricacies through experimental exploration.

CONCLUSION

In summary, this study used two-sample MR to investigate the causal relationships between four distinct iron status biomarkers (ferritin, transferrin, transferrin saturation, and serum iron) and ER^+ and ER^- breast cancer. The findings revealed a positive correlation between ferritin and ER^- breast cancer, a negative correlation between serum iron and ER^- breast cancer, and no causal relationship between transferrin, transferrin saturation, and ER^- breast cancer. Moreover, there was no causal association found between the four iron biomarkers and ER^+ breast cancer. Rigorous assessments, including Cochran's Q statistic, MR-Egger regression, funnel plots, and leave-one-out analysis, confirmed the stability and reliability of the MR results. Future research endeavors should delve into these indicators, especially ferritin and serum iron, through both clinical experiments and expanded MR studies. Additionally, a validation in populations beyond those of European descent is essential for a more comprehensive understanding.

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Availability of Data and Materials:
The datasets generated and analyzed during this study are available in the IEU OpenGWAS project database (https://gwas.mrcieu.ac.uk/).

Declaration of Interest:
None.

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