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Research article

# A cause-effect relationship between uterine diseases and breast cancer: A bidirectional Mendelian randomization study

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ABSTRACT

*Objective:* To explore the cause-effect relationship between uterine diseases (UDs) and breast cancer (BC) and underlying mechanism of the cause-effect relationship, enhance understanding of the association between BC and UDs.

*Methods:* A two-sample bidirectional Mendelian randomization (MR) analysis was conducted. We obtained summary statistics data from GWAS for BC, endometriosis, endometrial cancer (EC), uterine leiomyoma (UL), uterine polyps (UP), and cervical cancer (CC). Independent SNPs were selected as instrumental variables (IVs) for each disease. The inverse variance weighted (IVW) method was primary used for estimating the causal association between UDs and BC. To further evaluate the consistency and dependability of the results, we also utilized the weighted median, weighted mode, simple mode, and MR-Egger methods, along with sensitivity analyses. Furthermore, a supplementary analysis focusing on the variants linked to BC and UDs was conducted. This involved identifying corresponding genes and subsequently performing KEGG/GO analyses to investigate potential molecular mechanisms.

*Results:* The results indicated significant associations between genetic susceptibility to endometriosis, EC, and UL with BC risk. The odds ratios (ORs) were as follows: endometriosis at 0.963 (95 % CI, 0.942–0.984; $p = 7.11e\text{-}5$), EC at 1.056 (95 % CI, 1.033–1.081; $p = 2.39e\text{-}6$), and UL at 1.027 (95 % CI, 1.006–1.048; $p = 0.010$). Conversely, the predisposition to BC inferred from genetic factors was markedly correlated with an elevated risk of EC indicated by an OR of 1.066 (95 % CI, 1.019–1.116; $p = 0.006$), and was correlated with UP risk (OR, 1.001,95 % CI, 1.000–1.002; $p = 0.001$).

Sensitivity analyses provided weak evidence for these effects, suggesting that the study's outcomes are consistent and trustworthy. Further analysis of the genetic variants associated with BC, and these related genes are enriched in Cellular senescence, GnRH secretion, Phosphatidylinositol signaling system, and so on.

*Conclusion:* This study corroborates the existence of a reciprocal causal relationship between BC and EC, as well as highlighting the substantial correlations between a genetic susceptibility to UL and endometriosis with BC. BC may exert their influence on EC and UP through Cellular senescence, GnRH secretion, and other pathways. These discoveries offer fresh perspectives on the genetic pathogenesis of BC and UDs, and can guide future experimental studies. Additionally, they lay down a groundwork for the development of tailored preventative and therapeutic strategies moving forward.

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## 1. Introduction

Breast cancer (BC) ranks as the most common cancer globally and is a primary contributor to death among women [1]. Additionally, there is a noticeable increase in the incidence of other malignancies in the female reproductive system, such as cervical cancer (CC) and endometrial cancer (EC) [1,2]. Often diagnosed at advanced stages, these female tumors have limited treatment options, leading to higher recurrence rates and lower survival rates [3]. Consequently, preventing and treating tumors in the female reproductive system pose significant challenges. The female malignancies exert a profound influence on both the physical health and mental state of women, notably diminishing their general life quality [4]. Moreover, these malignancies have a unique location in the female reproductive system, affecting both the patients' health and lineage transmission [5]. However, the precise mechanisms underlying their development and progression are still unclear, and the risk factors associated with the diseases continue to evolve [6]. Besides malignancies, endometriosis, uterine leiomyomas (UL) and uterine polyps (UP) are common non-cancerous gynecological uterine diseases (UDs). Emerging epidemiological evidence suggested that women with these conditions tend to exhibit an increased susceptibility to hormone-associated cancers, encompassing BC, EC, CC, and ovarian cancer (OC) [7–9]. Although the etiology and natural history of these conditions differ, accurate differential diagnosis is necessary due to potential overlapping symptoms [10]. Therefore, it is crucial to understand the disease's etiology, identify high-risk populations, promote early detection, implement proactive intervention strategies, and provide effective treatment for the prevention and management of breast and gynecological malignancies.

BC can be classified into four major subtypes according to hormonal receptor expression and histopathological indicators: luminal A, luminal B, HER2-overexpressing, and triple-negative BC (TNBC). Approximately 75 % of BC are estrogen receptor (ER)-positive, with the luminal subtypes (A and B) accounting for a large proportion of these ER-positive cases and generally exhibiting high ER expression. Estrogen has been demonstrated to facilitate the advancement of BC via ER-mediated signaling cascades [11,12]. The primary source of estrogen varies between premenopausal and postmenopausal women. In premenopausal women, the ovaries are the major source. Genetic factors have been found to influence the occurrence of both breast and ovarian cancers. Genome-wide association studies (GWAS) have identified over 100 BC susceptibility loci and more than 20 OC susceptibility loci, with approximately 5 loci being associated with the susceptibility of both cancers [13]. The uterus and breast are key organs of the female reproductive tract, and there are also potential associations between them. A systematic review had underscored the substantial impact of sialyltransferases, notably ST6Gal-I, in the progression of gynecological tumors, encompassing cancers of the cervix, uterus, and breast [14]. Additionally, the presence of GnRH2 and its corresponding receptor has been observed in peripheral reproductive organs, including the ovaries, the uterus, and the mammary glands. Furthermore, GnRH2 and its receptor have been identified across a spectrum of human reproductive malignancy cells [15]. Auxiliary hormonal treatments, including tamoxifen and fulvestrant that inhibit estrogen/ER-mediated signaling, constitute the primary therapeutic approach for individuals with ER-positive BC [16]. However, the agonistic effects of tamoxifen on the endometrium can contribute to endometrial proliferation, hyperplasia, polyp formation, and cancer. Consequently, prolonged administration of tamoxifen has been linked to an elevated risk of EC or UP [17]. In light of their interconnection and potential shared mechanisms, understanding the cause-effect relationship between UDs and BC holds great promise for advancing the prevention, early detection, and management of UDs and BC. Further investigation is required to unravel the complexities of this cause-effect relationship and explore its potential clinical applications.

The intricate web of genetic and environmental elements contributes to the development of these female malignancies, but the underlying genetic basis remains incompletely understood. Previous research on the association between BC and UDs has limitations, such as reliance on case-control or observational designs, small sample sizes, and the potential for reverse causation. Therefore, the causal relationship between BC and UDs remains uncertain, emphasizing the necessity for additional research. Prospective studies and randomized controlled trials provide valuable insights into causality, but conducting such studies can be time-consuming, resource-intensive, and ethically challenging.

Mendelian randomization (MR) is a research approach employed to discern potential causal links between exposures and outcomes [18]. This methodology leverages genetic variants, specifically single nucleotide polymorphisms (SNPs), that correlate with the factor of interest, employing them as instrumental variables (IVs) to infer causality [19]. The random distribution of alleles ensures that the estimated causal effects remain theoretically unaffected by confounding factors and reverse causation [20]. IVs can be derived from readily accessible summary statistics obtained from GWAS, which are extensively employed in Two-Sample Mendelian Randomization (2SMR) [21].

This study aimed to comprehensively evaluate the bidirectional causal effects between breast cancer (BC) and a range of uterine diseases (UDs), including endometriosis, endometrial cancer (EC), uterine leiomyoma (UL), uterine polyps (UP), and cervical cancer (CC). Hence, a 2SMR analysis was employed. Furthermore, a supplementary analysis was conducted to explore the potential molecular mechanisms underlying the observed associations. This involved identifying the genetic variants associated with both BC and UDs, and subsequently performing KEGG and GO analyses on the corresponding genes. Additionally, the study investigated the differential relationships between BC subtypes (ER+ and ER-) and EC. The findings from these comprehensive investigations have the potential to guide future research, inform clinical practice, and ultimately contribute to improved public health outcomes by expanding our comprehension of the intricate dynamics between UDs and BC.

## 2. Materials and methods

### 2.1. Study design

A bidirectional 2SMR design was employed to investigate the causal relationship between uterine diseases and breast cancer (Fig. 1). The MR analysis was based on the following assumptions: 1) the SNPs derived from GWAS served as IVs and were associated with the exposures of interest; 2) the IVs were not correlated with confounding factors that might skew the causal inferences; 3) the effect of IVs on the outcome was exclusively transmitted via the exposure being studied. The bidirectional MR analysis adhered to the STROBE-MR guidelines [22,23].

### 2.2. Data source

The research data utilized in this investigation were sourced from the IEU OPEN GWAS PROJECT [24], an open-access database accessible at https://gwas.mrcieu.ac.uk/(accessed on June 13, 2023). Prominent SNPs associated with uterine diseases and breast cancer was selected, including endometriosis, EC, UL, UP, and CC, as genetic IVs for this bidirectional two-sample MR analysis. The necessary ethical clearance for the primary research was secured, confirming adherence to ethical protocols. To minimize potential biases stemming from population heterogeneity, this analysis was restricted to the European population. Detailed information regarding the data sources are presented in Table 1.

### 2.3. Selection of IVs

To meet the assumptions of MR, a set of criteria were applied for IV selection: 1) Inclusion of SNPs was predicated on their robust correlation with the exposure factors, with a genome-wide significance threshold of $p < 5 \times 10^{-8}$; 2) LD clumping was performed with criteria of $R^2 < 0.001$ and physical genetic distance >10,000 kb to select independent SNPs; 3) SNPs with minor allele frequency (MAF) < 0.03 were removed; 4) The PhenoScanner GWAS repository was leveraged to detect and eliminate SNPs correlated with possible confounders, including alcohol intake and smoking behavior; 5) MR Steiger test was utilized to exclude the SNPs exhibiting reverse causation [25].

It is important to note that including fewer than 10 independent SNPs as IVs may reduce the statistical power of MR analysis [19]. To address this concern, a relaxed instrument threshold ($p < 1 \times 10^{-5}$) was employed, which has been used in previous studies, to ensure an adequate number of SNPs, particularly in cases where traits had limited SNP coverage.

### 2.4. Statistical analysis

#### 2.4.1. Two-sample MR analysis

The principal methodology employed to assess the causality of the exposure's impact on the outcome was the inverse variance weighted (IVW) [18]. An OR value > 1 signified that the exposure acted as a risk factor, whereas an OR < 1 implied a protective role for the exposure. To bolster the reliability of the findings, an outlier detection process was implemented via the MR-PRESSO method [26]. Additionally, weighted median method, MR-Egger regression, weighted mode method, and simple mode method were used to validate the robustness of the IVW results [27].

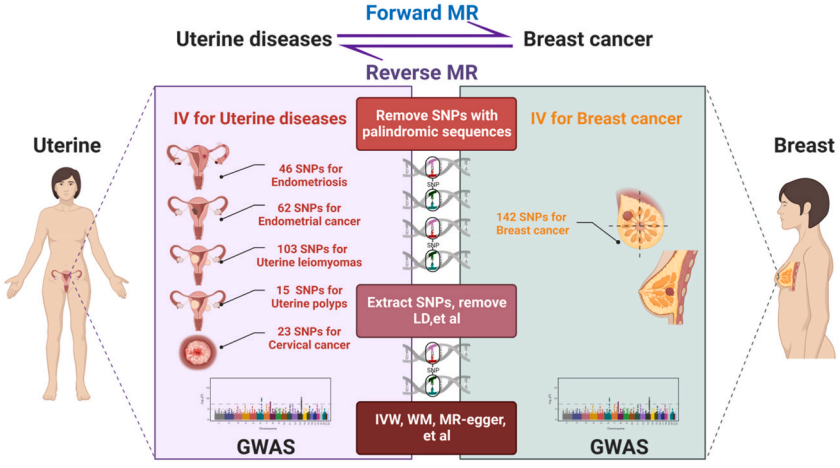


**Fig. 1.** Schematic representation of the MR procedure.

**Table 1**
Details of data sources.

| Exposures/Outcomes | GWASID | Ethnicity | Sample size (cases/controls) | SNPs Number |
|---|---|---|---|---|
| Endometriosis | finn-b-N14_ENDOMETRIOSIS | European | 8,288/68,969 | 16,377,306 |
| Endometriosis cancer | ebi-a-GCST006464 | European | 12,906/108,979 | 9,470,555 |
| Uterine leiomyomas | finn-b-CD2_BENIGN_LEIOMYOMA_UTERI | European | 18,060/105,519 | 16,379,784 |
| Uterine polyps | ukb-b-12944 | European | 5,419/457,591 | 9,851,867 |
| Cervical cancer | finn-b-C3_CERVIX_UTERI_EXALLC | European | 1,648/99,321 | 16,378,927 |
| Breast cancer | ieu-a-1126 | European | 122,977/105,974 | 10,680,257 |
| ER- Breast cancer | ieu-a-1128 | European | 21,468/105,974 | 10,680,257 |
| ER + Breast cancer | ieu-a-1127 | European | 69,501/105,974 | 10,680,257 |
| HPV E7 Type 16 | prot-c-2623_54_4 | European | 997 | 501,428 |
| HPV E7 Type 18 | prot-c-2624_31_2 | European | 997 | 501,428 |

#### *2.4.2. Horizontal pleiotropy analysis*

The MR-Egger regression analysis was conducted to detect any horizontal pleiotropy, where a p-value below 0.05 suggests its possible occurrence. Furthermore, if the intercept term in the MR-Egger regression is close to zero, it indicates a lack of evidence for horizontal pleiotropy.

#### *2.4.3. Heterogeneity testing*

To investigate potential heterogeneity due to different genetic variants, Cochran's Q statistic was applied to evaluate heterogeneity. A significant p-value from the Cochran's Q test at less than 0.05 would signal heterogeneity among the results, suggesting that the causal effects may vary among the IVs. If heterogeneity was detected, the IVW method under a multiplicative random effects framework was utilized to provide the effect size estimates [28].

#### *2.4.4. Sensitivity analysis*

To further assess the sensitivity, a leave-one-out approach was implemented. Specifically, each genetic variant (SNP) was systematically removed, and the resultant estimates were recomputed without the omitted variant. These recalculated estimates were subsequently contrasted with the aggregate estimate. Such an analysis is instrumental in discerning the sway of each individual SNP on the comprehensive causal inference and in substantiating the stability of the outcomes.

#### *2.4.5. Reverse MR analysis*

To assess whether uterine diseases have a causal effect on breast cancer, reverse MR analysis was performed via the aforementioned MR analysis methods. Each individual uterine-related disease was considered as the outcome variable, and breast cancer was analyzed as the exposures in separate analyses. The results of this study are depicted through ORs and 95 % confidence intervals (CIs), with a threshold at $\alpha = 0.05$. The entire suite of statistical computations was carried out via R version 4.1.3, and the software packages TwoSample MR and MR-PRESSO were utilized.

#### *2.4.6. GO/KEGG analysis*

Moreover, compiled SNPs for UDs and BC have been, identifying those SNPs with a consistent direction of effect on the outcome respectively. Then we created a Venn diagram to visualize the data and obtained the common SNPs, for which we searched their associated genes in the NCBI database (https://www.ncbi.nlm.nih.gov/). Subsequently, GO and KEGG enrichment analysis were performed in order to assist us in understanding the potential mechanisms of progression and pathogenesis between BC and UDs. Venn diagram and GO/KEGG results was portrayed by VennDiagram and ggplot2 (version 3.3.6).

## 3. Results

### *3.1. Selection of IVs*

A total of 46, 62, 103, 15, and 23 distinct significant SNPs were pinpointed for endometriosis, EC, UL, UP, and CC, respectively. However, some SNPs were excluded because they contained palindromic structures, suggested reverse causality, or were linked to possible confounding factors. After excluding the above SNPs, we performed causal estimation using 34, 55, 77, 13, and 21 SNPs for endometriosis, EC, UL, UP, and CC, respectively (Tables S1–5).

Notably, during the assessment of UP and BC, the orientation of the OR in the MR-Egger analysis was deviated from other methods. To address this issue, the p-value threshold was decreased to $p < 1e^{-8}$ [29]. Consequently, only 1 SNPs remained identified as IVs in the UP to BC analysis, which could not undergo further MR analysis (Table S6). Therefore, an investigation of UP on BC in the forward MR analysis was not conducted. Additionally, an additional 142 SNPs were screened from the human genotype-phenotype association database that were associated with BC based on hypotheses 1, 2, and 3. Furthermore, PhenoScanner was utilized to assess the SNP dataset and removed 4 SNPs due to their association with confounding variables. As a result, 138 SNPs were selected as genetic proxies for BC (Fig. 1, Table S7).

### 3.2. The causal effect of uterine diseases on breast cancer

Initially, the genetic correlation between each uterine disease and the risk of BC was estimated. The MR analyses results are depicted in Fig. 2 and Supplementary Figures. The findings indicated a substantial link between genetically anticipated BC and heightened susceptibility to endometriosis in the IVW model, with an OR of 0.963 (95 % CI, 0.942–0.984; $p = 7.11^{e-5}$). Consistent results were observed in the other three analyses, although statistical significance was not reached in the MR-Egger analysis. The MR-PRESSO analysis found no evidence of outliers, indicating the robustness and reliability of the results (Table S10). Furthermore, the Cochran Q test revealed no significant heterogeneity across the individual SNP estimates (Q = 29.4; $p = 0.598$), and the MR-Egger regression analysis showed no signs of pleiotropy (intercept $p = 0.5$; Table 2).

The IVW model results outcomes demonstrated a notable link between BC and EC (OR, 1.056 [95 % CI, 1.033–1.081]; $p = 2.39e\text{-}6$), indicating a shared genetic predisposition for the two diseases. Besides, a positive correlation was observed between BC and UL (OR, 1.027 [95 % CI, 1.006–1.048]; $p = 0.010$). The analysis did not uncover any significant heterogeneity, pleiotropy, or outliers. (Table 2 and Table S8).

However, the IVW analysis did not provide evidence of a link between genetic propensity for BC and CC. Similar to the other analyses, no statistically significant pleiotropy or heterogeneity was observed in the CC analysis (Table 2 and Table S8). It should be emphasized that additional research is necessary to substantiate the association between BC and CC, as more research is required in this area.

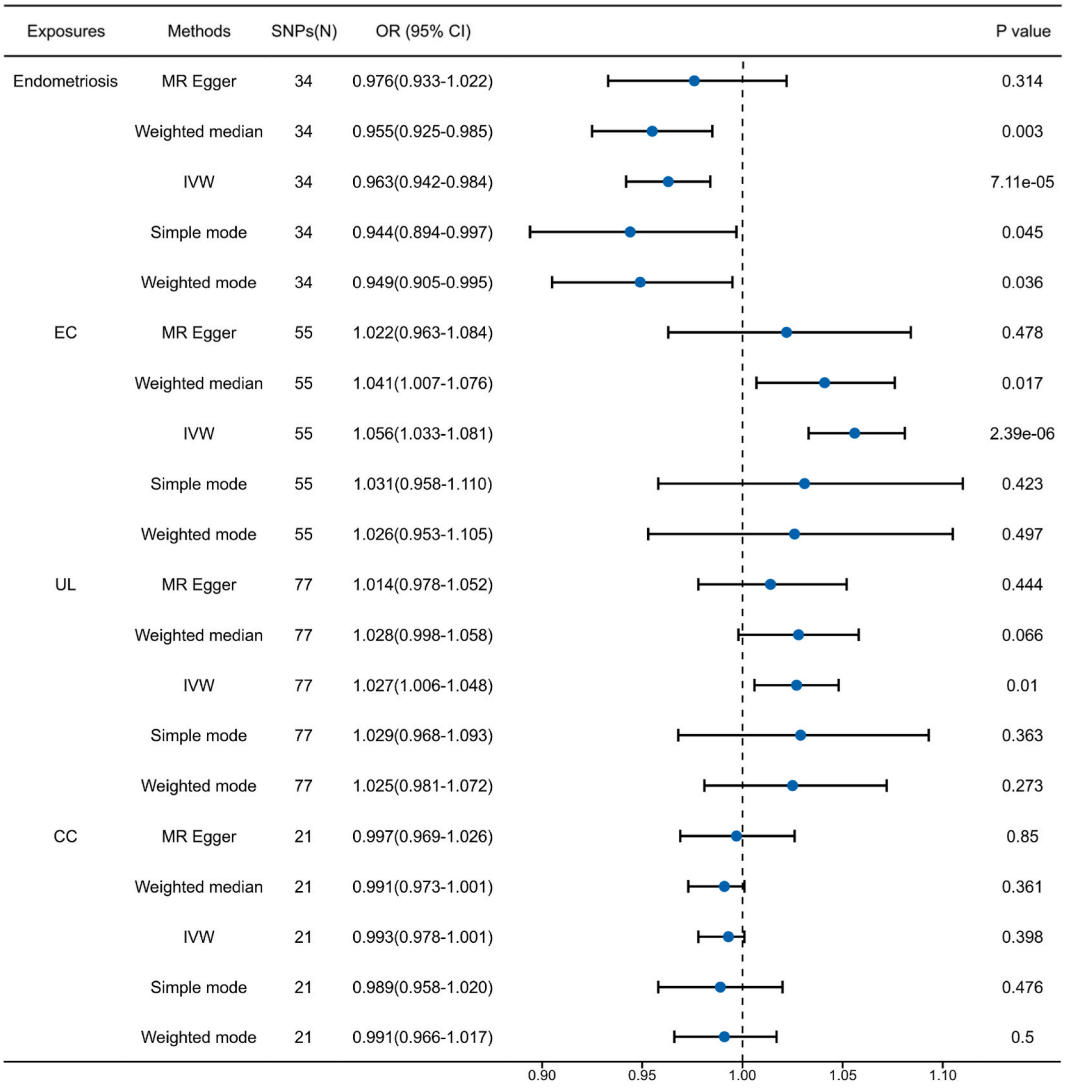


**Fig. 2.** Associations of uterine diseases with risk of breast cancer.

**Table 2**
Associations of UDs with risk of BC in sensitivity analyses.

| Uterine diseases | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| | Q | Q df | Q *P* value | Q | Q df | Q *P* value | Egger intercept | SE | *P* value |
| Endometriosis | 29.4 | 32 | 0.598 | 29.9 | 33 | 0.623 | −0.002 | 0.003 | 0.500 |
| Endometrial cancer | 57.8 | 53 | 0.301 | 59.4 | 54 | 0.285 | 0.004 | 0.004 | 0.236 |
| Uterine leiomyoma | 88.5 | 75 | 0.136 | 89.3 | 76 | 0.141 | 0.002 | 0.002 | 0.427 |
| Cervical cancer | 27.6 | 19 | 0.091 | 27.7 | 20 | 0.115 | −0.001 | 0.004 | 0.761 |

### *3.3. The causal effect of breast cancer on uterine diseases*

In the reverse-direction MR analyses, MR-PRESSO analysis was utilized to detect potential outliers that could potentially introduce bias. Outliers were detected in the analysis of endometriosis, EC, UL, and UP, indicating the presence of IVs that may influence the associations (Table 3 and Table S 9,11). The results of the reverse-direction MR analyses are shown in Fig. 3 and Supplementary Figures.

After correcting for these outliers, a noteworthy association was identified between the genetic predisposition to EC and BC was observed. This correlation remained significant in the IVW model, boasting an OR of 1.066 (95 % CI, 1.019–1.116; $p = 0.006$). Regarding the association between UP and BC (OR, 1.001,95 % CI, 1.000–1.002; $p = 0.001$), although the OR was close to 1, the p-value was less than 0.05. This suggests that the observed association may indeed be real. Nevertheless, the interpretation of these outcomes requires prudence, and they should be in conjunction with other evidence and studies for a more comprehensive evaluation.

On the other hand, no suggestive associations were found between genetic susceptibility to BC and other uterine diseases, including endometriosis, UL, or CC. These findings suggest that the genetic factors influencing BC may not affect on the developing of these specific UDs. However, the MR-Egger and Simple mode analyses suggested a potential association between BC and UL, with ORs of 0.895 (95 % CI, 0.817–0.981; $p = 0.020$) and 0.843 (95 % CI, 0.716–0.993; $p = 0.043$), respectively. The analysis indicated no significant pleiotropy or heterogeneity in the UL analysis (Table 3 and Table S9). Interestingly, these findings contrast with the results of the forward MR analysis, where UL was negatively correlated with BC. It is worth noting that additional research is essential for validating the link between BC and UL.

The Cochran Q test exposed no notable heterogeneity among the individual SNPs' estimates, signifying uniformity in the findings across various SNPs. Additionally, the MR-Egger regression analysis found no considerable pleiotropy for the five UDs analyzed, suggesting that no sizeable genetic determinants were concurrently influencing both BC and the UDs. (Table 3 and Table S9).

### *3.4. Sensitivity analysis*

Furthermore, extensive sensitivity analyses was executed to substantiate the causal link between UDs and BC. In addition, leave-one-out analyses was conducted to reinforce the observed causality and assess the possible influence of each IV on the outcomes. Such analysis revealed that the causality between UDs and BC was not predominantly influenced by any single SNP. (**Supplementary figures**).

### *3.5. GO/KEGG analyses*

To further investigate these relationships, genetic variant analysis was conducted. When BC was as the outcome event, 43 SNPs for endometriosis were picked out, 19 SNPs for EC, and 30 SNPs for UL. Detailed information on these genetic variants can be found in Table S12. However, no common SNPs were found among these three exposures. On the other hand, when BC was considered as the exposure, 60 SNPs for EC and 59 SNPs for UP were selected as outcome events. The details of BC-related genetic variants and their effects on the two subtypes of UDs can be found in Table S13. Then SNPs that consistently showed a positive effect on both EC and UP (beta.outcome >0) were identified. These common SNPs were visually represented using a Venn diagram in Fig. 4A. Further analysis involved the identification of 11 key genes corresponding to the 26 SNPs associated with BC. To explore the functional aspects of these disease-gene sets, GO and KEGG pathway enrichment analysis were conducted. The results revealed that these related genes are enriched in Cellular senescence, GnRH secretion, Phosphatidylinositol signaling system, and so on, as shown in Fig. 4B.

**Table 3**
Associations of BC with risk of UDs in sensitivity analyses.

| Uterine diseases | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| | Q | Q df | Q *P* value | Q | Q df | Q *P* value | Egger intercept | SE | *P* value |
| Endometriosis | 121.8 | 120 | 0.436 | 122.3 | 121 | 0.450 | 0.003 | 0.004 | 0.493 |
| Endometrial cancer | 110.2 | 109 | 0.448 | 110.6 | 110 | 0.466 | −0.002 | 0.003 | 0.575 |
| Uterine leiomyoma | 104.5 | 102 | 0.412 | 107.9 | 103 | 0.352 | 0.006 | 0.003 | 0.074 |
| Uterine polyps | 112.6 | 106 | 0.313 | 112.6 | 107 | 0.338 | <0.001 | <0.001 | 0.963 |
| Cervical cancer | 138.6 | 123 | 0.159 | 138.7 | 124 | 0.173 | −0.003 | 0.009 | 0.780 |

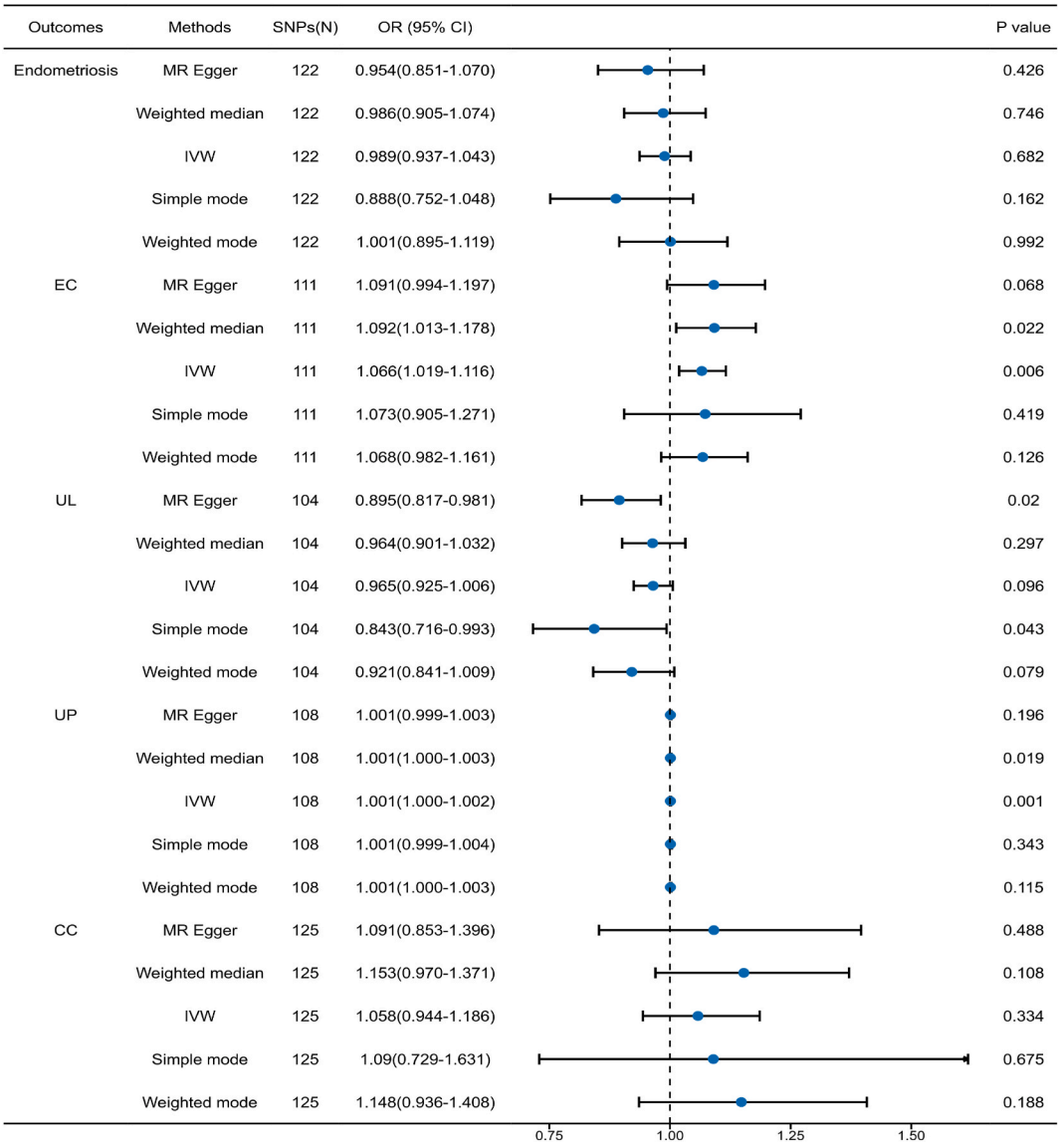


**Fig. 3.** Associations of breast cancer with risk of uterine diseases.

### *3.6. The causal effect of ER- and ER + breast cancer on endometrial cancer*

To further investigate the association between different subtypes of BC and EC, ER + BC and ER- BC were selected for exposure to further analyze their association with EC. After correcting for these outliers, a significant association between EC and ER + BC was observed. This association remained significant in the IVW model, with an OR of 1.070 (95 % CI, 1.016–1.127; $p = 0.010$). And no suggestive associations were found between genetic susceptibility to ER- BC and EC (Fig. 5).

The Cochran Q test exposed no notable heterogeneity among the individual SNPs' estimates, signaling that the findings were congruent across various SNPs. Moreover, the MR-Egger regression analysis found no considerable pleiotropy, implying the absence of influential genetic factors (Table S14). In addition, a suite of sensitivity analyses was executed to affirm the causal relationship (**Supplementary figures**).

## 4. Discussion

Previous epidemiological studies have provided evidence of a co-occurrence of UDs and BC [30], supporting the cause-effect relationship between UDs and BC. However, the depth mechanisms underlying these associations remain poorly understood. In this study, publicly available genetic data was utilized to investigate the causal relationships between UDs and BC. Using the bidirectional

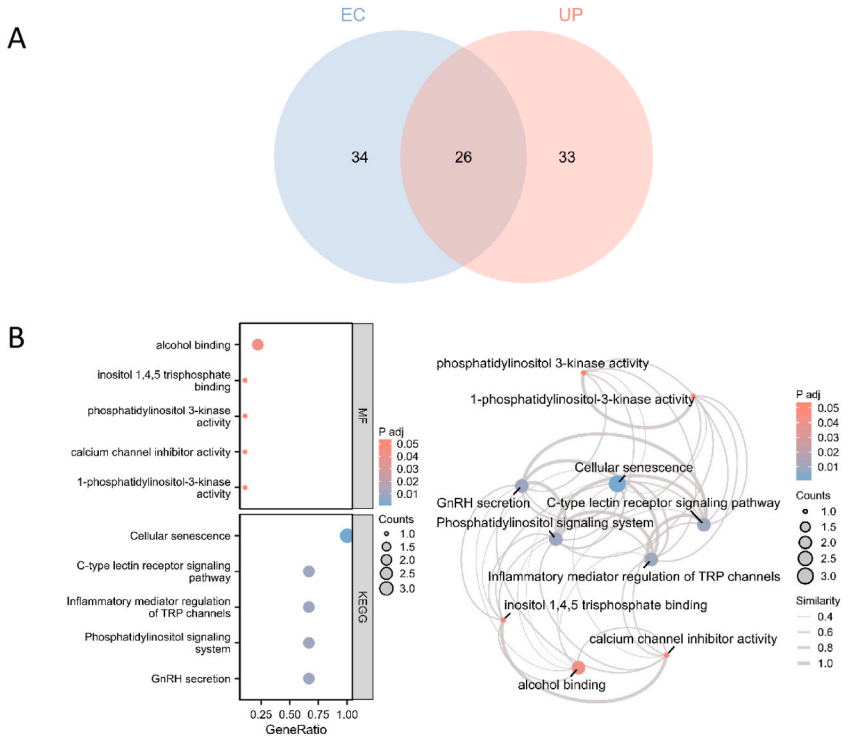


**Fig. 4.** Enrichment analysis of BC-related genes (A)Venn diagram of common genes (B) GO/KEGG enrichment analysis.

| Exposures | Methods | SNPs(N) | OR (95% CI) | P value |
|---|---|---|---|---|
| ER- BC | MR Egger | 27 | 0.933(0.760-1.146) | 0.515 |
| | Weighted median | 27 | 1.046(0.943-1.160) | 0.393 |
| | IVW | 27 | 1.066(0.991-1.146) | 0.086 |
| | Simple mode | 27 | 0.899(0.728-1.111) | 0.331 |
| | Weighted mode | 27 | 0.939(0.782-1.127) | 0.503 |
| ER+ BC | MR Egger | 84 | 1.035(0.926-1.158) | 0.544 |
| | Weighted median | 84 | 1.027(0.955-1.104) | 0.477 |
| | IVW | 84 | 1.070(1.016-1.127) | 0.01 |
| | Simple mode | 84 | 1.064(0.931-1.216) | 0.366 |
| | Weighted mode | 84 | 1.040(0.956-1.132) | 0.361 |

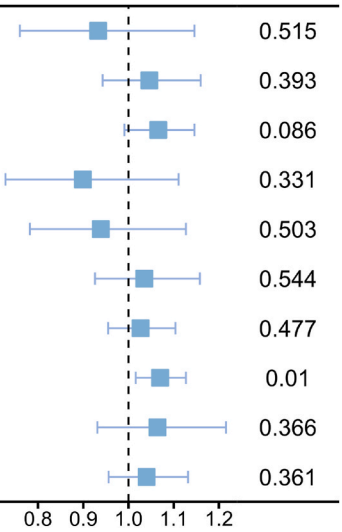


**Fig. 5.** Associations of ER-breast cancer and ER + breast cancer with risk of endometrial cancer.

2SMR approach, genetic variants were leveraged as IVs to assess causal effects while minimizing biases. This study yielded intriguing findings concerning the association between specific UDs and BC (Fig. 6).

In the forward MR analysis, a noteworthy inverse association between genetically anticipated endometriosis and the likelihood of BC was observed. This unexpected result suggests that endometriosis may potentially have a protective effect against BC development. However, prior research has reported inconsistent results and diverse interpretations regarding the link between endometriosis and BC. A number of studies have suggested that women afflicted with endometriosis might be at an elevated risk of BC development, while others have suggested a potential protective effect of endometriosis against BC. The inconsistencies could be attributed to differences

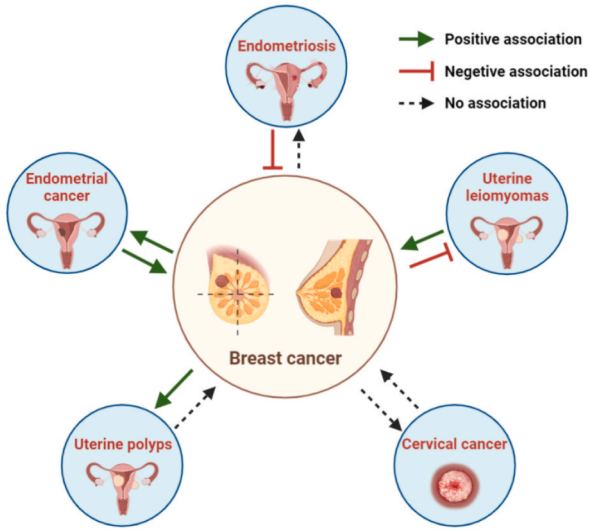


**Fig. 6.** The association between specific uterine diseases and breast cancer.

in study designs, sample sizes, and the populations under investigation. Variations in methodologies and criteria for diagnosing endometriosis and BC among different studies may contribute to the divergent associations observed. Furthermore, the age has also been a point of discrepancy among studies [31–33]. The underlying biological mechanisms linking these two conditions remain poorly understood and require further investigation.

Moreover, these findings highlighted a substantial positive association between BC and EC, as well as a suggestive positive genetic correlation between BC and UL. It is well-known that EC, UL, and BC are estrogen-dependent tumors, with estrogen playing a crucial role in their development and progression [34]. Interestingly, the positive association between EC and BC contradicted these finding of a negative association between endometriosis and BC. These findings diverge from previous studies that consistently demonstrated an increased risk of EC in individuals with endometriosis [32]. It is also noteworthy that there is comorbidity between UL and endometriosis, which should be considered when managing these conditions [27]. The unexpected findings from this study pointed to a multifaceted interplay between these conditions, underscoring the necessity for additional studies to confirm and decipher the potential mechanisms driving these connections, as well as to resolve the existing discrepancies.

In contrast, this analysis yield no indication of a causal link between BC and UP or CC. CC and BC exhibit distinct etiological mechanisms. CC is predominantly attributed to infection with human papillomavirus (HPV) [35], whereas BC arises from intricate interactions involving genetic and biological factors. Furthermore, an in-depth analysis was conducted to explore the potential mutual correlation between high-risk HPV types 16 and 18 and BC. These findings consistently demonstrated the absence of any causal relationship between these specific subtypes and BC (Supplementary figures and Table S15). Consequently, this study reveals no significant correlation between these two malignancies. Additional research is necessary to validate these results and explore potential genetic links between BC and CC. Considering UP, it is advisable to conduct further investigation into this association once a larger GWAS dataset for UP becomes available.

In the reverse-direction MR analyses, a noteworthy correlation was identified between EC and BC was observed. As for the tie between the genetic propensity for UP and BC, although the OR was close to 1, the p-value was less than 0.05, suggesting a possible association. Tamoxifen, functioning as a selective estrogen receptor modulator (SERM), is frequently utilized in the treatment of BC as an adjuvant therapy. However, tamoxifen's agonistic effects on the endometrium can contribute to endometrial proliferation, hyperplasia, polyp formation, and cancer. Prolonged administration of tamoxifen has been linked to an increased likelihood of developing EC or UP [36]. The KEGG enrichment analysis unveiled GnRH secretion as a potential mechanism. These findings align with observations suggesting a plausible association between tamoxifen use and an elevated risk of EC or UP. Interestingly, it has also been noted in clinical practice that BC patients who have not received tamoxifen treatment can still present with endometrial abnormalities. In order to further investigate the potential association between the use of SERMs and EC, the association between ER + BC cases treated with SERMs and ER- BC cases without SERM treatment was specifically examined. Consistent with previous findings, these results showed a significant association between ER + BC and EC, indicating it as a risk factor with an OR greater than 1. Conversely, no association was found between ER- BC and EC, which aligns with the previously reported conclusion that long-term use of tamoxifen is linked to a heightened susceptibility of EC. These findings provide further support for the notion that the association between SERMs, ER + BC, and EC warrants careful consideration.

In contrast, no significant associations were found between BC and other UDs, including endometriosis, UL, or CC. These findings suggested that the genetic factors influencing BC may not impact the developing of these specific UDs. However, the MR-Egger and Simple mode analyses suggested a potential association between BC and UL. It is worth noting that previous research has reported a higher prevalence of UL in BC patients [37], and the forward MR analysis identified UL as a risk factor for BC. However, in the reverse MR analysis, BC showed a negative association with UL, resulting in conflicting results. Moreover, in previous MR analysis, a genetic

correlation between EC and UL has been found [38]. These conflicting findings highlight the complexity of the relationship between BC and UL, which requires further investigation and validation.

The uterus and breasts play vital roles in the hormonal regulation of various physiological processes. Hormonal imbalances can lead to the development of UDs and hormone-related cancers, including BC [39,40]. Enhancing our understanding of the interplay between BC and UDs will lead to improved clinical practices for individuals affected by these conditions. Further research in the field of BC and estrogen-related diseases holds promise in unraveling the complex connections between these conditions, exploring shared risk factors, and understanding the underlying biological mechanisms.

## 5. Limitations and prospects

However, it is crucial to recognize the constraints inherent in MR analysis. Firstly, this analysis exclusively utilized GWAS data from individuals of European descent, which may restrict the applicability of the results to other ethnic groups. It is advisable that forthcoming studies aim for a more comprehensive validation across a broader spectrum of populations to ascertain the global relevance of the identified associations. Secondly, the present study conducted a 2SMR analysis supplemented by sensitivity analyses, it did not incorporate multivariable analyses to evaluate the impact of established comorbid conditions, such as physical activity or sleep patterns, on the observed relationship. This limits the comprehension of potential confounding factors. While the PhenoScanner GWAS database was used to identify and exclude possible confounders like alcohol consumption and smoking, the value of future research implementing multivariable analyses would be to thoroughly assess and corroborate the findings, ensuring a more meticulous scrutiny of this potential limitation. Moreover, due to the limited number of SNPs and their corresponding genes found in NCBI, there may be a potential bias in the GO/KEGG analysis. Therefore, further research, including large-scale epidemiological studies and functional studies at the cellular and molecular levels, is warranted to validate the findings and unravel the comprehensive interplay between UDs and BC, thus enhancing understanding for preventative strategies and refining prognostic capabilities. To sum up, while the current study offers important evidence suggesting a possible causal relationship between UDs and BC, it is imperative that future studies tackle the limitations discussed to bolster the robustness and applicability of the results.

## 6. Conclusion

In conclusion, this bidirectional MR study revealed intriguing associations between UDs and BC. A potential protective effect of endometriosis against BC development and a positive association between BC and EC were observed. Additionally, there was evidence of a shared genetic basis between BC and UL. However, the reverse analysis showed conflicting results for the association between BC and UL. BC may exert their influence on EC and UP through Cellular senescence, GnRH secretion, Phosphatidylinositol signaling system, and other pathways. These findings present a fresh viewpoint for exploring the genetic origins of the BC and UDs, and they pave the way for subsequent experimental research. Moreover, they also contributed valuable new insights into the causal links between UDs and BC, enhancing the understanding of the knowledge of their etiology and informing strategies for prevention, diagnosis, and management.

## Ethics statement

This study did not require ethics approval because the data used had already been granted ethical approval by the original studies.

## Data availability statement

The datasets that were created and/or analyzed for this study can be obtained from the corresponding author upon request for legitimate reasons.

## CRediT authorship contribution statement

**Zhipeng Liu:** Writing – original draft. **Min Jiang:** Data curation. **Taiyu Wang:** Resources. **Fang Li:** Data curation. **Yinxing Zhu:** Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38130.

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