CRP, IL-1α, IL-1β, and IL-6 Levels and the Risk of Breast Cancer: A Two-Sample Mendelian Randomization Study

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Abstract

Background

Epidemiological studies have reported a positive association between inflammation and cancer risk. However, the association between inflammation and breast cancer (BC) risk remains unclear. Here, we performed a Mendelian randomization study to investigate the etiological role of inflammation in BC risk.

Methods

We acquired data regarding C-reactive protein (CRP), interleukin (IL)-1α, IL-1β, and IL-6 expression and BC related to single nucleotide polymorphisms (SNPs) from two larger consortia (the genome-wide association studies (GWAS) and the Breast Cancer Association Consortium (BCAC)). Next, we conducted the two-sample Mendelian randomization (MR) study for investigating the relationship of the abovementioned inflammatory factors with the incidence of BC.

Results

Genetically predicted CRP levels did not increase BC incidence (odds ratio (OR) 1.06, 95% confidence interval (CI) 0.98–1.12, P = 0.2059). IL-6 was not related to BC incidence (OR 1.05, 95% CI 0.95–1.16, P = 0.3297). It showed no association between genetically predicted IL-1α levels and BC incidence (OR 1.01, 95% CI 0.99–1.03, P = 0.2167). IL-1β levels were not related to overall BC incidence (OR 1.07, 95% CI 0.98–1.17, P = 0.1140); however, in subgroup analysis, genetically predicted IL-1β levels increased ER + BC incidence (OR 1.15, 95% CI 1.03–1.27, P = 0.0088), but no correlation was found with ER-BC (OR 1.00, 95% CI 0.86–1.18, P = 0.9510).

Conclusions

Our study suggested that CRP, IL-1α, and IL-6 levels were not related to BC incidence; however, genetically predicted IL-1β levels were found to increase ER + BC susceptibility.

Introduction

The number of newly diagnosed breast cancer (BC) cases among women are currently exceeding those of lung cancer; thus, making BC the leading cause of global cancer incidence(Sung et al. 2021). Chronic inflammation has been considered to be associated with tumorigenesis of various cancers such as colorectal cancer (Nadeem et al. 2020), cervical cancer (Hong et al. 2017), and liver cancer (Li et al. 2021). However, the association of chronic inflammation with BC susceptibility remains unknown. C-reactive protein (CRP), a serum marker for chronic inflammation, is produced in the liver under the stimulation of interleukin (IL)-1 and IL-6 (Slaats et al. 2016; Crusz and Balkwill 2015). Studies on the

pathological characteristics of many bacterial diseases have revealed that CRP, IL-1, and IL-6 expression in plasma is correlated with each other (Oberhoffer et al. 1999). Therefore, CRP, IL-1, and IL-6 have been used as inflammatory markers to investigate the relationship of inflammation with BC susceptibility. The association of CRP with BC susceptibility remains controversial. Meta-analyses and systematic reviews suggest that increased CRP levels in serum are related to the occurrence of BC (Guo et al. 2015; Chan et al. 2015); in contrast, several clinical studies have shown no relationship between CRP and BC susceptibility (Allin, Bojesen, and Nordestgaard 2009; Zhang et al. 2007). Observational studies involve many uncontrollable factors that can lead to inaccurate results, such as residual confounding factors (e.g., obesity possibly plays an important role in regulating the association of CRP with BC susceptibility) or reverse causality (e.g., breast cancer may lead to chronic inflammation).

IL-1 and IL-6, as upstream stimulators of CRP, have critical roles in BC genesis. IL-1 family members, such as IL-1α and IL-1β, are important factors that mediate inflammation. IL-6 is highly expressed in human BC tissues and cells, and it activates glycoprotein (gp) 130-regulated pathways (MAPK and JAK/STAT pathways) to participate in cellular proliferation, survival, and differentiation. Many in vitro assays have revealed that IL-1 and IL-6 play a role in promoting the growth of BC cells (Chavey et al. 2007; Pantschenko et al. 2003; Masjedi et al. 2018; Dethlefsen, Højfeldt, and Hojman 2013). HER2-overexpressing BC cells show enhanced IL-1α production, which acts as a proinflammatory signal to activate additional signaling sequences (such as IL-6) and trigger the STAT3 and NF-κB pathways for generating and maintaining chronic inflammation and cancer stem cells (CSCs), thus promoting tumor occurrence and development (Liu et al. 2018). However, there is a lack of clinical studies supporting the association of IL-1/IL-6 with BC susceptibility. Consequently, for providing evidence on the effect of inflammation on BC etiology while minimizing the influence of confounding factors, our present study aimed to explore the causal relationship between CRP, IL-1, IL-6, and BC risk from a genetic perspective.

Recently, Mendelian randomization (MR) method has been proven an effective approach to perform causal association analysis. The concept of MR was first proposed by Katan in 1986; this concept relies on the use of genetic variants that have a strong relationship with exposure and are termed as instrumental variables (IVs) to predict how exposure affects the outcome. After the gametes are formed, they follow the MR inheritance law that parental alleles are randomly allocated to their offspring; thus, the association between genes and outcome remains unchanged even in the presence of confounders such as behavioral factors, puerperal environment, reverse causality, and socioeconomic status (Emdin, Khera, and Kathiresan 2017; Davies, Holmes, and Smith 2018). A previously published genome-wide association study (GWAS) provides an opportunity for a two-sample MR (TSMR) study that can improve statistical power and precision. TSMR uses two-sample summary data, which represent exposure-associated and outcome-related genetic variations.

Based on the role of chronic inflammation in the etiology of BC disease, we performed a TSMR analysis to assess the causal relationship between CRP, IL-1α, IL-1β, and IL-6 levels and BC risk.

Methods

Exposure data sources

CRP data sources

Summary data for CRP-associated single nucleotide polymorphisms (SNPs) were acquired from the largest GWAS study (including 204,402 European individuals), which was a meta-analysis of HapMap and 1000 Genomes imputed data that included 88 studies. The analysis excluded data from individuals with autoimmune diseases, those taking immunomodulatory agents, and those with CRP levels differing from the mean value by 4 SD or more. All the participating studies were approved by their respective institutional review boards (Ligthart et al. 2018).

IL-6 and IL-1β data sources

Summary data for IL-6-and IL-1β-related SNPs were obtained from the INTERVAL study, including 3,301 normal subjects, nested based on 50,000 blood donors' genetic biological resources at 25 centers from England. The Affymetrix Axiom UK Biobank Genotyping Array was used for genotyping. Each subject provided informed consent for participation. This work was approved by the National Research Ethics Service (Sun et al. 2018).

IL-1α data sources

Summary data for IL-1α-associated SNPs were derived from the genetic study of up to 700 maternal and infant cytokines/chemokines, of which 42% were European ancestry (Traglia et al. 2018). The study used the Affymetrix Axiom (Affymetrix 2011) EUR array for genotyping. All the studies were approved by the institutional review board.

Summary data on BC

According to the expression of estrogen receptor (ER), BC is divided into ER + BC and ER-BC, which exhibit different biological behaviors. Thus, they will be discussed in subgroups. Summary data for BC-associated SNPs, including 122,977 cases (69501 ER + cases and 21468 ER-cases) and 105974 controls, were acquired from the Breast Cancer Association Consortium (BCAC) (Michailidou et al. 2017). The study project was from European ancestry. Genotyping was performed using the 1000 Genomes Project (Phase 3) reference panel. SNPs with minor allele frequency (MAF) ≤ 0.5% and imputation quality score < 0.3 were excluded. All participating studies were approved by the appropriate ethical review boards. Moreover, all subjects provided informed consent to participate.

Statistical Analyses

The present study is a TSMR study. As shown in Fig. 1, the research of this study is based on three hypotheses: (1) IVs and exposure factors are strongly correlated (association); (2) IVs and confounders are not correlated (independence); and (3) IVs and the outcome are not directly related, and the effect on the outcome can only be demonstrated by exposure (exclusion restriction criterion). In a TSMR study,

exposure-related IVs and outcome-related IVs are obtained from two independent samples (e.g., a GWAS of exposure-related SNPs and a GWAS of outcome-related SNPs) from the same ethnic group. Compared to a single-sample Mendelian randomization study, TSMR involves a larger sample size and thus can obtain greater power. Currently, TSMR is widely used because of the availability of public data from a large number of GWAS collaborative groups worldwide.

To select the IVs that are strongly correlated with exposures (first MR assumption), this study implemented quality control for selecting potentially helpful SNPs. First, a genome-wide significance analysis was conducted to identify SNPs related to exposures (IL-1α, IL-1β, IL-6, and CRP) ($p < 5e-08$). Second, the exposure-related helpful SNPs should not be within linkage disequilibrium (LD), because SNPs strongly related to LD possibly induce bias in outcomes. The present work implemented a clumping procedure ($r^2 < 0.001$, threshold of distance = 10000 kb). Third, the low instrumental bias was assessed by F statistic, where F statistic of < 10 confirms that the selected genetic variants do not meet the strong correlation between IVs and exposure (Staiger and Stock 1994). F statistic was calculated using the formula: Beta2/SE2, where beta and se represent genetic association with the exposure and standard deviation, respectively. In addition, when choosing the IVs for exposures, the following conditions also need to be considered: First, we eliminated SNPs whose minor allele frequency (MAF) was lower than 0.01 in the process of extracting outcome information from IVs. Second, we eliminated recurrent SNPs from those chosen helpful SNPs during harmonization for ensuring that the effect of SNPs on exposure and the effect of identical SNPs on outcome were corresponding to the identical allele.

For CRP-associated SNPs, inverse-variance weighted (IVW), weighted median, and MR-Egger approaches were used for inferring causality, the most important of which was the IVW method. IVW is a method for meta-aggregating the effects of multiple loci in the process of MR analysis(Burgess, Butterworth, and Thompson 2013). The application of IVW is based on the concept that all SNPs are valid IVs and are completely independent of each other. On the basis of IVW, we additionally modified the MR-Egger approach. Compared to the IVW method, the core of this method is to consider the existence of the intercept term in the weighted linear regression. Simultaneously, the intercept term is used to measure the pleiotropy among the IVs, and the slope estimates the causal effect in an unbiased manner (Bowden, Davey Smith, and Burgess 2015). The weighted median method is the median values obtained by sorting all individual SNP effect values by weight. Weighted median can be a robust estimate when at least 50% of the genetic variation meets the MR core assumptions (Bowden et al. 2016). For IL-6-, IL-1α-, and IL-1β-associated SNPs, we used the Wald ratio for determining estimates for one SNP (Burgess, Small, and Thompson 2017).

Sensitivity analysis

To satisfy independence of the MR study (second MR assumption), after a comprehensive lookup of the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/), the significant associations of the selected SNPs with BC risk factors ($P < 1e-08$) were excluded, including BRCA1/2 gene mutation, childbearing, breastfeeding, mammographic density, height, obesity, alcohol intake, physical inactivity, age of menarche, and menopause (Britt, Cuzick, and Phillips 2020).

Experimental conditions, analytical platforms, and different study subjects may contribute to heterogeneity, leading to biased causal effect estimates. To meet the exclusion restriction criterion of the MR study (third MR assumption), based on MR-Egger and IVW analyses, our study used the Cochran *Q* statistic to conduct a heterogeneity test for detecting heterogeneity in causal estimate. $P > 0.05$ indicated the absence of heterogeneity within those enrolled IVs, and thus, we applied the fixed-effects MR estimates in such models; else, we used the random-effects MR estimates.

When MR-Egger, IVW, Wald ratio, and weighted median approaches were used to investigate causality, there may be other unknown confounders that were not conducive to biased estimates of genetic diversity and causal effects. We determined horizontal pleiotropy based on the intercept of MR-Egger and its p-values. If the MR-Egger regression intercept was close to 0 (< 0.1) and $P > 0.05$, we considered that there was no evidence of horizontal pleiotropy in the test. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) method was used for better validating potential outliers and horizontal pleiotropy (Verbanck et al. 2018). We set 5000 distributions as the parameter during MR-PRESSO analyses.

After the assessment of heterogeneity and horizontal pleiotropy, we performed a sensitivity analysis of the eligible SNPs by using the leave-one-out method. This method aims to re-estimate the causal effect by sequentially removing one SNP at each time in order to determine the SNP that greatly affects causal effect estimates. The results are considered to be reliable if the overall error bars do not change significantly after excluding each SNP.

Statistical power

According to the online calculation method (https://shiny.cnsgenomics.com/mRnd/) of the MR statistical power reported by Brion et al. (Brion, Shakhbazov, and Visscher 2013), α(type I error) was 0.05 and K (case composition ratio) was 54%. To calculate R^2 for the SNP instrument, we used the following formula: $2\times EAF\times(1-EAF)\times beta^2$(Papadimitriou et al. 2020), where EAF and beta represent effect allele frequency and predicted genetic impact on exposure, respectively. R^2xz (proportion of variance associated with CRP explained by SNPs) was 3.98%, while R^2xz (proportion of variance associated with IL-6 explained by SNPs) was 1.06%. Based on the meta-analysis of clinical observational trials, the RR value of CRP for BC in the European population was OR = 1.12 (Guo et al. 2015), and the risk ratio of IL-6 for BC was OR = 1.13 (Il'yasova et al. 2005). We used the number of BC summary data (n = 228,951, 122,977 cases, and 105974 controls) as the sample size. Our MR study had a high power (≥ 80%), and the results are shown in Supplementary Table S3. Statistical power for IL-1α and IL-1β was not calculated because of the lack of clinical studies on the association between IL-1α and IL-1β levels and BC risk.

The present study used the GWAS data to verify whether there was a causal relationship between CPR, IL-1α, IL-1β, and IL-6 levels and BC risk. All data were available online, and the data were analyzed with TwoSampleMR package version 0.5.6 and R version 4.1.0. $P < 0.05$ was considered to be statistically significant. In multiple testing, the link between exposure and outcome phenotype was considered to be

statistically significant when the Bonferroni-corrected P-value was < 0.05/N (N represents exposure factor number).

Results

MR estimates for CRP, IL-1α, IL-1β, and IL-6 levels with BC risk

MR estimates for CRP and BC susceptibility. There was almost no evidence that suggested the association of genetically predicted CRP level with all BC (Table 1) (OR 1.05, 95% CI 0.98–1.12, P = 0.2059), ER + BC risk (OR 1.07, 95% CI 0.98–1.17, P = 0.1340), and ER-BC (OR 1.04, 95% CI 0.94–1.15, P = 0.4396). Twelve SNPs were excluded by a comprehensive lookup of the PhenoScanner (Table 2). Two SNPs (rs4656849 and rs7121935) were excluded after removing LD. Three palindromic SNPs (rs2293476, rs10240168, and rs6485751) were excluded. F-statistics for CRP-associated SNPs ranged from 53 to 2408, indicating a strong correlation between IVs and exposure. CRP-associated SNPs could explain 3.98% of the total genetic variation. Supplementary Table S1 shows detailed information on CRP-associated SNPs. Supplementary Table S2 presents the summarized results of CRP and BC. Supplementary Figs.S1–S6 exhibit forest and scatter plots for the relationship of CRP with BC susceptibility.

Table 1
The MR estimate results of CRP, IL-1α, IL-1β, and IL-6 association with breast cancer risk

Exposure	nsnp	All breast cancer		ER + breast cancer		ER-breast cancer	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
CRP							
MR-Egger	36	0.99 (0.89,1.11)	0.8989	1.03 (0.90,1.18)	0.6868	1.00 (0.86.1.17)	0.9721
Weighted median	36	1.02 (0.96,1.09)	0.4946	1.07 (0.99,1.16)	0.0870	1.05 (0.93.1.19)	0.3956
Inverse variance weighted	36	1.05 (0.98,1.12)	0.2059	1.07 (0.98,1.17)	0.1340	1.04 (0.94.1.15)	0.4396
IL-1α							
Wald ratio	1	1.01 (0.99,1.03)	0.2167	1.01 (0.99,1.03)	0.2509	1.00 (0.97,1.03)	0.8569
IL-1β							
Wald ratio	1	1.07 (0.98,1.17)	0.1140	1.15 (1.03,1.27)	0.0088	1.00 (0.86,1.18)	0.9510
IL-6							
Wald ratio	1	1.05 (0.95,1.16)	0.3297	1.08 (0.95,1.22)	0.2312	0.97 (0.81,1.16)	0.7155

MR: Mendelian randomization, CRP: C-reactive protein, IL-1α: interleukin-1α, IL-1β: interleukin-1β, IL-6: interleukin-6, nsnp: number of single nucleotide polymorphism, OR: odds ratio, CI: confidence interval.

Table 2
Twelve SNPs excluded by a comprehensive lookup of the PhenoScanner

SNPs	Trait in PhenoScanner
rs1260326	Type II diabetes (Gaulton et al. 2015), Height (Wood et al. 2014),Alcohol consumption(Clarke et al. 2017),Age at menopause(Day et al. 2015)
rs12995480	Overweight (Berndt et al. 2013), Age at menarche(Perry et al. 2014)
rs1490384	Type II diabetes adjusted for BMI (Scott et al. 2017),Height(Lango Allen et al. 2010),Menarche age at onset(Pickrell et al. 2016),Age at menarche(Perry et al. 2014)
rs3134899	Height (Astle et al. 2016)
rs1558902	Menarche age at onset (Heard-Costa et al. 2009), Overweight (Berndt et al. 2013),Type II diabetes(Morris et al. 2012)
rs12960928	Type II diabetes(Scott et al. 2017), Height(Wood et al. 2014), Overweight(Berndt et al. 2013)
rs4420638	Type II diabetes (Teslovich et al. 2010)
rs12202641	Height(Wood et al. 2014)
rs10832027	Age at menarche(Perry et al. 2014)
rs7310409	Height(Lango Allen et al. 2010), Type II diabetes(Gaulton et al. 2015)
rs1800961	Type II diabetes(Gaulton et al. 2015)
rs6001193	Age at menopause(Day et al. 2015)

MR estimates for IL-1α with BC risk. Weak evidence was noted for the association of genetically predicted IL-1α level with all breast cancer (Table 1) (OR 1.01, 95% CI 0.99–1.03, P = 0.2167). In subgroup analysis, little evidence supported that IL-1α was related to ER + BC susceptibility (OR 1.01, 95% CI 0.99–1.03, P = 0.2509) and ER-BC susceptibility (OR 1.00, 95% CI 0.97–1.03, P = 0.8569). F-statistics for the IL-1α-associated SNP was 33.67, indicating that the SNP was unlikely to be affected by the weak instrument bias. IL-1α-associated SNPs could explain 2.38% of the total genetic variation. Genome-wide significant SNP loci for IL-1α along with F-statistics and R^2 values are shown in Supplementary Table S1. The summary data for IL-1α and BC are shown in Supplementary Table S2.

MR estimates for IL-1β with BC risk. There was evidence of a causal relationship between IL-1β level and ER + BC risk (OR 1.15, 95% CI 1.03–1.27, P = 0.0088). This finding was statistically significant when a Bonferroni-corrected threshold was used ($p < 0.0125$). Inverse results were found with all BC (OR 1.07, 95% CI 0.98–1.17, P = 0.1140) and ER-BC (OR 1.00, 95% CI 0.86–1.18, P = 0.9510). One palindromic SNP (rs13402561) was excluded. IL-1β associated with SNP whose F-statistics was 34.20, was strongly correlated with exposure. The IL-1β-associated SNP could explain 1.02% of the total genetic variation. Genome-wide significant SNP loci for IL-1β along with F-statistics and R^2 are shown in Supplementary Table S1. Summary data for IL-1β and BC are presented in Supplementary Table S2.

MR estimates for IL-6 with BC risk. MR estimates showed no significant relationship between genetically predicted IL-6 level and all BC (Table 1) (OR 1.05, 95% CI 0.95–1.16, P = 0.3297). Subgroup analysis demonstrated less evidence of a causal relationship between IL-6 and ER + BC risk (OR 1.08, 95% CI 0.95–1.22, P = 0.2312) or ER-BC risk (OR 0.97, 95% CI 0.81–1.16, P = 0.7155). F-statistics for the IL-6-associated SNP was 31, indicating that the SNP was unlikely to be affected by weak instrument bias. The IL-6-associated SNPs could explain 1.06% of the total genetic variation. Genome-wide significant SNP loci for IL-6 along with F-statistics and R^2 values are shown in Supplementary Table S1. Summary data for IL-6 and BC are presented in Supplementary Table S2.

Sensitivity Analyses

Heterogeneity test showed some significant differences for causal estimation between CRP level and all BC risk (IVW, Q (df) 86.04 (35), P = 3.47E-06; MR-Egger, Q (df) 82.29 (34), P = 6.97E-06), ER + BC (IVW, Q (df) 95.04 (35), P = 1.89E-07; MR-Egger, Q(df) 93.60 (34), P = 1.79E-07), and ER-BC (IVW, Q (df) 50.06 (35), P = 0.0475; MR-Egger, Q (df) 49.54 (34), P = 0.0414); thus, random-effects MR estimates were applied to these models. Heterogeneity test could not be performed for IL-1α, IL-1β, and IL-6 because of only one SNP.

Regarding horizontal pleiotropy in MR estimates for CRP and BC, the results demonstrated no evidence of horizontal pleiotropy effects based on the evaluation by the MR-Egger intercept and its p-values (All BC, intercept = 0.0036, P = 0.2220; ER + BC, intercept = 0.0026, P = 0.4745; ER-BC, intercept = 0.0024, P = 0.5527). The MR-PRESSO method did not find any potential pleiotropy in MR estimation of a causal relationship between CRP level and BC risk, but it found some outliers in all BC or ER + BC. For all BC, following the exclusion of SNPs (rs13233571) in the restrictive MR analysis, a significant causal relationship between CRP and all BC risk was not observed (OR 1.06, 95% CI 0.99–1.13, P = 0.1020, Table 3). The leave-one-out, scatter plot and forest plot were shown in Supplementary Figs.S7–S9. For ER + BC, following the exclusion of SNPs (rs1051338, rs2064009, and rs9271608) in the restrictive MR analysis, a similar result was observed for ER + BC (OR 1.06, 95% CI 0.99–1.15, P = 0.1119, Table 3). The leave-one-out, scatter plot and forest plot were shown in Supplementary Figs.S10–S12.Outlier SNPs were not found after the application of MR-PRESSO method in ER-BC. Because exposure has only one SNP as the IV, which included IL-1α, IL-1β, and IL-6, the MR-Egger intercept and MR-PRESSO methods could not be used for evaluating horizontal pleiotropy. However, we searched the PhenoScanner website and found no correlation between these IVs and other confounding factors.

Table 3
Results of the MR-PRESSO method applied after excluding CRP genetic variants associated with BC risk (P < 0.05)

	All BC *				ER + BC **			
	Beta	SE	P-value	OR (95% CI)	Beta	SE	P-value	OR (95% CI)
IVW (initial)	0.0457	0.0362	0.2059	1.05 (0.98,1.12)	0.0683	0.0456	0.1340	1.07 (0.98,1.17)
IVW (after outlier removal)	0.0558	0.0341	0.1020	1.06 (0.99,1.13)	0.0608	0.0382	0.1119	1.06 (0.99,1.15)
IVW: Inverse-variance weighted, SE: standard error, OR: odds ratio, CI: confidence interval.								
ER-BC absence of Outlier SNPs								
*Outlier SNPs: rs13233571.								
**Outlier SNPs: rs1051338, rs2064009, rs9271608.								

The results of the Leave-one-out sensitivity test showed that regardless of which CRP-associated SNP was removed, no significant change was observed in the results, indicating that the MR results were very robust (Fig. 2). Unfortunately, the Leave-one-out sensitivity test could not be used to verify the robustness of IL-1α, IL-1β, and IL-6 because of only one SNP.

Discussion

Our TSMR analysis of more than 122,977 patients with BC together with 105,974 normal controls suggested that chronic inflammation did not play an etiological role in BC susceptibility. In subgroup analyses, some evidence was noted wherein genetically predicted IL-1β expression elevated ER + BC susceptibility. Sensitivity analyses also showed that IVs affected outcomes only through exposure, rather than confounding and other pathways; thus, implying no pleiotropy.

As reported in recent studies, some lifestyle, social, and environmental factors may accelerate the occurrence of systemic chronic inflammation (SCI), which subsequently leads to the development of various diseases, including cancers (Furman et al. 2019). Serum CRP can be used as a chronic inflammatory biomarker; thus, reflecting the state of SCI. Clinical trials have demonstrated no association between serum CRP levels and BC susceptibility (Zhang et al. 2007; Allin, Bojesen, and Nordestgaard 2009); thus, supporting the findings of this work. Likewise, prior MR studies suggest that CRP is not related to BC susceptibility (Robinson, Martin, and Yarmolinsky 2020; Allin et al. 2010). However, meta-analyses have shown that the upregulated expression of CRP in serum leads to an increased BC risk (Guo et al. 2015; Chan et al. 2015; Wang et al. 2015), and this is possibly affected by reverse causality or

certain confounders such as physical activities, intake of nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, and smoking.

The “upstream” proinflammatory factors IL-1/IL-6 play an essential role in initiating inflammation which further triggers the production of inflammatory factors, including CRP. IL-6 is suggested to accelerate BC progression in most cellular assays; however, our study did not find a relationship between genetically predicted elevation of IL-6 and increased BC susceptibility; thus, showing consistency with previous studies (Heikkilä et al. 2009; Il'yasova et al. 2005). Similar results were obtained from a meta-analysis on the association of IL-6 gene polymorphisms with BC susceptibility (Yu et al. 2010). Previous studies have also confirmed that IL-6 gene polymorphisms are associated with BC susceptibility (Hefler et al. 2005), possibly because of different races (Slattery et al. 2014). According to our results, genetically predicted IL-1β levels increased ER + BC risk. To the best of our knowledge, the present MR work is the first to investigate the relationship of IL-1β content with BC susceptibility. Previous meta-analyses have shown that IL-1β expression is related to a higher BC risk, although the relationship of IL-1β with ER status has not been specifically analyzed (Liu et al. 2010). Both ER + BC cells and ER-BC cells show different IL-1β signaling pathways, which may be due to different IL-1 receptors on the cell surface (Liu and Gudas 2002). IL-1β directly affects the transcriptional activation of ER-alpha (Speirs et al. 1999). IL-1 dysregulation contributes to the occurrence, development, and migration of cancers, and therefore, IL-1 blockers have become increasingly popular in clinical trials of patients with cancers. Anakinra (Kineret), a recombinant, nonglycosylated IL-1Ra that negatively regulates IL-1α and IL-1β, was used to treat patients with metastatic BC (NCT01802970); however, the results have not yet been published. IL-1α is less stimulatory than IL-1β in stimulating liver CRP production; however, it appears to trigger inflammation by stimulating the NF-κB pathway (Dúcka et al. 2021). In our present study, no causal relationship was observed between IL-1α and BC susceptibility. This is different from the results of previous studies (Slattery et al. 2014; Han et al. 2010), which might be due to different study populations.

Our present study has some limitations. First, we selected European ancestry as our objects for reducing population stratification bias; consequently, the results cannot be applied to populations with different genetic backgrounds. Second, we were unable to stratify patients according to menopausal status or severity of CRP because of the lack of individual data. Third, we used only genetic tools to assess the causal relationship between inflammatory biomarkers and BC risk. Therefore, our results need to be treated with caution, as individuals can adapt to genetic changes through compensatory mechanisms, and the etiological role of other inflammatory factors in BC needs to be further investigated.

Conclusion

Our present study aimed to investigate the relationship of inflammation with BC susceptibility by using the two-sample Mendelian randomization method. The results indicate that genetically predicted IL-1β levels increase the susceptibility of ER + BC, whereas the levels of CRP, IL-1α, and IL-6 were not related to BC susceptibility. To the best of our knowledge, the present MR study is the first to investigate the

relationship of IL-1β with BC susceptibility, and the results suggest the different etiological effects of IL-1β on ER + BC as compared to that on ER-BC, which deserves further study.

Abbreviations

BC	breast cancer
BCAC	the Breast Cancer Association Consortium
CSCs	cancer stem cells
CI	confidence interval
CRP	C-reactive protein
ER	estrogen receptor
EAF	effect allele frequency
GWAS	the genome-wide association study
IL-1a	interleukin -1a
IL-1b	interleukin -1b
IL-6	interleukin -6
IVs	instrumental variables
IVW	inverse-variance weighted
LD	linkage disequilibrium
MR	Mendelian randomization
MR-PRESSO	Mendelian randomization pleiotropy residual sum and outlier
MAF	minor allele frequency
OR	odds ratio
SNP	single nucleotide polymorphism
TSMR	two-sample Mendelian randomization

Declarations

Ethics approval

Not applicable

Consent for publication

All authors have contributed to, read, and approved this submitted manuscript in its current form.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files. The GWAS summary statistics for CRP, IL-1b, IL-6, and breast cancer (including ER+ breast cancer and ER- breast cancer) is available in the the OpenGWAS database(https://gwas.mrcieu.ac.uk/). The GWAS summary statistics for IL-1a is available in the GWAS Catalog (https://www.ebi.ac.uk/gwas/).

Conflict of interest

None declared.

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Author contributions

YC, SC, and WL conceived the idea for the study. YC, ZZ, RW and DZ obtained the genetic data. SC, XW, LC, and XZ performed the data analyses. YC, WZ, MZ, YW and HM interpreted the results of the data analyses. All authors wrote the manuscript. All authors read and approved the final manuscript.

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Figures

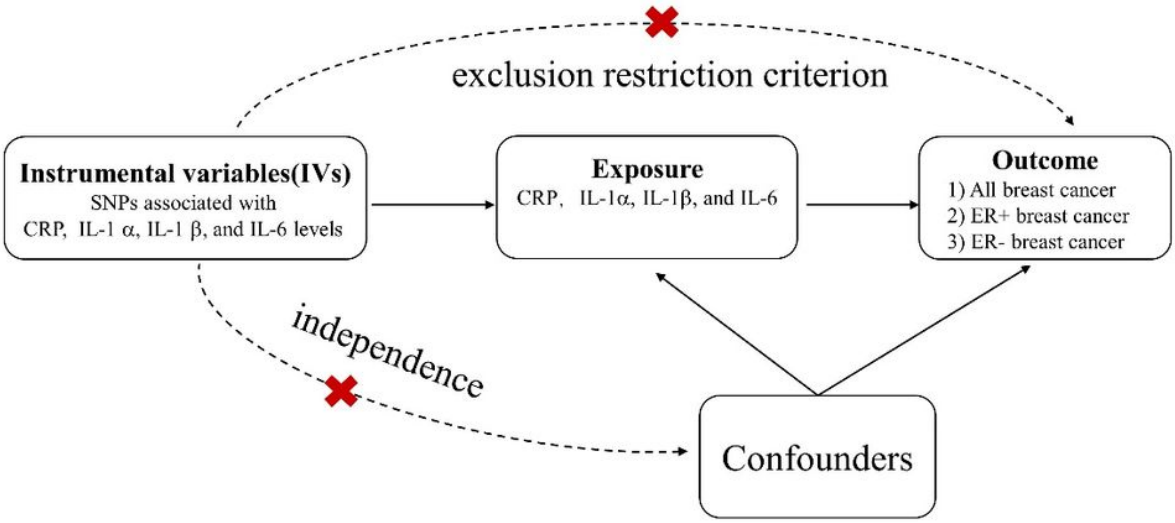

Figure 1

Basic assumptions in designing MR study. SNP, single nucleotide polymorphism; CRP, C-reactive protein.

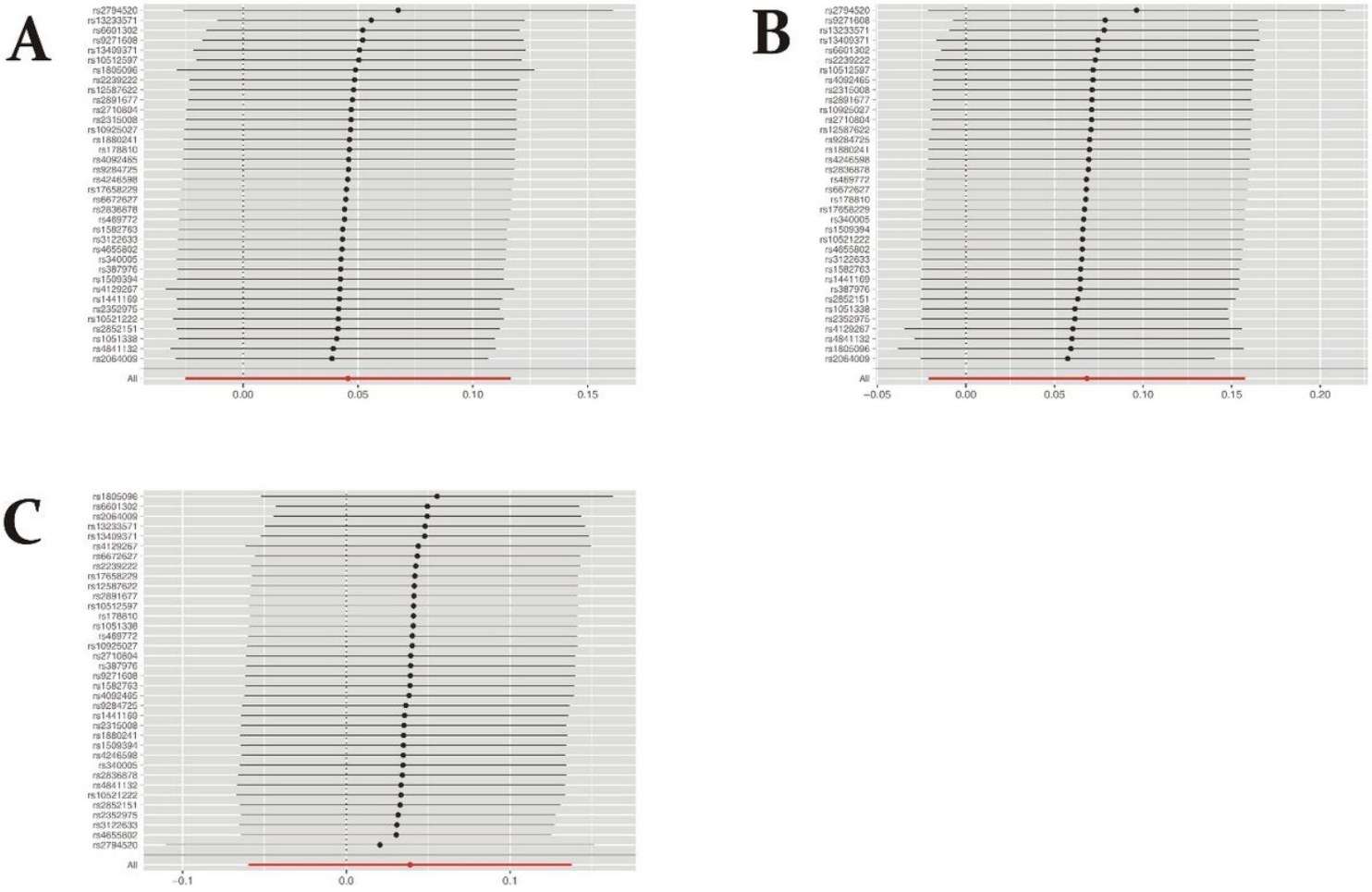

Figure 2

MR sensitivity analyses for the association between CRP and BC. (A) All BC. (B) ER+ BC. (C) ER-BC. Regardless of which SNP was removed, all points were to the right side of 0, indicating that the MR results were very robust.

Supplementary Files

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