



OPEN Genetic determinants of inflammatory cytokines and their causal relationship with inflammatory disorders of breast: a two-sample Mendelian randomization study

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This study utilized two-sample MR to investigate causality between genetically predicted inflammatory markers and the risk of IDB. This research leveraged publicly available GWAS summary statistics to collect data on inflammatory cytokines and IDB. The IVW method was primarily employed for causal inference, supplemented by weighted median, mode-based estimation, and MR-Egger regression. Stringent sensitivity methods included Cochran's Q test, MR-Egger regression, MR-PRESSO, and leave-one-out analyses to assess the robustness of the findings. This study selected 452 instrument variables (IVs) related to inflammatory factors. The IVW analysis revealed that GROa and RANTES/CCL5 exhibited causal relationships with IDB. Additionally, after removing outliers, significant causal associations were observed for IL-1ra and IL-9. Notably, the causal associations of RANTES/CCL5 and IL-9 with IDB remained significant after FDR correction. Upon integrating the findings from all sensitivity analyses, it is unlikely that heterogeneity and pleiotropy substantially influenced the observed relationships, underscoring the robustness of our findings. Our MR analysis identified the causal roles of specific inflammatory cytokines such as GROa, RANTES/CCL5, IL-1ra and IL-9 in the development of IDB. These findings deepen our understanding of the complex regulatory mechanisms involving inflammation in breast diseases and suggest directions for future research on biological pathways linking inflammation with IDB.

Keywords Inflammatory cytokines, Inflammatory disorders of breast, Mendelian randomization, Causal association, Genetic analyses

Inflammatory disorders of the breast (IDB) encompass a range of conditions characterized by inflammation, which can present with symptoms such as pain, heat, and redness. These disorders can be debilitating, leading to prolonged morbidity and varying in severity from benign to aggressive malignancies^{1,2}. The spectrum of IDB can be categorized into infectious mastitis, non-infectious mastitis, and mastitis associated with underlying malignancy^{2,3}. Additionally, they may manifest with nonspecific symptoms that can complicate early diagnosis and necessitate appropriate treatment^{4,5}. Recognizing the risk factors for inflammatory breast disorders is essential for timely diagnosis and intervention, which are critical for enhancing patient outcomes.

Inflammatory cytokines, which include chemokines, growth factors, interleukins, and other related molecules, are integral regulators of the immune response^{6,7}. They have been identified as key players in the pathogenesis of various diseases, including inflammatory breast disorders^{6–8}. A growing body of observational evidence suggests a significant association between specific inflammatory cytokines and the development of IDB. For example, Ibrahim et al. reported that cytokine array profiling of cancer-associated adipose tissue ex-vivo cultures from obese inflammatory breast cancer (IBC) patients revealed a significantly higher secretion of a panel of

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28 cytokines compared to non-IBC patients⁹. Li and co-workers employed cytokine microarray detection to discern a pronounced upregulation in the expression levels of cytokine factors, notably interleukin-1 β (IL-1 β), monokine-induced by γ -interferon (MIG), macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and tumor necrosis factor receptor 2 (TNF RII), in patients diagnosed with idiopathic granulomatous mastitis (IGM) relative to control subjects¹⁰. Furthermore, Iwase and colleagues elucidated that within the top 15 canonical pathways activated in IBC, the IL-7 signaling pathway, in conjunction with pathways such as ERK/MAPK and PDGF, is intricately linked to the estrogen receptor signaling pathway, thereby distinguishing it from non-IBC cases¹¹. Despite these suggestive associations, establishing a definitive causal link between inflammatory cytokines and inflammatory breast disorders is challenging due to the limitations inherent in observational studies, such as confounding factors and the possibility of reverse causality.

Mendelian randomization (MR) is an innovative epidemiological method that utilizes naturally occurring genetic variations as instrumental variables to infer causality. This approach takes advantage of the random distribution of genetic variants during meiosis, which reduces many of the biases found in traditional observational research^{12,13}. MR provides a robust framework for investigating the potential causal effects of inflammatory factors on inflammatory breast disorders, free from the influence of confounding or reverse causality. This study employs a two-sample MR design to explore the causal relationship between inflammatory cytokines and IDB, focusing on the impact of these cytokines on the development of the disorders. By integrating genome-wide association study (GWAS) data on 41 inflammatory cytokines and outcomes related to inflammatory breast disorders, our investigation aims to provide a more reliable basis for causal inference than purely observational studies.

Methods

Study design

Our MR study, as depicted in Fig. 1, adheres to the MR-STROBE guidelines¹⁴ and is designed to explore the potential causal associations between inflammatory cytokines and IDB. The two-sample MR approach is predicated on three key assumptions. Assumption 1: the instrumental variables (IVs) directly affect the exposure; Assumption 2: IVs are not associated with confounders; Assumption 3: IVs influence risk of the outcome directly through the exposure, not through other pathways¹⁵.

Data sources

Outcome data for IDB were procured from the FinnGen Consortium, encompassing 1,880 cases and 211,699 controls. Data for the 41 inflammatory cytokines were sourced from a prior investigation¹⁶, encompassing chemokines, growth factors, interleukins, and additional cytokines. Table S1 provides a comprehensive overview. All data originated from peer-reviewed studies or publicly accessible GWAS summary data, with ethical approval and informed consent already obtained. This study did not necessitate separate ethical clearance.

Instrumental variable selection

Genetic instrumental variables were initially sought for each cytokine and IDB trait with a stringent significance threshold of $P < 5 \times 10^{-8}$. However, due to insufficient single nucleotide polymorphisms (SNPs) meeting this threshold, the criterion was adjusted to a more lenient significance level of $P < 5 \times 10^{-6}$. These selected SNPs were independently associated with their respective exposures within populations of European ancestry¹⁷. SNPs with substantial linkage disequilibrium (LD; $r^2 < 0.001$ within a 10,000 kb window) were excluded, retaining only the SNP with the most pronounced P-value¹⁸. In cases where an IV was absent in the outcome summary data, proxy SNPs with high LD with the original IV ($R^2 > 0.8$) were identified. The predictive power of each SNP as an IV was quantified using the F-statistic, calculated as follows: $F = R^2 \times (N - 2) / (1 - R^2)$, where R^2 is the proportion of variance in the exposure explained by the SNP in the IV, ensuring adequate predictive strength ($F > 10$)¹⁹. Finally, we used an online web tool (<https://sb452.shinyapps.io/power/>) to calculate the statistical power of each cytokine. GWAS Catalog (<https://www.ebi.ac.uk/gwas/>) was utilized to further assess whether the IVs might be associated with confounding factors or risk factors for IDB²⁰.

Mendelian randomization analysis

The random-effects inverse variance weighted (IVW) method was utilized as the primary analytical technique to estimate the causal impact of inflammatory factors on IDB, with Odds Ratios (OR) and corresponding 95% Confidence Intervals (CI) being calculated²¹. To ensure the robustness of the findings, alternative MR methods were employed, including MR-Egger regression, weighted median, and weighted mode estimators. The MR-Egger method, which accounts for an intercept term, provides unbiased causal effect estimates even in the presence of potential pleiotropic bias²². The weighted median method presupposes that half of the IVs are valid, thus estimating the causal link between exposure and outcome²³.

Sensitivity analysis

Sensitivity analyses were conducted to assess potential violations of the MR assumptions due to horizontal pleiotropy. Cochran's Q statistic was calculated to measure heterogeneity in effect sizes, with a P-value > 0.05 indicating low heterogeneity and suggesting that the variation among IV estimates is random and minimally impactful on the IVW results²⁴. The MR-Egger regression was used to evaluate the influence of horizontal pleiotropy on the estimated association, with a nonsignificant intercept term indicating the absence of pleiotropy that could bias the results²⁵. Additionally, the MR pleiotropy residual sum and outlier (MR-PRESSO) method was employed to identify and exclude outlier SNPs ($P < 0.05$)²⁶. Steiger tests were incorporated to examine causal

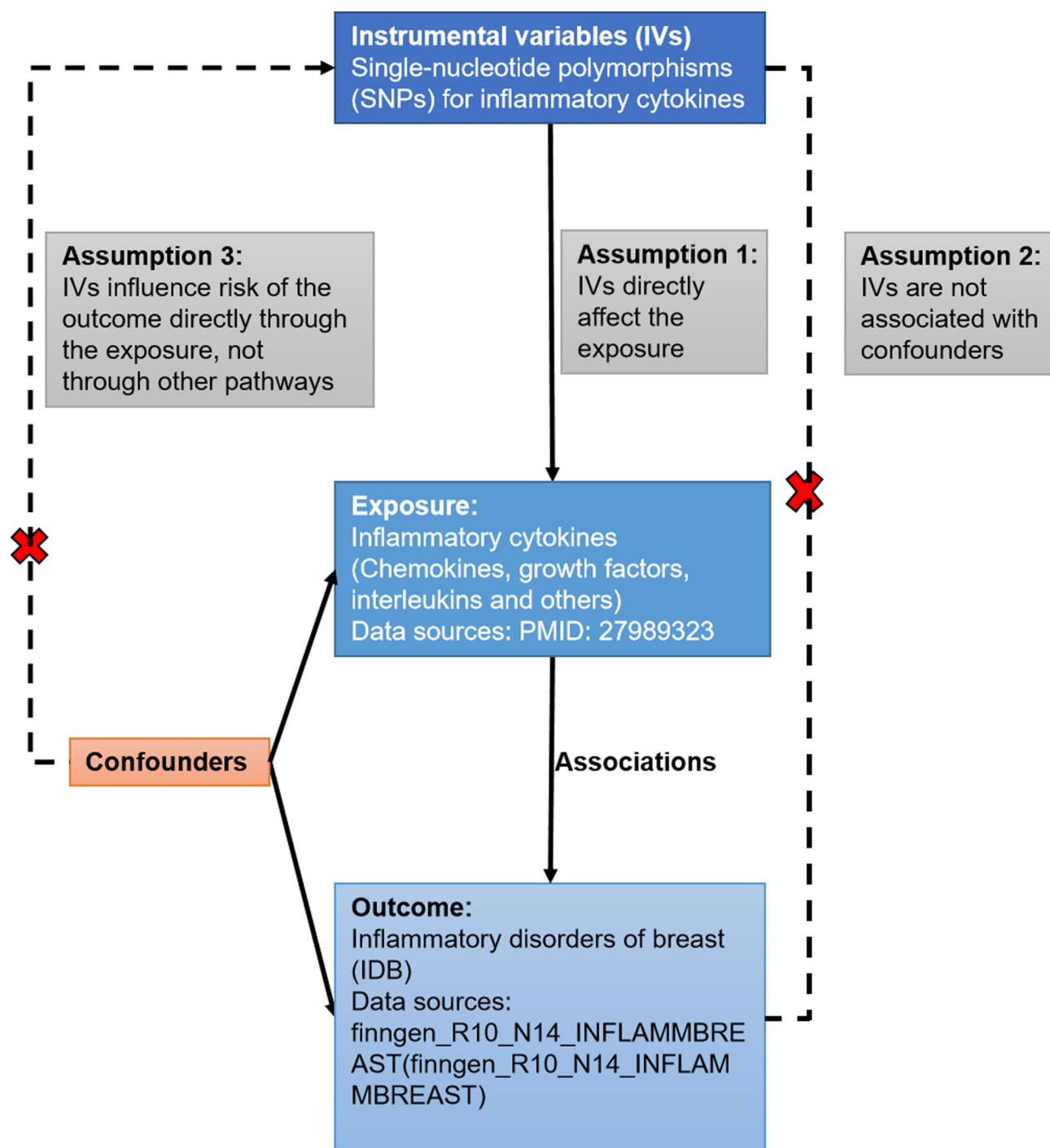


Fig. 1. Workflow of MR study revealing causal relationship between inflammatory cytokines and inflammatory disorders of the breast. MR, Mendelian randomization.

directions. Leave-one-out analyses were performed to ensure the robustness and consistency of the findings, demonstrating that the conclusions remain stable when individual genetic variants are sequentially omitted²⁷.

In this study, we applied both unadjusted and adjusted thresholds for statistical significance. An unadjusted P-value threshold of $P < 0.05$ was used for initial significance testing. To account for multiple comparisons, P-values were further adjusted using the Benjamini-Hochberg method, maintaining a significance threshold of $P < 0.05$ for FDR control. To bolster the interpretability of our findings, we employed visualization techniques such as scatter plots and diagrams illustrating the results of sensitivity analyses. The computational framework for all analyses was established using the “TwoSampleMR” package within the R statistical environment, specifically version 4.0.5, ensuring a robust and standardized approach to our data evaluation.

Results

Instrumental variable selection

In our MR analysis, after rigorous quality control measures, 452 SNPs were identified as IVs for inflammatory cytokines as exposures (Table S2). For IDB as an outcome, 17 SNPs (rs145902143, rs80341932, rs118158560, rs9450351, rs10892381, rs13143163, rs116615337, rs6900267, rs9793308, rs74966328, rs111913416, rs11700536, rs56134659, rs10903540, rs115360066, rs73479333, rs112783231) not matched in the summary and were excluded. The mean F-statistic for the IVs was 39.14, with a minimum of 11.16 and a maximum of 788.95, indicating no weak instrument bias in our analysis and the post hoc statistical power analysis. The power analysis showed moderate power ($\geq 50\%$) for RANTES/CCL5, GROa, and SDF1a in detecting significant associations (Table S3). IVs related to confounding factors were excluded from the analysis (Table S4). Notably, after excluding IVs associated with confounding factors, there were insufficient SNPs for further analysis between P10, MCP3, IL-12p70, IL-8, and IDB.

Causal effects of inflammatory cytokines on IDB

The IVW analysis indicated a negative causal relationship between GROa and the risk of IDB (OR 0.86, 95% CI 0.74–0.99, $P=0.04$, FDR=0.099), although MR Egger, weighted mode, and weighted median methods did not establish a causal association (all $P>0.05$) (Table 1; Table S5; Figure 2 A; Figure 3 A). A similar negative causal relationship was observed for RANTES/CCL5 (OR 0.83, 95% CI 0.71–0.98, $P=0.026$, FDR=0.048), supported by the weighted median (OR 0.78, 95% CI 0.623–0.987, $P=0.0329$, FDR=0.048) (Table 1; Table S5; Figs. 2B and 3B). Cochran's Q test did not detect significant heterogeneity, and MR-Egger analysis showed no evidence of directional pleiotropy affecting risk estimates for the associations between GROa, RANTES/CCL5, and IDB (Table 2). The MR-presso test did not identify any outlier SNPs or horizontal pleiotropy (Table 3).

Additionally, primary analysis did not reveal significant causal associations between IL-1ra (OR 0.92, 95% CI 0.65–1.30, $P=0.633$, FDR=0.633), IL-9 (OR 0.87, 95% CI 0.59–1.26, $P=0.453$, FDR=0.566) and IDB, with their associations showing significant heterogeneity (Tables 1 and 2). After the removal of outliers (rs11869294 for IL-1ra, rs61867538 for IL-9) identified through MR-PRESSO (Table 3), the causal associations between IL-1ra (OR 0.78, 95% CI 0.62–0.99, $P=0.038$, FDR=0.095) (Table 1; Figs. 2C and 3C), IL-9 (OR 0.68, 95% CI 0.52–0.89, $P=0.005$, FDR=0.013) and IDB turned significant (Table 1; Figs. 2D and 3D). In addition, the associations between IL-1ra and IL-9 with IDB exhibited no evidence of significant heterogeneity after outliers were removed (Table 2). However, the association between IL-13 and IDB remained insignificant even after the removal of outliers (rs12623722 and rs27949) according to the leave-one-out analysis, even though no pleiotropy existed after their removal (Tables 1 and 2). In addition, as IL-10 exhibited significant heterogeneity (Table 2), its association with IDB were examined via random effect IVW method. The results indicated that IL-10 was not causally associated with IDB via fixed-effect IVW (OR 1.17, 95% CI 0.79–1.75, $P=0.432$, FDR=0.719) or random-effect IVW method (OR 1.17, 95% CI 0.79–1.75, $P=0.432$, FDR=0.719) (Table 1).

No other inflammatory factors investigated showed a statistically significant relationship with IDB (Table 1). Sensitivity analyses revealed no evidence of heterogeneity, pleiotropy, or outlier SNPs. (Tables 2 and 3).

Through MR Steiger tests, we confirmed the consistency in causal directions of all inflammatory factors on IDB (Table 4). The symmetrical distribution of funnel plots in Fig. 4A–D, suggesting that the estimates of the relationships between GROa, RANTES/CCL5, IL-1ra and IL-9 and IDB were not influenced by any single outlier SNP. The consistency of their associations was further confirmed by leave-one-out sensitivity analyses, as depicted in Fig. 5A–D, with no significant alteration in the observed relationships upon the exclusion of any single SNP, underscoring the reliability of our study's conclusions.

Discussion

The findings from this MR study shed new light on the causal relationship between inflammatory cytokines and the development of IDB. By bypassing the biases often encountered in traditional observational studies, our research presents compelling evidence for potential causal relationships between specific cytokines and the risk of IDB. Notably, the negative causal associations observed for GROa, RANTES/CCL5, IL-1ra and IL-9 suggest that these cytokines may exert a protective influence against the onset of IDB, a finding that merits further exploration.

GROa, also known as CXCL1, is a member of the CXC chemokine family. It mainly acts as a chemoattractant, especially for neutrophils, and is involved in inflammation, angiogenesis, and tumorigenesis. While direct studies linking GROa specifically to IDB are limited, its involvement in inflammatory breast cancer implies potential relevance. For instance, research shows that GROa exerts pro-survival and anti-apoptotic effects on breast cancer cells, which are crucial for chemoresistance and radioresistance²⁸. It also induces the migration and epithelial-to-mesenchymal transition (EMT) of breast cancer cells by activating the extracellular signal-regulated kinase (ERK) MAPK pathway, leading to increased expression of matrix metalloproteinase 2 (MMP2) and MMP9²⁹. Additionally, GROa plays a role in angiogenesis by acting directly on endothelial cells and indirectly by increasing VEGF expression in breast cancer cells³⁰. In contrast, our study revealed that GROa served as a protective factor for IDB, though the OR was relatively low, suggesting its limited role in IDB. The discrepancy may stem from the fact that IDB may differ significantly from inflammatory breast cancer. The protective effect observed in our study might indicate that GROa contributes to a different immune response or cellular environment in IDB, potentially mitigating inflammation or promoting tissue repair rather than exacerbating tumor growth. This discrepancy highlights the complexity of chemokine functions and suggests that the dual roles of GROa in both promoting and protecting against disease processes warrant further investigation to fully understand its implications in various pathological contexts.

Exposure	Outcome	N.SNP	Method	OR (95% CI)	P	FDR adjusted P
CTACK/CCL27	Inflammatory disorders of breast	9	IVW	1.01 (0.83–1.24)	0.904	0.94
CTACK/CCL27	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	1.01 (0.83–1.24)	0.904	0.94
Eotaxin	Inflammatory disorders of breast	13	IVW	0.98 (0.78–1.24)	0.892	0.892
Eotaxin	Inflammatory disorders of breast	13	IVW (multiplicative random effects)	0.98 (0.78–1.24)	0.892	0.892
GROa	Inflammatory disorders of breast	7	IVW	0.86 (0.74–0.99)	0.04	0.099
GROa	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	0.86 (0.75–0.98)	0.026	0.099
MCP1	Inflammatory disorders of breast	5	IVW	0.88 (0.63–1.23)	0.458	0.938
MCP1	Inflammatory disorders of breast	5	IVW (multiplicative random effects)	0.88 (0.63–1.23)	0.458	0.938
MCP3	Inflammatory disorders of breast	2	IVW	0.91 (0.72–1.14)	0.408	0.408
MCP3	Inflammatory disorders of breast	2	IVW (multiplicative random effects)	0.91 (0.83–0.99)	0.033	0.067
MIG	Inflammatory disorders of breast	13	IVW	0.86 (0.74–1.00)	0.054	0.135
MIG	Inflammatory disorders of breast	13	IVW (multiplicative random effects)	0.86 (0.75–0.99)	0.039	0.135
MIP1a	Inflammatory disorders of breast	4	IVW	1.00 (0.76–1.30)	0.99	0.99
MIP1a	Inflammatory disorders of breast	4	IVW (multiplicative random effects)	1.00 (0.82–1.22)	0.986	0.99
MIP1b	Inflammatory disorders of breast	18	IVW	0.89 (0.76–1.04)	0.135	0.338
MIP1b	Inflammatory disorders of breast	18	IVW (multiplicative random effects)	0.89 (0.78–1.01)	0.072	0.338
RANTES/CCL5	Inflammatory disorders of breast	11	IVW	0.83 (0.71–0.98)	0.026	0.048
RANTES/CCL5	Inflammatory disorders of breast	11	IVW (multiplicative random effects)	0.83 (0.72–0.96)	0.011	0.048
SDF1a	Inflammatory disorders of breast	8	IVW	0.75 (0.55–1.01)	0.059	0.098
SDF1a	Inflammatory disorders of breast	8	IVW (multiplicative random effects)	0.75 (0.60–0.94)	0.012	0.058
Bngf	Inflammatory disorders of breast	6	IVW	0.92 (0.72–1.16)	0.478	0.478
Bngf	Inflammatory disorders of breast	6	IVW (multiplicative random effects)	0.92 (0.72–1.16)	0.478	0.478
FGFBasic	Inflammatory disorders of breast	7	IVW	0.99 (0.72–1.36)	0.951	0.951
FGFBasic	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	0.99 (0.74–1.33)	0.948	0.951
GCSF	Inflammatory disorders of breast	9	IVW	0.84 (0.66–1.07)	0.16	0.2
GCSF	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	0.84 (0.67–1.05)	0.129	0.2
HGF	Inflammatory disorders of breast	7	IVW	0.93 (0.68–1.28)	0.672	0.672
HGF	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	0.93 (0.72–1.22)	0.614	0.672
MCSF	Inflammatory disorders of breast	9	IVW	0.94 (0.82–1.07)	0.325	0.666
MCSF	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	0.94 (0.82–1.06)	0.307	0.666
PDGFbb	Inflammatory disorders of breast	12	IVW	0.91 (0.71–1.16)	0.435	0.706
PDGFbb	Inflammatory disorders of breast	12	IVW (multiplicative random effects)	0.91 (0.71–1.16)	0.435	0.706
SCF	Inflammatory disorders of breast	7	IVW	0.81 (0.57–1.14)	0.226	0.377
SCF	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	0.81 (0.57–1.14)	0.226	0.377
SCGFb	Inflammatory disorders of breast	14	IVW	1.00 (0.86–1.15)	0.966	0.966
SCGFb	Inflammatory disorders of breast	14	IVW (multiplicative random effects)	1.00 (0.86–1.15)	0.966	0.966
VEGF	Inflammatory disorders of breast	8	IVW	1.20 (0.96–1.50)	0.106	0.264
VEGF	Inflammatory disorders of breast	8	IVW (multiplicative random effects)	1.20 (0.97–1.49)	0.095	0.264
IL-10	Inflammatory disorders of breast	8	IVW	1.17 (0.79–1.75)	0.432	0.719
IL-10	Inflammatory disorders of breast	8	IVW (multiplicative random effects)	1.17 (0.79–1.75)	0.432	0.719
IL-12p70	Inflammatory disorders of breast	3	IVW	1.04 (0.69–1.57)	0.858	0.962
IL-12p70	Inflammatory disorders of breast	3	IVW (multiplicative random effects)	1.04 (0.74–1.45)	0.824	0.962
IL-13	Inflammatory disorders of breast	10	IVW	1.03 (0.82–1.29)	0.831	0.831
IL-13(after outliers removal)	Inflammatory disorders of breast	8	IVW	0.90 (0.75–1.08)	0.247	0.617
IL-13	Inflammatory disorders of breast	10	IVW (multiplicative random effects)	1.03 (0.82–1.29)	0.831	0.831
IL-16	Inflammatory disorders of breast	9	IVW	1.03 (0.86–1.22)	0.762	0.974
IL-16	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	1.03 (0.91–1.16)	0.676	0.974
IL-17	Inflammatory disorders of breast	11	IVW	0.97 (0.73–1.30)	0.863	0.863
IL-17	Inflammatory disorders of breast	11	IVW (multiplicative random effects)	0.97 (0.73–1.30)	0.863	0.863
IL-18	Inflammatory disorders of breast	13	IVW	1.07 (0.94–1.22)	0.288	0.719
IL-18	Inflammatory disorders of breast	13	IVW (multiplicative random effects)	1.07 (0.95–1.21)	0.269	0.719
IL-1b	Inflammatory disorders of breast	5	IVW	0.93 (0.71–1.21)	0.575	0.784
IL-1b	Inflammatory disorders of breast	5	IVW (multiplicative random effects)	0.93 (0.75–1.14)	0.474	0.784
IL-1ra	Inflammatory disorders of breast	7	IVW	0.92 (0.65–1.30)	0.633	0.633
IL-1ra (after outliers removal)	Inflammatory disorders of breast	6	IVW	0.78 (0.62–0.99)	0.038	0.095
IL-1ra	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	0.92 (0.65–1.30)	0.633	0.633
Continued						

Exposure	Outcome	N.SNP	Method	OR (95% CI)	P	FDR adjusted P
IL-2	Inflammatory disorders of breast	8	IVW	0.94 (0.75–1.18)	0.603	0.854
IL-2	Inflammatory disorders of breast	8	IVW (multiplicative random effects)	0.94 (0.75–1.18)	0.603	0.854
IL-2ra	Inflammatory disorders of breast	6	IVW	1.22 (0.98–1.52)	0.075	0.125
IL-2ra	Inflammatory disorders of breast	6	IVW (multiplicative random effects)	1.22 (1.04–1.43)	0.014	0.07
IL-4	Inflammatory disorders of breast	9	IVW	1.12 (0.85–1.46)	0.424	0.557
IL-4	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	1.12 (0.88–1.41)	0.363	0.557
IL-5	Inflammatory disorders of breast	4	IVW	1.00 (0.69–1.45)	0.999	0.999
IL-5	Inflammatory disorders of breast	4	IVW (multiplicative random effects)	1.00 (0.69–1.45)	0.999	0.999
IL-6	Inflammatory disorders of breast	4	IVW	0.81 (0.45–1.46)	0.487	0.812
IL-6	Inflammatory disorders of breast	4	IVW (multiplicative random effects)	0.81 (0.45–1.46)	0.487	0.812
IL-7	Inflammatory disorders of breast	8	IVW	0.87 (0.74–1.01)	0.075	0.188
IL-7	Inflammatory disorders of breast	8	IVW (multiplicative random effects)	0.87 (0.74–1.01)	0.075	0.188
IL-8	Inflammatory disorders of breast	3	IVW	0.97 (0.61–1.54)	0.9	0.9
IL-8	Inflammatory disorders of breast	3	IVW (multiplicative random effects)	0.97 (0.61–1.54)	0.9	0.9
IL-9	Inflammatory disorders of breast	6	IVW	0.87 (0.59–1.26)	0.453	0.566
IL-9 (after outliers removal)	Inflammatory disorders of breast	5	IVW	0.68 (0.52–0.89)	0.005	0.013
IL-9	Inflammatory disorders of breast	6	IVW (multiplicative random effects)	0.87 (0.59–1.26)	0.453	0.566
IFNg	Inflammatory disorders of breast	7	IVW	1.23 (0.90–1.68)	0.2	0.5
IFNg	Inflammatory disorders of breast	7	IVW (multiplicative random effects)	1.23 (0.95–1.58)	0.113	0.5
MIF	Inflammatory disorders of breast	9	IVW	0.94 (0.78–1.13)	0.503	0.503
MIF	Inflammatory disorders of breast	9	IVW (multiplicative random effects)	0.94 (0.80–1.11)	0.456	0.503
TNFa	Inflammatory disorders of breast	5	IVW	1.02 (0.81–1.29)	0.866	0.866
TNFa	Inflammatory disorders of breast	5	IVW (multiplicative random effects)	1.02 (0.89–1.17)	0.771	0.866
TNFb	Inflammatory disorders of breast	5	IVW	0.97 (0.80–1.17)	0.741	0.9
TNFb	Inflammatory disorders of breast	5	IVW (multiplicative random effects)	0.97 (0.80–1.17)	0.741	0.9
TRAIL	Inflammatory disorders of breast	17	IVW	0.93 (0.84–1.03)	0.162	0.295
TRAIL	Inflammatory disorders of breast	17	IVW (multiplicative random effects)	0.93 (0.86–1.01)	0.079	0.295

Table 1. Results of MR analysis of inflammatory factors and inflammatory disorders of the breast by inverse variance weighted method.

RANTES/CCL5 serves a dual function as a T cell chemoattractant and an immune-regulatory molecule. It exerts its effects by signaling through specific G Protein-Coupled Receptors (GPCRs), namely CCR1, CCR3, and CCR5. Research by Maillard and colleagues has indicated that the biological impacts of RANTES/CCL5 are contingent upon the syndecan-4/PKCα signaling pathway³¹. Here, our findings indicated that RANTES/CCL5 was negatively associated with IDB risks, albeit with a relatively low OR, suggesting its limited role in this condition. It is conceivable that in IDB, RANTES/CCL5 may exert a protective influence by modulating inflammatory-associated cellular signaling pathways, which could involve the inhibition of pro-inflammatory cytokines such as TNF-α and IL-6s³². In addition, RANTES/CCL5 is involved in the recruitment of regulatory T cells (Tregs) to sites of inflammation. Tregs play a vital role in suppressing excessive immune responses and maintaining immune tolerance³³. By attracting these cells, RANTES can help mitigate the inflammatory processes characteristic of IBD. However, further research is required to elucidate the detailed mechanisms by which RANTES/CCL5 inhibits IDB.

IL-1ra is a crucial anti-inflammatory cytokine that plays a significant role in regulating inflammatory responses by competitively binding to the IL-1 receptor (IL-1R). Our findings reveal that IL-1ra was negatively associated with the risk of IDB after outliers removal. Elevated levels of IL-1 are often observed in inflammation, contributing to inflammatory disorders. However, there has been limited report on the direct connection of IL-1ra and IDB. It can be postulated that dysregulation of the IL-1/IL-1ra axis can lead to exacerbated inflammatory responses in various diseases, including those affecting breast tissue. For instance, insufficient IL-1ra may fail to counteract the effects of elevated IL-1 levels, potentially leading to persistent inflammation and contributing to the pathogenesis of IDB^{34,35}. On the other hand, IL-9 exerts a protective effect against IDB after outliers were removed. IL-9 is a pleiotropic cytokine primarily produced by CD4+ T helper cells and stimulates the growth of various immune cells. Although specific studies directly connecting IL-9 to IBD are limited, its roles in inflammation and immune modulation suggest potential implications. Given that IL-9 is involved in promoting inflammation through its effects on T cells and mast cells, it may influence the inflammatory microenvironment characteristic of IBD³⁶. Despite these insights, further longitudinal studies are essential to elucidate the mechanisms involving GROα, RANTES/CCL5, IL-1ra and IL-9 in IDB and to assess their potential as therapeutic targets. Our findings contribute to the understanding of the complex interplay of inflammatory cytokines with breast diseases, emphasizing the need for ongoing research to clarify their individual roles and mechanisms of action.

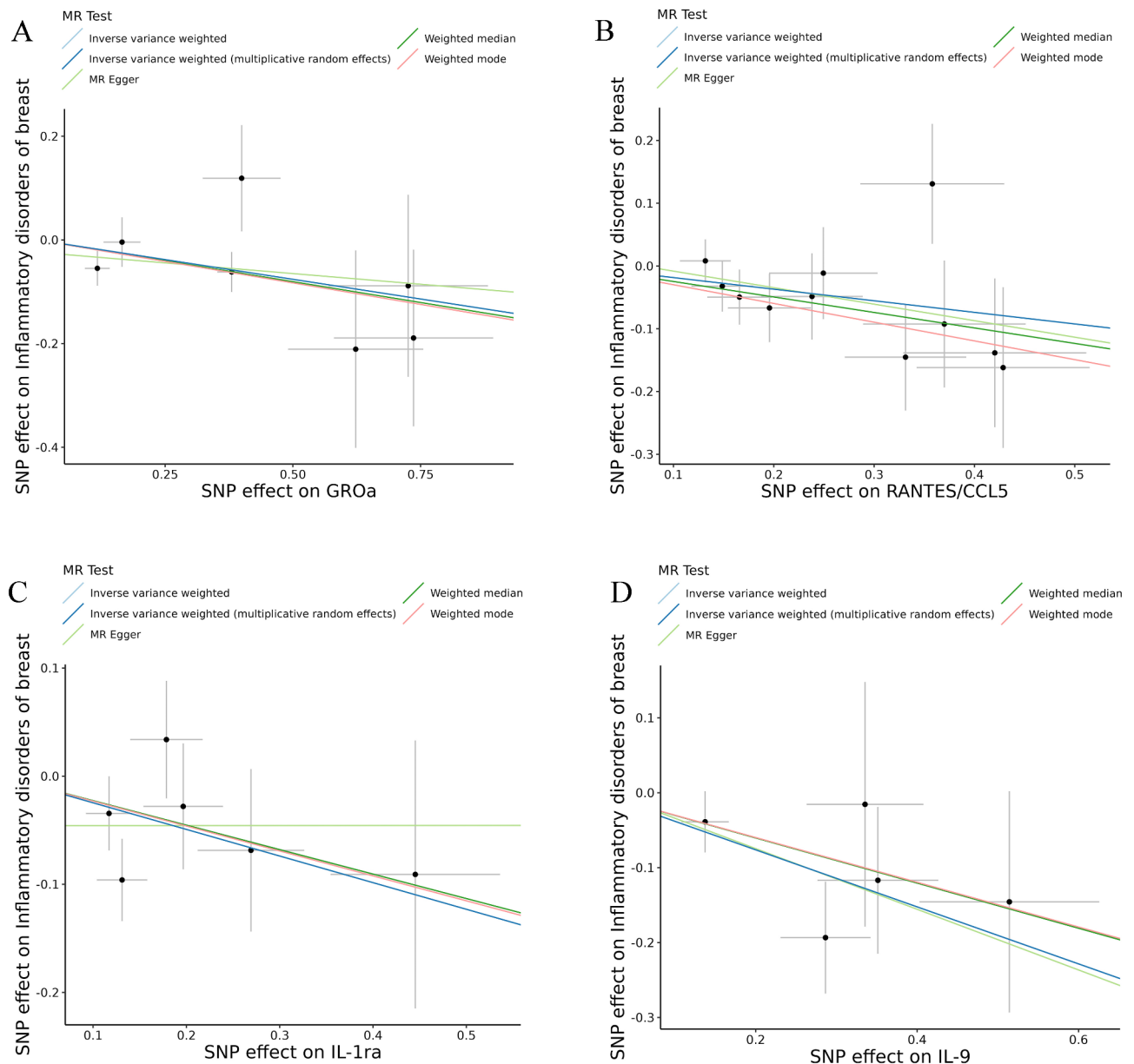


Fig. 2. Scatter plots of Mendelian randomization models: exploring the potential associations between IDB and GROa (A), RANTES/CCL5 (B), IL-1ra (C), and IL-9 (D). SNP, single-nucleotide polymorphism; IDB, inflammatory disorders of the breast; MIG, Monokine induced by gamma interferon; PDGFbb, Platelet-derived growth factor BB.

Our research also failed to identify a causal relationship between IDB and other inflammatory mediators, such as IL-1 β , MIG, IL-4, IL-10, MIP1, etc. However, the plausibility of a relationship between these factors and IDB remains a subject of interest. For instance, Li et al. utilized cytokine microarray detection to measure and analyze differentially expressed cytokine factors between patients with IGM and control subjects. Their findings revealed a significant increase in the expression of cytokines in IGM patients compared to controls, including IL-1 β , MIG, MIP1 α , MIP1 β , and TNF RII¹⁰. Additionally, Du et al. employed univariate and multivariate analysis to demonstrate that IL-4, IL-10, and INF- α were independent diagnostic factors for abscess formation in granulomatous lobular mastitis (GLM). They further developed a predictive model for GLM abscess formation based on inflammatory markers, offering a novel strategy for the early diagnosis and treatment of GLM during the purulent phase³⁷. Similarly, Mohamed et al. found that cytokine profiling of CD14+ cells isolated from IBC patients showed a marked increase in the secretion of TNF- α , MCP1/CC-chemokine ligand 2, IL-8, and IL-10, compared to those from non-IBC patients⁶. Rubbo and his team investigated immune markers in subclinical mastitis (SCM) breast milk samples, finding higher levels of inflammatory markers (TNF- α , IL-6, IL-8, IL-17, RANTES, etc.) and Th1-related cytokines (IL-2R, IL-12p40/70, IFN- α , IFN- γ , CXCL-9, and IP-10) associated with SCM, which was observed in 23% of women³⁸.

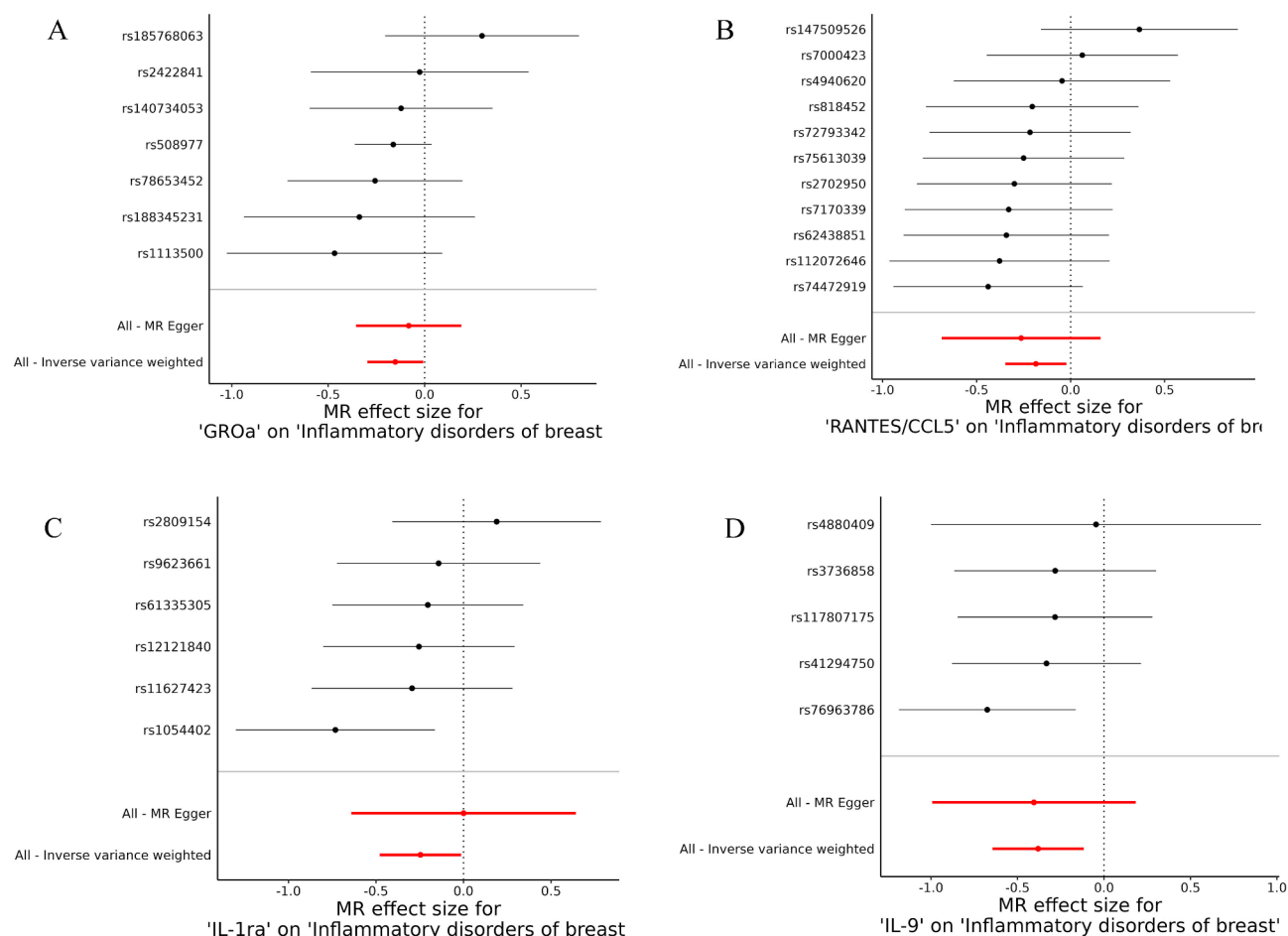


Fig. 3. Forest plot of MR effect size for potential relationship between IDB and GROa (A), RANTES/CCL5 (B), IL-1ra (C), and IL-9 (D). SNP, single-nucleotide polymorphism; IDB, inflammatory disorders of the breast; MR, Mendelian randomization; MIG, Monokine induced by gamma interferon; PDGFbb, Platelet-derived growth factor BB.

Collectively, these studies underscore the necessity for continued research into the interplay between inflammatory mediators and IDB. While our study suggests that inflammatory cytokines such as GROa, RANTES/CCL5, IL-1ra and IL-9 are associated with the development of IDB, it is essential to consider the clinical translation of these findings. The potential for these cytokines to serve as biomarkers for disease progression and as therapeutic targets in clinical settings should be considered in the following research. Such efforts could explore the use of specific anti-cytokine therapies, which have already shown promise in other inflammatory conditions.

Our study benefits from the comprehensive evaluation of a wide array of inflammatory cytokines, providing a nuanced perspective on cytokine effects on breast diseases. However, the limitations of this study have to be addressed. Firstly, caution is advised when extending the conclusion of this study to other populations as this study was solely based on European ancestry. Future research should aim to include diverse ethnic groups to offer a more comprehensive understanding of the causal relationships being investigated. Secondly, the number of IVs employed in the analysis varied from 3 to 20, potentially impacting the MR findings due to the restricted amount of IVs. However, this is unlikely to mislead the study as the F-statistics of each IV exceeds 10. Thirdly, the absence of individual information hinders further categorization of patients into finer subgroups based on disease stages. Lastly, the statistical power for other exposure factors, aside from RANTES/CCL5 and GROa, was relatively low, thereby leading to an increased probability of type II errors.

Exposure	Outcome	Heterogeneity		Pleiotropy		
		Q statistic (IVW)	P value	MR-Egger Intercept	P-value	FDR adjusted P
Bngf	Inflammatory disorders of breast	5.890782	0.316992	0.215377	0.123891	0.89
CTACK/CCL27		10.52622	0.230015	0.027011	0.639173	0.89
Eotaxin		12.5145	0.405292	0.078429	0.231944	0.89
FGFBasic		5.311584	0.504515	-0.01577	0.747763	0.91
GCSF		6.840304	0.553957	-0.01599	0.582013	0.89
GROa		5.096754	0.531465	-0.02369	0.581207	0.89
HGF		4.23177	0.645344	0.066929	0.317448	0.89
IFNg		3.910784	0.688749	-0.02399	0.59938	0.89
IL-10		14.40009	0.044506	-0.15234	0.27802	0.89
IL-12p70		1.294086	0.523592	0.114192	0.59696	0.89
IL-13		16.19971	0.062827	0.086658	0.047779	0.89
IL-13(after outliers removal)		3.81921	0.80035	-0.00997	0.856636	0.86
IL-16		4.186159	0.839949	0.005637	0.886199	0.96
IL-17		17.88231	0.056982	-0.03092	0.590841	0.89
IL-18		11.09133	0.521108	-0.00881	0.782075	0.92
IL-1b		2.455979	0.652535	0.051358	0.359925	0.89
IL-1ra		15.9157	0.014214	-0.07686	0.425043	0.89
IL-1ra (after outliers removal)		5.02955	0.41232	-0.04573	0.46217	0.46
IL-2		11.59005	0.114872	-0.01693	0.690366	0.89
IL-2ra		2.620788	0.758204	-0.05135	0.284533	0.89
IL-4		6.178035	0.627297	0.002505	0.950761	0.97
IL-5		5.856269	0.118816	0.124205	0.152635	0.89
IL-6		5.765054	0.123617	-0.17525	0.172896	0.89
IL-7		8.872339	0.261952	-0.00237	0.966518	0.97
IL-8		4.02884	0.133398	-0.05013	0.705856	0.89
IL-9		13.83134	0.016717	-0.05274	0.698576	0.89
IL-9 (after outliers removal)		2.00165	0.73546	0.006519	0.93338	0.93
MCP1		6.658821	0.155054	-0.03034	0.698291	0.89
MCP3		0.151204	0.697387			NA
MCSF		7.418987	0.492174	0.018049	0.675086	0.89
MIF		6.456698	0.596215	0.033474	0.428221	0.89
MIG		10.44591	0.576902	-0.01764	0.669046	0.89
MIP1a		1.720783	0.632323	-0.04058	0.641851	0.89
MIP1b		11.7253	0.816479	-0.0047	0.850641	0.95
PDGFbb		14.75395	0.194041	0.064898	0.077553	0.89
RANTES/CCL5		7.618724	0.666024	0.01815	0.702314	0.89
SCF		7.578613	0.270628	-0.04757	0.288481	0.89
SCGFb		18.29263	0.146721	-0.01327	0.665449	0.89
SDF1a		3.921198	0.788808	-0.00206	0.949251	0.97
TNFa		1.344666	0.853753	-0.02905	0.530992	0.89
TNFB		6.761108	0.149064	-0.06994	0.163841	0.89
TRAIL		10.16858	0.857678	0.004127	0.833061	0.95
VEGF		6.573237	0.474623	0.023268	0.679887	0.89

Table 2. Heterogeneity and horizontal Pleiotropy analysis of the relationship between inflammatory factors and inflammatory disorders of the breast.

Exposure	Outcome	Raw		Outlier corrected		Global P	Number of outliers	Distortion P
		OR (CI%)	P	OR (CI%)	P			
CTACK/CCL27	Inflammatory disorders of breast	1.01 (0.83–1.24)	0.91	NA (NA - NA)	NA	0.211		
Eotaxin		0.98 (0.78–1.24)	0.89	NA (NA - NA)	NA	0.406		
GROa		0.86 (0.75–0.98)	0.07	NA (NA - NA)	NA	0.645		
MCP1		0.88 (0.63–1.23)	0.5	NA (NA - NA)	NA	0.284		
MIG		0.86 (0.75–0.99)	0.06	NA (NA - NA)	NA	0.589		
MIP1a		1.00 (0.82–1.22)	0.99	NA (NA - NA)	NA	0.649		
MIP1b		0.89 (0.78–1.01)	0.09	NA (NA - NA)	NA	0.812		
RANTES/CCL5		0.83 (0.72–0.96)	0.03	NA (NA - NA)	NA	0.68		
SDF1a		0.75 (0.60–0.94)	0.04	NA (NA - NA)	NA	0.805		
Bngf		0.92 (0.72–1.16)	0.51	NA (NA - NA)	NA	0.341		
FGFBasic		0.99 (0.74–1.33)	0.95	NA (NA - NA)	NA	0.549		
GCSF		0.84 (0.67–1.05)	0.17	NA (NA - NA)	NA	0.572		
HGF		0.93 (0.72–1.22)	0.63	NA (NA - NA)	NA	0.644		
MCSF		0.94 (0.82–1.06)	0.34	NA (NA - NA)	NA	0.405		
PDGFBb		0.91 (0.71–1.16)	0.45	NA (NA - NA)	NA	0.229		
SCF		0.81 (0.57–1.14)	0.27	NA (NA - NA)	NA	0.277		
SCGFb		1.00 (0.86–1.15)	0.97	NA (NA - NA)	NA	0.151		
VEGF		1.20 (0.97–1.49)	0.14	NA (NA - NA)	NA	0.541		
IL-10		1.17 (0.79–1.75)	0.46	NA (NA - NA)	NA	0.053		
IL-13		1.03 (0.82–1.29)	0.84	NA (NA - NA)	NA	0.073		
IL-13 (after outliers removal)		0.90 (0.78–1.03)	0.16	NA (NA - NA)	NA	0.806		
IL-16		1.03 (0.91–1.16)	0.69	NA (NA - NA)	NA	0.838		
IL-17		0.97 (0.73–1.30)	0.87	NA (NA - NA)	NA	0.079		
IL-18		1.07 (0.95–1.21)	0.29	NA (NA - NA)	NA	0.454		
IL-1b		0.93 (0.75–1.14)	0.51	NA (NA - NA)	NA	0.8		
IL-1ra		0.92 (0.65–1.30)	0.65	0.78 (0.62–0.99)	0.09	0.018	1:rs11869294	0.476
IL-1ra (after outliers removal)		0.78 (0.62–0.99)	0.018	NA (NA - NA)	NA	0.46		
IL-2		0.94 (0.75–1.18)	0.62	NA (NA - NA)	NA	0.159		
IL-2ra		1.22 (1.04–1.43)	0.06	NA (NA - NA)	NA	0.818		
IL-4		1.12 (0.88–1.41)	0.39	NA (NA - NA)	NA	0.641		
IL-5		1.00 (0.69–1.45)	1	NA (NA - NA)	NA	0.19		
IL-6		0.81 (0.45–1.46)	0.54	NA (NA - NA)	NA	0.197		
IL-7		0.87 (0.74–1.01)	0.12	NA (NA - NA)	NA	0.344		
IL-9		0.87 (0.59–1.26)	0.49	0.68 (0.57–0.82)	0.02	0.022	1:rs61867538	0.457
IL-9 (after outliers removal)		0.68 (0.57–0.82)	0.02	NA (NA - NA)	NA	0.744		
IFNg		1.23 (0.95–1.58)	0.16	NA (NA - NA)	NA	0.723		
MIF		0.94 (0.80–1.11)	0.48	NA (NA - NA)	NA	0.604		
TNFa		1.02 (0.89–1.17)	0.79	NA (NA - NA)	NA	0.857		
TNFB		0.97 (0.80–1.17)	0.76	NA (NA - NA)	NA	0.211		
TRAIL		0.93 (0.86–1.01)	0.1	NA (NA - NA)	NA	0.877		

Table 3. MR-PRESSO analysis of the relationship between inflammatory factors and inflammatory disorders of the breast.

Exposure	Outcome	snp_r2.exposure	snp_r2.outcome	correct_causal_direction	steiger_pval	FDR adjusted <i>P</i>
CTACK/CCL27	Inflammatory disorders of breast	0.064648	4.94E-05	TRUE	1.45E-51	4.58E-51
Bngf	Inflammatory disorders of breast	0.041725	3.04E-05	TRUE	1.49E-32	1.97E-32
VEGF	Inflammatory disorders of breast	0.039879	4.30E-05	TRUE	2.16E-59	8.85E-59
MIF	Inflammatory disorders of breast	0.065745	3.23E-05	TRUE	4.19E-51	1.23E-50
TRAIL	Inflammatory disorders of breast	0.177862	5.68E-05	TRUE	0	0
TNfb	Inflammatory disorders of breast	0.112971	3.25E-05	TRUE	1.19E-41	2.21E-41
TNfa	Inflammatory disorders of breast	0.033457	6.43E-06	TRUE	2.09E-26	2.60E-26
SDF1a	Inflammatory disorders of breast	0.030073	3.51E-05	TRUE	3.23E-38	5.75E-38
SCGFb	Inflammatory disorders of breast	0.128598	8.57E-05	TRUE	2.18E-107	2.98E-106
SCF	Inflammatory disorders of breast	0.019604	4.42E-05	TRUE	3.79E-33	5.35E-33
IL-16	Inflammatory disorders of breast	0.064373	2.00E-05	TRUE	2.58E-50	6.61E-50
RANTES/CCL5	Inflammatory disorders of breast	0.077154	5.89E-05	TRUE	2.54E-58	9.46E-58
PDGFbb	Inflammatory disorders of breast	0.045289	7.29E-05	TRUE	9.24E-77	7.58E-76
MIP1b	Inflammatory disorders of breast	0.081181	6.53E-05	TRUE	4.08E-142	8.37E-141
MIP1a	Inflammatory disorders of breast	0.025427	8.06E-06	TRUE	1.46E-20	1.57E-20
MIG	Inflammatory disorders of breast	0.080421	6.63E-05	TRUE	3.36E-65	1.72E-64
MCSF	Inflammatory disorders of breast	0.248776	3.93E-05	TRUE	4.35E-55	1.49E-54
MCP3	Inflammatory disorders of breast	0.051587	3.91E-06	TRUE	3.37E-11	3.46E-11
MCP1	Inflammatory disorders of breast	0.026356	3.55E-05	TRUE	3.79E-45	7.77E-45
IL-12p70	Inflammatory disorders of breast	0.011524	6.21E-06	TRUE	5.91E-21	6.55E-21
IP10	Inflammatory disorders of breast	0.006476	1.09E-06	TRUE	1.68E-06	1.68E-06
IL-18	Inflammatory disorders of breast	0.125189	6.19E-05	TRUE	8.95E-104	9.17E-103
IL-17	Inflammatory disorders of breast	0.032474	8.40E-05	TRUE	1.15E-50	3.15E-50
IL-13	Inflammatory disorders of breast	0.063647	7.62E-05	TRUE	4.18E-49	1.01E-48
IL-10	Inflammatory disorders of breast	0.024428	7.34E-05	TRUE	1.13E-37	1.92E-37
IL-8	Inflammatory disorders of breast	0.019847	1.90E-05	TRUE	5.85E-16	6.15E-16
IL-6	Inflammatory disorders of breast	0.013603	3.15E-05	TRUE	3.97E-23	4.52E-23
IL-1ra	Inflammatory disorders of breast	0.043379	7.74E-05	TRUE	9.26E-34	1.41E-33
IL-1b	Inflammatory disorders of breast	0.033195	1.30E-05	TRUE	6.51E-25	7.63E-25
HGF	Inflammatory disorders of breast	0.019265	2.07E-05	TRUE	1.47E-33	2.15E-33
IL-9	Inflammatory disorders of breast	0.036695	7.21E-05	TRUE	1.53E-28	1.97E-28
IL-7	Inflammatory disorders of breast	0.096206	6.03E-05	TRUE	2.17E-73	1.48E-72
IL-5	Inflammatory disorders of breast	0.034051	2.95E-05	TRUE	1.90E-25	2.29E-25
IL-4	Inflammatory disorders of breast	0.028806	4.05E-05	TRUE	2.98E-48	6.80E-48
IL-2ra	Inflammatory disorders of breast	0.042557	4.16E-05	TRUE	3.48E-34	5.48E-34
IL-2	Inflammatory disorders of breast	0.057603	7.95E-05	TRUE	3.08E-43	6.01E-43
IFNg	Inflammatory disorders of breast	0.02136	2.60E-05	TRUE	1.70E-34	2.78E-34
GROa	Inflammatory disorders of breast	0.090317	4.37E-05	TRUE	5.45E-71	3.19E-70
GCSF	Inflammatory disorders of breast	0.029391	5.07E-05	TRUE	1.37E-47	2.95E-47
FGFBasic	Inflammatory disorders of breast	0.020782	2.49E-05	TRUE	4.53E-33	6.19E-33

Table 4. MR-Steiger analysis of the causal directions between inflammatory factors and inflammatory disorders of the breast.

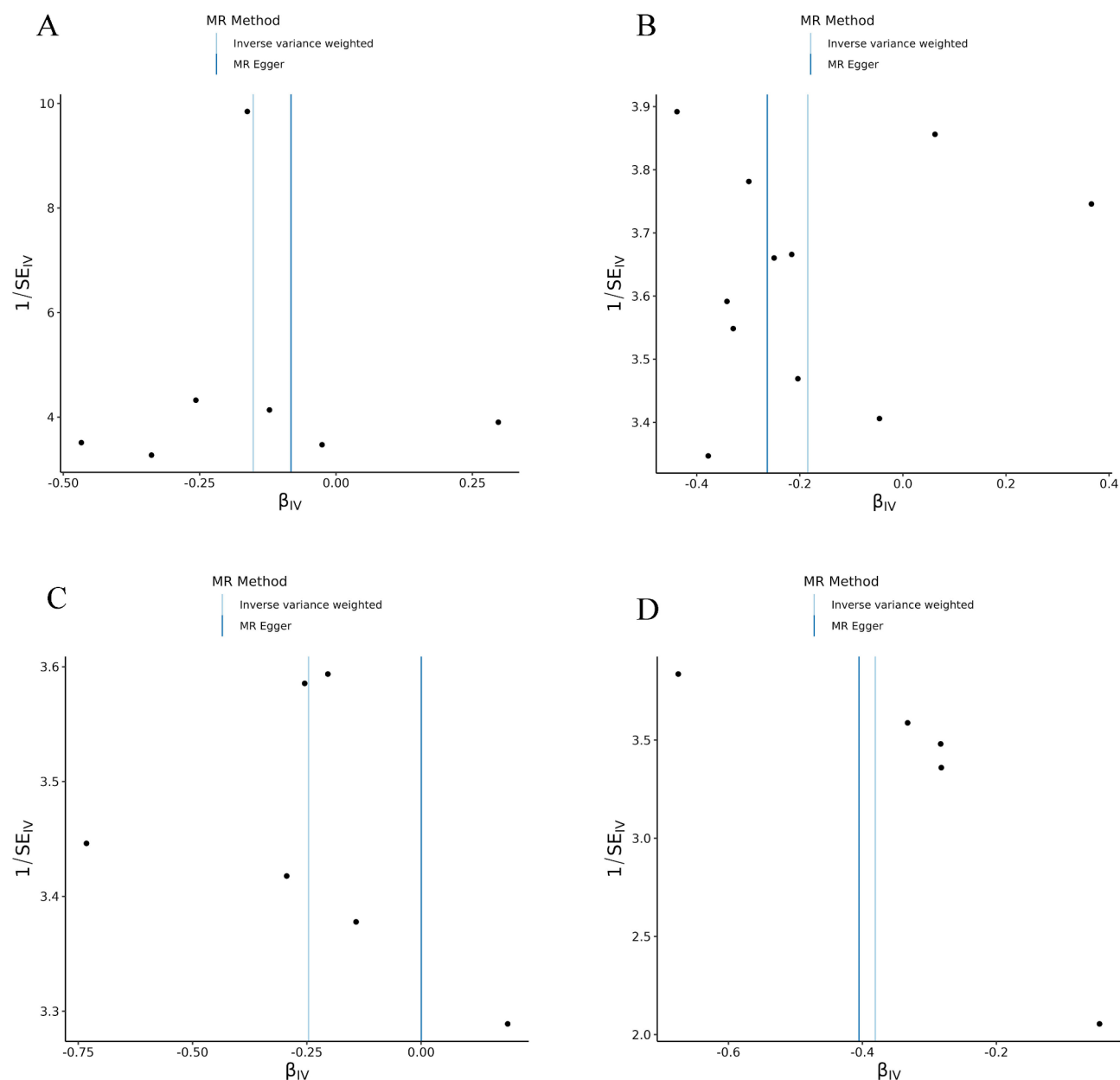


Fig. 4. Funnel plot of IVW model and MR-Egger model for potential relationship between IDB and GROa (A), RANTES/CCL5 (B), IL-1ra (C), and IL-9 (D). SNP, single-nucleotide polymorphism; IDB, inflammatory disorders of the breast; MR, Mendelian randomization; MIG, Monokine induced by gamma interferon; PDGFbb, Platelet-derived growth factor BB.

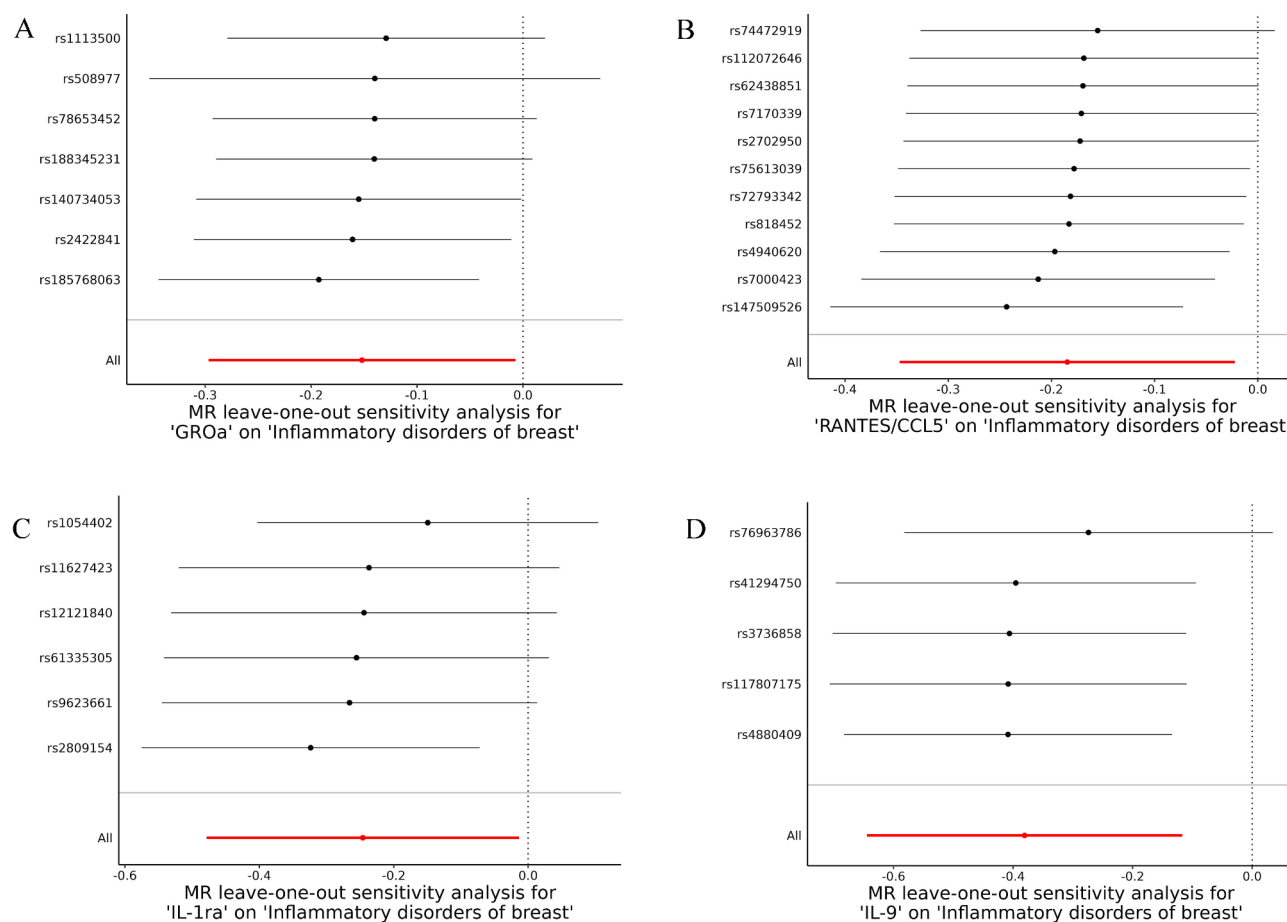


Fig. 5. MR leave-one-out sensitivity analysis for potential relationship between IDB and GROa (A), RANTES/CCL5 (B), IL-1ra (C), and IL-9 (D). IDB, inflammatory disorders of the breast; MR, Mendelian randomization; MIG, Monokine induced by gamma interferon; PDGFbb, Platelet-derived growth factor BB.

Data availability

All data generated or analyzed during this study are included in this article and supplementary information files.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This article is a mendelian randomization study. The data for this study were obtained from publicly available databases and published literature data and does not require ethical approval and written informed consent.

Additional information

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