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ORIGINAL RESEARCH

Differential Causal Effects of Common Analgesics on Breast Cancer Risk and Survival: Evidence from Mendelian Randomization

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Background: The causal effects of widely used analgesics—paracetamol(acetaminophen), aspirin (acetylsalicylic acid), and ibuprofen—on breast cancer risk and survival remain uncertain. This Mendelian randomization (MR) study investigated their causal relationships with breast cancer incidence, mortality, and estrogen receptor (ER)-subtype heterogeneity.

Methods: Using two-sample MR, genetic instruments for analgesic use were derived from UK Biobank GWAS (N=457,547). Outcome data included breast cancer incidence (122,977 cases/105,974 controls), ER-subtypes (ER+: 69,501 cases; ER−: 21,468 cases), and survival statistics. Inverse-variance weighted (IVW) analyses were primary, supplemented by MR-Egger, weighted median/mode, and sensitivity analyses (MR-PRESSO, leave-one-out). Bidirectional MR assessed reverse causation.

Results: Genetically predicted paracetamol use increased overall breast cancer risk (IVW OR=3.26, 95% CI:1.60–6.63, *p*FDR=0.005) and ER+ subtype risk (OR=3.65, 1.79–7.45, *p*FDR=0.003). Aspirin use showed no association with incidence but improved overall survival (HR=0.0036, 0.0001–0.1218, *p*FDR=0.016). Ibuprofen demonstrated no significant associations with risk or survival. Subtype-specific survival analyses were null. No reverse causation was detected (all $p > 0.05$). Sensitivity analyses confirmed robustness, with minimal pleiotropy (MR-Egger intercept $p > 0.05$) and consistent effects after outlier correction.

Conclusion: This MR study links a genetic predisposition to paracetamol use with increased breast cancer risk (especially ER+), and to aspirin use with improved survival. These divergent findings point to drug-specific mechanisms, warranting caution with long-term paracetamol use and further study of aspirin's therapeutic potential. Clinical decisions should balance analgesic benefits against these potential cancer-related outcomes.

Keywords: breast cancer, paracetamol, aspirin, ibuprofen, Mendelian randomization, nonsteroidal anti-inflammatory drugs, pharmacoepidemiology

Introduction

Breast cancer remains the most common malignancy and a leading cause of cancer-related deaths among women globally, with an estimated 2.3 million new cases diagnosed annually.[1] Despite advances in early detection and treatment, breast cancer heterogeneity presents significant challenges for prevention and management strategies.[2] Identification of modifiable risk factors and potential chemopreventive agents therefore remains a key priority in breast cancer research.

Inflammation has been recognized as an enabling characteristic in cancer development and progression. Chronic inflammation can contribute to tumor initiation, promotion, and metastasis through various mechanisms, including the production of reactive oxygen species, cytokines, and growth factors.[3] This understanding has generated considerable interest in the potential role of anti-inflammatory medications in cancer prevention and treatment.

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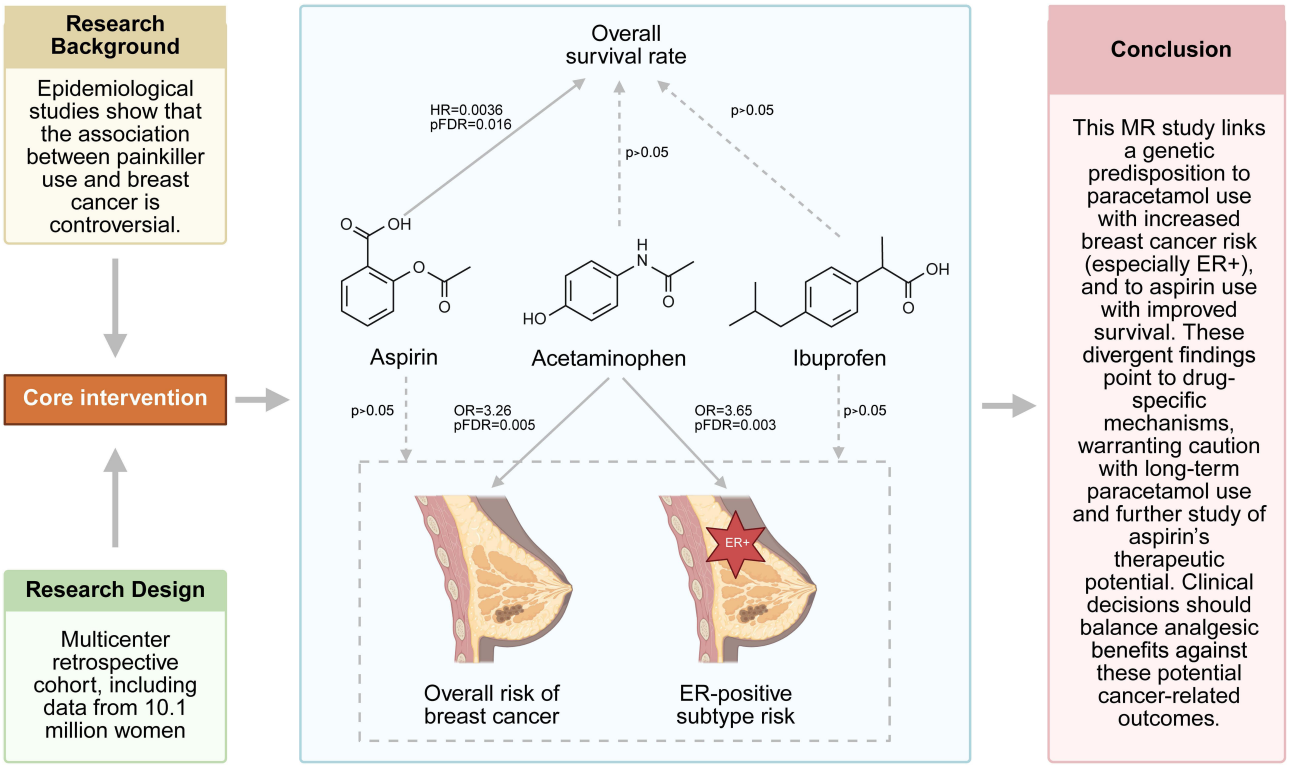
Graphical Abstract



Paracetamol (acetaminophen), aspirin (acetylsalicylic acid), and ibuprofen are among the most widely used over-the-counter analgesic medications worldwide.[4] While paracetamol primarily acts through inhibition of cyclooxygenase enzymes in the central nervous system with weak anti-inflammatory properties, aspirin and ibuprofen belong to the nonsteroidal anti-inflammatory drugs (NSAIDs) class that inhibit cyclooxygenase enzymes in peripheral tissues, reducing prostaglandin synthesis and inflammation.[5,6]

Observational studies examining the relationship between NSAID use and breast cancer risk have yielded inconsistent results. Some studies suggest a protective effect of aspirin and ibuprofen on breast cancer incidence,[7–10] while others report null or even harmful associations.[11–13] Similarly, evidence regarding NSAID use and breast cancer survival is conflicting.[14,15] These inconsistencies likely stem from methodological limitations inherent to observational studies, including residual confounding, recall bias, and reverse causation.[9]

Breast cancer is a heterogeneous disease with distinct molecular subtypes that demonstrate different risk factors, treatment responses, and prognoses.[16] The most fundamental classification is based on estrogen receptor (ER) status, with ER-positive (ER+) tumors generally having better outcomes than ER-negative (ER-) tumors.[17,18] The effects of NSAIDs may differ across these subtypes due to varying inflammatory pathways and hormone receptor interactions, yet few studies have examined such heterogeneity.

Mendelian randomization (MR) is an analytical method that uses genetic variants as instrumental variables to investigate causal relationships between exposures and outcomes.[19] By leveraging the random allocation of genetic variants at conception, MR can minimize confounding and reverse causation biases that plague traditional observational studies.[20] The two-sample MR approach, which uses summary statistics from separate GWAS for exposures and outcomes, further enhances statistical power and convenience.[21]

To date, few studies have applied MR to investigate the causal relationship between the use of paracetamol, aspirin, and ibuprofen, the most widely used over-the-counter analgesics, and breast cancer prognosis.[15] Furthermore, most previous

studies did not examine the differential effects of these drugs on breast cancer subtypes or survival outcomes.[22–24] Understanding these relationships may have important implications for preventive and therapeutic strategies for breast cancer management.

In this study, we used a bidirectional two-sample MR approach to investigate the causal relationships between genetically predicted use of paracetamol, aspirin, and ibuprofen and breast cancer risk, including subtype-specific analyses (ER-positive and ER-negative) and survival outcomes. We also performed reverse MR analyses to examine potential reverse causation, assessing whether genetic predisposition to breast cancer influences the use of these medications.

Methods

Study Design

We employed a two-sample Mendelian randomization approach to investigate the causal relationships between the use of paracetamol, aspirin, and ibuprofen and breast cancer outcomes. MR is an epidemiological method that uses genetic variants as instrumental variables to infer causality between an exposure and an outcome. By leveraging the principle of random assortment of genes from parents to offspring during meiosis—a process analogous to random allocation in a clinical trial—MR can significantly reduce biases from confounding factors and reverse causation that often limit traditional observational studies.

The validity of any MR study rests on three core assumptions, which in the context of our study are: (1) The Relevance Assumption: The selected genetic variants (instruments) must be robustly and reliably associated with the exposure; (2) The Independence/Exclusion Assumption: The genetic variants must not be associated with any confounders of the exposure-outcome relationship (eg, factors like smoking or alcohol use that could influence both analgesic use and breast cancer risk). (3) The Restriction Assumption: The genetic variants must affect the outcome (breast cancer risk or survival) only through their effect on the exposure (analgesic use) and not via any other biological pathway. This is also known as the "no horizontal pleiotropy" assumption.[25] (Figure 1).

Our study utilizes a two-sample MR design: one for the exposure (analgesic use) and one for the outcome (breast cancer). This approach increases statistical power and allows us to leverage large-scale, publicly available data. We also conducted bidirectional MR analyses to assess for potential reverse causality, investigating whether a genetic predisposition to breast cancer influences the use of these analgesics. It is worth noting that according to item 1 and 2 of Article

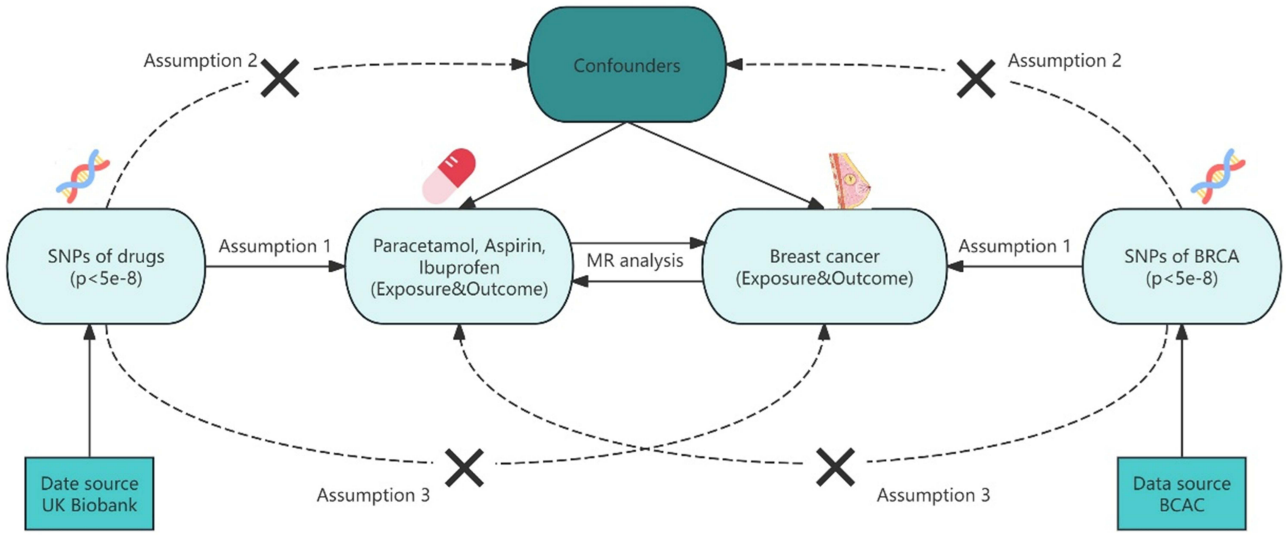


Figure 1 The workflow of the MR analysis in the present study. MR instrumental variable assumptions: Assumption 1: The instruments are associated with exposure; Assumption 2: The instruments are not associated with measure or unmeasured confounders; Assumption 3: The instruments influence the outcome only through the exposure.

Abbreviations: MR, Mendelian randomization; BRCA, breast cancer; BCAC, breast cancer association consortium.

32 of "the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects", this study is exempt from ethical review and approval.

Data Sources

Genetic Instruments for the Use of Paracetamol, Aspirin, and Ibuprofen

Genetic instruments for the use of paracetamol, aspirin, and ibuprofen were obtained from genome-wide association studies (GWAS) conducted within the UK Biobank, a large-scale biomedical database containing genetic and health information from approximately 500,000 participants aged 40–69 years recruited across the United Kingdom between 2006 and 2010.[26] Specifically, we used summary statistics from GWAS of self-reported regular use of paracetamol (ukb-b-17595), aspirin (ukb-b-7137), and ibuprofen (ukb-b-8888), each including data from 457,547 participants of European ancestry and approximately 9.85 million genetic variants (Table 1). The two-sample MR design leverages summary statistics from separate consortia, which may have different recruitment periods. This is a standard and valid approach, provided the underlying populations share a similar genetic ancestry, as is the case here with both cohorts being of predominantly European descent.

For each drug, we selected independent genetic variants (single nucleotide polymorphisms, SNPs) strongly associated with the exposure ($p < 5 \times 10^{-8}$) and with low linkage disequilibrium ($r^2 < 0.001$). Given that various confounding factors are closely related to the pathogenesis of breast cancer, we excluded SNPs that were significantly associated with body mass index, alcohol consumption, and smoking at the genome-wide level. The data related to confounding factors were obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas) and GWAS summary data (https://gwas.mrcieu.ac.uk/). After excluding confounding SNPs, we identified 17–18 SNPs for paracetamol, 9–10 SNPs for aspirin, and 5–6 SNPs for ibuprofen as instrumental variables. The F-statistics for all instruments exceeded 10, indicating sufficient strength to minimize weak instrument bias.[27]

Breast Cancer Outcome Data

Outcome data were obtained from the Breast Cancer Association Consortium (BCAC), which conducted a comprehensive GWAS meta-analysis of breast cancer.[28] This dataset includes summary statistics from 122,977 breast cancer cases and 105,974 controls of European ancestry (228,951 total participants). For subtype analyses, we used data on 69,501 ER+ cases and 105,974 controls (175,475 total participants) and 21,468 ER- cases and 105,974 controls (127,442 total participants). For survival analyses, we utilized GWAS summary statistics for overall breast cancer survival (ieu-a-1165), ER+ breast cancer survival (ieu-a-1164), and ER- breast cancer survival (ieu-a-1163)[29] (Table 1).

Table 1 Summary Information on Exposure and Outcome GWAS Data

GWAS id	Trait(s)	Data Sources	Trait(s)	Sample Size	nSNPs	Year	PMID
ukb-b-17595	Paracetamol	UK Biobank	European	457,547	9,851,867	2018	30305743
ukb-b-7137	Aspirin	UK Biobank	European	457,547	9,851,867	2018	30305743
ukb-b-8888	Ibuprofen	UK Biobank	European	457,547	9,851,867	2018	30305743
ieu-a-1126	Breast cancer	BCAC	European	228,951	10,680,257	2017	29059683
ieu-a-1127	ER+ Breast cancer	BCAC	European	175,475	10,680,257	2017	29059683
ieu-a-1128	ER- Breast cancer	BCAC	European	127,442	10,680,257	2017	29059683
ieu-a-1165	Breast cancer (Survival)	BCAC	European	37,954	12,940,150	2015	25890600
ieu-a-1164	ER+ Breast cancer (Survival)	BCAC	European	23,059	8,714,606	2015	25890600
ieu-a-1163	ER- Breast cancer (Survival)	BCAC	European	6,881	8,828,662	2015	25,890,600

Abbreviations: nSNPs, Single nucleotide polymorphisms number; BCAC, breast cancer association consortium; ER, estrogen receptor.

Statistical Analysis

We performed primary MR analyses using the inverse variance weighted (IVW) method, which provides the most precise estimates when all MR assumptions are met.[30] To assess potential violations of the third MR assumption (no horizontal pleiotropy), we employed several complementary approaches: MR-Egger regression,[31] weighted median,[32] and weighted mode[33] methods. MR-Egger can detect and correct for directional pleiotropy, while weighted median and weighted mode methods provide consistent estimates even when a proportion of the genetic instruments are invalid.

Heterogeneity among the causal effects estimated by individual SNPs was assessed using Cochran's Q statistic for the IVW method and Rucker's Q' statistic for MR-Egger.[34] A significant Q statistic ($p < 0.05$) indicates potential heterogeneity, which may arise from pleiotropy. We also examined the MR-Egger intercept as a test for directional pleiotropy, with a non-zero intercept ($p < 0.05$) suggesting the presence of pleiotropy.[31]

For the primary analyses, we estimated the causal effects of each drug on overall breast cancer risk, ER+ breast cancer risk, and ER- breast cancer risk. Secondary analyses focused on breast cancer survival outcomes. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for risk analyses, while regression coefficients (β) were reported for survival analyses. To address potential reverse causation, we conducted bidirectional MR analyses examining the causal effects of breast cancer risk on NSAID use.

All statistical analyses were performed using the TwoSample MR[35] and Mendelian Randomization[36] packages in R version 4.0.3. Multiple testing was accounted for using the false discovery rate (FDR) method, with an adjusted p-value < 0.05 considered statistically significant.

Sensitivity Analyses

We conducted several sensitivity analyses to assess the robustness of our findings. First, we performed leave-one-out analyses to evaluate whether individual SNPs disproportionately influenced the results. Second, we applied the MR-PRESSO method to detect and correct for horizontal pleiotropy by identifying and removing outlier SNPs.[37]

Ethical Considerations

This study used publicly available summary statistics data with no individual-level information. The original studies providing these data obtained appropriate ethical approval and participant consent.[26,28]

Results

Paracetamol Use and Breast Cancer Risk

Genetically predicted paracetamol use showed significant associations with increased risk of overall breast cancer in the IVW analysis (OR=3.26, 95% CI: 1.60–6.63, p=0.001, FDR-adjusted p=0.005) (Figures 2, 3A, 4A, and Supplementary Table 1). This association was also observed with the weighted median method (OR=3.08, 95% CI: 1.30–7.30, p=0.011, FDR-adjusted p=0.097), but not with the MR-Egger (OR=3.93, 95% CI: 0.10–156.54, p=0.478) or weighted mode methods (OR=1.02, 95% CI: 0.16–6.42, p=0.986). The MR-Egger intercept did not indicate significant directional pleiotropy (intercept=−0.00118, p=0.920), although there was evidence of heterogeneity among the SNPs (Q=26.93, p=0.042) (Table 2 and Supplementary Table 1).

Subtype analyses revealed that genetically predicted paracetamol use was significantly associated with increased risk of ER+ breast cancer (IVW: OR=3.65, 95% CI: 1.79–7.45, p=0.0004, FDR-adjusted p=0.003) (Figures 2, 3B, 4B and Supplementary Table 1). This association remained significant in the weighted median analysis (OR=2.71, 95% CI: 1.07–6.90, p=0.036, FDR-adjusted p=0.163). No significant pleiotropy was detected by the MR-Egger intercept (intercept=0.011, p=0.360), and heterogeneity was not significant (Q=21.43, p=0.207) (Table 2 and Supplementary Table 1).

For ER- breast cancer, the association with genetically predicted paracetamol use did not reach statistical significance in the IVW analysis (OR=2.75, 95% CI: 0.95–7.97, p=0.063, FDR-adjusted p=0.190) (Figure 2 and Supplementary Table 1). Results from alternative MR methods were also non-significant, with no evidence of pleiotropy (MR-Egger intercept=0.007, p=0.693) or significant heterogeneity (Q=18.19, p=0.253) (Table 2 and Supplementary Table 1).

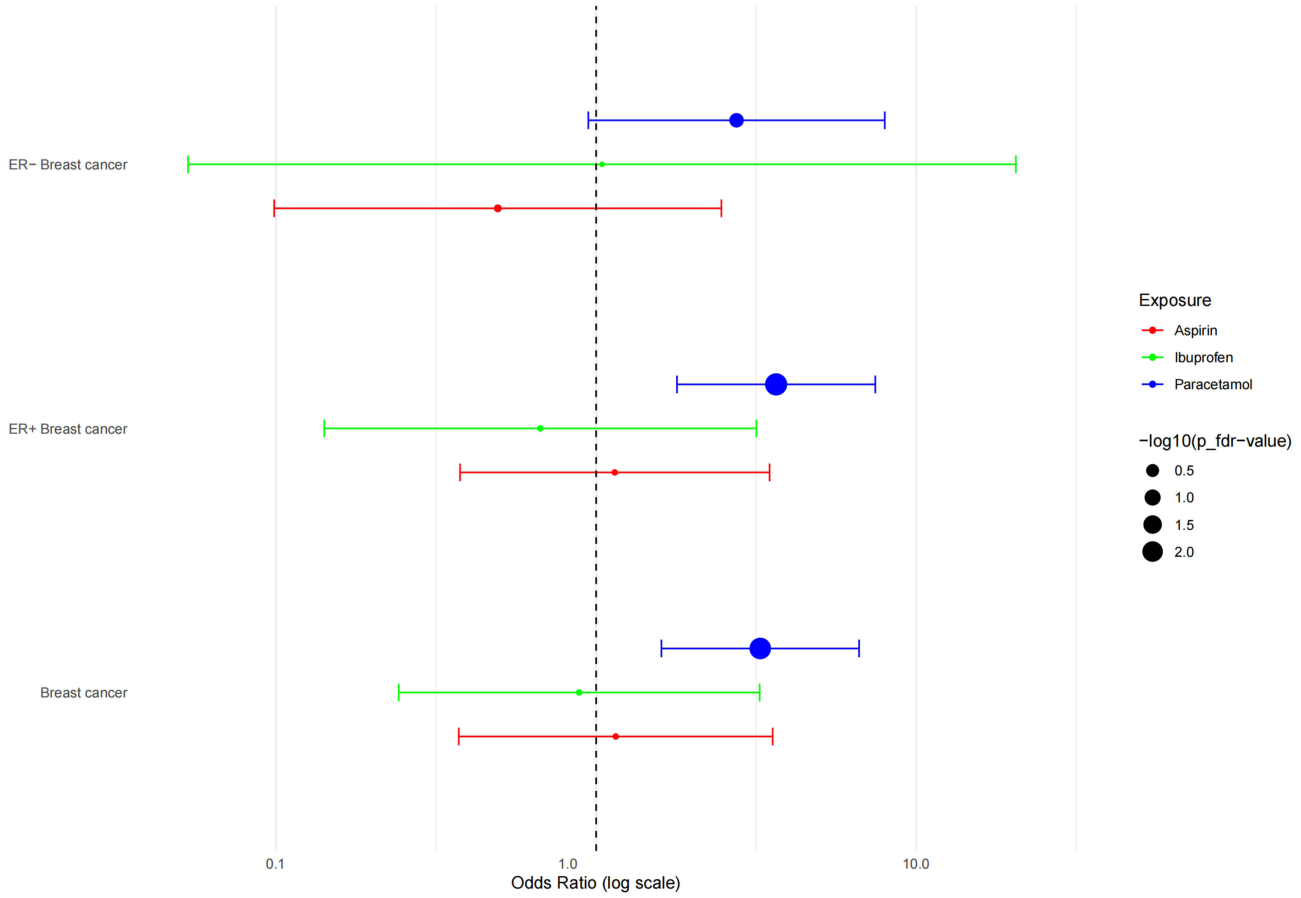


Figure 2 Two-sample Mendelian randomization analysis results for paracetamol, ibuprofen, aspirin, and breast cancer risk.

Aspirin Use and Breast Cancer Risk

Genetically predicted aspirin use showed no significant association with overall breast cancer risk in the IVW analysis (OR=1.15, 95% CI: 0.37–3.56, p=0.806, FDR-adjusted p=0.978) (Figure 2 and Supplementary Table 1). Results from alternative MR methods were similarly non-significant, with the MR-Egger intercept showing no evidence of directional pleiotropy (intercept=0.005, p=0.647). However, there was some evidence of heterogeneity among the SNPs (Q=14.73, p=0.065) (Table 2 and Supplementary Table 1).

Similarly, no significant associations were observed between genetically predicted aspirin use and ER+ breast cancer risk (IVW: OR=1.14, 95% CI: 0.38–3.48, p=0.813, FDR-adjusted p=0.978) or ER- breast cancer risk (IVW: OR=0.49, 95% CI: 0.10–2.46, p=0.389, FDR-adjusted p=0.874) (Figure 2 and Supplementary Table 1). None of the alternative MR methods yielded significant results, and no evidence of directional pleiotropy was detected for either subtype (Table 2 and Supplementary Table 1).

Ibuprofen Use and Breast Cancer Risk

Genetically predicted ibuprofen use showed no significant association with overall breast cancer risk in the IVW analysis (OR=0.89, 95% CI: 0.24–3.24, p=0.854, FDR-adjusted p=0.961) (Figure 2 and Supplementary Table 1). Results from alternative MR methods were also non-significant, with no evidence of directional pleiotropy (MR-Egger intercept=0.022, p=0.631) or heterogeneity (Q=0.93, p=0.920).

For breast cancer subtypes, no significant associations were observed between genetically predicted ibuprofen use and either ER+ breast cancer risk (IVW: OR=0.67, 95% CI: 0.14–3.17, p=0.613, FDR-adjusted p=0.978) or ER- breast cancer risk (IVW: OR=1.04, 95% CI: 0.05–20.47, p=0.978, FDR-adjusted p=0.978) in the primary analyses (Figure 2

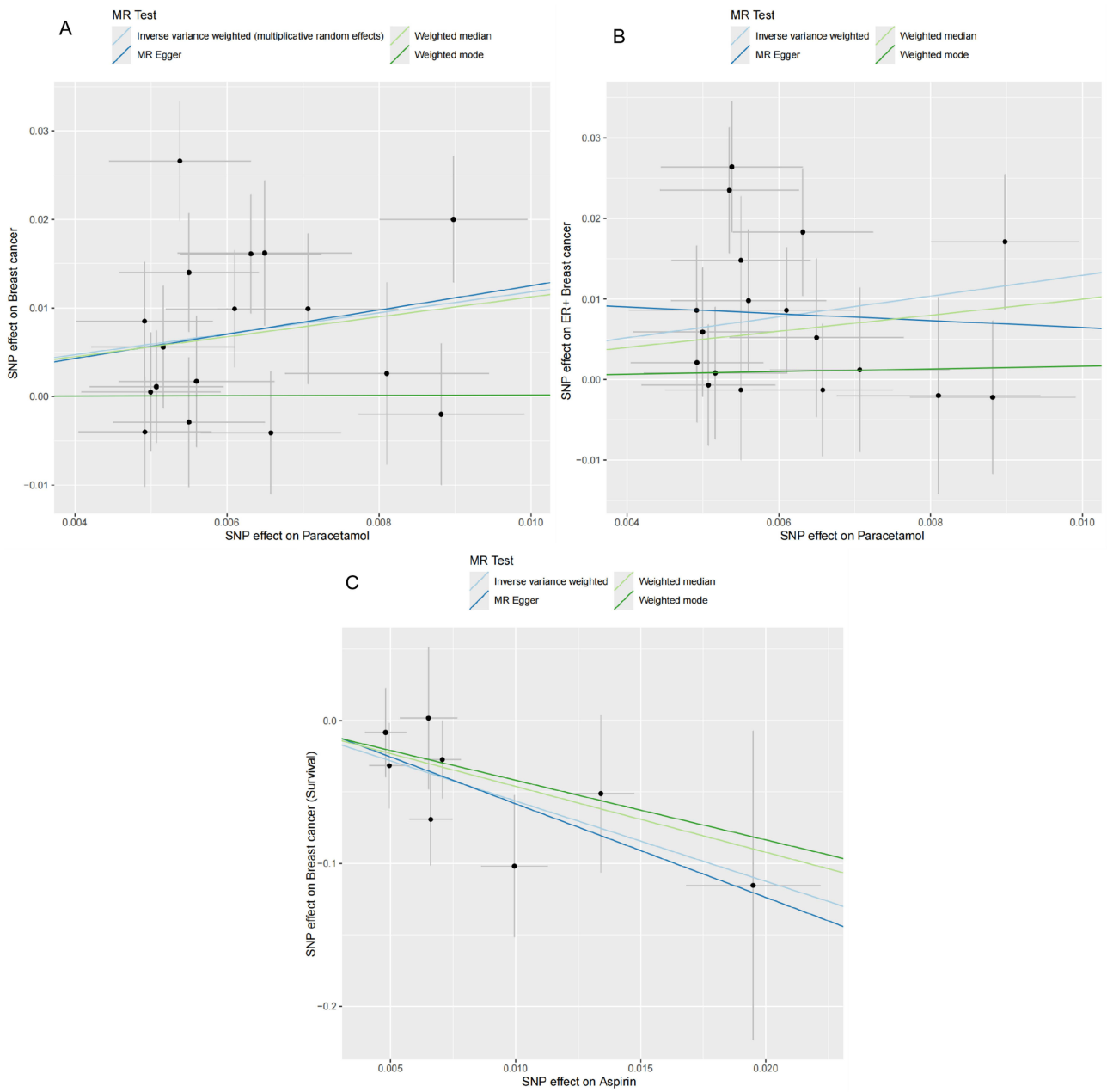


Figure 3 Scatter plots of the two-sample Mendelian randomization analysis. (**A**) Paracetamol and breast cancer risk. (**B**) Paracetamol and ER+ breast cancer risk. (**C**) Aspirin and breast cancer survival. The slope of the line indicates the causal relationship. Each color represents a different MR method. An upward tilt of the slope indicates a positive correlation, while a downward tilt indicates a negative correlation.

and Supplementary Table 1). Interestingly, the MR-Egger analysis suggested a potential protective effect of ibuprofen on ER- breast cancer (OR=1.35×10^-13, 95% CI: 4.10×10^-22-4.42×10^-5, p=0.041, FDR-adjusted p=0.373), although this finding should be interpreted with caution given the wide confidence interval and lack of consistency across methods (Table 2 and Supplementary Table 1).

Use of Paracetamol, Aspirin, and Ibuprofen and Breast Cancer Survival

In analyses of breast cancer survival outcomes, genetically predicted aspirin use showed a significant association with improved overall breast cancer survival (IVW: HR=0.0036, 95% CI: 0.0001 to 0.1218, p=0.002, FDR-adjusted p=0.016) (Figures 3C, 4C, 5 and Supplementary Table 2). This association remained significant in the weighted median analysis

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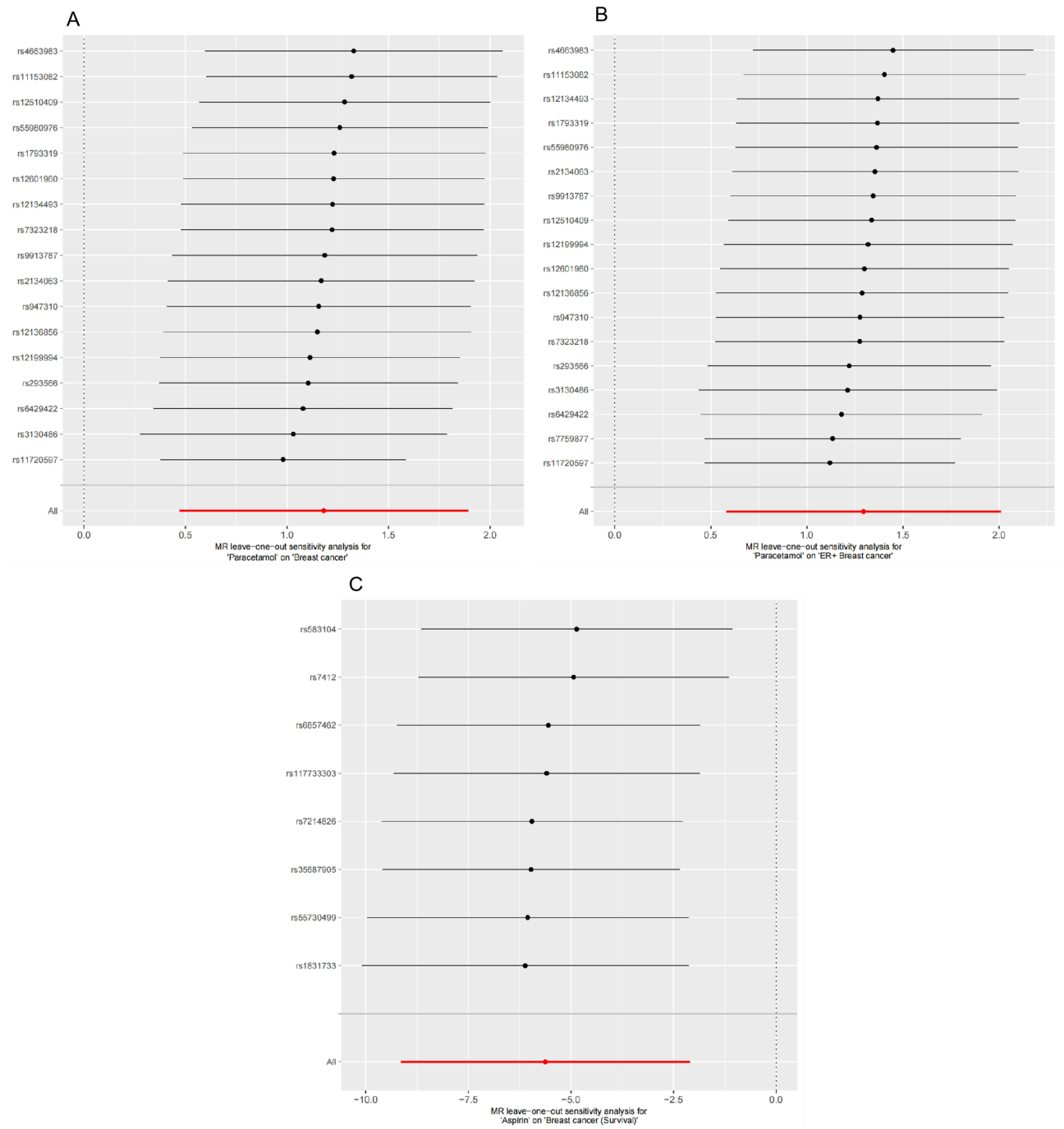

Figure 4 Leave-one-out plots of the two-sample Mendelian randomization analysis. (**A**) Paracetamol and breast cancer risk. (**B**) Paracetamol and ER+ breast cancer risk. (**C**) Aspirin and breast cancer survival. By systematically excluding each SNP, we assessed whether the causal relationship between the exposure and the outcome was robust and reliable, and no obvious bias was observed.

(HR=0.0100, p=0.042, FDR-adjusted p=0.339). The MR-Egger approach showed a similar direction of effect (HR=0.0014, p=0.236), with no evidence of directional pleiotropy (intercept=0.007, p=0.848) or significant heterogeneity (Q=3.22, p=0.864) (Table 3 and Supplementary Table 2).

No significant associations were observed between genetically predicted aspirin use and either ER+ breast cancer survival (IVW: HR=0.0648, 95% CI: 0.0002 to 24.8608, p=0.367, FDR-adjusted p=0.917) or ER- breast cancer survival (IVW: HR=0.3787, 95% CI: 0.0029 to 48.9607, p=0.696, FDR-adjusted p=0.985) (Figure 5, Table 3 and Supplementary Table 2).

Table 2 Heterogeneity and Pleiotropy Tests

Exposure	Outcome	Method	nSNPs	Q	Q_df	Q_pval	Egger_Intercept	se	pval	Global Test P value
Paracetamol	Breast cancer	IVW	17	26.93	16	0.042				0.052
Paracetamol	Breast cancer	MR Egger	17	26.91	15	0.030	−0.00118	0.011562	0.920	
Paracetamol	ER+ Breast cancer	IVW	18	21.43	17	0.207				0.228
Paracetamol	ER+ Breast cancer	MR Egger	18	20.30	16	0.207	0.010711	0.011354	0.360	
Paracetamol	ER- Breast cancer	IVW	17	18.19	15	0.253				0.329
Paracetamol	ER- Breast cancer	MR Egger	17	18.39	16	0.301	0.006964	0.017277	0.693	
Aspirin	Breast cancer	IVW	9	14.73	8	0.065				0.084
Aspirin	Breast cancer	MR Egger	9	14.26	7	0.047	0.005374	0.011234	0.647	
Aspirin	ER+ Breast cancer	IVW	9	10.04	8	0.262				0.282
Aspirin	ER+ Breast cancer	MR Egger	9	9.97	7	0.190	0.002355	0.01122	0.840	
Aspirin	ER- Breast cancer	IVW	10	12.35	9	0.194				0.218
Aspirin	ER- Breast cancer	MR Egger	10	11.53	8	0.174	0.013013	0.017246	0.472	
Ibuprofen	Breast cancer	IVW	5	0.93	4	0.920				0.927
Ibuprofen	Breast cancer	MR Egger	5	0.64	3	0.886	0.021508	0.04033	0.631	
Ibuprofen	ER+ Breast cancer	IVW	5	3.37	4	0.498				0.501
Ibuprofen	ER+ Breast cancer	MR Egger	5	3.18	3	0.365	−0.02128	0.049831	0.698	
Ibuprofen	ER- Breast cancer	IVW	6	9.63	5	0.087				0.113
Ibuprofen	ER- Breast cancer	MR Egger	6	0.72	4	0.949	0.137527	0.046084	0.041	

Notes: Pval > 0.05 and Global Test P value > 0.05 indicate no significant pleiotropy. Q_pval < 0.05 indicates the presence of heterogeneity.
Abbreviations: nSNPs, Single nucleotide polymorphisms number; IVW, inverse variance weighted; ER, estrogen receptor.

Genetically predicted paracetamol use showed no significant associations with overall breast cancer survival (IVW: HR=0.2855, 95% CI: 0.0146 to 5.5798, p=0.409, FDR-adjusted p=0.917), ER+ breast cancer survival (IVW: HR=0.7475, 95% CI: 0.0142 to 39.4069, p=0.886, FDR-adjusted p=0.985), or ER- breast cancer survival (IVW: HR=0.4149, 95% CI: 0.0004 to 397.8971, p=0.802, FDR-adjusted p=0.985) (Figure 5, Table 3 and Supplementary Table 2).

Similarly, genetically predicted ibuprofen use showed no significant associations with overall breast cancer survival (IVW: HR=1.0520, 95% CI: 0.0057 to 194.5131, p=0.985, FDR-adjusted p=0.985), ER+ breast cancer survival (IVW: HR=0.1402, 95% CI: 0.0001 to 355.4397, p=0.623, FDR-adjusted p=0.917), or ER- breast cancer survival (IVW: HR=6.1984, 95% CI: 0.0015 to 25016.3728, p=0.667, FDR-adjusted p=0.985) (Figure 5, Table 3 and Supplementary Table 2).

Bidirectional MR Analyses

To assess potential reverse causality, we conducted bidirectional MR analyses examining the causal effects of breast cancer risk on NSAID use. No significant associations were observed in either direction for any of the NSAID-breast cancer pairs. For example, genetically predicted breast cancer risk showed no significant effect on paracetamol use (IVW: OR=1.00, 95% CI: 0.997–1.003, p=0.914), aspirin use (IVW: OR=0.998, 95% CI: 0.995–1.000, p=0.101), or ibuprofen use (IVW: OR=1.002, 95% CI: 0.999–1.004, p=0.195) (Supplementary Figure 1, Tables 3 and 4). These findings suggest that reverse causality is unlikely to explain our main results.

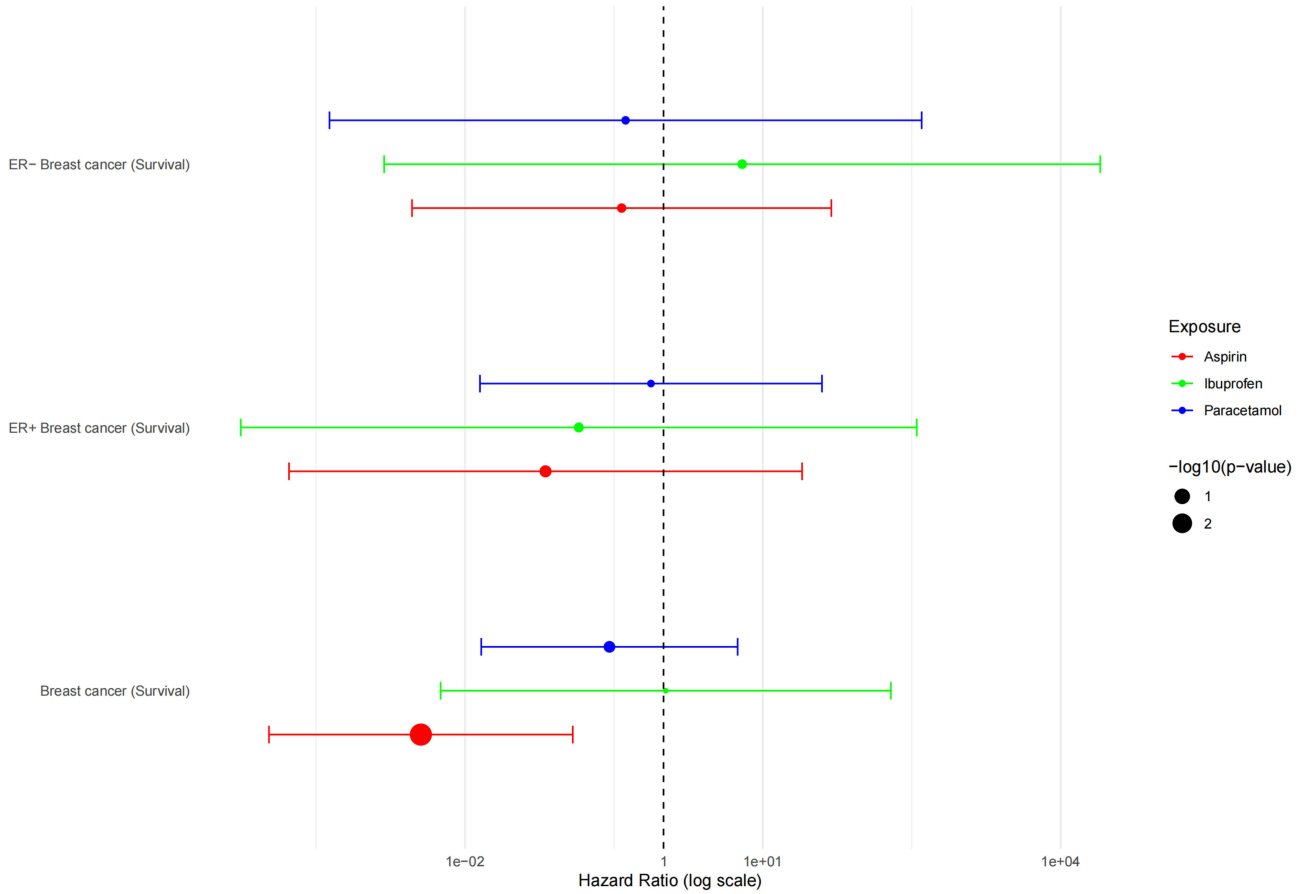

Figure 5 Two-sample Mendelian randomization analysis results for the use of paracetamol, ibuprofen, aspirin, and breast cancer survival.

Sensitivity Analyses

Leave-one-out analyses did not identify any individual SNPs with disproportionate influence on the main results. The MR-PRESSO method detected and corrected for potential outliers, but the corrected estimates remained consistent with the primary analyses (Tables 2 and 3, Figures 3 and 4 and Supplementary Table 4).

Table 3 Heterogeneity and Pleiotropy Tests

Exposure	Outcome	Method	nSNPs	Q	Q_df	Q_pval	Egger _Intercept	se	pval	Global Test pval
Paracetamol	ER- Breast cancer (Survival)	IVW	8	1.08	7	0.993				0.992
Paracetamol	ER- Breast cancer (Survival)	MR Egger	8	0.87	6	0.990	0.084412	0.186067	0.666	
Paracetamol	ER+ Breast cancer (Survival)	IVW	15	6.47	14	0.953				0.961
Paracetamol	ER+ Breast cancer (Survival)	MR Egger	15	6.17	13	0.940	−0.03907	0.071272	0.593	
Paracetamol	Breast cancer (Survival)	IVW	12	8.67	11	0.653				0.656
Paracetamol	Breast cancer (Survival)	MR Egger	12	8.51	10	0.579	0.019736	0.049886	0.700	
Aspirin	ER- Breast cancer (Survival)	IVW	27	2.46	26	0.99				0.99
Aspirin	ER- Breast cancer (Survival)	MR Egger	27	2.40	25	0.99	0.006807	0.030138	0.823	

(*Continued*)

Table 3 (Continued).

Exposure	Outcome	Method	nSNPs	Q	Q_df	Q_pval	Egger _Intercept	se	pval	Global Test pval
Aspirin	ER+ Breast cancer (Survival)	IVW	6	0.57	5	0.989				0.993
Aspirin	ER+ Breast cancer (Survival)	MR Egger	6	0.55	4	0.969	−0.01064	0.067034	0.882	
Aspirin	Breast cancer (Survival)	IVW	8	3.22	7	0.864				0.887
Aspirin	Breast cancer (Survival)	MR Egger	8	3.18	6	0.786	0.007363	0.036743	0.848	
Ibuprofen	ER- Breast cancer (Survival)	IVW	9	0.83	8	0.999				0.999
Ibuprofen	ER- Breast cancer (Survival)	MR Egger	9	0.79	7	0.998	0.014085	0.073563	0.854	
Ibuprofen	ER+ Breast cancer (Survival)	IVW	5	0.20	4	0.995				0.997
Ibuprofen	ER+ Breast cancer (Survival)	MR Egger	5	0.20	3	0.978	−0.00583	0.096307	0.956	
Ibuprofen	Breast cancer (Survival)	IVW	5	2.20	4	0.699				0.727
Ibuprofen	Breast cancer (Survival)	MR Egger	5	1.63	3	0.654	−0.04854	0.06407	0.503	

Notes: Pval > 0.05 and Global Test P value > 0.05 indicate no significant pleiotropy. Q_pval < 0.05 indicates the presence of heterogeneity.
Abbreviations: nSNPs, Single nucleotide polymorphisms number; IVW, inverse variance weighted; ER, estrogen receptor.

Discussion

This two-sample Mendelian randomization study provides novel insights into the causal relationships between commonly used analgesics and breast cancer outcomes. Our findings suggest that genetically predicted paracetamol use is associated with increased risk of overall breast cancer and ER+ breast cancer specifically. We also observed that genetically predicted aspirin use may improve overall breast cancer survival, while no significant associations were detected between genetically predicted ibuprofen use and breast cancer outcomes in our primary analyses.

It is critical to interpret the magnitude of our effect estimates with caution. The large odds ratio for paracetamol risk (OR=3.26) and the very small hazard ratio for aspirin survival (HR=0.0036) may seem implausibly extreme when compared to typical effect sizes from observational studies. This is because MR estimates reflect the effect of a lifelong genetic predisposition towards an exposure, representing a maximal, lifelong contrast between "users" and "non-users." As such, the primary value of these estimates lies in their direction and statistical robustness, which provide evidence for a causal relationship, rather than their precise numerical value, which is not directly translatable to the effect of taking a specific dose of the drug. The consistency of our findings across multiple MR methods, the strength of our genetic instruments, and especially the divergent effects observed across the three different analgesics, lend credibility to the conclusion that these are drug-specific pharmacological effects rather than methodological artifacts.

The association between genetically predicted paracetamol use and increased breast cancer risk (OR=3.26 for overall breast cancer; OR=3.65 for ER+ breast cancer) represents a concerning finding that warrants careful consideration. Although paracetamol has long been regarded as having better safety compared to non-steroidal anti-inflammatory drugs, and has been recommended by the World Trade Organization as the preferred painkiller for treating mild to moderate pain,[38] our research results have raised questions about its long-term impact on the pathogenesis of breast cancer. The stronger association with ER+ breast cancer suggests potential interaction with hormonal pathways. This aligns with emerging evidence that paracetamol may have endocrine-disrupting properties.[39] Harnagea-Theophilus et al demonstrated that paracetamol induces breast cancer cell proliferation via estrogen receptor.[12] Furthermore, paracetamol metabolism generates N-acetyl-p-benzoquinone imine (NAPQI), which can induce oxidative stress and DNA damage when glutathione stores are depleted, potentially contributing to carcinogenic processes.[40]

Our finding regarding aspirin use and improved breast cancer survival (HR=0.0036) is concordant with several observational studies suggesting beneficial effects of aspirin on cancer outcomes. A meta-analysis by Huang et al of 16 studies found

that post-diagnosis aspirin use was associated with reduced breast cancer-specific mortality (HR=0.69).[41] The anti-cancer effects of aspirin likely involve both cyclooxygenase (COX)-dependent and COX-independent mechanisms. Aspirin irreversibly inhibits COX-1 and modifies the activity of COX-2, reducing prostaglandin synthesis and subsequent inflammation.[42] Additionally, aspirin may inhibit platelet activation, suppress NF-κB signaling, and directly induce apoptosis in cancer cells.[43,44] These mechanisms could collectively impair cancer progression and metastasis, explaining the observed survival benefit. However, the lack of significant associations with subtype-specific survival in our study suggests that these effects may be complex and influenced by various tumor and host factors.

The absence of significant associations between genetically predicted ibuprofen uses and breast cancer outcomes in our primary analyses contrasts with some observational studies suggesting protective effects of non-aspirin NSAIDs.[45] This discrepancy may reflect differences in study design, potential confounding in observational studies, or context-dependent effects of ibuprofen. The intriguing finding from MR-Egger analysis suggesting a potential protective effect of ibuprofen on ER- breast cancer, although not robust across methods, merits further investigation given the generally worse prognosis associated with this breast cancer subtype and the limited treatment options available.

One critical finding from our bidirectional MR analyses was the absence of evidence for reverse causation. This strengthens the interpretation that the observed associations represent causal effects of analgesic use on breast cancer outcomes rather than the influence of breast cancer or associated factors on medication use patterns. This addresses a major limitation of traditional observational studies in this field.

The biological plausibility of our findings is supported by the distinct pharmacological profiles of these medications. Paracetamol primarily exerts its analgesic effects through central mechanisms with modest peripheral anti-inflammatory activity.[5] Its potential to increase breast cancer risk may relate to its effects on prostaglandin synthesis in specific tissues, endocrine-disrupting properties, or metabolic activation to reactive species. In contrast, aspirin's irreversible inhibition of COX enzymes, antiplatelet effects, and direct influences on cancer-related signaling pathways could explain its potential benefit for cancer survival.[5] Ibuprofen, as a reversible non-selective COX inhibitor, has different pharmacokinetic and pharmacodynamic properties that may result in distinct effects on cancer pathogenesis.[5]

Our study has several strengths compared to previous research. The MR approach helps minimize confounding and reverse causation that have limited observational studies. The use of large sample sizes from established consortia provides robust statistical power. Our comprehensive sensitivity analyses help assess the validity of our findings, while the investigation of specific breast cancer subtypes and survival outcomes offers a more nuanced understanding of these relationships than many previous studies.

Nevertheless, important limitations must be acknowledged. First, our genetic instruments reflect a self-reported propensity for "regular use" of these medications from the UK Biobank, which lacks a standardized definition and does not include data on specific dosages, frequency, or duration of use. Consequently, our findings cannot establish a dose-response relationship and should be interpreted as the causal effect of a genetic tendency towards regular use. Second, the GWAS cohorts for each analgesic are not mutually exclusive, and participants may use more than one type of analgesic. However, the distinct causal effects we identified for each drug—with paracetamol increasing risk, aspirin improving survival, and ibuprofen showing null effects—strongly suggest that our findings are drug-specific and not driven by a general "analgesic user" phenotype. Third, while we excluded SNPs associated with common confounders, residual pleiotropy cannot be completely ruled out, as evidenced by some heterogeneity in our results. Third, our study was conducted primarily in populations of European ancestry, potentially limiting generalizability to other ethnic groups. Fourth, the genetic variants associated with medication use may capture not only the direct pharmacological effects but also the underlying conditions for which these medications are prescribed (ie, confounding by indication, a form of pleiotropy). However, the highly divergent causal estimates we observed provide strong evidence against this. If a common underlying condition like chronic pain or inflammation were driving the results, we would expect a similar directional association for all three analgesics. The distinct patterns observed are far more consistent with drug-specific biological mechanisms.

The clinical implications of our findings require careful consideration. The association between paracetamol use and increased breast cancer risk, if confirmed, could influence recommendations for long-term analgesic choices, particularly among women with additional breast cancer risk factors. This is especially relevant given paracetamol's widespread use and general perception as a safe analgesic option. However, these potential risks must be balanced against paracetamol's

established benefits for pain management and its favorable cardiovascular and gastrointestinal safety profile compared to NSAIDs.[46]

For aspirin, the potential survival benefit observed in our study adds to the growing evidence supporting its role in cancer management. Recent clinical guidelines have begun to acknowledge the potential cancer-preventive effects of aspirin, though primarily in the context of colorectal cancer prevention.[46] Our findings suggest that aspirin's benefits may extend to breast cancer outcomes, supporting further investigation of its role in breast cancer survivorship care. However, the optimal timing, dosage, and patient selection remain unclear, and the known risks of aspirin, including gastrointestinal bleeding and hemorrhagic stroke, necessitate careful individualized risk-benefit assessment.

The lack of clear associations for ibuprofen suggests that its effects on breast cancer may be neutral, though this should not be interpreted as evidence of safety without further research. Current evidence does not support using or avoiding ibuprofen specifically for breast cancer prevention or management, and its use should continue to be guided by approved indications and individual patient factors.

Future research should focus on validating these findings in diverse populations and elucidating the biological mechanisms underlying the observed associations. Prospective clinical trials specifically designed to evaluate the effects of these medications on breast cancer outcomes at different dosages and durations would provide more definitive evidence to guide clinical recommendations. Additionally, studies examining potential interactions between analgesic use and other breast cancer risk factors, treatments, or genetic susceptibilities could help identify patient subgroups most likely to benefit from specific medication strategies.

Conclusion

In conclusion, this Mendelian randomization study provides evidence suggesting that commonly used analgesics have distinct, and even opposing, causal effects on breast cancer. A lifelong genetic predisposition towards regular paracetamol use may increase the risk of ER+ breast cancer, while a similar predisposition to aspirin use may substantially improve overall survival. The divergent nature of these findings strongly suggests they are driven by drug-specific mechanisms rather than a single shared confounding pathway, such as the indication for use. While these genetic estimates require cautious interpretation, they highlight the need to evaluate the long-term cancer-related safety of common analgesics. Further research to validate these causal links and quantify dose-response relationships is essential to inform personalized pain management strategies.

Abbreviations

BC, breast cancer; BCAC, Breast Cancer Association Consortium; BMI, body mass index; CI, confidence interval; ER, estrogen receptor; FDR, false discovery rate; GWAS, genome-wide association study; HR, hazard ratio; IVs, instrumental variables; IVW, inverse-variance weighted; LD, linkage disequilibrium; MR, Mendelian randomization; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; PR, progesterone receptor; SNPs, single nucleotide polymorphisms; STROBE-MR, Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization; TSMR, two-sample Mendelian randomization; UKB, UK Biobank; WM, weighted median.

Ethical Approval

This study was exempt from institutional ethical review, as it used anonymized, publicly available data that pose no risk to human subjects, in accordance with Article 32 (items 1 and 2) of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (issued by the National Health Commission of China, 18 February 2023).

Informed Consent

This study did not require written informed consent from participants as it solely involved secondary data analysis of previously published genome-wide association studies (GWAS) data. All data utilized in this research were anonymized and publicly available, ensuring that no identifiable personal information was accessed or used. The ethical considerations

and consent processes of the original studies were thoroughly reviewed to confirm compliance with relevant ethical guidelines and standards.

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Author Contributions

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

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Disclosure

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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