RESEARCH ARTICLE

Causal relationship between other diseases of digestive system and breast cancer: A 2-sample Mendelian randomization study

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Abstract

Objective: To assess the causality between other diseases of digestive system and breast cancer.

Method: The 2-sample Mendelian randomisation study was conducted in 2023 at the First Affiliated Hospital of Bengbu Medical University, Bengbu, China, using data obtained from genome-wide association studies. A 2-sample Mendelian randomisation was done and inverse variance weighting was the major method to estimate the causality. The supplementary methods were Mendelian randomisation-Egger, weighted median, simple mode and weighted mode. Sensitivity analysis, heterogeneity and pleiotropy tests were used for ensuring the reliability of the findings.

Results: A causality was noted between other diseases of the digestive system and breast cancer utilising inverse variance weighting method (inverse variance-weighted odds ratios (ORIVW)=0.030429786, penalized inverse-variance weighted (PIVW) =0.024714444). The outcomes of supplementary methods were in line with that of inverse variance weighting method. In the reverse Mendelian randomisation analysis, there was no clear causal relationship between other diseases of the digestive system and breast cancer (p>0.05). Multivariate Mendelian randomisation analysis demonstrated that after adjusting for the influence of oestrogen, other diseases of the digestive system still had a significant direct impact on BC.

Conclusion: A causal relationship was noted between other diseases of the digestive system and breast cancer.

Keywords: Breast cancer, Digestive system, Oestrogen, Mendelian randomisation. (JPMA 75: S-119 [Suppl. 02]; 2025)

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Introduction

As a globally common cancer in female, most breast cancer (BC) deaths are caused by recurrence or distant tumour metastasis.[2] BC is a complex disease that is the result of multifactorial processes triggered by genetics, gene expression, or environmental changes.[3] Due to the implementation and advancement of various treatment methods, including radical surgery, radiotherapy, chemotherapy, targetted therapy and hormone therapy, the prognosis of BC patients has greatly improved.[4] However, many BC patients still experience recurrence, metastasis and treatment resistance.[5] Risk factors for BC include obesity, smoking and unbalanced dietary intake.[6] Studies have shown that BC patients have a higher detection rate of digestive diseases, including digestive system tumours, functional dyspepsia, gastrointestinal inflammation, and others.[7-12] Common digestive system diseases contain inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), autoimmune hepatitis, cholecystitis and pancreatitis.[13-15] It has been reported that IBD patients have an increased risk of developing BC, and first-degree relatives of IBD patients are more likely to develop BC.[16] In addition, studies have shown that the occurrence and clinicopathological characteristics of BC are closely related to the imbalance of intestinal flora, which can influence the occurrence and development of BC via modulating oestrogen metabolism and immune function.[17] Apparent differences in the gut microbiota between BC patients and healthy people have been documented, suggesting that they take part in BC progression.[18] Nevertheless, the causality between other diseases of digestive system and BC is unclear.

Mendelian randomisation (MR) refers to a genetic epidemiological method widely utilised in causal reasoning.[19,20] Traditional epidemiological causal inference is hampered by reverse causality and confounding factors, resulting in insufficient evidence. By utilising genetic variants, like single nucleotide polymorphisms (SNPs), which can alter disease risk factors, MR studies can enhance causal reasoning about exposure-outcome relations.[21-23] In comparison with standard multivariate regression methods, MR has been confirmed to be more reliable in terms of measurement error, confusion and reverse causation.[24] This is so because alleles are fixed at birth and would not alter as the disease progresses.[25] Additionally, since genetic variants are distributed at conception in random, they cannot be affected by bias and confounding factors (including postnatal environment, socioeconomic status, and behavioural factors) that may affect the outcomes of observational studies.[26] Therefore, MR can be

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regarded as a randomised controlled trial (RCT).[27] By identifying numerous genetic variants that are closely linked to specific traits in biology, and the availability of aggregated data on the relationship among exposure, disease, and genetic variants from a wide range of genome-wide association studies (GWAS), researchers can assess genetic associations using a wide range of datasets.[28]

The current study was planned to use MR analysis to explore the causality between other diseases of the digestive system and BC.

Materials and Methods

The 2-sample MR study was conducted at the First Affiliated Hospital of Bengbu Medical University, Bengbu, China, in 2023, and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)-MR statement Figure 1A-B).[29]

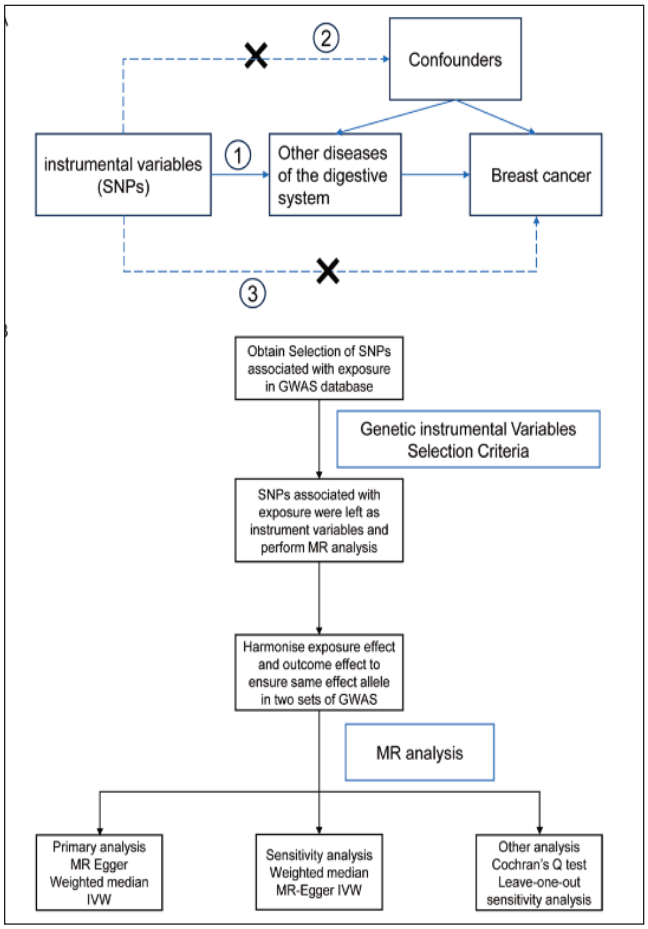

Figure-1: Research principles and schematic design of a two-sample Mendelian randomisation (MR) study. (A) The three assumptions for MR analysis include relevance assumption, exclusive restrictions, and independence assumption. (B) The flowchart of the current study.
SNPs: Single nucleotide polymorphisms, IVW: Inverse variance weighted.

GWAS data of other digestive diseases was acquired from a GWAS analysis of 361,194 participants of European ancestry by Zhang et al.[30] GWAS data of BC was obtained from a GWAS analysis by Michailidou K et al. that had 33,832 subjects of European ancestry, including 15,748 cases and 18,084 controls.[31] GWAS data for oestrogen was acquired from a GWAS analysis conducted by Litton et al. with 10,534,735 cases of samples.[1] The data came from publicly available repositories and did not require any further ethical approval or patient consent.

The MR study required the hypotheses that instrumental variables were strongly correlated with exposure, not with exposure and outcome, or only with exposure and outcome.[32]

The screening criteria for the exposure instrumental variables had several elements. The primary screening criterion for SNP in GWAS was $p<5\times10\text{-}8$. To avoid results bias, SNPs with linkage disequilibrium $r^2<0.01$ together with a clump distance >10,000kb window were excluded.[33] The instrumental variables in GWAS data were extracted from the screened SNPs. F-statistic was used to calculate the potency of each SNP as an instrumental variable, with F<10 indicating that the genetic variation adopted may subject the results to some bias[34] and then it could be removed to avoid affecting the results. F-statistic was based on the formula:[35]

$$F=(N-k-1)/k\times R^2/(1-R^2)$$

The F-statistic was calculated from the sample size (n), the number of instrumental variables (k), as well as the variance explained by instrumental variables (R^2).[35] The design formula of R^2 was: $R^2=2\times$(1-major allele frequency [MAF]) $\times$ MAF $\times$ beta (β).[2] MAF was the minimum allele frequency while β was the allele effect value.

To evaluate the causal effect of exposure on outcome, 2-sample MR methods containing inverse variance weighted (IVW), MR-Egger, weighted median (WM) method, simple mode as well as WM were employed. IVW method is considered to be a more robust method for MR analysis.[36] Therefore, in the absence of pleiotropy, IVW was the main MR analysis method in the current study, and the others served as supplements. Reverse causality was evaluated in the same way.

Sensitivity analysis was conducted through various methods, such as heterogeneity test, pleiotropy test, and the leave-one-out test. Cochran's Q test was a method utilised for assessing heterogeneity of MR analysis. P<0.05 in the Cochran's Q test reflected the presence of significant heterogeneity. The highly heterogeneous results were evaluated using the IVW random effect model to assess the

magnitude of causal effects. Cochran's Q test could only detect the presence or absence of heterogeneity. I2 statistic (I2 represents the percentage of the total variation in the effect size between studies that can be attributed to heterogeneity rather than sampling error) was used for reflecting the proportion of heterogeneity, with I2=0 indicating that each study was completely homogeneous. MR-Egger method was used to perform a pleiotropy test on instrumental variables. P<0.05 suggested notable horizontal pleiotropy of genetic variation. The leave-one-out sensitivity test calculated the MR outcomes, excluding one instrumental variable at a time, to measure whether SNPs influenced the relation between other diseases of the digestive system and BC. If a significant difference was found between the MR effect estimation and the total effect estimation followed by removing a certain instrumental variable, the MR effect estimation was considered sensitive to the SNP.

The IVW method, the primary research method, used a meta-analysis that combined Wald ratios of individual SNPs to produce accurate estimates. WM, together with MR-Egger, served as additional tests for MR estimation. The WM method was more effective when half of the genetic variation was not effective. The MR-Egger relied on the hypothesis that "instrument strength is independent of direct effects" and that exposure and results were independent. P<0.05 indicated the presence of horizontal pleiotropy.

Cochran's Q test was employed for examining the heterogeneity of SNPs included in each analysis. If the Cochran's Q test was statistically significant, it indicated significant heterogeneity. MR-Egger regression test was employed to analyse the horizontal pleiotropy of genetic variation.[37] Finally, leave-one-out sensitivity test was employed to estimate whether the outcomes are strongly driven via a single SNP.

All data calculations and statistical analysis were implemented in R version 4.3.1 using the two-sample MR package.[38] All statistical tests were conducted by bilateral tests, in which the SNP loci generated by GWAS studies had statistical significance $p<5\times10\text{-}8$, and the SNP sites generated by other statistical tests had statistical significance $p<0.05$.

Results

In the current study, SNPs in linkage disequilibrium were excluded according to the screening criteria of instrumental variables, and SNPs related to other diseases of the digestive system were included in the instrumental variables after matching with the GWAS data of BC (Table 1). The F value of the instrumental variables of these indicators was >10, implying that the SNPs included in the study were mostly strong instrumental variables, and the underlying bias resulted by weak instrumental variables was limited.

MR results for two-sample analysis, MR-Egger, WM, IVW, simple mode as well as WM were utilised for analysis. The IVW model confirmed the significant causal relationship between BC and other diseases of the digestive system (Table 2). Other digestive system diseases could cause an increased risk of BC (ORIVW=0.030429786, PIVW= 0.024714444). There was a linear relationship between instrumental variables and the risk effects of other digestive system diseases and BC under the five models (Figure 2A). The slope represented the the strength of the linear dependence of the instrumental variable on the exposure effect and the outcome effect. Finally, the forest plot of effect values of instrumental variables for other diseases of the digestive system on outcomes also confirmed the finindgs (Figure 2B).

Sensitivity analysis and Cochran's Q test were employed for detecting the heterogeneity of MR-Egger and IVW outcomes. There was a heterogeneity between MR results of other diseases of the digestive system and BC (Table 3). Through the leave-one-out analysis, the instrumental variable loci were removed one by one to analyse the causal effect on other diseases of the digestive system and

Table-1: Instrumental variables for other diseases of the digestive system and for breast cance (BC) risk.

Exposure	SNP	beta.exposure	*p*val.exposure	FSTAT
Other diseases of the digestive system	rs2854275	0.00614991	4.38E-41	180.2436772
Other diseases of the digestive system	rs3132449	0.00540662	7.47E-30	128.831082
Other diseases of the digestive system	rs9274247	0.00363802	3.06E-21	89.51564145
Other diseases of the digestive system	rs9469573	0.00510294	1.45E-09	36.59814535

SNPs: Single nucleotide polymorphisms, FSTAT: F statistics.

Table-2: Instrumental variables for other diseases of the digestive system and for breast cance (BC) risk.

Exposure	Method	number of SNPs	β (95% CI)	OR (95% CI)	p-value
Other diseases of the digestive system	MR Egger	4	-7.443117312	0.000585457	0.412459102
Other diseases of the digestive system	Weighted median	4	-3.069055938	0.046465	0.096832225
Other diseases of the digestive system	Inverse variance weighted	4	-3.492333339	0.030429786	0.024714444
Other diseases of the digestive system	Simple mode	4	-2.183346585	0.11266386	0.461321103
Other diseases of the digestive system	Weighted mode	4	-2.667019947	0.069458908	0.336743838

β: MR analysis effect coefficients, OR: Odds ratio, CI: Confidence interval, SNP: Single nucleotide polymorphism.

BC (Figure 3A, Table 4). There was not much deviation between the combined effects of the remaining SNPs and the overall results. The scatter plot showed that the scatter points of IVW model of other diseases of the digestive system were relatively symmetrical (Figure 3B), suggesting that the results did not have potential bias.

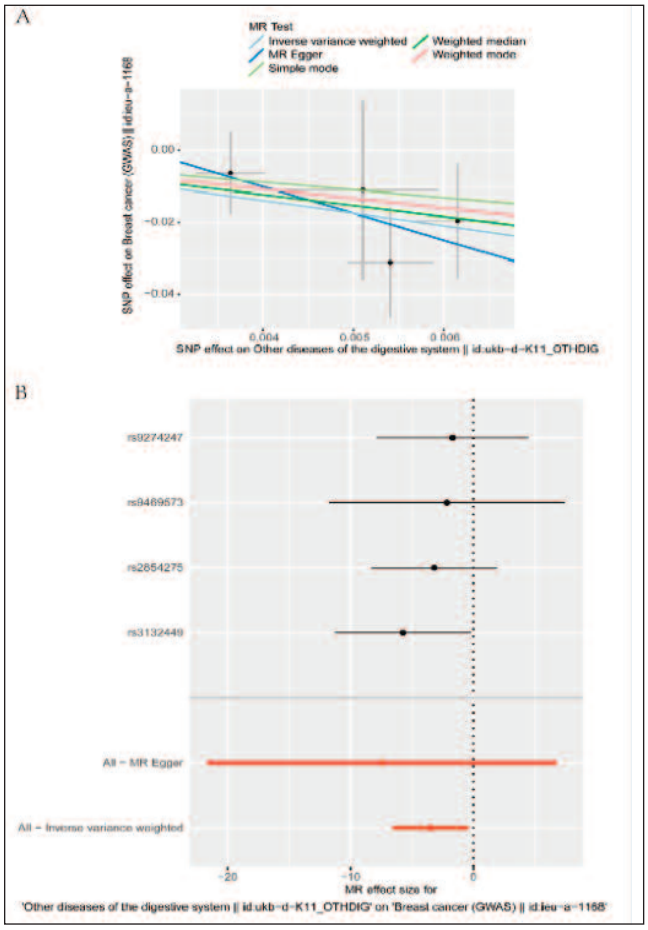

Figure-2: MR analysis results of causality between other diseases of the digestive system and breast cancer (BC), and effect estimation using single SNP locus analysis. (A) Scatter plot showing the effects of SNP on BC under various methods. (B) Forest plot of the effect values of various instrumental variables on outcomes of other diseases of digestive system.
SNPs: Single nucleotide polymorphisms, MR: Mendelian randomisation, GWAS: Genome-wide association studies.

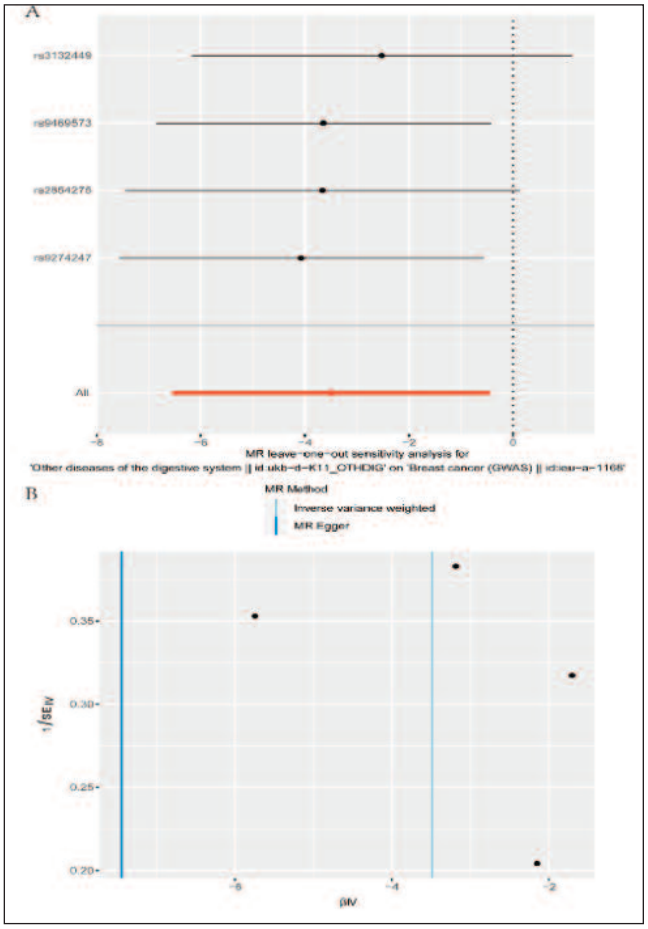

Figure-3: The leave-one-out analysis and funnel plots. (A) The leave-one-out analysis of causality between other diseases of the digestive system and breast cancer (BC) by each instrumental variable. (B) Funnel plot for heterogeneity test of other diseases of the digestive system.
MR: Mendelian randomisation, GWAS: Genome-wide association studies.

Table-3: Cochran Q test results.

Exposure	Method	Q	Q df	Cochran Q p-value	I2 (%)
Other diseases of the digestive system	MR Egger	0.729930803	2	0.69422067	63.5
Other diseases of the digestive system	Inverse variance weighted	1.041330989	3	0.791252462	65.2

Q: Test statistic of Cochran Q, Q df: Degree of freedom of Q, MR-Egger: Mendelian randomisation-Egger.
I2 statistics reflect the proportion of heterogeneity attributed to instrumental variables in total variability.

Table-4: Cochran Q test results.

Exposure	SNP	β	SE	*p*-vlaue
Other diseases of the digestive system	rs2854275	-3.66061067	1.935604781	0.058598002
Other diseases of the digestive system	rs3132449	-2.521655572	1.860163384	0.175223376
Other diseases of the digestive system	rs9274247	-4.066532451	1.787744644	0.022925621
Other diseases of the digestive system	rs9469573	-3.642802157	1.639911674	0.02632791
Other diseases of the digestive system	All	-3.492333339	1.555025221	0.024714444

SNPs: Single nucleotide polymorphisms, SE: Standard error.

MR-Egger intercept test was used for pleiotropy test, and the outcomes manifested that the intercept terms of other diseases of the digestive system were significantly greater (p>0.05), implying that the causal inference could be not influenced by horizontal pleiotropy (Table 5).

For MR analysis of the risk of BC in relation to other diseases of the digestive system, MR-Egger, WM, IVW were used. The reverse causal MR analysis illustrated that the risk of BC had no causal effect on other diseases of the digestive

Table-5: Horizontal pleiotropy test of the relationship between other diseases of the digestive system and breast cancer (BC).

Exposure	MR-Egger intercept	Standard error	*p*-value
Other diseases of the digestive system	0.019751153	0.035394276	0.632952845

MR-Egger: Mendelian randomisation-Egger.

Table-6: Mendelian randomisation (MR) analysis of breast cancer (BC) risk on other diseases of the digestive system.

Exposure	Method	Number of SNPs	Beta. Exposure	*p*-alue
Breast cancer	Inverse variance weighted	60	9.55E-05	0.8581228

SNPs: Single nucleotide polymorphisms.

Table-7: Multivariate Mendelian randomisation (MR) analysis.

Model	Exposure	Number of SNPs	OR (95% CI)	*p*-value
Model 1	Breast cancer anti-oestrogen resistance protein 3	2	0.982383236	0.22798644
Model 1	Other diseases of the digestive system	3	0.086854862	0.009783348

SNPs: Single nucleotide polymorphisms, OR: Odds ratio, CI: Confidence interval.

system (p>0.05) (Table 6).

In multivariate MR analysis, oestrogen exposure was added for multivariate MR analysis. Model 1 was corrected for indirect effects of oestrogen, and it was displayed that other digestive system diseases still possessed significant direct effects on BC (Table 7).

Discussion

The current study adopted GWAS dataset for 2-sample MR analysis to evaluate the causality between other digestive system diseases and the risk of BC. The outcomes demonstrated that other diseases of digestive system were a risk factor for BC. When exploring the causal relationship between other diseases of digestive system and BC, digestive system diseases were only identified as potential risk factors for BC.

In recent years, some epidemiological studies have pointed out the connection between digestive system diseases and BC. A retrospective study suggested that the incidence rate of gastrointestinal diseases was 44.4% in non-elderly BC patients and 57% in elderly BC patients.[39] Additionally, a study showed an elevated incidence of BC 8-11 years after diagnosis of IBD, suggesting that IBD may be significantly linked to the risk of BC in IBD patients in specific age groups.[40] A case-control study showed that 22 relatives of Crohn's patients were diagnosed with BC, including 18 mothers. In the control group, 8 relatives were diagnosed with BC, including 5 mothers. Mothers of Crohn's patients had a higher risk of BC relative to the control group.[41] Overall, these observational studies collectively suggest that diseases of the digestive system may increase the risk of BC. In the current study, the causal relationship between other diseases of the digestive system and BC was further investigated by MR analysis. MR analysis estimates causality via linking the exposure and the outcome, and reduces the risk of confounding factors of traditional observational studies.[42] GWAS was used for assessing the causality between other diseases of digestive system and BC. MR analysis was performed using GWAS data and a notable causal relationship was discovered between other diseases of digestive system and BC (ORIVW=0.030429786, PIVW= 0.024714444). The reverse causal MR analysis results showed that the risk of BC had no causal effect on other digestive system diseases. In addition, multiple sensitivity analysis was implemented to assess and adjust the impact of horizontal pleiotropy on the outcomes. All analyses demonstrated that the findings were consistent and reliable. Thus, there is reason to believe that there is a strong correlation between other digestive diseases and BC. Consistently, Guo et al. also performed a 2-sample bidirectional MR study and indicated a bidirectional causal effect of IBD and BC.[43]

A multivariate MR study was conducted to validate the results and explore possible mediators. Oestrogen is thought to be a pathogenic factor of hormone receptor (HR)-positive BC and has a crucial role in promoting tumour growth.[44] A cohort study has shown that a relative excess of oestrogen during pregnancy is linked to an elevated risk of BC in daughters born to such mothers.[45] The current study proved that after adjusting for the effect of oestrogen, other digestive system diseases still had a significant direct impact on BC. Likewise, Chapadgaonkar et al. indicated that the gut microbiota enhances the availability of oestrogen in the host during digestion and homeostasis, which may influence the incidence of hormone-induced BC.[46]

There are several explanations for the important causal relationship between digestive system diseases and BC. Many cancers are caused by infection, chronic irritation and inflammation.[47] Researches have shown that long-term impacts of the inflammatory response are associated with adverse outcomes of BC.[48] Long-term chronic inflammation may participate in tumour development and metastasis by producing inflammatory mediators and participating in angiogenesis and EMT process.[49] In addition, changes in gut microbiota are a key factor in the development of digestive system diseases.[50] The imbalance of gut microbiota has also been proven to be closely related to BC progression.[51] Therefore, it is very important to elucidate the pathogenesis of digestive diseases and BC.

The current study has limitations. The limited data on individuals of non-European ancestry may have had a certain impact on the generalisability of the findings. In addition, the limited number of SNPs available in the MR analysis may have led to bias in the results.

Conclusion

The 2-sample MR study indicated a causality between other diseases of digestive system and BC, which provided support for digestive system diseases as a risk factor for BC.

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Conflict of Interest: None.

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