Body Mass Index and Causal Relationships with Four Characteristic Female Cancers: A Two-Sample Mendelian Randomization Study

YIN Yulai[1*], ZHANG Xiaoyu[2]

[1]Cangzhou Central Hospital, Hebei Medical University,Cangzhou 061000 , China
[2]Department of Thyroid and Breast Surgery III, Cangzhou Central Hospital,Cangzhou 061000, China
*Corresponding author Email: 1972688583@qq.com

Abstract: This study employs a two-sample Mendelian randomization (MR) approach to investigate the causal relationship between increased Body Mass Index (BMI) and four characteristic female cancers. BMI and data pertaining to the four characteristic female cancers were obtained from the GWAS database. Single nucleotide polymorphisms (SNPs) were selected as instrumental variables (IVs) based on assumptions. The PhenoScanner method was utilized to eliminate SNPs associated with confounding factors. Five Mendelian randomization analysis methods, including inverse variance-weighted (IVW), were employed for two-sample Mendelian randomization analysis. Cochran Q and Rücker Q heterogeneity tests were conducted using IVW and MR-Egger methods. Egger-intercept method was employed for pleiotropy testing, and stepwise exclusion testing for sensitivity analysis. F-values were calculated to assess the presence of weak IV bias. Genetically predicted increase in BMI was causally associated with reduced risk of breast cancer (OR=0.648, 95% CI: 0.535-0.783, P=7.74e-06), and increased risk of endometrial cancer (OR=1.534, 95% CI: 1.195-1.970, P=7.84e-04). There was insufficient evidence to suggest a causal relationship between genetically determined BMI increase and other characteristic female cancers studied. Increased Body Mass Index may potentially decrease the risk of female breast cancer while increasing the risk of endometrial cancer. There is inadequate evidence to indicate a significant impact of increased BMI on the occurrence risk of other characteristic female cancers studied. Further research is warranted to elucidate these findings.

Key words: Mendelian Randomisation, Body Mass Index, breast cancer, endometrial cancer, ovarian cancer, cervical cancer

1.Background:

Cancer poses a significant threat to human health, exerting a critical influence on prognosis and quality of life. As female populations increasingly prioritize their health, preventing cancer occurrence becomes a pivotal measure for enhancing women's overall well-being[1]. Among all malignancies, female breast cancer, endometrial cancer, ovarian cancer, and cervical cancer are characteristic cancers specific to the

female population, constituting significant threats to women's health[2].

Body Mass Index (BMI) is an internationally recognized metric for evaluating individual obesity, calculated as body weight (kg) divided by the square of height (m) [3-5]. With improving living standards worldwide, dietary constraints have diminished, coupled with a lack of physical exercise in a majority of populations, resulting in a gradual increase in the global obese population. Data shows that since 1975, the global obesity count has nearly tripled, and research indicates higher mortality rates in countries with a larger proportion of obese individuals. Additionally, studies suggest an association between obesity and the incidence of certain cancers.

Increased BMI may elevate cholesterol, low-density lipoprotein, and very low-density lipoprotein levels in the blood. The carcinogenic effect of cholesterol may manifest through the Hedgehog pathway. In healthy individuals, this pathway is largely inactive, but cholesterol binding to the G-protein-coupled receptor Smoothened (Smo) can activate it[6]. Once activated, this pathway influences the survival, proliferation, and migration of tumor stem cells. Furthermore, some scholars suggest an inverse correlation between the use of statin drugs, which lower cholesterol and low-density lipoprotein, and cancer incidence. However, this conclusion is speculative and uncertain. Therefore, this study conducts a two-sample Mendelian randomization research to explore the causal relationship between increased BMI and the risk of characteristic female cancers.

Mendelian Randomization Study

Mendelian Randomization (MR) is a commonly employed epidemiological research method in recent years[7-11], primarily based on single nucleotide polymorphisms (SNPs) to infer causal relationships between exposure and disease outcomes through genetic variation. In MR studies, phenotype-associated genetic variations are utilized as instrumental variables for exposure, allowing for causal inferences of exposure-outcome associations. Genetic variations adhere to the rules of random segregation from parents to offspring and are determined by genetic variations at conception, thus making them less susceptible to population confounding factors in traditional observational studies. Currently, Mendelian randomization has been applied in various medical disciplines. For instance, in the field of nutrition, Paul Carter et al.'s Mendelian randomization study demonstrates a positive causal association between coffee consumption and the occurrence of certain cancers[12]. In epidemiology, Shili Xue et al. investigated the causal relationship between serum uric acid levels and 136 health outcomes, revealing that elevated serum uric acid levels were only confirmed exposure factors for gout and kidney stones. This demonstrates the crucial role of Mendelian randomization studies in exploring causal relationships between exposure and outcomes in medical research. Furthermore, a randomized clinical trial related to BMI and breast cancer suggests that compared to women of normal weight, overweight and obese women have an increased risk of invasive breast cancer. BMI $\geqslant$35.0 kg/m^2 is an independent risk factor for estrogen receptor-positive and progesterone receptor-positive breast cancer, but is unrelated to estrogen receptor-negative breast cancer. It was also found that changes in weight during the follow-up period (increase or decrease) were unrelated to breast cancer[13]. Therefore, caution is needed in generalizing and extrapolating the impact of increased BMI on the occurrence of different

types of cancer.This study conducts a two-sample Mendelian randomization analysis to explore the causal relationship between increased BMI and four characteristic female cancers: breast cancer, endometrial cancer, ovarian cancer, and cervical cancer.

2.Methods

The data utilized in this study were sourced from previously published research or public databases, specifically the GWAS (Genome-Wide Association Studies) database. Consequently, ethical committee approval was not required.

2.1 Exposure and Outcome Measurement

The exposure variable was BMI increase, encoded as "ebi-a-GCST90095039" in the GWAS dataset, which encompassed a mixed population dataset comprising 330,793 samples. To minimize the impact of linkage disequilibrium (LD), we opted for single nucleotide polymorphisms (SNPs) meeting established genome-wide significance thresholds ($P<5\times10\text{-}8$, $r2\leqslant0.001$, adhering to Hardy-Weinberg equilibrium (H-W), genetic distance <10000kb) as instrumental variables (IVs). The F-values of IVs were computed, ensuring that IVs with F>10 were incorporated into the study to mitigate biases stemming from weak instrumental variables. Instrumental variable selection criteria entailed that the instrumental variables exclusively exert influence on the outcome through the exposure variable BMI increase; instrumental variables do not influence the outcome through confounding factors; and instrumental variables do not directly impact the outcome, as depicted in Figure 1.

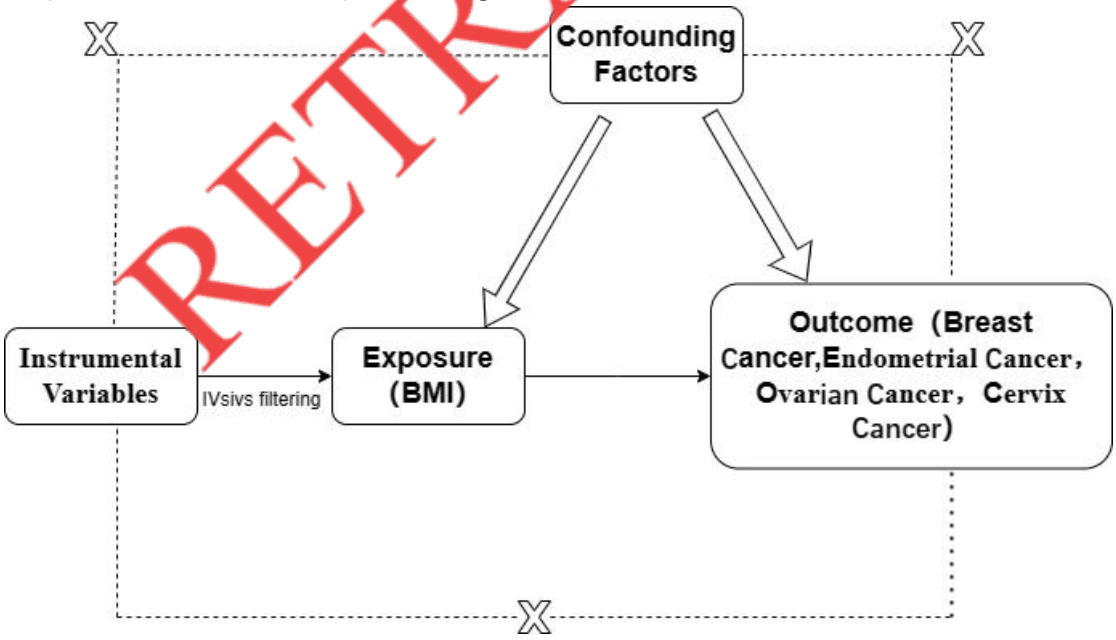

Figure 1: Instrumental Variables Selection Principle

The outcome factors comprise four distinct female-specific cancer types, namely breast cancer, endometrial cancer, ovarian cancer, and cervical cancer, with their respective GWAS data codes being "ieu-a-1131", "ebi-a-GCST90018838", "ebi-a-GCST90018888", and "ukb-b-918". All data have been

derived from a meta-analysis of GWAS studies. Detailed information regarding the data is provided in Table 1. Our study solely relies on published GWAS results and does not involve individual-level data. All summary data for exposures and outcomes were obtained from the publicly accessible Genome-Wide Association Studies (GWAS) database (https://gwas.mrcieu.ac.uk/).

Exposure	Outcome	Cases(n)	Controls(n)	Size(n)
BMI		-	-	330793
	Breast Cancer	14910	17588	32498
	Endometrial Cancer	2188	237839	240027
	Ovarian Cancer	1588	244932	246520
	Cervical Cancer	3175	459835	463010

Table 1: Exposure and Outcome Data Information

2.2 Mendelian Randomization

The MR analysis was conducted on the MR base online platform (https://app.mrbase.org/). This study explores causal relationships between exposure and outcome using a two-sample MR framework. Specifically, SNP exposures (Body Mass Index) and SNP outcomes (female-specific cancers: breast, endometrial, ovarian, and cervical) were incorporated to investigate the causal relationship between BMI and these four female-specific cancers. To eliminate SNPs associated with confounding factors, this study employed the PhenoScanner method. The Mendelian randomization analysis employed five MR analysis methods, with the Inverse Variance Weighting (IVW) method serving as the primary approach. Heterogeneity testing was performed using both the IVW and MR Egger methods. If Cochran's Q test yielded a P-value < 0.05, it indicated heterogeneity among single nucleotide polymorphisms. The final Mendelian randomization analysis was conducted using the random effects model of the IVW method. The MR-Egger-intercept method was used to test for horizontal pleiotropy.

3. Results

3.1 Heterogeneity Testing

Heterogeneity tests were performed using the Inverse Variance Weighting (IVW) method and the MR-Egger method, employing Cochran Q and Rücker Q tests. The results revealed significant heterogeneity among internal SNPs in the breast cancer and endometrial cancer groups ($P < 0.05$). Conversely, heterogeneity within internal SNPs in the ovarian cancer and cervical cancer groups was not substantial ($P > 0.05$). Consequently, the final Mendelian randomization analysis for the breast cancer and endometrial cancer groups employed the random effects model of the IVW method, while the ovarian cancer and cervical cancer groups utilized the fixed effects model of the IVW method. Refer to Table 2 for detailed results.

	IVW（P-value）	MR-Egger(P-value)

Breast Cancer	0.0008528	0.0008864
Endometrial Cancer	0.0287088	0.0269904
Ovarian Cancer	0.8860324	0.8826452
Cervical Cancer	0.0535872	0.0464912

Table 2: Heterogeneity Testing Results

3.2 Pleiotropy Analysis

The Egger-intercept method was employed to assess horizontal pleiotropy. The results indicated that there was no significant association between the causal relationship of the exposure and outcome factors for breast cancer, endometrial cancer, ovarian cancer, and cervical cancer, and horizontal pleiotropy. This implies that Mendelian randomization analysis can be conducted without concern for horizontal pleiotropy. Refer to Table 3 for the results of the horizontal pleiotropy test.

	Egger-intercept(P-value)
Breast Cancer	0.3308104
Endometrial Cancer	0.4706562
Ovarian Cancer	0.4070501
Cervical Cancer	0.9090576

Table 3: Horizontal Pleiotropy Test Results

3.3 Sensitivity Analysis

The funnel plots representing the causal association between BMI increase as the exposure factor and the outcome factors of the four female-specific cancers display a generally symmetrical distribution when using individual SNPs as instrumental variables. This suggests a low likelihood of potential bias in the causal association (see Figure 4). Sensitivity analysis using the leave-one-out method revealed that after sequentially excluding each SNP, the results of the IVW analysis for the remaining SNPs were similar to the analysis including all SNPs (see Figure 5). No SNPs were identified to have a significant impact on the exposure and outcome factors.

3.4 Mendelian Randomization Analysis Results

A two-sample Mendelian randomization study was conducted to investigate the causal relationship between BMI increase as the exposure factor and four female-specific cancers: breast cancer, endometrial cancer, ovarian cancer, and cervical cancer. Five MR analysis methods were employed, with the Inverse Variance Weighting (IVW) method serving as the primary approach. Odds ratios (OR), 95% confidence intervals, and P-values were calculated for each of the four groups. The results are presented in Table 4. A causal relationship was observed between BMI increase and breast cancer as well as endometrial cancer. The P-values for almost all five MR analysis methods were less than 0.05, indicating robustness and reliability in the study results. Refer to Figures 2, 3, 4, and 5 for visual representations of the results.

	OR(Odds Ratio)	95%CI	P-value
Breast Cancer	0.648	0.535-0.783	7.74e-06*
Endometrial Cancer	1.534	1.195-1.970	7.84e-04*
Ovarian Cancer	0.901	0.695-1.168	0.4296406
Cervical Cancer	1.000	0.999-1.002	0.7945332

Table 4: Mendelian Randomization Analysis Results using IVW Method(The table presents the results of Mendelian randomization analysis using the Inverse Variance Weighting (IVW) method. Statistically significant differences (P<0.05) are indicated with an asterisk (*). The results show the odds ratio (OR), 95% confidence interval, and P-value for each cancer type).

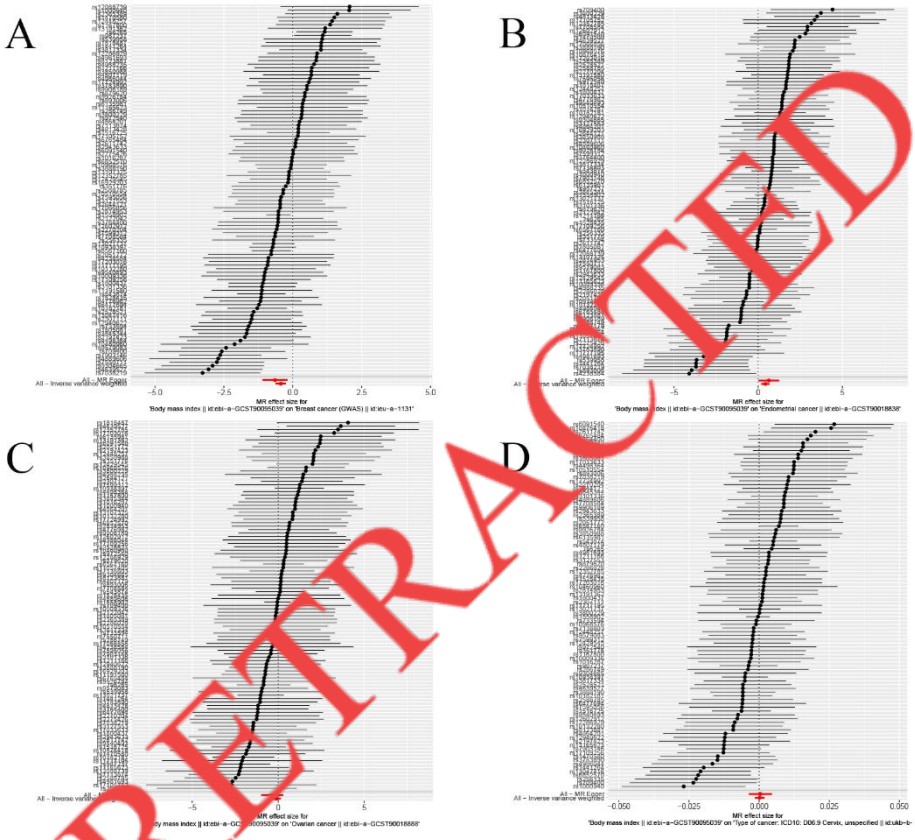

Figure 2: Forest Plots of Two-Sample Mendelian Randomization Results

A. Forest plot depicting the results for the group of female breast cancer as the outcome factor.

B. Forest plot displaying the results for the group of endometrial cancer as the outcome factor.

C. Forest plot illustrating the results for the group of ovarian cancer as the outcome factor.

D. Forest plot showing the results for the group of cervical cancer as the outcome factor.

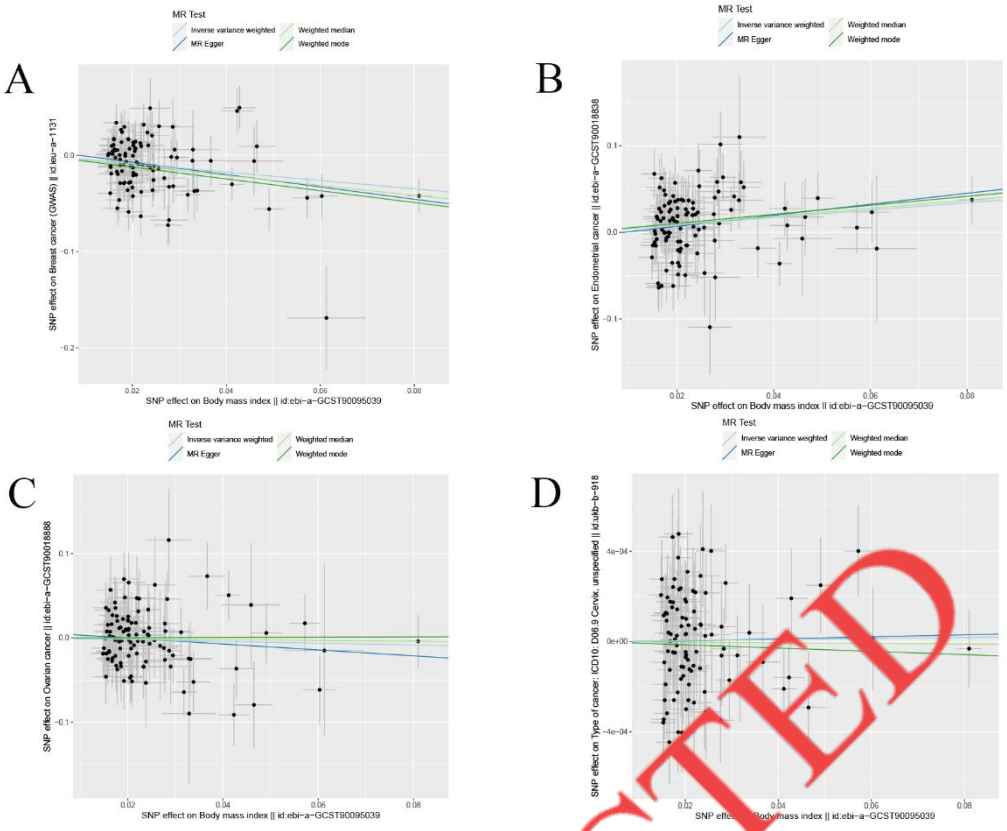

Figure 3: Scatter Plots of Two-Sample Mendelian Randomization Results

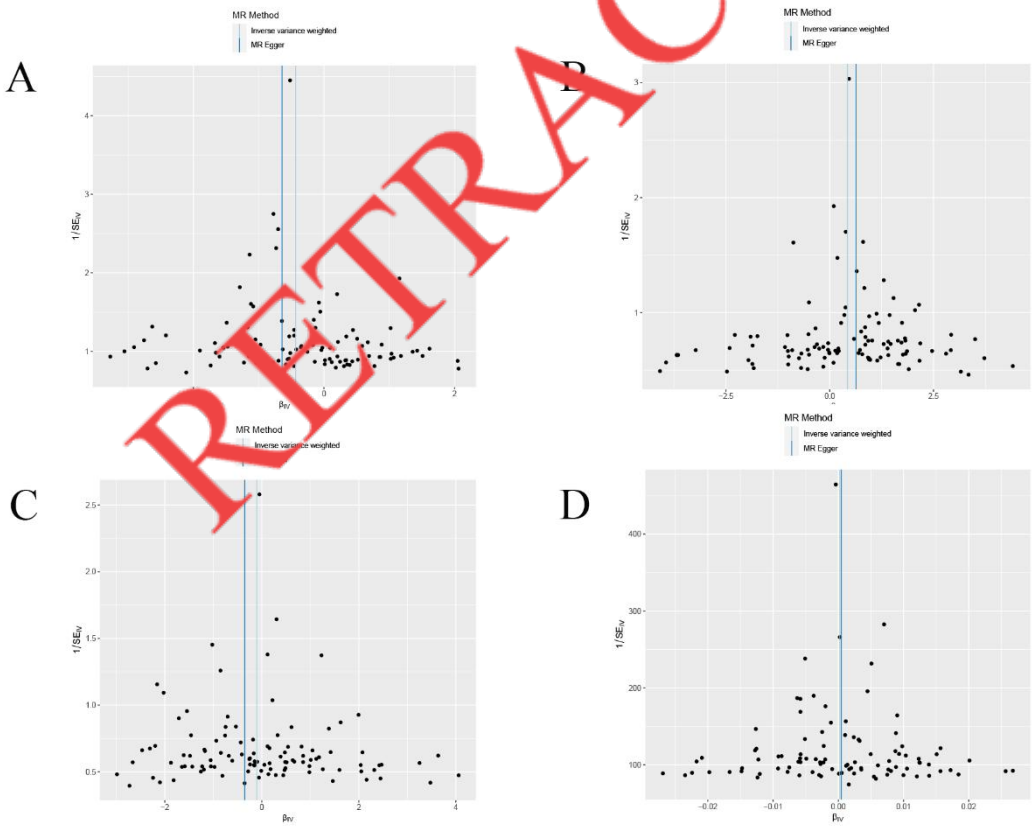

Figure 4: Funnel Plots of Two-Sample Mendelian Randomization Results

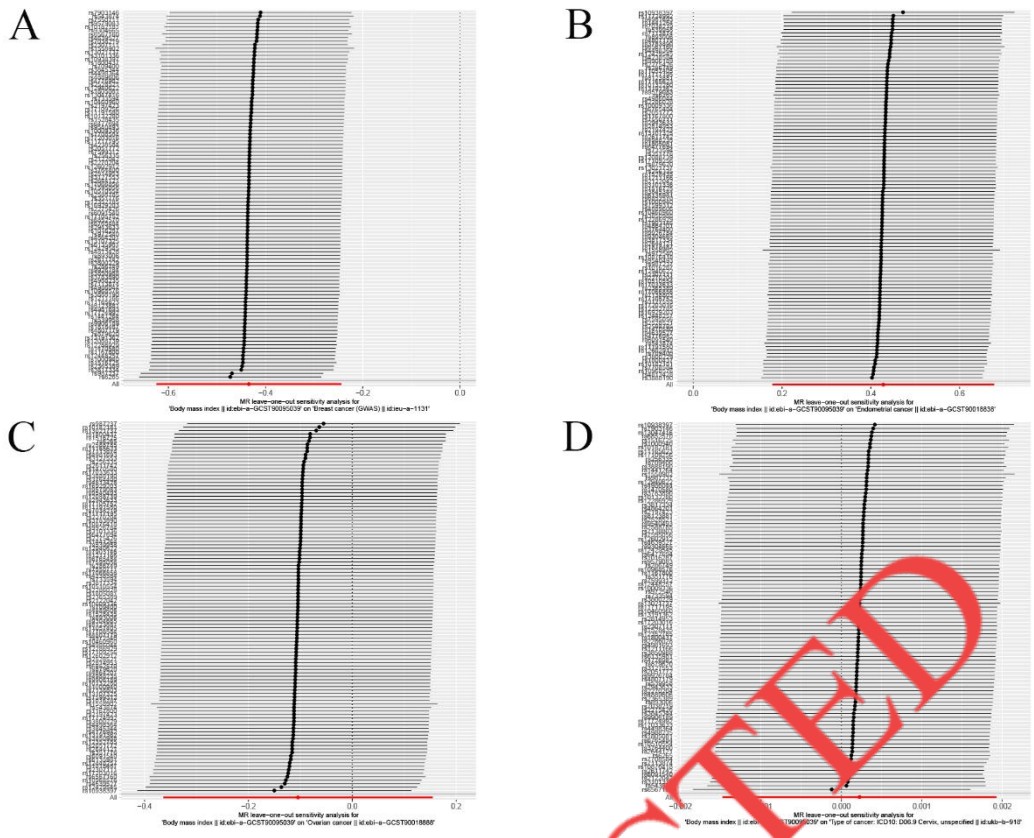

Figure 5: "Leave-On[illegible]-Out" Plots

4.Discussion

This study utilized two-sample Mendelian randomization analysis to establish a negative causal association between BMI increase exposure and female breast cancer outcomes (OR=0.648, 95%CI: 0.535-0.783, P=7.74e-06). This suggests that the genetic variation associated with increased BMI may serve as a protective factor against breast cancer in females. However, this finding appears contradictory to the conclusion by Huang et al. that central obesity may elevate the risk of breast cancer in perimenopausal women. Additionally, the study's results indicate that the impact of central obesity on breast cancer risk may vary depending on the different estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 statuses.

Research conducted by Manuel Picon-Ruiz et al. supports a negative correlation between BMI increase and breast cancer risk in premenopausal women, which aligns with the results of this study. Furthermore, a meta-analysis incorporating nine studies suggests a negative correlation between premenopausal breast cancer risk and obesity (RR=0.98, 95%CI: 0.97-0.99). Another large-scale meta-analysis covering over 2.5 million women and 7930 cases of premenopausal breast cancer indicates that with every 5kg/m^2 increase in BMI, the risk of premenopausal breast cancer decreases by approximately 8% (RR=0.92, 95%CI: 0.88-0.97, P=0.001). Discrepancies in conclusions between different studies may be attributed to variations in hormone receptor statuses.Existing research results predominantly affirm a negative correlation between BMI increase and breast cancer incidence. However, for Asian women, there appears to be a positive correlation between BMI increase and breast

cancer incidence.

The study's Mendelian randomization analysis also demonstrated a positive causal association between BMI increase exposure and female endometrial cancer outcomes. The tumorigenic mechanisms associated with BMI increase might involve obesity-induced local and systemic pro-inflammatory cytokines, promoting tumor angiogenesis, and stimulating the most malignant cancer stem cell populations to drive cancer cell growth, invasion, and metastasis. Alternatively, BMI increase may lead to higher levels of cholesterol, low-density lipoprotein, and very-low-density lipoprotein in the blood. The pro-carcinogenic effect of cholesterol may manifest through the Hedgehog pathway, where the binding of cholesterol to the G protein-coupled Smoothened receptor (Smo) activates the pathway, subsequently leading to the survival, proliferation, and migration of tumor stem cells. Studies indicate that the impact of BMI increase on endometrial cancer risk surpasses that of any other cancer type[14]. A meta-analysis comprising 30 prospective studies demonstrates that for every 5kg/m^2 increase in BMI, the risk of endometrial cancer rises by 54% (95%CI: 47%-61%). This conclusion aligns with the findings of this study and is largely consistent with current research, with little academic dissent regarding the positive causal association between BMI increase and endometrial cancer.

Notably, BMI increase did not exhibit a causal association with ovarian and cervical cancer in this study. However, numerous other studies suggest positive associations between BMI increase and various cancers, as well as other disease outcomes[5,15-20]. Therefore, maintaining a healthy BMI through balanced nutrition holds significant importance in cancer and disease prevention. Given that this study is based on a two-sample Mendelian randomization analysis focused on female-specific cancers, there are limitations to the generalization of its conclusions. Therefore, large-scale, multicenter case-control studies and prospective cohort studies are crucial for verifying the causal relationship between BMI increase and female-specific cancers. This, in turn, would provide scientific recommendations for the prevention of female-specific cancers and weight management.

Conclusion:

In summary, genetically predicted BMI increase was found to have a clear negative causal association with female breast cancer, and a clear positive causal association with endometrial cancer. However, BMI increase did not show a causal association with ovarian or cervical cancer. The study is subject to limitations including potential confounders and biases, and further Mendelian randomization and clinical studies are needed to confirm the precise causal relationships between exposure and outcomes.

5. Conclusion

In summary, this study identified clear causal associations between genetically predicted BMI increase

and female breast cancer (negative) and endometrial cancer (positive) using two-sample Mendelian randomization analysis. However, there was no observed causal relationship between BMI increase and ovarian or cervical cancer. The study's results are subject to limitations, including potential confounders and biases, and further research is needed to confirm the causal relationships between exposure and outcomes.

Declarations:

Ethical Approval
The data for this study were obtained from the GWAS public database and the patients' personal information was anonymised, so it does not require ethical committee approval as it does not involve personal privacy or informed consent.

Consent for publication
Not applicable

Availability of data and materials
All data covered in this study were obtained from the GWAS public database.

Competing interests
The author(s) declare that they have no competing interests.

Funding
No funding sources

Authors' contributions
Y.Y. wrote the main manuscript text and prepared Figures 1-5,X.Z.prepared Tables1-4. All authors reviewed the manuscript.

Acknowledgements
Thanks to Mr Xiaoyu ZAHNG for his outstanding contribution to this article.

References

[1] PAREDES F, WILLIAMS H C, SAN MARTIN A. Metabolic adaptation in hypoxia and cancer[J]. Cancer Lett, 2021, 502:133-142.
[2] GIANNAKEAS V, LIM D W, NAROD S A. The risk of contralateral breast cancer: a SEER-based analysis[J]. Br J Cancer, 2021, 125(4):601-610.
[3] LARSSON S C, BURGESS S. Causal role of high body mass index in multiple chronic diseases: a systematic review and meta-analysis of Mendelian randomization studies[J]. BMC Med, 2021, 19(1):320.

[4] MA M, ZHI H, YANG S, et al. Body Mass Index and the Risk of Atrial Fibrillation: A Mendelian Randomization Study[J]. Nutrients, 2022, 14(9).
[5] RIDNER S H, DIETRICH M S, STEWART B R, et al. Body mass index and breast cancer treatment-related lymphedema[J]. Support Care Cancer, 2011, 19(6):853-7.
[6] JENG K S, SHEEN I S, LEU C M, et al. The Role of Smoothened in Cancer[J]. Int J Mol Sci, 2020, 21(18).
[7] BOWDEN J, HOLMES M V. Meta-analysis and Mendelian randomization: A review[J]. Res Synth Methods, 2019, 10(4):486-496.
[8] KIM M S, KIM W J, KHERA A V, et al. Association between adiposity and cardiovascular outcomes: an umbrella review and meta-analysis of observational and Mendelian randomization studies[J]. Eur Heart J, 2021, 42(34):3388-3403.
[9] LI P, WANG H, GUO L, et al. Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study[J]. BMC Med, 2022, 20(1):443.
[10] SEKULA P, DEL GRECO M F, PATTARO C, et al. Mendelian Randomization as an Approach to Assess Causality Using Observational Data[J]. J Am Soc Nephrol, 2016, 27(11):3253-3265.
[11] XU J, ZHANG S, TIAN Y, et al. Genetic Causal Association between Iron Status and Osteoarthritis: A Two-Sample Mendelian Randomization[J]. Nutrients, 2022, 14(16).
[12] YUAN S, CARTER P, MASON A M, et al. Coffee Consumption and Cardiovascular Diseases: A Mendelian Randomization Study[J]. Nutrients, 2021, 13(7).
[13] PICON-RUIZ M, MORATA-TARIFA C, VALLE-GOFFIN J J, et al. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention[J]. CA Cancer J Clin, 2017, 67(5):378-397.
[14] HAZELWOOD E, SANDERSON E, TAN V Y, et al. Identifying molecular mediators of the relationship between body mass index and endometrial cancer risk: a Mendelian randomization analysis[J]. BMC Med, 2022, 20(1):125.
[15] NEUHOUSER M L, ARAGAKI A K, PRENTICE R L, et al. Overweight, Obesity, and Postmenopausal Invasive Breast Cancer Risk: A Secondary Analysis of the Women's Health Initiative Randomized Clinical Trials[J]. JAMA Oncol, 2015, 1(5):611-21.
[16] PARK B. Changes in weight and waist circumference during menopausal transition and postmenopausal breast cancer risk[J]. Int J Cancer, 2022, 150(9):1431-1438.
[17] VENKATESH S S, FERREIRA T, BENONISDOTTIR S, et al. Obesity and risk of female reproductive conditions: A Mendelian randomisation study[J]. PLoS Med, 2022, 19(2):e1003679.
[18] WANG Y B, YANG L, DENG Y Q, et al. Causal relationship between obesity, lifestyle factors and risk of benign prostatic hyperplasia: a univariable and multivariable Mendelian randomization study[J]. J Transl Med, 2022, 20(1):495.
[19] GUCALP A, TRAINA T A, EISNER J R, et al. Male breast cancer: a disease distinct from female breast cancer[J]. Breast Cancer Res Treat, 2019, 173(1):37-48.
[20] WHITE K K, PARK S Y, KOLONEL L N, et al. Body size and breast cancer risk: the Multiethnic Cohort[J]. Int J Cancer, 2012, 131(5):E705-16.