Genetically Predicted Circulating Levels of Antioxidants and Risk of Breast and Ovarian Cancer

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ABSTRACT

Evidence from observational studies for the effects of circulating antioxidants on the risk of breast and ovarian cancer was inconsistent. To elucidate the potential causal association of circulating antioxidants on the risk of breast and ovarian cancer, we carried out a two-sample Mendelian randomization (MR) study. The instrumental variables as proxies of genetic liability to circulating antioxidants were obtained from several published data. Summary-level data of breast and ovarian cancer were obtained from genome-wide association study (GWAS) conducted by the Breast (122,977 cases and 105,974 controls) and the Ovarian (25,509 cases and 40,941 controls) Cancer Association Consortiums. MR analyses were mainly performed using the inverse variance–weighted tests. Sensitivity analyses were further conducted to assess heterogeneity and horizontal pleiotropy. No evidence of causal association between genetically predicted circulating antioxidants and breast cancer and its histotypes was discovered as assessed by absolute levels [β-carotenoid: OR, 0.98; 95% confidence interval (CI), 0.92–1.05; $P = 0.627$; lycopene: OR, 0.99; 95% CI, 0.95–1.03; $P = 0.532$; retinol: OR, 0.87; 95% CI, 0.49–1.55; $P = 0.645$; ascorbate: OR, 1.00; 95% CI, 0.99–1.00; $P = 0.123$] and metabolites (α-tocopherol: OR, 0.88; 95% CI, 0.65–1.19; $P = 0.394$; γ-tocopherol: OR, 1.00; 95% CI, 0.87–1.16; $P = 0.978$; retinol: OR, 1.02; 95% CI, 1.00–1.04; $P = 0.070$; ascorbate: OR, 0.99; 95% CI, 0.91–1.06; $P = 0.703$). Similarly, no beneficial effect of genetic determinants of circulating antioxidants on ovarian cancer and its histotypes was found. Our study might not indicate a protective role of circulating antioxidants on the breast or ovarian cancer risk.

Prevention Relevance: Although this study does not find that circulating antioxidants are protective against breast and ovarian cancer, it is still possible that a high intake of antioxidant-rich foods containing other potentially beneficial components could be cancer preventative.

Introduction

Breast cancer and ovarian cancer persist as two of the most common cancers in women worldwide (1). Although breast and ovarian cancer usually present as distinct clinical entities, the recent explosion of large-scale omics research has uncovered many overlaps in these two hormone-dependent cancers, particularly the genetic and epigenetic alterations (2). Moreover, there also exist some common lifestyle-related modifiable factors, such as hormone use, alcohol and tobacco use, diet, and physical activity (3). In addition, increasing evidence indicates that oxidative stress may play a significant role in multiple stages of the carcinogenesis process of the two cancers. Increased level of oxidative DNA damage promotes the initiation of cancer, leading to irreversible DNA changes (4). Furthermore, reactive oxygen species (ROS) can interact with cell receptors, regulate signal pathways, as well as destroy relevant mechanisms related to reproduction, apoptosis, and angiogenesis, thereby affecting the cancer progression (5). It has been reported that the alteration of the breast cell genome may be caused by the oxidative attack of ROS generated by estrogen-induced oxidative stress and proliferation of damaged cells (6, 7). Therefore, antioxidants that have the effect of clearing ROS may be useful in cancer prevention and treatment.

Epidemiologic evidence regarding the effects of antioxidants, whether measured in dietary intake or circulating concentration, on risk of breast and ovarian cancer remains controversial (8–16). Intake of β-carotene significantly associated with lowering of breast cancer risk was indicated by a meta-analysis of 10 studies that included 18,191 cases among 775,765 subjects (14). Nevertheless, a comprehensive pooled analysis indicated that dietary intake of vitamin A, C, and E had no protective impact on lowering the risk of breast cancer (10). For ovarian cancer, a pooled analysis involving 4,882 cases and 443,179 participants based on 10 case–control studies and 5 cohort studies found that dietary vitamin A intake had a protective effect on ovarian cancer risk (15). A recent meta-analysis of 10 cohorts and 11 case–control studies (4,553 cases, 437,689 controls) showed no significant association between

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dietary vitamin C and ovarian cancer risk (16). Due to lack of data, observational epidemiologic research results cannot be compared with clinical randomized controlled trials. The inconsistency in the results of previous observational studies may be due to the following reasons. In previous related studies, many confounding factors such as smoking, age at menarche, age at menopause, alcohol intake, breastfeeding, BMI, height, hormone replacement therapy have been adjusted, although different adjustments were selected in each article. Moreover, unmeasured or residual confounding should also be considered. Reverse causality in the case–control study is also a possible explanation as patients with breast and ovarian cancer are more prone to increase their antioxidant intake after diagnosis (17). Therefore, better methods are needed to further explore the causal relationship between circulating antioxidants and the risk of breast and ovarian cancer.

MR method uses genetic variations as instruments of exposure (18). One advantage of MR over traditional research methods is that it effectively avoids confounding factors and reverse causality. However, to achieve this, it must satisfy the following three hypotheses: (i) genetic variations have strong associations ($P < 5\times10^{-8}$) with various types of antioxidants; (ii) genetic variation has no relationship with potential confounding factors such as estrogen, alcohol intake, diet habits, and smoking or antioxidants; and (iii) genetic variation is independent of other pathways and only affects the outcome through exposure (18). This study utilized MR methods to infer the causal relationship of antioxidants on breast and ovarian cancer as well as histotypes. The data of genetically predicted circulating antioxidants on breast and ovarian cancer are all from genome-wide association studies (GWAS). Here we mainly studied the potentially causal effects of genetically determined antioxidants of vitamin A (retinol), vitamin C (ascorbate), vitamin E (α-and γ-tocopherol), and carotenoids (lycopene, β-carotene) on the risk of breast and ovarian cancer as well as histotypes.

Materials and Methods

Genetic variants for antioxidants

Participant flow chart and analysis plan using two-sample MR is shown in **Fig. 1**. This study makes use of summary-level data; therefore, no ethical approval or consent to participate was required. The instrumental variables (IV) on genetic predisposition to circulating antioxidants concentration are in accordance with the latest article on MR of antioxidants by Luo and colleagues (19). In this article, we studied six antioxidants: vitamin A (retinol), vitamin C (ascorbate), vitamin E (α- and γ-tocopherol), and carotenoids (lycopene, β-carotene). Antioxidants derived from both circulating antioxidants and

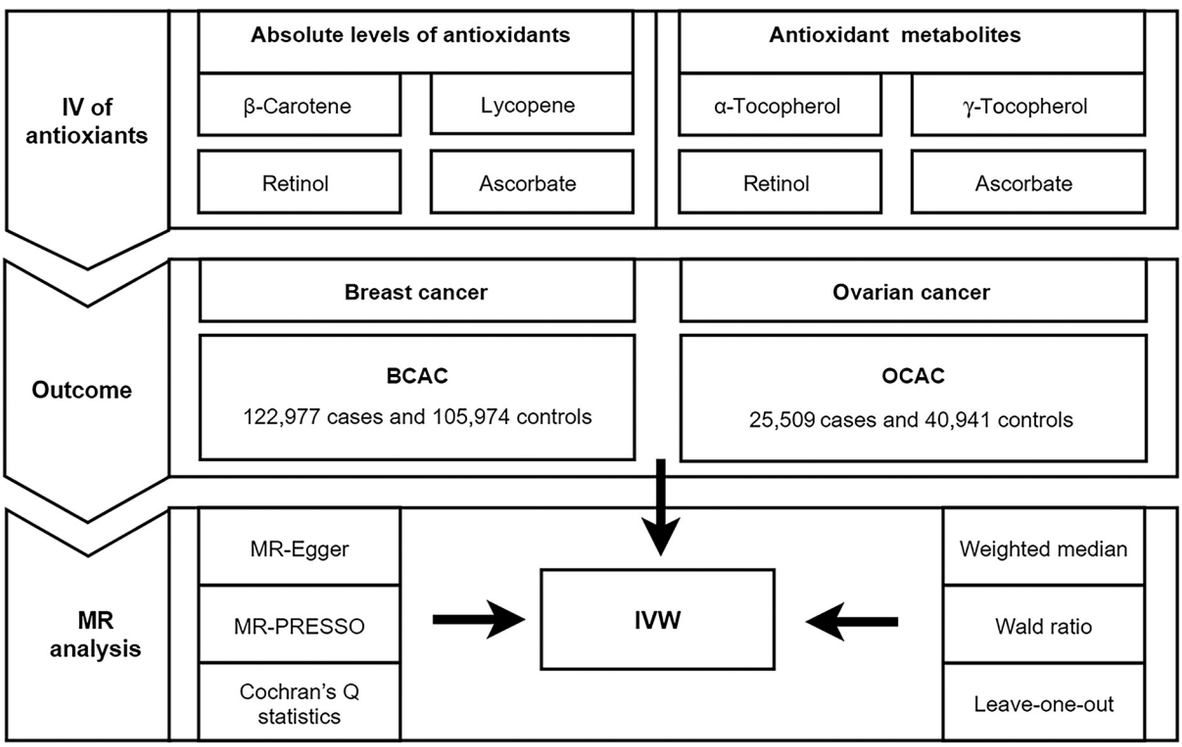

Figure 1.

Participants flow chart and analysis plan using MR. Summary statistics from SNP phenotypes were obtained from GWAS consortium datasets. IV, instrumental variable; IVW, inverse variance weighted; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger regression method; PRESSO, Pleiotropy Residual Sum and Outlier; BCAC, Breast Cancer Association Consortium; OCAC, Ovarian Cancer Association Consortium.

corresponding circulating metabolites in plasma or serum are considered. Therefore, absolute levels of antioxidants include β-carotene, lycopene, retinol, and ascorbate, while for diet-derived antioxidant metabolites, α-tocopherol, γ-tocopherol, retinol, and ascorbate are included (Supplementary Table S1).

Absolute circulating antioxidants

We selected summary data of three SNPs for β-carotene from a GWAS involving 2,344 subjects of the Nurses' Health Study [$P < 5\times10^{-8}$; Linkage disequilibrium (LD) < 0.2 as indicated in the study; ref. 20]. Summary data of five variants were obtained from a published GWAS on circulating lycopene level conducted in 441 older Amish adults ($P < 5\times10^{-6}$; LD < 0.001; ref. 21). One SNP of lycopene, rs6108801, is related to uterine tumors of unknown location. Finally, four SNPs for lycopene were considered as instrumental variables. Two SNPs used as proxies of circulating retinol levels were detected from a GWAS including 5,006 participants (22). Regrettably, only one single SNP of ascorbate was identified among 15,000 individuals (23). Details of SNPs of absolute circulating antioxidants were shown in Supplementary Table S2.

Circulating antioxidant metabolites

SNPs associated with each metabolite at the suggestive genome-wide significance level ($P < 1\times10^{-5}$) in populations of European ancestry were included (22, 24). Finally, eleven SNPs of α-tocopherol ($n = 7{,}276$), thirteen SNPs of γ-tocopherol ($n = 5{,}822$), fourteen SNPs of ascorbate ($n = 2{,}063$), and twenty-four SNPs of retinol ($n = 1{,}957$) were identified as circulating antioxidant metabolites. When LD is present (LD > 0.001), the variation with the smallest P value was retained. To eliminate the confounding factors related to exposure, we manually excluded each tool variable one by one in the PhenoScanner (25) and GWAS catalog (26). One SNP of α-tocopherol, rs11992435, is related to age at menarche which was ruled out from instrumental variables (Supplementary Table S3).

Data sources for breast and ovarian cancer

We extracted GWAS summary data on breast cancer from the Breast Cancer Association Consortium (BCAC) that including 122,977 breast cancer cases and 105,974 controls subjects of European ancestry (27). According to the presence or absence of estrogen receptor (ER), breast cancer was further divided into two histotypes, estrogen receptor–positive (ER^+) breast cancer (69,501 cases and 105,974 controls) and ER-negative (ER^-) breast cancer (21,468 cases and 105,974 controls; ref. 27). For ovarian cancer, the genetic data in relation to ovarian cancer was obtained from an epithelial ovarian cancer (EOC) GWAS of European subjects (25,509 cases and 40,941 controls) conducted by the Ovarian Cancer Association Consortium (OCAC; ref. 28). EOC can be further divided into invasive ovarian cancer (22,406 cases) and borderline ovarian cancer (3,103 cases). Invasive ovarian cancer can be further divided into high-grade serous (13,037 cases), low-grade serous (1,012 cases), endometrioid (2,810 cases), clear cell (1,366 cases), mucinous (1,417 cases), and other EOC (2,764 cases) according to the biological characteristics of cells. Borderline histotypes can be further divided into serous borderline (1,954 cases) and mucinous borderline (1,149 cases).

Statistical analysis

The two most critical variables for calculating statistical power are R^2 and F-statistic. R^2 of the regression equation indicates the extent to which IVs can explain exposure (29). The SNPs of absolute antioxidant levels instruments explained 0.9% to 30.1% of phenotypic variability, while the SNPs of antioxidants metabolites instruments explained 3.3% to 18.6% of phenotypic variability. R^2 for each SNP was either calculated using an online tool at http://cnsgenomics.com/shiny/mRnd/ or obtained from the original study (30). To solve a key shortcoming of weak instrumental variable bias in MR research, we utilized F-statistic to evaluate all exposures and used a standard of F-statistic > 10 to filter instrumental variables.

MR analysis

To elucidate the potential effects of antioxidants on breast and ovarian cancer, we conducted a two-sample MR study. The key MR analysis was conducted by the inverse-variance weighted (IVW) approach. When only one single SNP can be used as instrument, Wald ratio estimate were used (31, 32). To ensure the statistical validity of the results, we adopted the following supplementary methods: MR-Egger regression is a commonly used MR analysis method. The obvious distinction from IVW is that it takes the intercept into consideration and uses the distance between the intercept and zero to evaluate the pleiotropy (33). If the intercept approaches zero, the MR-Egger regression model is basically consistent with the results of IVW, and it means that there may be horizontal pleiotropy between these IVs inversely. Weight Median estimator is the median SNP ratio method of individual weighted empirical distribution function instead of simple median estimation (34). We also apply MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) to test and rectify any potential outliers that may have pleiotropic deviations (35, 36). In addition to the above methods, we also conducted several sensitivity analyses. We used Cochran Q statistics method to test the heterogeneity (36). We used the MR Egger intercept test to assess the horizontal pleiotropy. We also used leave-one-out sensitivity analysis to eliminate the IVs one by one and calculate the MR results of the remaining IVs to see whether a certain SNP drives MR results. All results were in a respective unit increment of measure for absolute circulating levels of antioxidants of β-carotene and retinol (natural log-transformed levels) lycopene (mg/dL), and ascorbate (mmol/L) or a 10-fold change for metabolites concentrations to be expressed as ORs with 95% CIs for breast cancer and ovarian cancer.

All the MR analysis was performed using the "TwoSampleMR" packages and "MRPRESSO" packages in R (version 4.0.3).

Exposure	MR methods	OR	(95% CI)	SE	*P* value	Heterogeneity Q	Heterogeneity *P* value	Pleiotropy Intercept	Pleiotropy *P* value
Absolute circulating antioxidants									
β-carotene	IVW	0.98	(0.92–1.05)	0.03	0.627	2.5	0.285		
	MR-Egger	0.87	(0.69–1.09)	0.12	0.444			0.015	0.471
	Weighted median	0.98	(0.91–1.05)	0.04	0.517				
Lycopene	IVW	0.99	(0.95–1.03)	0.02	0.532	4.2	0.242		
	MR-Egger	1.02	(0.96–1.08)	0.03	0.591			−0.013	0.345
	Weighted median	0.98	(0.95–1.02)	0.02	0.356				
	MR-PRESSO	0.99	(0.95–1.03)	0.02	0.576				
Retinol	IVW	0.87	(0.49–1.55)	0.29	0.645	3.5	0.061		
Ascorbate	Wald ratio	1.00	(0.99–1.00)	0.00	0.123				
Circulating antioxidant metabolites									
α-tocopherol	IVW	0.88	(0.65–1.19)	0.16	0.394	17.5	0.042		
	MR-Egger	0.82	(0.42–1.61)	0.34	0.581			0.002	0.833
	Weighted median	0.99	(0.72–1.38)	0.17	0.973				
	MR-PRESSO	0.88	(0.57–1.18)	0.16	0.416				
γ-tocopherol	IVW	1.00	(0.87–1.16)	0.07	0.978	13.1	0.216		
	MR-Egger	0.96	(0.72–1.29)	0.15	0.807			0.002	0.763
	Weighted median	0.99	(0.83–1.18)	0.09	0.887				
	MR-PRESSO	1.00	(0.86–1.15)	0.07	0.978				
Retinol	IVW	1.02	(1.00–1.04)	0.01	0.070	29.6	0.100		
	MR-Egger	1.02	(0.97–1.08)	0.03	0.383			−0.001	0.825
	Weighted median	1.03	(1.00–1.05)	0.01	0.029				
	MR-PRESSO	1.02	(1.00–1.04)	0.01	0.084				
Ascorbate	IVW	0.99	(0.91–1.06)	0.04	0.703	43.2	4.2E−5		
	MR-Egger	1.02	(0.89–1.18)	0.07	0.786			−0.004	0.571
	Weighted median	0.99	(0.93–1.06)	0.03	0.822				
	MR-PRESSO	0.99	(0.91–1.06)	0.04	0.507				

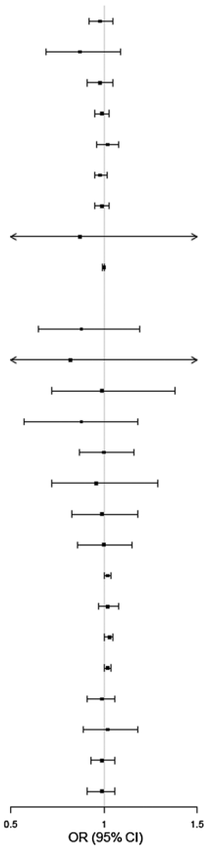

Figure 2.
MR estimates from each method of assessing the causal effects of genetically predicted circulating antioxidants on breast cancer. IVW, inverse-variance weighted; MR, Mendelian randomization; PRESSO, Pleiotropy RESidual Sum and Outlier; OR, odds ratio; CI, confidence interval; SE, standard error.

Data and materials availability

Genetic instruments can be obtained from individual reference papers (DOI: 10.1016/j.jacc.2020.10.048). GWAS summary data for BCAC and OCAC were publicly available and downloaded from the corresponding consortium sites (http://bcac.ccge.medschl.cam.ac.uk/bcacdata/oncoarray and http://ocac.ccge.medschl.cam.ac.uk/).

Results

Absolute circulating antioxidants with breast and ovarian cancer

We displayed the SNPs used as proxies for genetically determined absolute circulating antioxidants in Supplementary Table S4 and S5 and their association with the risk of breast cancer (**Fig. 2**), ovarian cancer (**Fig. 3**), and the histotypes, including ER^+ breast cancer, ER^- breast cancer, high-grade serous, low-grade serous, endometrioid, clear cell, mucinous, serous borderline, and mucinous borderline ovarian cancer (Supplementary Fig. S1–S9). In general, we found no evidence of the causal relationship between genetically predicted absolute circulating antioxidants and breast and ovarian cancer as well as histotypes. The IVW method indicated that there was no association between genetically predicted absolute circulating antioxidants and risk of breast cancer [β-carotenoid: OR, 0.98; 95% confidence interval (CI), 0.92–1.05; $P = 0.627$; lycopene: OR, 0.99; 95% CI, 0.95–1.03; $P = 0.532$; retinol: OR, 0.87; 95% CI, 0.49–1.55; $P = 0.645$; ascorbate: OR, 1.00; 95% CI, 0.99–1.00; $P = 0.123$]. Results obtained from other MR analyses coincided with those of the IVW method. When the associations with ovarian cancer were investigated, results from the IVW analysis showed that genetic liability to absolute circulating antioxidants were not casually associated with risk of ovarian (β-carotenoid: OR, 1.02; 95% CI, 0.90–1.16; $P = 0.771$; lycopene: OR, 0.98; 95% CI, 0.92–1.05; $P = 0.546$; retinol: OR, 0.82; 95% CI, 0.19–3.57, $P = 0.795$; ascorbate: OR, 1.01; 95% CI, 1.00–1.02, $P = 0.094$). Results obtained from other MR analyses are in accordance with those of the IVW method. Furthermore, the Cochran Q test revealed no

Exposure	MR methods	OR	(95% CI)	SE	*P* value	Heterogeneity Q	Heterogeneity *P* value	Pleiotropy Intercept	Pleiotropy *P* value
Absolute circulating antioxidants									
β-carotene	MR-Egger	1.09	(0.62–1.91)	0.29	0.824			−0.008	0.858
	Weighted median	1.02	(0.88–1.18)	0.08	0.830				
	IVW	1.02	(0.90–1.16)	0.07	0.771	1.6	0.460		
Lycopene	MR-Egger	0.93	(0.83–1.04)	0.06	0.345			0.022	0.396
	Weighted median	0.96	(0.89–1.04)	0.04	0.350				
	IVW	0.98	(0.92–1.05)	0.03	0.546	3.0	0.397		
	MR-PRESSO	0.98	(0.92–1.04)	0.03	0.578				
Retinol	IVW	0.82	(0.19–3.57)	0.75	0.795	5.3	0.021		
Ascorbate	Wald ratio	1.01	(1.00–1.02)	0.01	0.094				
Circulating antioxidant metabolites									
α-tocopherol	MR-Egger	1.90	(0.72–5.00)	0.49	0.229			−0.015	0.394
	Weighted median	1.12	(0.60–2.09)	0.32	0.728				
	IVW	1.28	(0.81–2.03)	0.24	0.296	7.8	0.557		
	MR-PRESSO	1.28	(0.85–1.71)	0.22	0.291				
γ-tocopherol	MR-Egger	1.36	(0.81–2.25)	0.26	0.271			−0.017	0.549
	Weighted median	1.23	(0.88–1.72)	0.17	0.227				
	IVW	1.18	(0.91–1.53)	0.13	0.218	8.1	0.621		
	MR-PRESSO	1.18	(0.94–1.41)	0.12	0.202				
Retinol	MR-Egger	1.01	(0.90–1.13)	0.06	0.849			−0.001	0.959
	Weighted median	1.00	(0.95–1.05)	0.03	0.966				
	IVW	1.01	(0.97–1.05)	0.02	0.703	33.6	0.039		
	MR-PRESSO	1.01	(0.97–1.05)	0.02	0.715				
Ascorbate	MR-Egger	1.04	(0.90–1.21)	0.08	0.586			0.001	0.924
	Weighted median	1.03	(0.91–1.16)	0.06	0.666				
	IVW	1.05	(0.96–1.14)	0.04	0.257	7.4	0.883		
	MR-PRESSO	1.05	(0.99–1.11)	0.03	0.168				

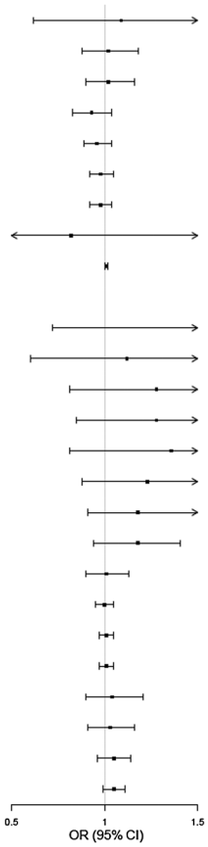

Figure 3.

MR estimates from each method of assessing the causal effects of genetically predicted circulating antioxidants on ovarian cancer. IVW, inverse-variance weighted; MR, Mendelian randomization; PRESSO, Pleiotropy RESidual Sum and Outlier; OR, odds ratio; CI, confidence interval; SE, standard error.

heterogeneity in effects among SNPs of absolute circulating antioxidants except for IVs of ascorbate. Moreover, MR-Egger intercepts estimator suggested no horizontal pleiotropy among IVs. Similarly, leaving-one-out analyses indicated that no single SNP drove MR results.

Circulating antioxidant metabolites with breast and ovarian cancer

We displayed the SNPs used as proxies for circulating antioxidant metabolites in Supplementary Tables S4 and S5 and their association with the risk of breast cancer (**Fig. 2**), ovarian cancer (**Fig. 3**), and histotypes (Supplementary Fig. S1-S9). Among the 13 γ-tocopherol-associated SNPs and 24 retinol-associated SNPs, 4 SNPs were not available in the dataset of breast and ovarian cancer. There was no evidence of causal effect of genetically predicted circulating antioxidant metabolites on the risk of breast and its histotypes conducted by the IVW method or other MR tests (α-tocopherol: OR, 0.88; 95% CI, 0.65–1.19; $P = 0.394$; γ-tocopherol: OR, 1.00; 95% CI, 0.87–1.16; $P = 0.978$; retinol: OR, 1.02; 95% CI, 1.00–1.04; $P = 0.070$; ascorbate: OR, 0.99; 95% CI, 0.91–1.06; $P = 0.703$).

For ovarian cancer, genetic-determined circulating antioxidant metabolites may not have a protective impact on reducing ovarian cancer risks using IVW method (α-tocopherol: OR, 1.28; 95% CI, 0.81–2.03; $P = 0.296$; γ-tocopherol: OR, 1.18; 95% CI, 0.91–1.53; $P = 0.218$; retinol: OR, 1.01; 95% CI, 0.97–1.05, $P = 0.703$; ascorbate: OR, 1.05; 95% CI, 0.96–1.14, $P = 0.257$). Results obtained from other MR analyses were in line with the IVW. The Cochran's Q estimator supported evidence of heterogeneity among IVs of α-tocopherol, retinol, and ascorbate. There was no indication of directional pleiotropy based on the MR-Egger intercept method (all $P > 0.05$). Moreover, the results of the Leave-one-out analysis revealed that no matter which SNP was removed, there was no fundamental impact on MR results, suggesting that our MR results are robust. Consistent with ovarian cancer, genetic determinants of circulating antioxidant metabolites had no protective influence on seven histotypes of ovarian cancer.

Discussion

In this study, we take full advantage of large GWAS summary data of breast cancer from BCAC (122,977 cases and 105,974 controls) and ovarian cancer from OCAC (25,509 cases and 40,941 controls) to investigate the causal relationship between genetically predicted circulating antioxidants and the risk of breast and ovarian cancer as well as their histotypes using the two-Sample MR approach. To the best of our knowledge, this is the first study adopting the MR approach to explore the potential causal relationship of antioxidants with the risk of breast and ovarian cancer as well as histotypes. Our findings indicate that genetically predicted circulating antioxidants may not have a beneficial role in lowering the incidence of breast cancer, ovarian cancer, or their histotypes in general.

Multiple observational studies have investigated the associations between antioxidants and the risk of breast or ovarian cancer. A pooled analysis of eight prospective studies comprising 3,055 cases with breast cancer and 3,956 controls suggested that higher circulating levels of α-carotene, β-carotene, and lycopene among women may reduce the risk of breast cancer (13). Similarly, a prospective cohort among 90,655 premenopausal women found that intake of vitamin A is likely to reduce the risk of breast cancer among smokers (8). A meta-analysis of 7 case–control studies and 3 prospective studies of 678,892 subjects including 6,127 cases of ovarian cancer suggested a weak inverse association between dietary lycopene consumption and ovarian cancer among postmenopausal women (9).

On the other hand, recent epidemiology studies have recently shown that diet-derived antioxidants were not protective against breast and ovarian cancer, which are consistent with our MR results. For example, a comprehensive pooled analysis indicated that dietary intake of vitamin A, C, and E had no protective role on lowering the risk of breast cancer (10). Furthermore, a comprehensive meta-analysis involving 10 cohort studies confirmed that the major carotenoids intakes of α-carotene, β-carotene, and lycopene during adults were not associated with a decreased risk of ovarian cancer (11). Similarly, the results from a pooled analysis of 10 cohort studies including 1,973 cases with ovarian cancer among 501,857 women suggested that dietary vitamins A, C, and E during adulthood did not play a major role in ovarian cancer risk (12). The inconsistency of our MR results with several previous observational studies may be explained by potential confounders (such as alcohol use, cigarette smoking) and reverse causality (people with cancer may be more prone to increased antioxidants intake). In addition, the duration of observation periods of different observational studies is inconsistent, and the dietary characteristics and habits of each study population are distinct.

There are several notable advantages in our research. First, the summary-level data of IVs of circulating antioxidants were obtained from the latest published articles and the summary data of breast and ovarian cancer were from two GWASs with large sample sizes. Thus, we had sufficient statistical power to discover even weak associations. Second, we use MR, a new research method, which can effectively avoid the drawbacks of traditional epidemiologic research, such as confounders and reverse causality. In addition, the IVs of antioxidants have two different sources, one is absolute circulating antioxidants, and another comes from their metabolites. The results of the two sources of retinol and ascorbic acid were consistent, confirming the robustness of our findings.

Nevertheless, our study has several potential limitations. First, the IVs for genetically predicted circulating antioxidants are derived from studies based on both females and males, while the GWAS data for breast and ovarian cancer was only conducted among female participants. It would be interesting and beneficial to explore the sex-specific association. However, we are unable to investigate whether the difference between males and females influences the results due to the lack of sex-specific data for antioxidants. Second, the number of instrumental variables for absolute circulating antioxidants is limited, especially for the SNPs of retinol and ascorbic acid. Third, although the heterogeneity test results of circulating metabolite antioxidants suggest the exist of potential pleiotropy, the pleiotropic test did not detect pleiotropy. Another potential limitation of MR research is pleiotropy when SNPs in relation to multiple factors (vertical pleiotropy) or pathways (horizontal pleiotropy). Although the hypothesis of instrumental variables cannot be fully verified, we have used some methods to ensure the validity of the IV as much as possible. We use different MR methods to detect potential pleiotropy, and exclude some known confounding factors and pathways, but not completely. The results of this study should be treated more cautiously. In addition, the level of circulating antioxidants for those in the highest and lowest quintiles of genetically predicted circulating antioxidants are 75–136.9 μg/dL (lycopene), 2,097–6,515 μg/L (β-carotene), 461–794 μg/L (retinol), and 21.22–60.14 μmol/L (ascorbate). We cannot ignore the possibility that null association was due to the small ranges of circulating antioxidants.

In conclusion, our study does not provide supportive evidence on protective roles of genetically predicted circulating antioxidants with the risk of breast cancer, ovarian cancer, or their histotypes. MR results are consistent with the results from the latest epidemiologic studies. Although our findings suggest that single antioxidants may not play a significant role in breast or ovarian cancer prevention, it is still possible that high intake of antioxidant-rich foods, which also contains many other potentially beneficial components, may play a role.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

H. Zhao: Conceptualization, resources, data curation, software, formal analysis, writing–original draft, writing–review and editing. **J. Zhu:** Conceptualization, resources, data curation, software, supervision, writing–review and editing. **L.A. TSE:** Writing–review and editing.

S. Kinra: Writing–review and editing. **Y. Li:** Conceptualization, supervision, writing–review and editing.

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