Effects of Glutamate and Aspartate on Prostate Cancer and Breast Cancer: A Mendelian Randomization Study

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Abstract

Background: Respectively, prostate cancer and breast cancer are the second most and most commonly diagnosed cancer in men and woman, and they account for major cancer-related deaths world-wide. Special attention aiming to find potentially effective early detection of, and intervention strategies against, prostate cancer (PCa) and breast cancer need to be paid.

Objective: Utilizing Mendelian randomization (MR), we aimed to estimate how genetically predicted glutamate and aspartate levels affected prostate and breast cancers development.

Methods: Single nucleotide polymorphisms (SNPs) were selected as instrumental variables (IVs) to predict the serum levels of glutamate and aspartate from the publicly available genome-wide association studies (GWASs), which were conducted to associate genetic variations with blood metabolite levels using comprehensive metabolite profiling in 1,960 adults and the glutamate and aspartate we chosen were two of 644 metabolites. The summary statistics for the largest and latest GWAS datasets for prostate cancer (61,106 controls and 79,148 cases) were from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium and datasets for breast cancer (113,789 controls and 133,384 cases) were from Breast Cancer Association Consortium (BCAC). The analyses were performed through two-sample MR method.

Results: Serum level of aspartate was positively associated with prostate cancer (Effect = 1.043; 95% confidence interval, 1.003 to 1.084; P = 0.034) and breast cancer (Effect = 1.033; 95% confidence interval, 1.004 to 1.063; P = 0.028); however, glutamate was neither associated with prostate cancer and breast cancer. The potential causal associations were robust to the sensitivity analysis.

Conclusions: Our study found that the level of serum aspartate could serve as a risk factor that contributed to the development of prostate and breast cancers. Efforts detailing the underlying mechanism(s) would be extremely valuable in early assessment/diagnosis and strategizing clinical intervention of both cancers.

Introduction

In cells, nutrients are the important supports for energy and biosynthesis of many molecules. Accumulating evidence from basic studies suggests that cancer cells continually change the way of nutrient utilization during malignancy development. For instance, under aerobic condition, cancer cells often exhibit upregulated glycolysis for their rapid growth (1); and they also use amino acids, in particular glutamine and aspartate, as anaplerotic nutrients for the tricarboxylic acid (TCA) cycle that is coupled with oxidative phosphorylation (2). Hence, cancer cells are generally vulnerable to nutrient deficiency, a feature that potentially provides new targets for cancer therapy (3). Indeed, given altered metabolism typical of cancer tissues (4), quite an ever-growing number of epidemiological studies have exploited metabolomics to research the etiology and figure out biomarkers for early detection, or progression of prostate cancer (5); however, little is known about causal associations between cancers and metabolites such as amino acids. Furthermore, at times the metabolic studies generate results that are not always consistent due to the differences in outcome examined, metabolomics platforms exploited, and characteristics and/or sizes of study populations.

MR analysis can avoid bias, along with misinterpretation of results, by removing potential confounding factors in traditional observational studies like socio-economic status, lifestyle (alcohol and smoking) and health status. Mendel's second law dictates that each pair of alleles undergoes independent assortment without interference from environmental factors. MR analysis widely utilizes the power of SNPs genetically associated with exposures as IVs to evaluate the causal associations between risk factors and disease outcomes (6). Two-sample MR analysis requires summary-level data from two independent GWASs for putative exposures and outcomes (7).

The prostate and breast cancers are almost the most frequently diagnosed cancers that also constitute a major cause of cancer-related deaths (8, 9). In western countries, prostate cancer is the most common form of cancer among men of 50yrs and older with a mortality-to-incidence ratio of 20% (10). As for breast cancer, the United States alone in 2017 recorded 255,180 new cases and 41,070 deaths (11). Thus, despite tremendous advancements over the previous decade, early detection/treatment of prostate and breast cancers is next to satisfaction.

Observationally, soy proteins, rich in glutamate and aspartate, are reported to lower the androgen levels but no large randomized controlled trials (RCT) have been conducted to test their health effects; in addition, animal experiment results suggest that glutamate and aspartate can decease the testosterone levels (12, 13), and diverse epidemiological studies suggest that consumption of soy, fruits, and vegetables are linked with reduced risk of recurrence and increased survival rate of prostate cancer and breast cancer (14–17). In a RCT of men, $_{D}$-aspartate can reduce testosterone (18). Apart from cancer-related studies, in patients with Parkinson's disease, the glutamate homeostasis is found to be impaired and the modifiability of glutamine/glutamate metabolism is known to impede cognitive decline (19–21).

Despite the above-listed studies, up to now the causal relationship between serum glutamate and aspartate levels and prostate and breast cancers remains elusive. Here, exploiting genetically instrumented glutamate and aspartate from GWAS (22) and large case-control studies of prostate and breast cancers with extensive genotyping (23, 24), an MR study was performed to estimate the causal effects of serum glutamate and aspartate levels and the development of prostate and breast cancers.

Subjects And Methods

Study Design and Data Sources

As shown in the Fig.1, a two-sample Mendelian Randomization approach was designed in this study. It is based on the assumption that instrumental variables are related to serum levels of glutamate and aspartate, but independent of the risk of cancer and cofounders.

The IVs were from the publicly available genome-wide association studies, which were a common, low-frequency and rare variants GWASs, and conducted in 1,960 adults to associate genetic variations with blood metabolites by comprehensive metabolite profiling. In our study, the glutamate and aspartate were chosen from the whole 644 metabolites according three longitudinal data collections. SNPs were removed as the call rate was less than 95%, the P value was above 10^{-6} and the minor allele frequency was less than 1%. Information of data on the association of SNPs with serum glutamate and aspartate and the association of SNPs with breast cancer and prostate cancer were obtained from the GWAS database (23, 24).

Genetic instruments for glutamate and aspartate

Genetic signatures, such as SNPs, strongly associated with glutamate or aspartate were obtained from a large GWAS study (22), in which the participants were of European origin. We selected SNPs reaching genome-wide association significance (5×10^{-8}) as IVs, which were not confounded by socio-economic status and lifestyle factors. Then pleiotropy was checked based on the hypothesis of MR approach (25), using PhenoScanner V2 website (www.phenoscanner.medschl.cam.ac.uk), to validate that the SNPs were associated with prostate or breast cancer solely via glutamate or aspartate, rather than other phenotypes.

In order to make sure the independent contribution of selected SNPs (r^2 < 0.005), LD-link website (https://ldlink.nci.nih.gov/, population: CEU) was used to perform a linkage disequilibrium (LD) test. And to evaluate the potential heterogeneity due to pleiotropy or other causes, we conducted the Cochran's Q-test (together with the I^2 statistic).

Genetic associations with prostate and breast cancers

Summary data for prostate cancer was extracted from the largest GWAS meta-analysis including 79,148 cases and 61,106 controls of European ancestry from PRACTICAL consortium (http://practical.icr.ac.uk/blog/) (24). Summary statistics for breast cancer was extracted from the latest and largest meta-GWAS from BCAC including 133,384 cases and 113,789 controls (http://bcac.ccge.medschl.cam.ac.uk/) (23). The participants were women of European ancestry. The written consent of participants was provided, and all the studies contributing data to our MR analysis were supported by the ethical review boards.

Statistical analysis

Major analyses of the association between glutamine and aspartate and cancers were performed by the inverse-variance-weighted (IVW) method to calculate the Wald ratio of each SNP and estimate each IV's combined causal effects, then the corresponding inverse variance was regarded as weights for meta-analysis (26). As IVs were greater than or equal to three SNPs, the estimate obtained were validated by the weighted median method (WM) in the sensitivity analysis (27). Otherwise, the Wald ratio was directly used. Then, MR-Egger approach was used to test the pleiotropy by estimating the deviation of MR-Egger intercepts from zero (28).

All statistical analyses were performed using the R Studio (R version 4.0.2) software and the R package "Mendelian Randomization".

Results

Genetic instruments for glutamate and aspartate

In a sensitivity analysis, 5 SNPs with no potential pleiotropy from the large GWASs of European ancestry (22) at genome-wide association significance level (5×10^{-8}) were obtained, including 1 SNP for glutamate and 4 SNPs for aspartate. However, the result of the linkage disequilibrium test on the LD-link website showed that rs139051778 and rs33966350 associated with aspartate were not independent (r^2 > 0.005). In fact, these two SNPs were within a same gene; thus, to get more reliable results, we needed to exclude rs139051778 for analysis, as rs33966350 with lower P value. The characteristics of all genetic variants were shown in Table 1.

The SNPs were from genes thought to be functionally relevant to the exposures. The SNP rs113141482 is on the gene *GPR158*, which encodes G protein-coupled receptor 158 (GPR158). GPR158 is a newly characterized cell surface protein that plays the same role, as other G-protein coupled receptors (GPCRs), on promoting prostate cancer (PCa) malignancy. Indeed, currently, the glutamate family member GPR158 is a therapeutic target for PCa (29). The SNP rs33966350 is loci on the gene *ENPEP* associated with blood pressure (35) and is related to aspartate, given that the gene is also relevant to the metabolism of aspartate in function: *ENPEP* encodes glutamyl aminopeptidase, catalyzing the cleavage of glutamate and aspartate from the N-terminal polypeptides.

Table 1. Characteristics of the instrumental variables for glutamate and aspartate and the causal associations between glutamate and aspartate with prostate and breast cancers

Exposure	SNPs	Gene	Chromosome	Effect allele	Association with exposure			Association with prostate cancer			Association w
					Beta[1]	SE[2]	P value	Beta[1]	SE[2]	P value	Beta[1]
Glutamate	rs239614	LOC102724355	21	G	0.28	0.06	0.000000047	0.008	0.0116	0.4895	0.011461117
Aspartate	rs33966350	ENPEP	4	A	-1.5	0.19	1.4E-17	-0.0425	0.0375	0.2565	-0.044261478
	rs113141482	GPR158	10	A	-0.98	0.19	0.000000001	-0.1032	0.0497	0.03787	-0.018497524
	rs189080637		3	A	-0.98	0.21	0.000000043	-0.037	0.042	0.3779	-0.048760895

[1]Beta, per allele effect on exposure or outcomes

[2]SE, standard error;

Associations with prostate and breast cancers

As shown the result of IVW analysis in Fig. 2, the genetically instrumented aspartate was positively associated with prostate and breast cancers after correction for multiple comparisons; While glutamate was not significantly associated with breast cancer and prostate cancer (Fig. 3).

The summary information of GWAS on outcomes was displayed in Table S1. And the genetic associations between serum levels of glutamate and aspartate and the outcomes were shown in Table 1.

Sensitivity Analysis

As the IVs (for aspartate) were three SNPs, WM, MR-Egger analysis and Cochran's Q test were performed to test the pleiotropy of aspartate, the results of which were similar to the estimated effects of IVW analyses in the Table 2 and Table 3. And the intercept values of the MR-Egger analysis did not significantly differ from zero. Therefore, no signs of directional pleiotropy and heterogeneity (Cochran's Q analysis) among these instruments were discovered, suggesting that the pleiotropy did not bias the results.

There was only one SNP at genome-wide association significance to predict serum level of glutamate, sensitivity analysis could not be performed and the Wald ratio was used directly (Table 4).

Table 2. Weighted median, MR-Egger analysis and Cochran's Q test for genetic associations between aspartate and prostate cancer

Method	Weighted median	MR-Egger		IVW	Cochran's Q test
		Estimate	Intercept		
Estimate (95% CI)	0.032 (-0.012, 0.077)	-0.042 (-0.232, 0.147)	0.106 (-0.126, 0.338)	0.042 (0.003, 0.081)	
P value	0.155	0.66	0.37	0.034	0.3932
Cochran's Q					1.868
I^2					0.00%

Table 3. Weighted median, MR-Egger analysis and Cochran's Q test for genetic associations between aspartate and breast cancer

Method	Weighted median	MR-Egger		IVW	Cochran's Q test
		Estimate	Intercept		
Estimate (95% CI)	0.031 (-0.001, 0.063)	0.015 (-0.122, 0.152)	0.021 (-0.148, 0.191)	0.032 (0.004, 0.061)	
P value	0.06	0.827	0.805	0.028	0.8021
Cochran's Q					0.441
I^2					0.00%

Table 4. IVW analysis for genetic associations between glutamate and prostate and breast cancers

Cancer	Estimate (95% CI)	P value
Prostate Cancer	0.029 (-0.053, 0.110)	0.49
Breast Cancer	0.041 (-0.020, 0.102)	0.186

Discussion

Balanced diet/nutrition intake constitutes a preventive strategy for cancer incidences that in turn impacts the development and progression of cancer. Amino acids, glutamine and aspartate in particular, are vital alternative nutrients for cellular energetics and biomass synthesis apart from glucose. With consistency of the implication of evolutionary biology theory, our finding suggested that aspartate and, potentially to a lesser extent, glutamate, might feedback to the endocrine system hence playing a role for the underlying risk for prostate cancer.

To the best of our knowledge, it is the first MR study to examine the potential causal effects of glutamate and aspartate on prostate and breast cancers. Genetically instrumented glutamate and aspartate can remove potential confounding factors in observational studies and make a difference of the effects of these two dietary programs, which are correlated and co-occur. And at the same time it can also minimize the measurement error in nutrition studies from self-reported dietary consumption (30). Furthermore, it is cost-efficient depending on large GWASs and case-control studies with extensive genotyping (31). The samples for MR analysis were from two completely separate GWASs, one sample for genetic variants on exposures (glutamate and aspartate) and the other sample for genetic variants on outcomes (prostate cancer and breast cancer), which means any correlation in the sample with the exposures is unlikely to be replicated in the sample with the clinical outcomes with prostate and breast cancers.

Strength notwithstanding, this study has several limitations. Firstly, our findings on serum aspartate were seemingly inconsistent with anti-cancer effect of aspartate in food, such as soy (16, 17, 32–34). A possible explanation was that the effects of serum glutamate and aspartate reflected endogenous exposures that maybe distinguish with exogenous dietary exposures; but, levels of serum glutamate and aspartate are likely affected by dietary consumption (35). Secondly, MR requires the genetic instruments associated with the exposures. There are maybe no confounders in the causal association of the genetic instruments with the outcomes and the genetic variants are no pleiotropy (36). As a result, here only 4 SNPs met the requirements, one for glutamate. The exposure glutamate was dropped in the sensitivity analysis because the IVs for glutamate were less than three SNPs and might decrease the reliability without MR-Egger and WM analysis. Thirdly, population aspect might affect MR study. The genetic associations in our study were from studies largely conducted in European descent with genomic control (30, 37), and the results might not be applied to other populations. Forth, we could not judge whether the causal associations varied as baseline levels of glutamate and aspartate. Fifth, a feasible nonlinear association between serum levels of glutamate and aspartate with prostate cancer and breast cancer requires individual-level data, rather than our recent summary statistics (38). Sixth, our estimates for prostate cancer and breast cancer were prone to be conservative when the associations were sex-specific or age-specific. Last, but not the least, the underlying pathways of the causal effects remained to be clarified.

Aspartate and glutamate belong to the arginine family, as well as asparagine, glutamine and arginine itself. They are interconvertible via complex metabolism in most mammals. In our findings, aspartate and glutamate were risk factors for prostate cancer and breast cancer development. Emerging evidence reveals that glutamine and interlinked asparagine metabolism may be critical for endothelial cell (EC) metabolism, as a regulator of angiogenesis (39). Therefore, the fact that the serum levels of aspartate and glutamine serving as a risk factor might be exerted via their relevant metabolites, asparagine and glutamine that are known to promote cancer cell proliferation and vessel sprouting; and, in one breast cancer model, asparagine bioavailability impacts the ratios of epithelial-to-mesenchymal-like tumor cells and tumor progression (40). In the epithelial-mesenchymal transition (EMT) and PCa progression, aspartate is a contributor and aspartate metabolism elevates with high levels of adenylosuccinate, arginosuccinate, malate, asparagine (41).

Another plausible mechanism underlying our findings is a link between aspartate and arginine via the urea cycle. The urea cycle detoxifies free ammonia in the livers of mammals, in which arginine is synthesized in two steps: citrulline and aspartate are used to synthesize argininosuccinate which is then converted to arginine. Arginine is a non-essential amino acid in adults but is necessary for fast-growing cells such as cancer cells. Currently Graboa et al. (42) have reported that arginine is crucial during malignancy development. Arginine deprivation has been a novel and promising approach to treat tumors that are not hepatocyte-derived thus unable to self-suffice for arginine owing to a lack of the urea cycle (43). However, the effects of glutamate and aspartate on human health are very complex. Some studies show that nutritional supplements, aspartate and glutamate, possess beneficial health and anti-oxidative effects. For example, aspartate can improve liver metabolism (44), and glutamate can modulate the body weight (45), regulate the release of hormones (46) and lipid metabolism (47), probably owing to its impact upon the TCA cycle and ATP production (48). Aspartate might also operate by lowering androgens (12), and high level of circulating androgens is a risk factor for prostate cancer, a notion for which there is, however limited, evidence in human studies (18).

Currently, the technique of ultra-high performance liquid chromatography-tandem mass spectrometry measuring levels of amino acids such as aspartate and glutamate has been well validated (49). It will be worthwhile to exploit more relevant genetic instruments if available. Our work, based on MR studies that constitute a tool for testing causation, cannot dictate the exact size/degree of causal effects (50) nor can replace clinical trials; however, the findings built on the ever-growing knowledge about the effects of glutamine and aspartate on prostate cancer and breast cancer development is for sure greatly relevant to dietary recommendations, along with providing guidance for cancer prevention as well as public health in general.

Declarations

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Authors' contributions

L.Z. and Y.L. conceived the study design and drafted the manuscript. Z.Y., J.L. and Y.S. participated in data extraction and data analysis, X.Z. and Z.Q. did the data checking and analysis. Y.L reviewed and edited the manuscript, and guided L.Z. and Y.L. to design and carry out the experiments. All authors read and agreed to the published version of the manuscript.

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Availability of data and materials

All data analyzed in this study are included in the manuscript and supplementary materials.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

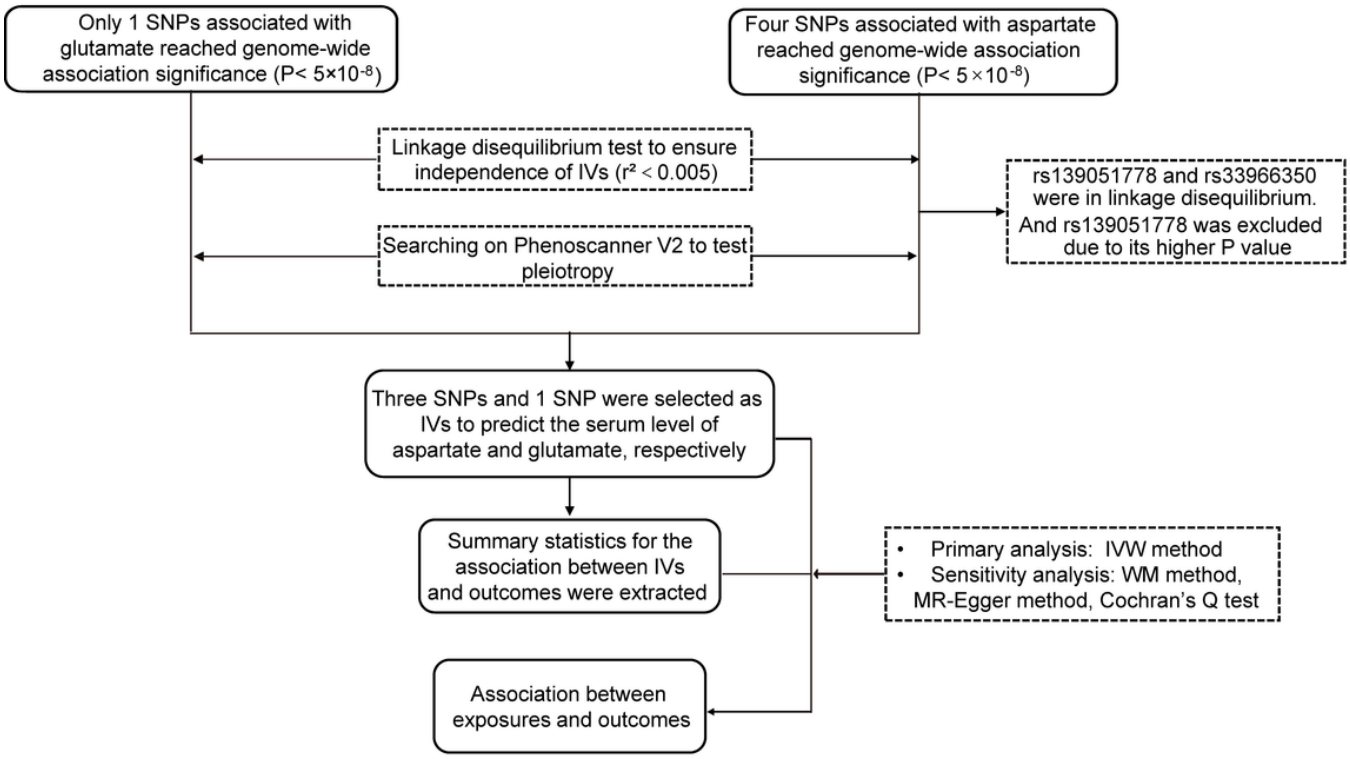

Figure 1

The flowchart of Mendelian Randomization analysis of serum levels of glutamate and aspartate and risk of development of breast cancer and prostate cancer.

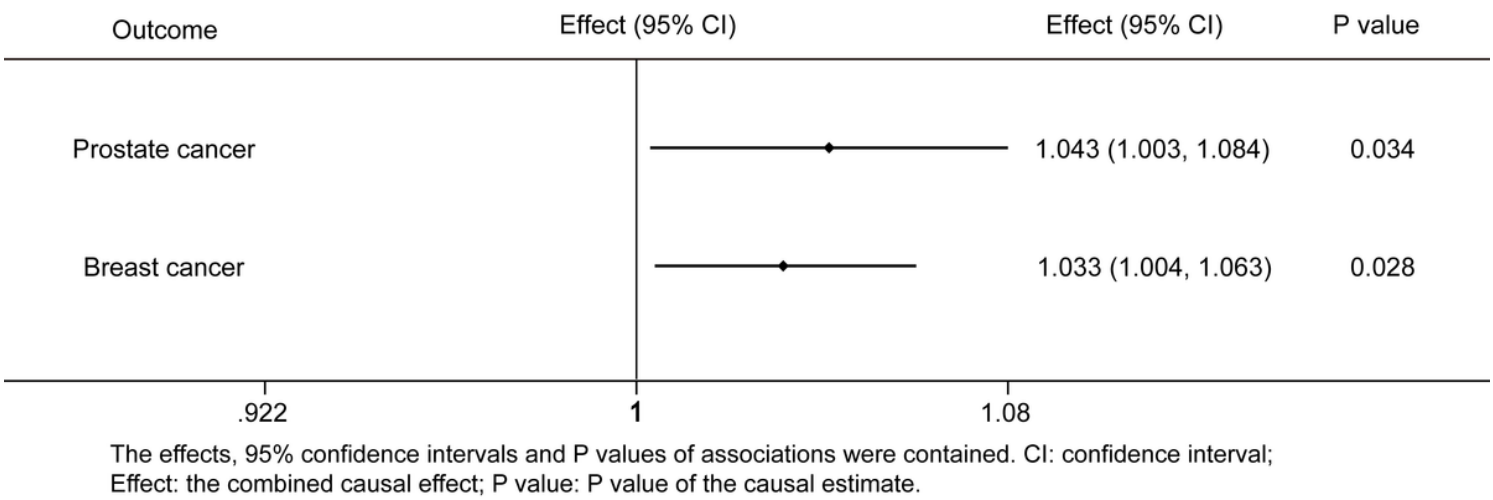

Figure 2

Causal associations between aspartate and prostate and breast cancers.

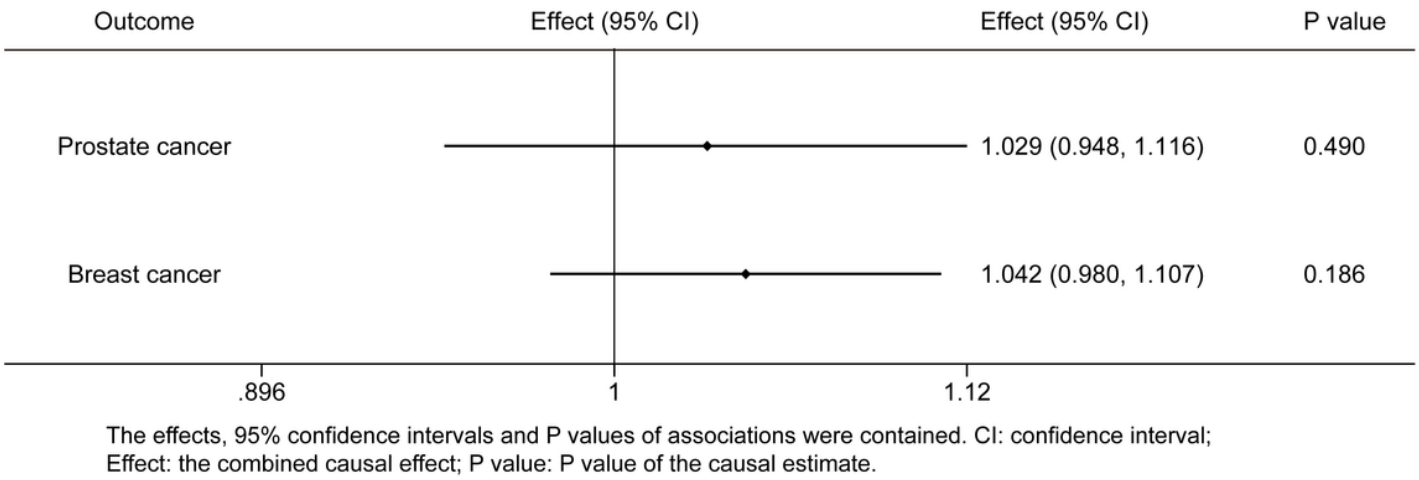

Figure 3

Causal associations between glutamate and prostate and breast cancers.

Supplementary Files

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- TableS1.xls