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The causal effect of serum amino acids on the risk of prostate cancer: a two-sample mendelian randomization study

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Prostate cancer (PCa) is the second most common malignancy affecting men globally. Recent advances in metabolomics have highlighted significant alterations in specific amino acid (AA) metabolism linked to PCa, indicating their potential utility in diagnosis and therapy. However, no direct causal association between serum AA levels and PCa risk has been established. A total of 35 patients with PCa and 30 individuals with benign prostatic hyperplasia (BPH) were recruited for this study. Targeted metabolomic analysis was performed using ultra-high-performance liquid chromatography-tandem mass spectrometry on serum samples. Two-sample Mendelian randomization (MR) was applied to explore potential causal links between serum AA levels and PCa risk, including mediator effects using dual-phase MR and assessing reverse causality through reverse MR. Results Targeted metabolomic profiling identified six amino acids—glutamate (Glu), Ser, histidine (His), arginine (Arg), aspartic acid (Asp), and glycine (Gly)—that showed significant area under the ROC curve in differentiating between BPH and PCa cases. Notably, Glu demonstrated an inverse association with PCa risk, distinct from the other AAs identified. However, definitive evidence supporting a causal relationship between low Glu levels and increased PCa risk was not observed. Our results suggest a protective role of Glu against PCa development, which may have implications for disease prognosis. Increasing dietary Glu intake may present a potential preventive or therapeutic approach for PCa.

Keywords Prostate cancer, Glutamate, Two-sample, Mendelian randomization, Protective factors

Abbreviations

AA	Amino acid.
AUC	Area under the ROC curve.
BPH	Benign prostatic hyperplasia.
BMI	Body Mass Index.
GWAS	Genome-wide association study.
MR	Mendelian randomization.
IVs	Instrumental variables.
IVW	Inverse Variance Weighted.
LD	Linkage disequilibrium.
OPLS-DA	Orthogonal partial least squares discriminant analysis.
PCa	Prostate cancer.
PSA	Prostate-specific antigen.
ROC	Receiver operating characteristic.
SNPs	Single nucleotide polymorphisms.
TCA	Tricarboxylic acid.

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UPLC-MS/MS	Ultra-high-performance liquid chromatography-tandem mass spectrometry.
VIP	Variable importance in the projection.
WME	Weighted Median Method.

According to the American Cancer Society's 2023 estimates, prostate cancer (PCa) is the most prevalent malignancy among men globally, ranking second in cancer-related mortality. It accounts for 29% of all new cancer diagnoses and 11% of cancer-related deaths in males worldwide[1,2]. PCa cells exhibit diverse behavior, ranging from indolent to highly invasive[3]. Current screening methods, including digital rectal examination, transrectal ultrasound, and serum prostate-specific antigen (PSA) testing[4,5], suffer from limitations such as false positives and negatives, complicating the differentiation between benign and malignant PCa[6]. Moreover, treatment options for advanced PCa, such as anti-androgen therapy and chemotherapy, often face resistance[7], underscoring the urgent need for improved diagnostic methods.

Recent advancements in metabolomics have enabled deeper exploration of metabolic alterations in PCa cells, revealing their pathophysiological mechanisms. PCa cells exhibit significant variations in amino acid (AA) metabolism and biosynthesis, which reflect their adaptation to hypoxia, oxidative stress, and heightened metabolic demands, thereby contributing to PCa progression[8,9]. Observational studies have identified associations between AAs and PCa risk, with case-control studies highlighting distinct AA profiles in patients compared to controls[10]. Emerging biomarkers for PCa have been identified, involving pathways related to ethanolamine, arginine (Arg), and branched-chain amino acids (BCAAs)[11]. Moreover, genetically inferred circulating alanine (Ala) levels have been linked to altered PCa risk[12], and elevated aspartate (Asp) levels have been suggested as potential contributors to PCa development[13]. This study employs targeted metabolomics to identify unique variations in AA composition among patients with PCa. However, the findings of observational epidemiological studies are prone to biases, including confounding and reverse causation. Therefore, establishing a causal link between altered AA metabolism and PCa risk requires a more robust approach.

Mendelian randomization (MR) offers a powerful genetic approach to address biases inherent in traditional epidemiological studies by using genetic variants associated with exposures as instrumental variables (IVs)[14]. This method mitigates issues of reverse causality and confounding due to the random allocation of genotypes during gamete formation[15,16]. In this study, we applied a two-sample MR design to investigate the potential causal relationships between AA levels and PCa risk, aiming to elucidate the genetic determinants of AA dynamics in PCa progression.

Methods

Study participants
The retrospective cross-sectional analysis was conducted at Xuzhou No. 1 People's Hospital and included 30 patients with benign prostatic hyperplasia (BPH) and 35 patients diagnosed with PCa. Patients were selected based on PSA screening and clinical evaluations performed by a urologist. The diagnosis of PCa was confirmed through either prostate biopsy or surgery following hospital admission. Exclusion criteria included a family history of hereditary diseases, significant hepatic or renal dysfunction, cardiovascular disorders, hematological conditions, concurrent systemic tumors, or recent use (within three months) of immunosuppressants, hormones, or lipid-lowering drugs. The study protocol was approved by the Ethical Review Committee of Xuzhou No. 1 People's Hospital (approval number xyyll[2023]153), and informed consent was obtained from all participants. All patient samples were fully de-identified to maintain privacy and comply with ethical standards. BPH diagnoses were confirmed by negative biopsy results, excluding the presence of PCa. Further participant details are summarized in Table 1.

Serum sample collection
Following subject enrollment and obtaining informed consent, participant body mass index (BMI) was recorded. All participants were instructed to fast after 10 p.m. (no food or water). Venous blood samples were collected at 6 a.m. the next morning, ensuring a fasting state. Collected blood samples were immediately centrifuged at 1500 g for 10 min at 4 °C to separate the serum. The obtained serum was stored at −80 °C until subsequent analyses.

Sample preparation for UPLC-MS/MS analysis
For ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis, the stored serum samples were first thawed at 4 °C and homogenized by vortexing for 10 s. Twenty AAs and internal standards (as shown in Table 2) were obtained from Hangzhou Hanko Biotechnology Co., Ltd. Mobile phases were prepared as follows: Reagent A: 2% formic acid in acetonitrile, used to enhance ionization efficiency during the mass spectrometry process. Reagent B: 0.1% formic acid in water, used to maintain appropriate pH levels during analysis. Reagent C (Buffer): 0.5 M ammonium acetate solution, used to stabilize the pH during sample preparation. Reagent D: Acetonitrile, used as a solvent for the mobile phase preparation. Mobile phase A consisted of 500 μL of reagent D mixed with 500 mL of purified water, while mobile phase B contained 500 μL of reagent D mixed with 500 mL of acetonitrile. To precipitate proteins, 50 μL of serum, 50 μL of internal standards, and 50 μL of pre-chilled ultra-pure water (4 °C) were combined. This mixture was centrifuged at 18,000 g for 5 min at 4 °C, and 10 μL of the supernatant was transferred to a new tube. Subsequently, 70 μL of reagent C (buffer) and 20 μL of reagent A were added, followed by a second centrifugation at 21,000 g for 10 min. The resulting supernatant was mixed with 990 μL of ultra-pure water, vortexed, and 100 μL of the mixture was transferred into a vial containing a 300 μL insert for analysis.

Parameters		BPH	PCa	*P*
No. of subjects		30	35	
Age (years)		72.60 ± 8.58	74.31 ± 6.78	0.360[a]
BMI (kg/m²)		23.12 ± 3.51	24.58 ± 3.47	0.099[b]
Smoking status, n (%)		1(3.3%)	2(5.7%)	0.648[c]
PSA (ng/mL)		16.12 ± 17.25	21.70 ± 17.16	0.060[a]
Gleason score	3 + 3	-	4	
	3 + 4	-	4	
	3 + 5	-	1	
	4 + 3	-	2	
	4 + 4	-	7	
	4 + 5	-	10	
	5 + 3	-	3	
	5 + 4	-	3	
	5 + 5	-	1	
Tumor size(cm³)		107.21 ± 69.87	119.83 ± 60.83	0.114[a]

Table 1. Characteristics of participants' pathology and clinical profile. Data were presented as Mean ± SEM; a: Mann-Whitney U test, b: unpaired Student' s t-test, c: chi-square test, and the statistically significant *P*-values were highlighted in **bold**.

Abbreviation	Common Name	Internal standards
Asn	Asparagine	L-Aspartate-d3
His	Histidine	DL-Histidine-d3
Arg	Arginine	L-Arginine-^{13}C6,15N4
Ser	Serine	L-13C3-Serine
Gly	Glycine	^{15}N;2-^{13}C-Glycine
Gln	Glutamine	L-Glutamine-d5
Lys	Lysine	L-Lysine-d4
Thr	Threonine	L-Threonine-d5
Ala	Alanine	$^2H_4$-Alanine
Glu	Glutamate	$^2H_2$-Glutamate
Pro	Proline	DL-Proline-d3
Cys	Cysteine	DL-Cystine-d6
Tyr	Tyrosine	$^{13}C_6$-Tyrosine
Val	Valine	$^2H_8$-Valine
Met	Methionine	$^2H_4$-Methionine
ILe	Isoleucine	$^2H_3$-Leucine
Leu	Leucine	$^2H_3$-Leucine
Phe	Phenylalanine	$^{13}C_6$-Phenylalanine
Trp	Tryptophan	L-Tryptophan-d3
Asp	Aspartic acid	L-Aspartate-d3

Table 2. 20 amino acids and internal standards.

AA extraction and analysis using UPLC-MS/MS

The prepared sample solution was analyzed using the UPLC-MS/MS system (Waters Xevo-TQS). The setup utilized the Waters Acquity UPLC® I Class along with a CORTECS UPLC C18 column (1.6 μm, 2.1 × 150 mm). The mass spectrometer (Waters XEVO® TQS) operated in positive ion mode. Samples were loaded onto a 96-well autosampler plate in a specific sequence: blank samples, linear gradient standards, quality control samples, and test samples. Analysis began with three runs of blank samples, followed by ascending testing standards, with quality control samples analyzed every twenty test samples. Mobile phase A was replaced every two days, in conjunction with ion source cleaning for the mass spectrometry unit. Calibration standard concentrations (C1 to C6) were set using the system software according to the values in the calibration table. The AA detection showed a strong linear relationship across a concentration range of 1.0–6000.0 nmol/L, with a linear determination coefficient (R^2) consistently greater than 0.99. Quantification limits for the compounds ranged between 1.0 and 10.0 nmol/L, with intra-day and inter-day precision (relative standard deviation) maintained below 15%.

Additionally, the coefficient of variation for both high and low-quality control samples of AAs was kept below 15%.

Clinical statistical analysis

To compare continuous data distributions, the chi-square test was employed. Data normality was expressed as the mean $\pm$ standard error of the mean (SEM). Statistical significance for AAs, age, BMI, and PSA levels was determined using the Mann-Whitney U test and unpaired Student's t-test, with Bonferroni correction applied for multiple comparisons. Diagnostic efficacy was assessed using receiver operating characteristic (ROC) curves, with the area under the curve (AUC) calculated in R. The Spearman method was used to analyze correlations among non-normally distributed bivariate variables. Orthogonal partial least squares discriminant analysis (OPLS-DA) was employed to improve group classification by excluding variables misaligned with the model. Key metabolites were identified by variable importance in projection (VIP) scores, with VIP values greater than 1 considered significant. Statistical significance was set at $P < 0.05$, with AUC values near 1 indicating high diagnostic accuracy, while values around 0.5 suggested accuracy comparable to random chance.

GWAS data sources

The genome-wide association study (GWAS) datasets for AAs and PCa were obtained from the IEU GWAS database (https://gwas.mrcieu.ac.uk/). The AA dataset included 122,776 participants, while the PCa dataset comprised 462,933 individuals. All participants were of European descent, with datasets ensuring non-overlapping independent samples. Figure 1 illustrates the overall framework of this study.

Selection of instrumental variables for MR analysis

In this MR analysis, three assumptions were crucial: (1) IVs must be strongly associated with the exposure (i.e., serum AA levels); (2) IVs should not be related to confounding factors; and (3) IVs should influence the outcome only through the exposure. Single nucleotide polymorphisms (SNPs) significantly associated with serum AAs were selected at a genome-wide significance level ($P < 5 \times 10^{-8}$). When the number of SNPs was insufficient, the P-value threshold was adjusted to 5e-6 for Arg, serine (Ser), and threonine (Thr), and to 5×10^{-5} for glutamate (Glu) and Asp. Linkage disequilibrium was assessed using the European population from the 1000 Genomes Project, and SNPs with $r^2 < 0.001$ and a physical distance greater than 10,000 kb were retained. SNPs with minor allele frequency < 0.01 and those with an F-statistic < 10 were excluded to avoid weak instrument bias. Additionally, MR Steiger analysis verified the causal direction for each SNP to exclude those indicative of reverse causation.

Statistical analysis

The primary method for evaluating the causal relationship between exposure and outcome was the IVW approach. In addition, two supplementary MR methods, namely the weighted median method (WMM) and MR-Egger regression, each predicated on distinct modeling assumptions, were applied. The objective of utilizing these varied MR approaches was to examine the consistency and dependability of the associations
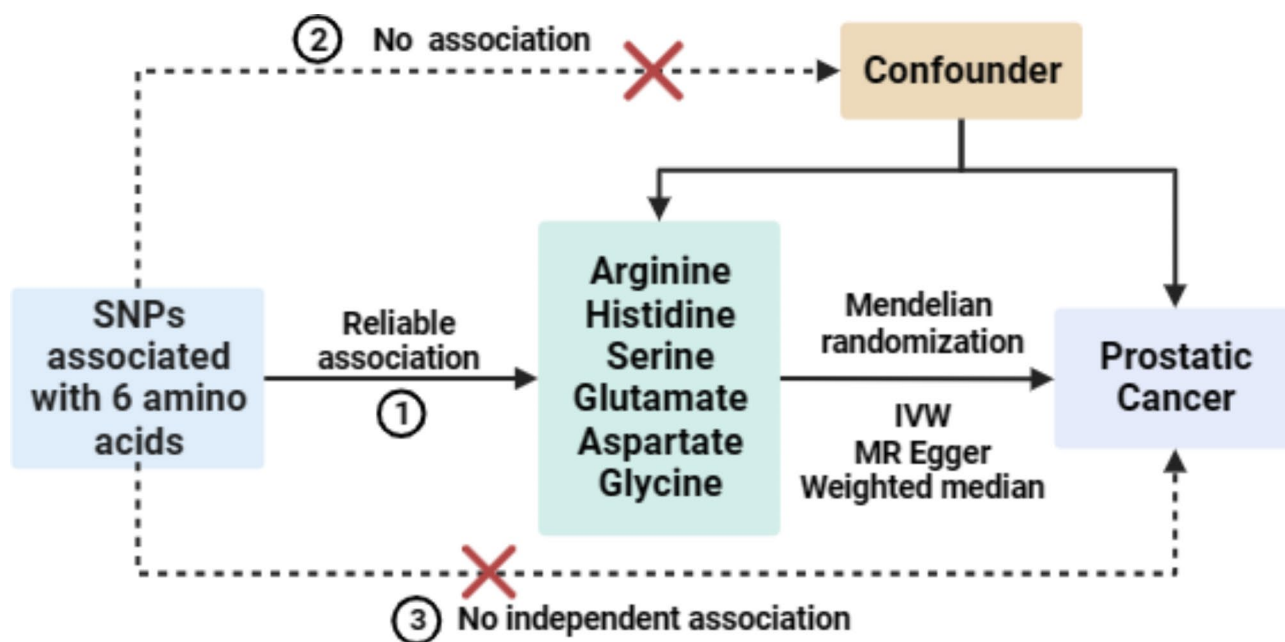
Fig. 1. Overview of the Study Design. Schematic representation of the study workflow, encompassing serum sample collection, targeted metabolomics analysis using UPLC-MS/MS, and MR analysis to investigate causal relationships between amino acid levels and PCa risk.

across different theoretical frameworks. Within the IVW framework, the Cochran's Q statistic was employed to probe the heterogeneity among the IVs, whereas the MR-Egger regression's intercept term served to investigate potential horizontal pleiotropy among the IVs. To explore the possibility of a causal impact of PCa on AA levels within the body, we applied the described MR methodologies. In this reverse MR setup, PCa functioned as the exposure with Glu considered the outcome. SNPs linked to PCa, acting as IVs, were identified with genome-wide significance ($P < 5 \times 10^{-8}$), ensuring no LD ($r^2 < 0.01$) with other SNPs over a 10,000 kb range. The entirety of these analyses was conducted utilizing R Language 4.2.1.

Results

Clinical attributes of participants

The study cohort included 65 subjects: 30 individuals with BPH and 35 with PCa. Clinical attributes of all participants are detailed in Table 1. Statistical analysis showed no significant differences in age, BMI, smoking status, tumor size, or PSA levels between the BPH and PCa groups. These findings highlight the limitations of PSA as a sole diagnostic marker, emphasizing the need for improved diagnostic approaches in urology.

Discriminatory disparities in AA composition among BPH and PCa patients

To investigate differences in serum AA profiles between BPH and PCa patients, we conducted a comparative analysis. This cross-sectional evaluation revealed that glycine (Gly), Arg, Glu, and histidine (His) levels were significantly lower in PCa patients compared to BPH controls. Conversely, higher levels of glutamine (Gln), Ala, Ser, aspartic acid (Asp), and phenylalanine (Phe) were observed in PCa patients (Fig. 2A). Using OPLS-DA, we were able to distinguish between the two groups based on these metabolic differences (Fig. 2B). Variables of VIP scores further confirmed the significance of metabolites such as Glu, Ser, His, Arg, Asp, Gly, and Thr in distinguishing BPH from PCa (Fig. 2C).
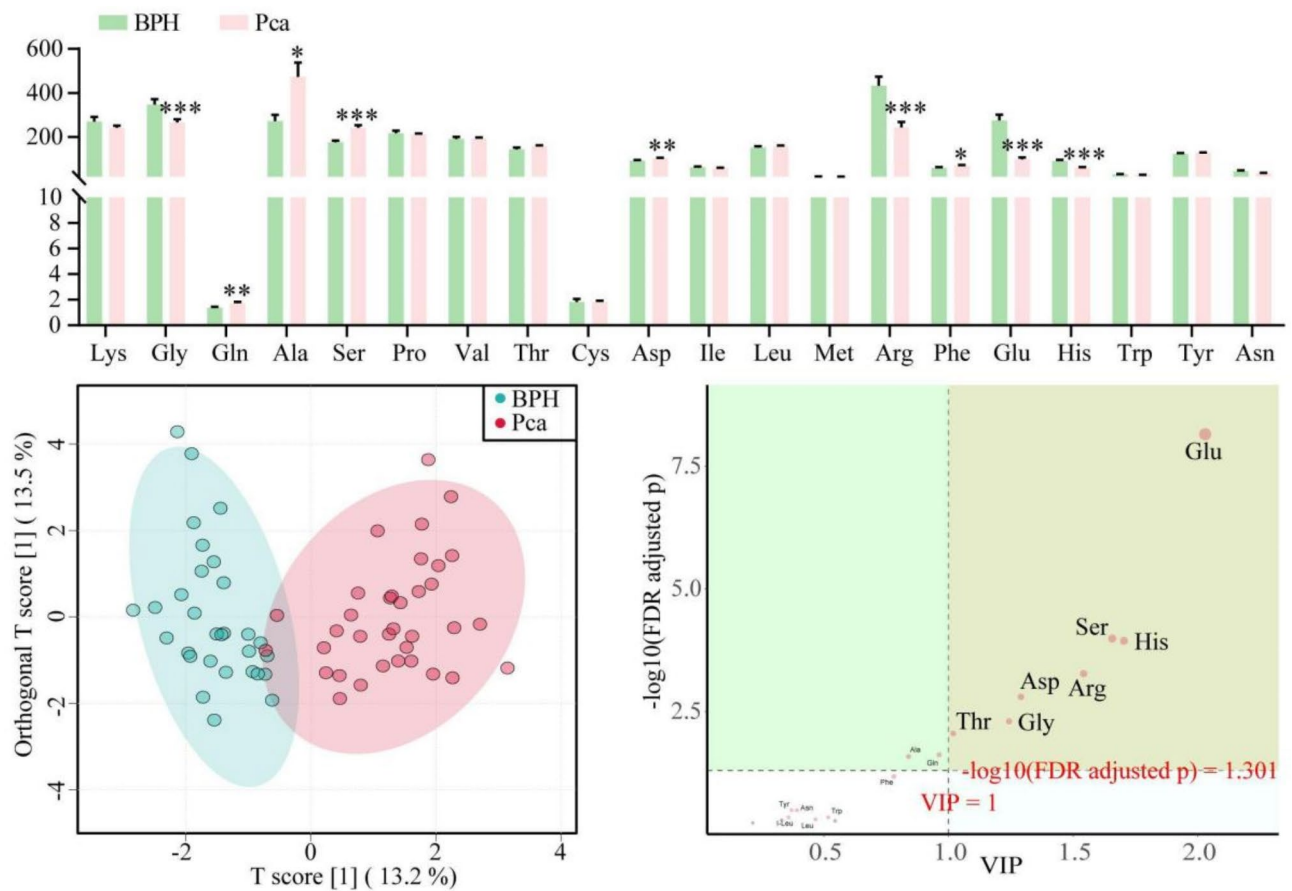
Fig. 2. Comparison of UPLC-MS/MS-based amino acid profiles between the BPH group and the PCa group reveals significant differences. **A** Comparison of serum AA concentrations between the BPH group and the PCa group, analyzed using the Mann-Whitney U test. Significant differences were observed for specific AAs, as indicated by *$P < 0.05$, ***$P < 0.001$. **B** OPLS-DA score plot illustrating the discrimination between serum AA profiles from the BPH group (blue) and the PCa group (red). **C** VIP scores derived from the OPLS-DA model, showing the importance of serum AAs in distinguishing between the two groups (VIP > 1 was considered significant). Sample sizes were $n = 30$ for the BPH group and $n = 35$ for the PCa group.

Diagnostic performance of potential AA biomarkers

We evaluated the diagnostic performance of six AAs (Glu, Ser, His, Arg, Asp, and Gly) as potential biomarkers using ROC curve analysis. The analysis showed strong diagnostic potential, with each AA achieving an AUC value above 0.6 and a P-value below 0.05 (Fig. 3). Glu exhibited particularly high sensitivity and specificity (AUC = 0.963, sensitivity = 82.9%, specificity = 96.7%), followed by Ser (AUC = 0.800, sensitivity = 82.9%, specificity = 80.0%), His (AUC = 0.813, sensitivity = 100%, specificity = 50.0%), Arg (AUC = 0.750, sensitivity = 51.4%, specificity = 90.0%), Asp (AUC = 0.731, sensitivity = 74.3%, specificity = 73.3%), and Gly (AUC = 0.739, sensitivity = 65.7%, specificity = 83.3%).

Two-sample MR analysis of AAs and PCa

Using the IVW approach for MR analysis, we investigated the potential causal associations between serum AAs and PCa risk. The results highlighted a significant causal relationship between Glu and PCa ($P < 0.05$), while no significant associations were found for other AAs ($P > 0.05$). Detailed results are presented in Tables 3 and 4. A focused MR analysis on the relationship between Glu and PCa confirmed a significant association ($P < 0.05$) through the IVW method, while results from the WMM were nearly significant ($P \approx 0.05$) with an odds ratio (OR) of less than 1 (Fig. 4A). An inverse correlation was observed between Glu levels and PCa risk (Fig. 4B).
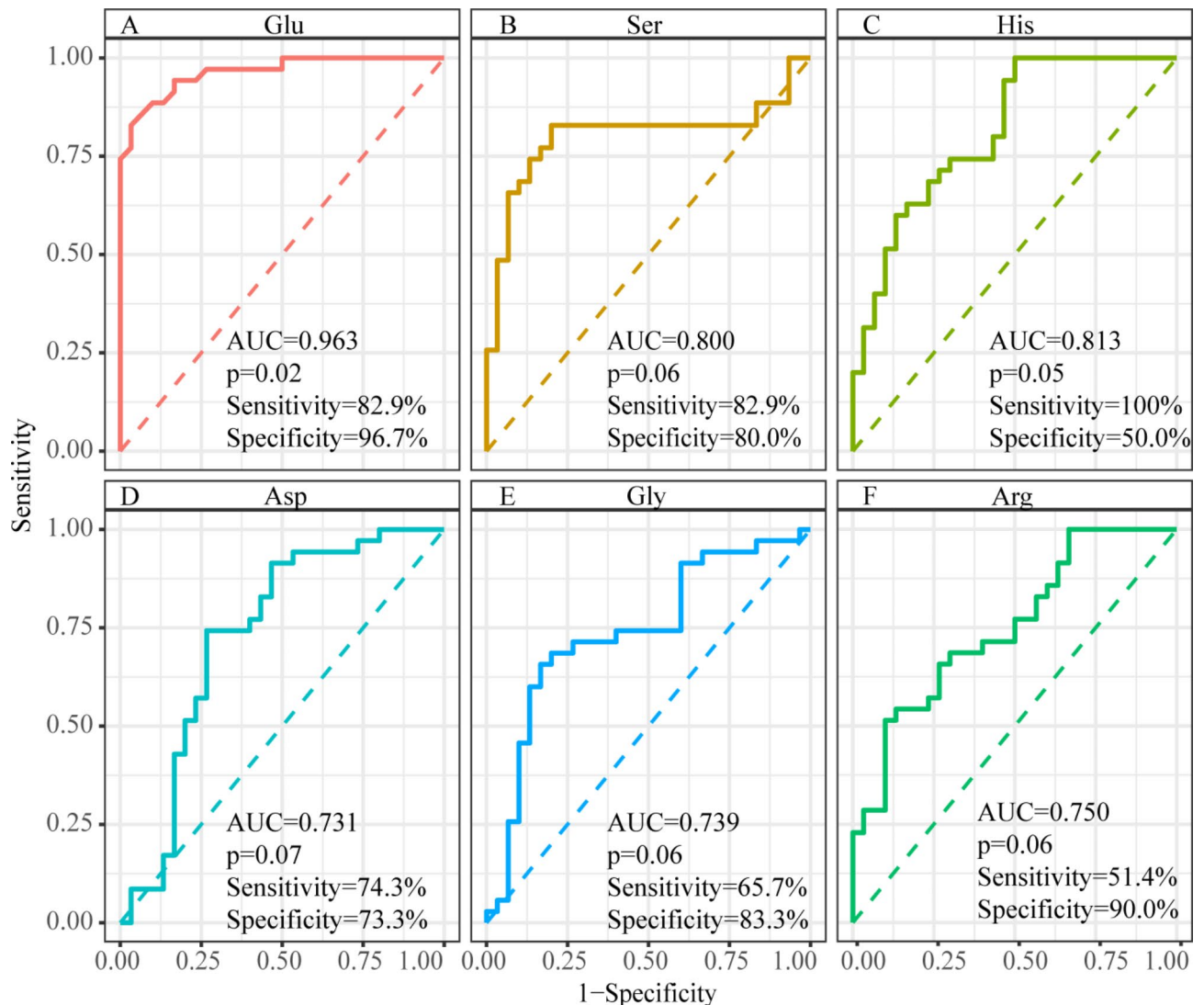

Fig. 3. Comparison of ROC curve analysis for selected amino acids reveals significant differences in their ability to discriminate between the BPH group and the PCa group. Evaluation of diagnostic performance for individual amino acids as potential biomarkers in distinguishing prostate cancer from benign prostatic hyperplasia. **A** Glutamate (Glu) (AUC = 0.963, sensitivity = 82.9%, specificity = 96.7%). **B** Serine (Ser) (AUC = 0.800, sensitivity = 82.9%, specificity = 80.0%). **C** Histidine (His) (AUC = 0.813, sensitivity = 100%, specificity = 50.0%). **D** Arginine (Arg) (AUC = 0.750, sensitivity = 51.4%, specificity = 90.0%). **E** Aspartic acid (Asp) (AUC = 0.731, sensitivity = 74.3%, specificity = 73.3%). **F** Glycine (Gly) (AUC = 0.739, sensitivity = 65.7%, specificity = 83.3%).

Exposure	ID	Population	Sample size	Number of SNPs	Year	Author
Arginine	met-a-347	European	7,528	2,545,579	2014	Shin
Histidine	met-d-His	European	114,895	12,321,875	2020	Borges CM
Serine	met-a-464	European	7,796	2,545,555	2014	Shin
Glutamate	met-a-466	European	7,804	2,545,537	2014	Shin
Aspartate	met-a-388	European	7,721	2,545,425	2014	Shin
Glycine	met-d-Gly	European	114,972	12,321,875	2020	Borges CM

Table 3. Summary of the amino acid data sets.

Exposure	ID	No. of SNPs	OR	95% CI	*P* value
Arginine	met-a-347	7	0.997	(0.987–1.008)	0.674
Histidine	met-d-His	9	1.001	(0.999–1.005)	0.193
Serine	met-a-464	13	0.996	(0.987–1.005)	0.415
Glutamate	met-a-466	31	0.996	(0.992–1.000)	**0.049**
Aspartate	met-a-388	16	1.001	(0.996–1.005)	0.649
Glycine	met-d-Gly	30	0.999	(0.998–1.000)	0.056

Table 4. MR analysis for 6 amino acids in blood associations with prostate cancer risk. The statistically significant *P*-values were highlighted in **bold**.
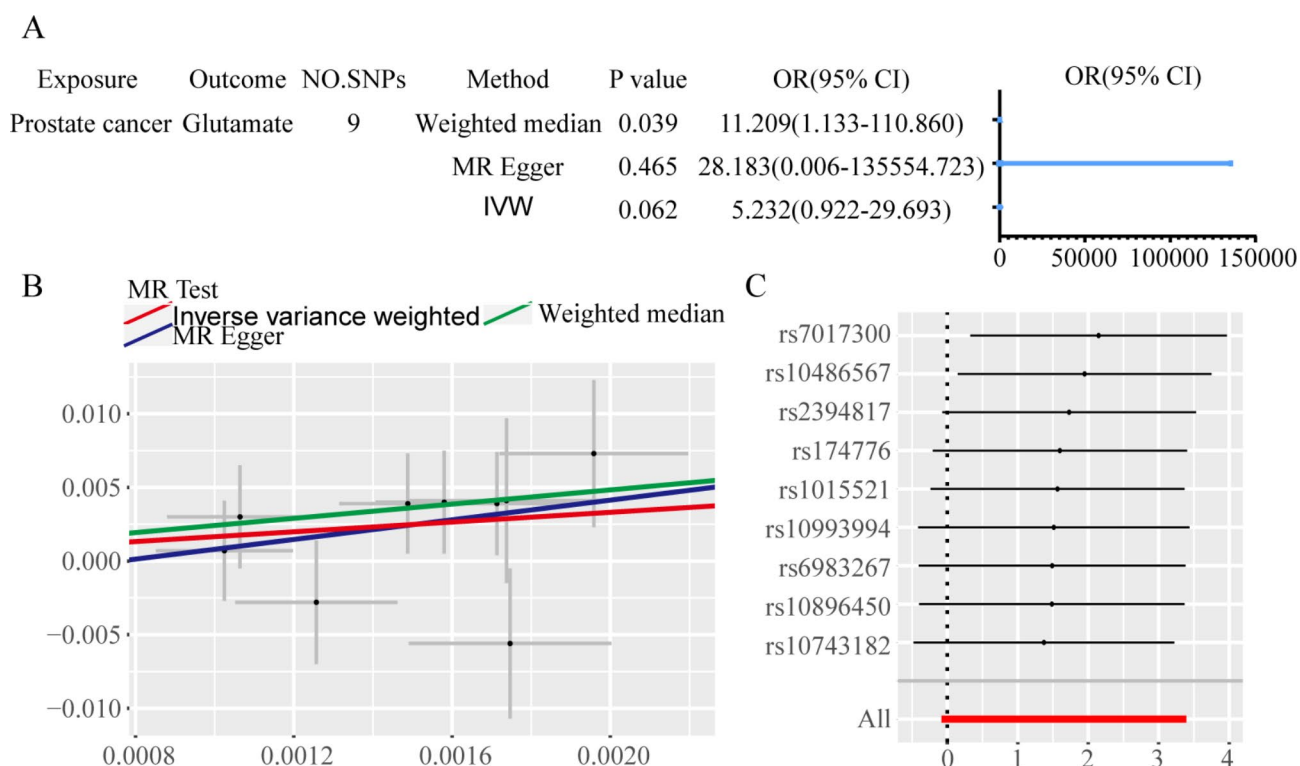
Fig. 4. Two-sample MR analysis results reveals the association between Glu levels and PCa risk. **A** Forest plot displaying MR estimates and 95% confidence intervals for the causal effect of Glu levels on PCa risk. **B** Scatter plot depicting the relationship between genetically predicted Glu levels and the risk of PCa using different MR methodologies (inverse-variance weighted, MR-Egger, and weighted median). **C** Leave-one-out sensitivity analysis indicating the stability of the association between Glu and PCa risk, demonstrating that no single SNP disproportionately influenced the results.

Leave-one-out analysis showed that no single SNP had an undue influence on the outcome (Fig. 4C). Sensitivity analyses, including the MR-Egger intercept and Cochran's Q test, produced *P*-values greater than 0.05, indicating minimal bias.
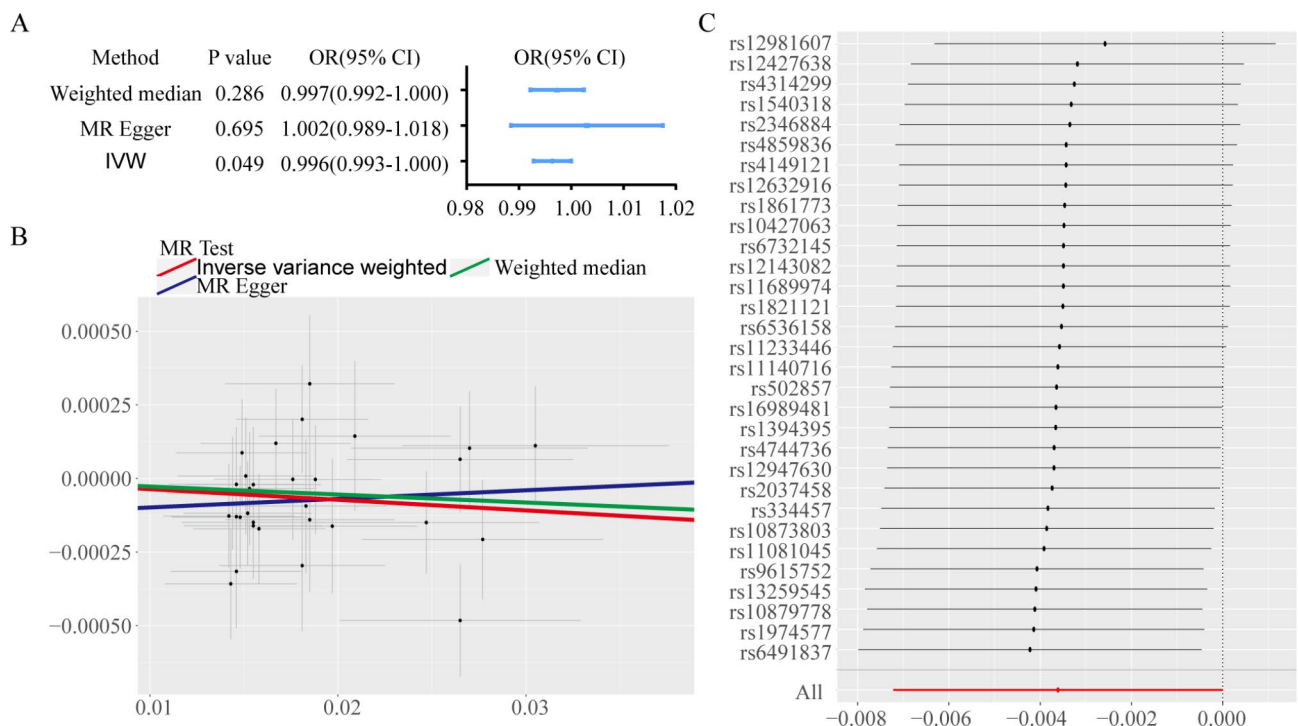
Reverse MR analysis of Glu and PCa

To explore a possible reverse causal relationship, we conducted an MR analysis positioning PCa as the exposure and Glu as the outcome. The results indicated no significant association between PCa and Glu levels (*P* > 0.05, Fig. 5A) and showed no apparent correlation (Fig. 5B). This finding was further corroborated by a leave-one-out analysis, which demonstrated the stability and robustness of the results against individual variations (Fig. 5C).

Discussion

Tumor cells thrive in harsh environments, including nutrient scarcity and hypoxia, while evading immune detection[17]. Traditional invasive biopsies, though accurate, often face challenges related to patient compliance and high costs. Non-invasive diagnostic methods, such as serum cancer embryonic antigen tests, lack sensitivity and specificity, making them unreliable for tumor detection[18,19]. Consequently, developing precise and broadly applicable techniques for tumor assessment remains crucial. Metabolomics, with its non-invasive nature, reliability, and cost-effectiveness, offers a promising approach by directly linking metabolic disturbances to tumor presence[9,20]. AAs are vital for cancer cell biosynthesis and survival[21]. Prior research has indicated the potential of AAs as biomarkers for enhancing PCa screening, emphasizing alterations in AA metabolism associated with PCa[11–13]. In this study, we analyzed serum AA profiles from patients with BPH and PCa using UPLC-MS/MS and identified significant differences in AA concentrations between the two groups. Specifically, our VIP analyses identified Glu, Ser, His, Arg, Asp, Gly, and Thr as key AAs, with ROC curve analysis highlighting Glu as a promising marker for distinguishing PCa from BPH (AUC = 0.963, sensitivity = 82.9%, specificity = 96.7%).

Glu, a nonessential AA, is crucial for metabolism, brain function, and immune response regulation[22–24]. Glu supplementation may offer health benefits due to its influence on the tricarboxylic acid (TCA) cycle and ATP production, impacting body weight regulation and hormone release[25,26]. Gln, synthesized from Glu, is abundant in blood and muscle tissues and plays an important role in cancer biology[9,27]. In PCa, mitochondrial DNA mutations often impair energy processes like TCA and oxidative phosphorylation, prompting tumor cells to rely on alternative metabolic pathways[28]. Oncogenes, such as Myc and androgen receptors, promote Gln uptake, influencing its metabolism and contributing to PCa cell proliferation[29]. Investigations into PCa cells have uncovered anomalies in Gln metabolism and the TCA cycle[29,30]. PC-3 PCa cells, characterized by high aggressiveness, exhibit increased Gln utilization, emphasizing the role of Gln in cancer progression[31]. In our study, patients with PCa exhibited decreased Glu and increased Gln levels, suggesting a potential protective effect

Fig. 5. Reverse MR analysis reveals the association between PCa as exposure and Glu as outcome. **A** Forest plot illustrating MR estimates and corresponding 95% CI values for the causal effect of PCa as an exposure on Glu levels. **B** Scatter plot showing the relationship between genetically predicted PCa levels and Glu concentrations using different MR approaches. **C** Leave-one-out sensitivity analysis for the reverse MR analysis, confirming the robustness of the findings by demonstrating minimal influence from individual SNPs.

of Glu against PCa. UPLC-MS/MS-based targeted metabolomics enabled the precise quantification of Glu and Gln, which are structurally similar, allowing for independent evaluation of their levels. Prior studies have linked elevated Glu levels to higher PCa mortality risk, while increased Gln may contribute to cancer progression and angiogenesis[13,32]. These findings support the role of Glu as a potential protective factor against PCa.

Our study employed MR to address biases and confounding factors common in traditional epidemiological research[33]. Previous MR studies have identified associations between genetically inferred serum levels of Ala and Asp with altered PCa risk[12,13]. In our study, MR analysis using the IVW approach indicated an inverse association between Glu levels and PCa risk, while no significant associations were found for the other AAs. A two-sample MR analysis, utilizing independent datasets for Glu and PCa, provided robust evidence for this association. Sensitivity analyses, including MR-Egger and Cochran's Q test, produced P-values greater than 0.05, demonstrating minimal bias. The leave-one-out analysis confirmed that no individual SNP unduly influenced the overall findings. In a reverse MR analysis exploring PCa as the exposure and Glu as the outcome, no significant causal relationship was found, further supporting our conclusions regarding Glu's protective effect. Early diagnosis is critical for effective PCa management, but traditional screening methods such as PSA testing are prone to overdiagnosis and overtreatment, complicating treatment decisions and affecting outcomes[34,35]. Although PSA has been a cornerstone of PCa diagnosis for decades, its specificity is compromised in elderly men with BPH, reducing its diagnostic accuracy[36,37]. In this context, we included BPH patients as the control group to identify metabolic differences that could serve as more reliable markers for distinguishing BPH from PCa. Our findings suggest that Glu could serve as an adjunct to PSA in early PCa screening and risk stratification, potentially enabling more tailored treatment strategies. However, a key limitation of this study is the lack of biopsy-confirmed BPH cases, which restricts the ability to fully explore the association between Glu levels and histopathological changes. Future studies should include pathological analyses to validate these associations.

In our MR analysis, results from the MR-Egger approach differed from those obtained using IVW and Weighted Median methods, highlighting potential issues such as pleiotropy. The MR-Egger method, while designed to estimate causal effects even with pleiotropic variants, is susceptible to biases introduced by invalid IVs or outliers. However, our findings indicated no substantial pleiotropy, as evidenced by the MR-Egger intercept ($P > 0.05$). This divergence underscores the complexity of genetic influences on AA levels and their association with PCa risk, emphasizing the need to consider the assumptions and limitations of each MR approach. This discussion serves to acknowledge these complexities and underscores the importance of using multiple methodologies to strengthen our conclusions. Additionally, our study did not directly measure the impact of physical activity on serum AA levels, which could be an influential factor. Although preliminary assessments showed no significant differences in activity levels between groups, incorporating more detailed measures of physical activity in future research may provide further insights into its role in cancer risk and AA metabolism.

Conclusions
Although AA dysregulation in cancer is well documented, identifying specific serum AAs as risk or protective factors has proven challenging. Our study suggests that lower serum Glu levels may be associated with a reduced risk of PCa, highlighting a potential protective role. These findings warrant further investigation and suggest that increasing Glu levels might represent a future strategy for PCa prevention and treatment.

Data availability
The published article includes all data generated or analyzed during the course of this study. The codes generated or utilized during the study are available upon request from the corresponding author.

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Author contributions

LM and QCW made equal contributions to this study. XLC and NNQ designed the study, while LM, QCW, SK and NNQ conducted experiments and analyzed data. LM, WQL, YJZ and WC collected clinical samples. LM, QCW, NNQ, and XLC finalized the manuscript. All authors listed in this manuscript contributed to data interpretation and approved the final version.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki. The present study was approved by the ethical review committee of Xuzhou No. 1 People's Hospital (approval number xyyll[2023]153), with all participants providing informed consent.

Additional information

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