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ORIGINAL RESEARCH

Genetically Predicted Circulating Concentrations of Alanine and Alanine Aminotransferase Were Associated with Prostate Cancer Risk

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Object: Prostate cancer is one of the leading malignancies in men worldwide. Previous observational studies have linked amino acids and transaminase with altered risk of prostate cancer. However, whether these associations were causal remained unclear. Therefore, we conducted a Mendelian randomization (MR) to assess their potential causal associations.

Methods: Summary-level data for prostate cancer were obtained from a meta-analysis of genome-wide association studies (GWAS) including 79,148 prostate cancer cases and 61,106 controls of European descent. Instrumental variables (IVs) of amino acids and alanine aminotransferase (ALT) were obtained from a GWAS of 86,507 European individuals and a GWAS of 312,572 participants from the UK Biobank, respectively. MR analyses were performed using inverse-variance-weighted (IVW), likelihood-based, MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test and MR-Egger regression.

Results: Genetically predicted circulating concentrations of alanine were associated with an increased risk of prostate cancer (odds ratio (OR): 1.16, 95% confidence interval (CI): 1.01–1.33, P=0.037 by IVW). Consistently, genetically predicted ALT was inversely associated with the risk of prostate cancer (OR: 0.43, 95% CI: 0.27–0.68, $P=3.28\times10^{-4}$ by IVW). MR-Egger regression did not indicate evidence of directional pleiotropy and sensitivity analyses yielded consistent associations.

Conclusion: Our study revealed that genetically predicted circulating alanine and ALT levels were associated with an altered risk of prostate cancer, suggesting their potential roles in the development of prostate cancer. Whether targeting alanine, ALT or its downstream effectors are helpful in reducing prostate cancer incidence warrants further investigation.

Keywords: alanine, alanine aminotransferase, amino acids, Mendelian randomization, prostate cancer

Introduction

Prostate cancer is the second most frequently diagnosed cancer type and the fifth leading cause of cancer death among men, with approximately 1.4 million new cases and 375,000 deaths worldwide in 2020.[1,2] Incidence rates of prostate cancer vary from 6.3 to 83.4 per 100,000 men across regions, with the highest rates found in Northern and Western Europe, Australia/New Zealand and Northern America, and the lowest rates in Asia and Northern Africa.[2] For a disease with a heavy burden such as prostate cancer, its etiology and pathogenesis remain largely unknown. Established risk factors for prostate cancer are limited to advanced age, family history of cancer and genetic factors.[3–5] Therefore, it is necessary to search for additional biomarkers, so as to aid in the prevention, diagnosis and treatment of prostate cancer.

Since the metabolic characteristics of cancer cells are different from those of normal cells, determination of metabolites, such as free amino acids (AAs), transaminase, in biological fluids may be valuable in the prevention,

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diagnosis or treatment of cancer.[6] Previous observational studies have linked the associations of amino acids with the risk of prostate cancer. For example, a prospective study of 523 prostate cancer patients and 523 matched controls found that serum levels of cysteine were associated with a reduced risk of prostate cancer, while levels of dipeptide leucyl glycine and glutamyl amino acids were associated with an increased risk of prostate cancer.[7] Another case–control study including 400 participants also found statistically significant differences in free amino acids profiles between patients with prostate cancer and controls.[8] In addition, transaminases, such as alanine aminotransferase (ALT), were found to be markedly elevated in serum samples from patients with prostate cancer, compared with controls.[9] However, Zhou et al reported that there were no differences in the serum levels of ALT in patients with prostate cancer compared with those diagnosed with benign prostatic hyperplasia.[10] Since the above findings were from traditional observational epidemiological studies, which may be vulnerable to bias such as confounding and reverse causation, it remains unclear whether these AAs and transaminases are causally associated with the risk of prostate cancer.

Mendelian randomization (MR) study is a genetic approach designed to overcome the biases of traditional epidemiological research by using genetic variants associated with exposure as instrumental variables (IV). Since genotypes are presumed to be randomly assigned during the process of gamete formation, MR study can effectively avoid the interference of reverse causality and are less susceptible to confounding factors.[11] Therefore, in the present study, we implemented a MR design to investigate the potential causal effects of AAs and related transaminases on the risk of prostate cancer, so as to provide genetic evidence on the potential roles of AAs and transaminases in the development of prostate cancer.

Methods

Data Sources

The overall design of this study is shown in Figure 1. MR analyses were performed based on summary-level data from publicly available genome-wide association studies (GWASs) of AAs, transaminases and prostate cancer, respectively. The detailed information of related GWASs is summarized in Supplementary Table 1. Briefly, summary-level data for prostate cancer were obtained from the Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome (PRACTICAL) Consortium,[12] which includes 79,148 prostate cancer cases and 61,106 controls of European descent. The effect estimate of each single-nucleotide polymorphism (SNPs) was obtained using fixed-

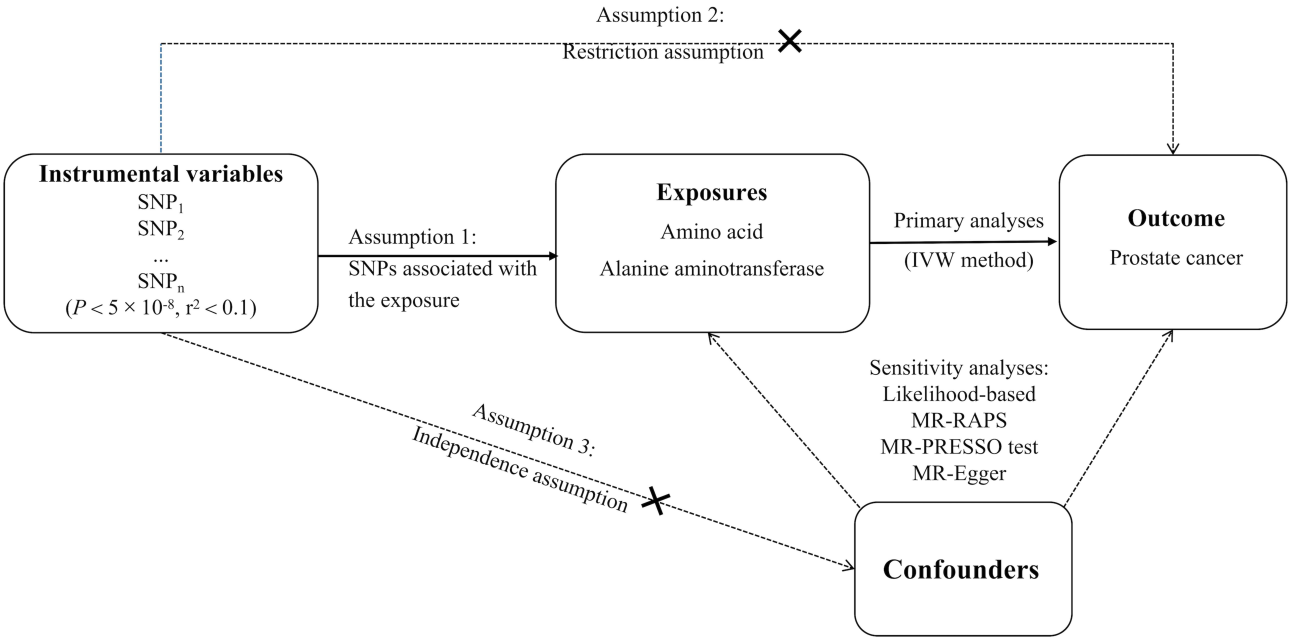

Figure 1 An overall design of the present study.

effects meta-analysis combining the summary statistics from OncoArray analysis and seven previous prostate cancer GWAS or high-density SNP panels of European ancestry imputed to the 1000 Genomes Project.

Selection of Instrumental Variables

Amino acid-associated SNPs used as genetic instruments were obtained from a GWAS of 86, 507 adult individuals with European ancestry from the Fenland cohort with those run in the EPIC-Norfolk and INTERVAL studies.[13] A total of 119 independent SNPs achieving genome-wide association threshold ($P<5\times10^{-8}$) and with linkage disequilibrium (LD) $r^2<0.1$ were identified for common AAs (16 for alanine, 9 for arginine, 3 for aspartate, 15 for glycine, 10 for glutamine, 8 for histidine, 4 for isoleucine, 6 for leucine, 9 for lysine, 1 for methionine, 7 for phenylalanine, 3 for proline, 5 for serine, 7 for threonine, 2 for tryptophan, 6 for tyrosine, and 6 for valine).[13] In addition, a total of 237 independent SNPs ($r^2<0.1$) associated with serum ALT were obtained from two GWASs of circulating biomarkers,[14] including 315,572 participants from the UK Biobank (UKBB). Association estimates were obtained assuming an additive genetic model on log 10-transformed ALT values, with adjustment for age, sex, and genetic principal components.

Statistical Analysis

We first calculated *F* statistics for SNPs used as IVs to measure the strength of the instruments, since IVs with an *F* statistic less than 10 were frequently labeled "weak instruments" and thus excluded from the MR analyses.

The potential causal associations between common AAs and the risk of prostate cancer were assessed using the inverse variance weighted (IVW) method as the primary analysis. This method first obtains the causal effect estimates based on a single genetic instrumental variable to calculate the Wald estimates,[11] and then meta-analyzed using a fixed- or random-effects model to generated a combined causal effect estimate which provide a constant estimate of the causal association between AAs and the risk of prostate cancer.[15] Cochran's Q test was used to test whether the estimate of the association between exposure and outcome was consistent across each individual SNP.[16] A fixed-effects model was utilized when no statistically significant heterogeneity was presented, otherwise the random-effects model was used to provide more conservative estimates.[17]

We further conducted several sensitivity analyses to evaluate the robustness of our main analyses. The likelihood-based method was used to evaluate the potential linear relationship between the exposure and the outcome, and the likelihood-based estimator expresses the causal increase in the outcome per unit change in the risk factor assuming a linear association between the risk factor and the outcome variables.[15] We also performed the Robust Adjusted Profile Score (MR-RAPS) with Huber loss function to estimate the causal effects, which considers the measurement error in SNP-exposure effects and is robust to systematic and idiosyncratic pleiotropy.[18] In addition, we conducted MR-Egger regression and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) to identify the potential horizontal pleiotropic effects of the SNPs.[19] The intercept term from MR-Egger regression analysis represents the average pleiotropic effect of a genetic variant. An intercept different from zero suggests evidence of directional pleiotropy.[20] MR-PRESSO analysis conducts a global test of heterogeneity by regressing the SNPs-outcome associations on the SNPs-exposure associations and comparing the observed distance of each SNP from the regression with the distance expected under the null hypothesis of no pleiotropy.

Moreover, we performed "leave-one-out" analyses to identify potential influential SNPs, by sequentially removing of each SNP from the IV set and rerunning MR analyses. Finally, considering the potential influence of pleiotropy on the association results, SNPs used as IVs were manually scanned for related secondary phenotypes using the GWAS Catalog https://www.ebi.ac.uk/gwas, last accessed on April 15, 2022). SNPs associated with other traits at the genome-wide significance level were excluded from the IV list and MR analyses were further performed using the remaining SNPs.

All statistical analyses were performed using R (version 4.1.0) with packages "MendelianRandomization" and "MR-PRESSO", unless otherwise noted. *P*-values <0.05 were considered statistically significant.

Results

The detailed information of the SNPs used as IVs for AAs and ALT and their association estimates with the risk of prostate cancer are listed in Supplementary Tables 2 and 3. We first calculated F-statistics to quantify the strength of the IVs by using

the following equation: $F = R^2 \times (N - 1 - K) / (1 - R^2) \times K$, in which R^2 represents the variance explained by the IVs and N indicates the sample size. R^2 was estimated according to minor allele frequency (MAF) and β-value.[21] The *F*-statistics for AAs and ALT ranged from 30.24 to 1035.80, satisfying the threshold of >10 (Supplementary Table 4).

As shown in Table 1, genetically predicted higher circulating levels of alanine were nominally associated with the risk of prostate cancer (odds ratio (OR) per 1 SD = 1.16, 95% confidence interval (CI): 1.01–1.33, *P*=0.037). Sensitivity analyses using maximum-likelihood methods (OR = 1.16, 95% CI: 1.01–1.34, *P*=0.034) and MR-RAPS (OR = 1.16, 95% CI: 1.05–1.28, *P*=0.005) supported a similar association as the main analysis. No outlier SNPs were detected using MR-PRESSO test, and the causal effect estimate between alanine and prostate cancer was similar (OR:1.16, 95% CI: 1.01–1.33, *P*=0.037). MR-Egger regression did not suggest evidence of potential directional pleiotropy (*P* for intercept =0.508) (Figure 2).

In addition, genetically predicted higher ALT levels were associated with a decreased risk of prostate cancer (OR=0.43, 95% CI: 0.27–0.68, $P = 3.28\times10^{-4}$, by IVW). The effect estimate was consistent in sensitivity analyses using the maximum likelihood (OR = 0.41, 95% CI: 0.26–0.66, $P=2.57\times10^{-4}$) method and MR-RAPS (OR=0.53, 95% CI: 0.41–0.69, $P=3.11\times10^{-6}$). MR-Egger regression did not suggest evidence of directional pleiotropy (*P* for intercept=0.067) (Figure 3). Fourteen outliers were detected using the MR-PRESSO test. After excluding these SNPs, subsequent MR analyses showed that the effect between ALT and prostate cancer did not change markedly (OR = 0.52, 95% CI: 0.36–0.77, $P = 9.70\times10^{-4}$). Moreover, we manually searched the SNPs used as IVs for ALT in the GWAS Catalog, and found 37 SNPs were associated with secondary traits (Supplementary Table 5). Using the remaining SNPs as IVs, the MR analysis suggested an inverse association between ALT and prostate cancer (OR = 0.40, 95% CI: 0.24–0.67, $P = 5.04\times10^{-4}$, by IVW) (Supplementary Table 6). Finally, we performed "leave-one-out" analyses to identify potential influencing SNPs. We found that the effect

Table 1 Associations Between Genetically Predicted Serum Concentrations of AAs and ALT with the Risk of Prostate Cancer

	No. of SNPs	OR (95% CI)	*P* for Association	*P* value for Cochran Q test	*P* Intercept from MR-Egger Regression
Alanine					
Inverse-variance weighted	16	1.16 (1.01–1.33)	0.037	0.009	
Maximum-likelihood	16	1.16 (1.01–1.34)	0.034		
MR-RAPS	16	1.16 (1.05–1.28)	0.005		
MR-PRESSO	16	1.16 (1.01–1.33)	0.037		
MR-Egger	16	/	/		0.508
ALT					
Inverse-variance weighted	237	0.43 (0.27–0.68)	3.28×10^{-4}	3.16×10^{-55}	
Maximum-likelihood	237	0.41 (0.26–0.66)	2.57×10^{-4}		
MR-RAPS	237	0.53 (0.41–0.69)	3.11×10^{-6}		
MR-PRESSO	223	0.52 (0.36–0.77)	9.70×10^{-4}		
MR-Egger	237	/	/		0.054
Arginine					
Inverse-variance weighted	9	0.96 (0.89–1.03)	0.368	0.061	
Maximum-likelihood	9	0.96 (0.87–1.05)	0.363		
MR-RAPS	9	0.97 (0.90–1.04)	0.407		
MR-PRESSO	9	0.96 (0.87–1.05)	0.394		
MR-Egger	9	/	/		0.355
Aspartate					
Inverse-variance weighted	3	0.99 (0.92–1.07)	0.874	0.480	
Maximum-likelihood	3	0.99 (0.92–1.10)	0.874		
MR-RAPS	3	0.99 (0.92–1.08)	0.877		
MR-Egger	3	/	/		0.231

(*Continued*)

Table 1 (Continued).

	No. of SNPs	OR (95% CI)	*P* for Association	*P* value for Cochran Q test	*P* Intercept from MR-Egger Regression
Glutamine					
Inverse-variance weighted	10	0.94 (0.82–1.08)	0.405	1.94×10^{-4}	
Maximum-likelihood	10	0.95 (0.82–1.08)	0.414		
MR-RAPS	10	0.95 (0.88–1.02)	0.153		
MR-PRESSO	9	0.92 (0.82–1.04)	0.22		
MR-Egger	10	/	/		0.892
Glycine					
Inverse-variance weighted	14	1.00 (0.90–1.12)	0.991	4.01×10^{-18}	
Maximum-likelihood	14	1.00 (0.90–1.12)	0.991		
MR-RAPS	14	1.01 (0.95–1.07)	0.791		
MR-PRESSO	11	1.00 (0.90–1.11)	0.971		
MR-Egger	14	/	/		0.124
Histidine					
Inverse-variance weighted	8	0.95 (0.86–1.05)	0.323	0.118	
Maximum-likelihood	8	0.95 (0.83–1.09)	0.441		
MR-RAPS	8	0.97 (0.87–1.08)	0.530		
MR-PRESSO	8	0.95 (0.83–1.08)	0.466		
MR-Egger	8	/	/		0.01
Isoleucine					
Inverse-variance weighted	4	1.07 (0.86–1.34)	0.533	0.370	
Maximum-likelihood	4	1.07 (0.86–1.35)	0.540		
MR-RAPS	4	1.06 (0.84–1.34)	0.620		
MR-PRESSO	4	1.07 (0.86–1.34)	0.585		
MR-Egger	4	/	/		0.432
Leucine					
Inverse-variance weighted	6	1.07 (0.83–1.38)	0.612	0.035	
Maximum-likelihood	6	1.07 (0.82–1.39)	0.606		
MR-RAPS	6	1.07 (0.90–1.27)	0.449		
MR-PRESSO	6	1.07 (0.83–1.38)	0.633		
MR-Egger	6	/	/		0.256
Lysine					
Inverse-variance weighted	9	1.05 (0.99–1.11)	0.145	0.386	
Maximum-likelihood	9	1.05 (0.98–1.11)	0.153		
MR-RAPS	9	1.05 (0.98–1.12)	0.104		
MR-PRESSO	9	1.05 (0.98–1.11)	0.195		
MR-Egger	9	/	/		0.849
Methionine					
Inverse-variance weighted	1	0.92 (0.70–1.21)	0.571	/	
Maximum-likelihood	1	0.92 (0.70–1.21)	0.572		
MR-RAPS	1	0.92 (0.69–1.24)	0.595		
Phenylalanine					
Inverse-variance weighted	7	0.99 (0.89–1.11)	0.937	0.454	
Maximum-likelihood	7	1.00 (0.84–1.11)	0.936		
MR-RAPS	7	0.99 (0.89–1.12)	0.986		
MR-PRESSO	7	0.99 (0.90–1.11)	0.938		
MR-Egger	7	/	/		0.328

(*Continued*)

Table 1 (Continued).

	No. of SNPs	OR (95% CI)	*P* for Association	*P* value for Cochran Q test	*P* Intercept from MR-Egger Regression
Proline					
Inverse-variance weighted	3	1.01 (0.95–1.08)	0.688	0.425	
Maximum-likelihood	3	1.01 (0.95–1.08)	0.688		
MR-RAPS	3	1.01 (0.95–1.08)	0.696		
MR-Egger	3	/	/		0.75
Serine					
Inverse-variance weighted	5	0.99 (0.88–1.10)	0.795	0.003	
Maximum-likelihood	5	0.99 (0.88–1.10)	0.793		
MR-RAPS	5	1.01 (0.96–1.07)	0.656		
MR-PRESSO	2	1.06 (1.05–1.07)	0.046		
MR-Egger	5	/	/		0.64
Threonine					
Inverse-variance weighted	7	1.06 (0.90–1.25)	0.509	4.5×10^{-5}	
Maximum-likelihood	7	1.06 (0.89–1.26)	0.496		
MR-RAPS	7	1.13 (0.99–1.30)	0.08		
MR-PRESSO	6	1.09 (1.04–1.36)	0.055		
MR-Egger	7	/	/		0.12
Tryptophan					
Inverse-variance weighted	2	1.06 (0.94–1.19)	0.357	0.993	
Maximum-likelihood	2	1.06 (0.94–1.19)	0.358		
MR-RAPS	2	1.06 (0.93–1.20)	0.377		
Tyrosine					
Inverse-variance weighted	6	1.08 (0.97–1.19)	0.159	0.428	
Maximum-likelihood	6	1.08 (0.97–1.19)	0.158		
MR-RAPS	6	1.08 (0.97–1.20)	0.171		
MR-PRESSO	6	1.08 (0.97–1.19)	0.214		
MR-Egger	6	/	/		0.115
Valine					
Inverse-variance weighted	6	1.04 (0.92–1.17)	0.561	0.594	
Maximum-likelihood	6	1.04 (0.92–1.17)	0.559		
MR-RAPS	6	1.04 (0.91–1.18)	0.573		
MR-PRESSO	6	1.04 (0.93–1.15)	0.528		
MR-Egger	6	/	/		0.264

Abbreviations: CI, confidence interval; MR, Mendelian randomization; OR, odds ratio; SNP, single-nucleotide polymorphism.

estimates of genetically predicted ALT with the risk of prostate cancer did not change substantially after excluding one single SNP at a time (Supplementary Figure 1).

Discussion

To the best of our knowledge, this study is the first MR analysis which assessed the associations of circulating AAs and ALT with the risk of prostate cancer using MR approach based on large-scale genetic data. We provided suggestive evidence that higher circulating alanine was associated with an increased risk of prostate cancer and higher ALT was associated with a decreased risk of prostate cancer, suggesting a potential causal role of alanine and ALT in the development of prostate cancer.

Amino acid metabolism is a crucial metabolic pathway for regulating growth, reproduction and immunity.[22] Inflammatory stimulation in patients with cancer may lead to changes in the collective demand for protein and energy,

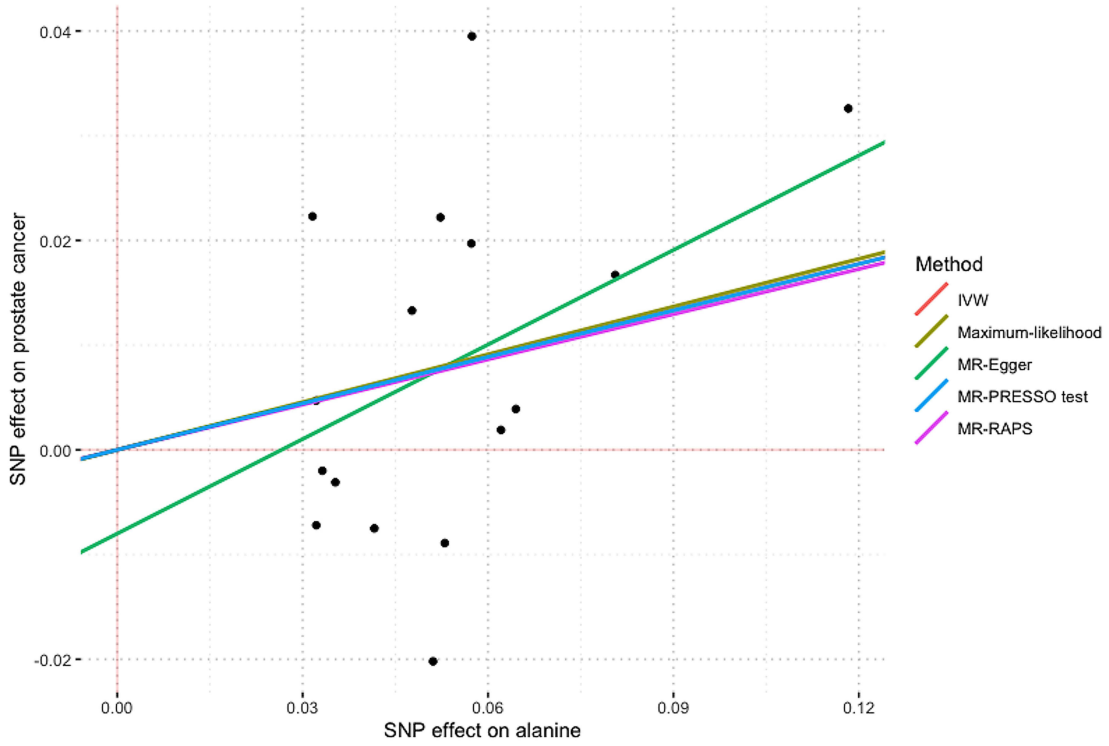

Figure 2 Association between genetically predicted alanine levels and risk of prostate cancer based on different MR methods.

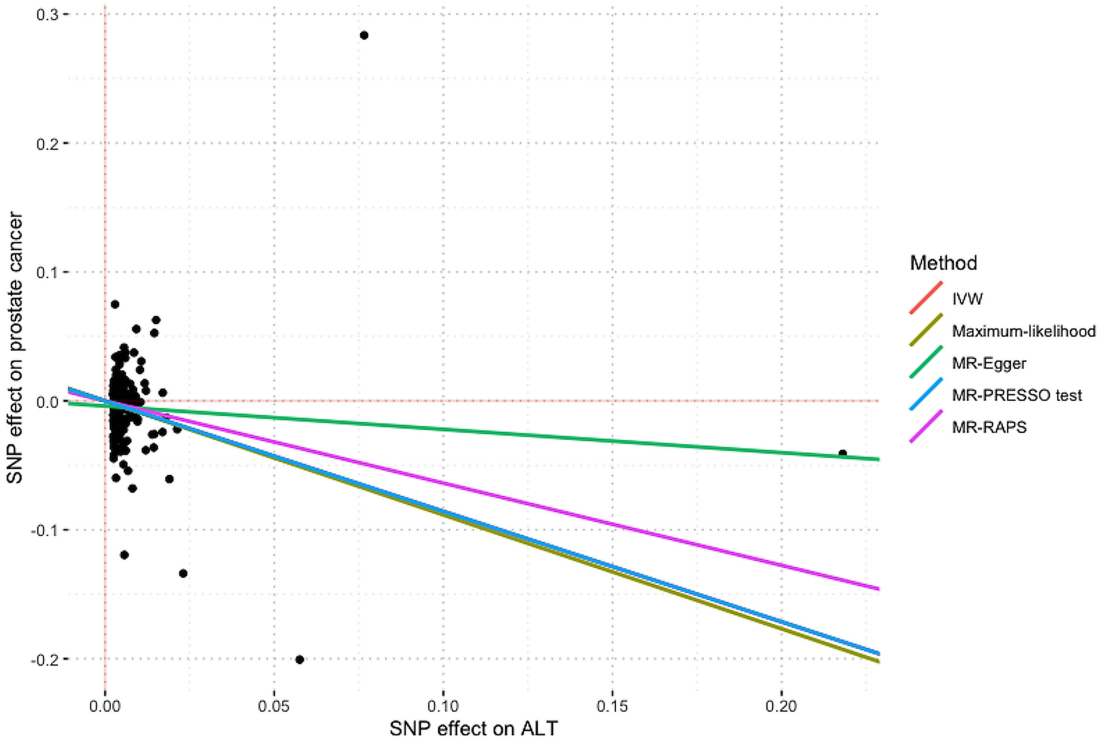

Figure 3 Association between genetically predicted ALT levels and risk of prostate cancer based on different MR methods.

accompanied by metabolic disorders.[23] A growing body of evidence has suggested that amino acids are potential biomarkers that could be used in cancer screening[24–27]; however, there was only a handful of studies with small sample sizes in prostate cancer. Our study used an MR design and provided evidence for potential causal associations of genetically predicted circulating levels of alanine with the risk of prostate cancer. This association was in the same direction as those observed in conventional epidemiological analyses. For example, in a case–control study, statistically significant differences in serum alanine levels were found in patients with prostate cancer compared to the control group (median (range): 384.8 (281.9–604.1) vs 479.9 (207.6–782.2); $P<0.001$).[28] Moreover, May-Britt et al measured alanine concentrations in 82 benign and 16 malignant prostate biopsies by spectroscopy and found that the concentrations of alanine were higher in prostate cancer biopsies than that in the benign prostate (mean ± SD: 0.26±0.07 mmol/kg vs 0.14 ±0.06 mmol/kg, $P<0.001$).[29] Our results, together with these observational findings, suggested a potential causal role of alanine in the development of prostate cancer.

In addition, we also found that ALT, one of the most common transaminases, which catalyzes the reversible conversion of alanine and 2-oxoglutaric acid to pyruvate and glutamate, was negatively associated with the risk of prostate cancer. Previous studies have indicated that ALT is involved in the conversion of alanine to pyruvic acid so as to promote gluconeogenesis.[30] Transaminases have been reported to be associated with the development of hepatocellular cancer, intrahepatic and extrahepatic cholangiocarcinoma.[31–33] Epidemiological studies have examined the association of ALT with the risk of prostate cancer, but the findings have been conflicting. For example, a case-cohort study found that ALT levels were not associated with the risk of prostate cancer (hazard ratio (HR): 0.89, 95% CI: 0.73–1.07, $P=0.212$).[34] A case–control study including 194 patients with prostate cancer and 210 controls did not find evidence for an association between ALT and risk of prostate cancer (mean ± SD: 20.08 ± 10.87 vs 21.71 ± 13.21),[10] whereas another cross-sectional study reported that prostate patients with poorly differentiated tumors had a higher ALT level (0.35ukat/l vs 0.39ukat/l) than had patients with well-differentiated clinical prostate cancer,[29] and Govand et al indicated that the activity of ALT was higher in patients with prostate cancer compared with controls (median and range, 16.25:11.38–29.05 vs 17.1:11.33–31.75).[9] These conflicting findings from conventional observational studies may because that these studies are susceptible to biases, such as reverse causation and residual confounding (eg, cancer staging, dietary, or molecular factors), which may confound the observational association estimates between ALT and prostate cancer. Nevertheless, evidence from a meta-analysis reported an inverse association between circulating levels of ALT and risk of overall cancer in European populations (RR per 1 SD=0.96, 95% CI: 0.94–0.99),[35] suggesting that ALT may be involved in carcinogenesis.

In the current study, we found evidence that higher ALT levels were associated with a decreased risk of prostate cancer using the IVW method. It has been suggested that lower ALT levels may reflect impaired synthesis in the liver. Since detoxification and lipid metabolism are closely related to hepatocytes, a sharp decrease in functional hepatocytes may increase the susceptibility of the body to toxins and metabolic disorders, and hepatocyte injury also affects the metabolism of androgens in the liver, which may lead to the occurrence and development of prostate cancer.[36,37] These findings support the rationale for using alanine and ALT as a promising target for prostate cancer screening and prevention. Further large-scale studies with a longitudinal design as well as in vitro and in vivo experiments are required to validate the causal nature of the associations between ALT and the risk of prostate cancer.

MR is a genetic epidemiological approach which uses SNPs that strongly associated with the exposure as IVs to estimate the potential causal relationship between the exposure and the outcome.[38] Since genotypes are presumed to be randomly allocated in the process of gamete formation, the introduction of IV model to a large extent solves the problem of confounding in observational studies, especially the bias effect of unmeasured confounders on causal inference.[39] The strong instruments we used in our study (ie, F-statistic much >10) should make it possible to reduce the potential bias caused by weak instrument as much as possible. Whether our results could apply to other ethnic groups remains to be confirmed, because the participants in this study involved only those of European descent. However, this greatly reduces the impact of demographic stratification bias on the results. Furthermore, for the result that was not statistically significant in this study, we cannot completely negate the association between these exposures and prostate cancer risk, since it may be caused by the small sample size and insufficient statistical power (<80%).

Conclusion

To sum up, our study suggested that alanine and ALT may play a causal role in the development of prostate cancer. Further studies are warranted to validate the observed associations and to elucidate the underlying mechanisms of alanine and ALT in risk of prostate cancer.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Statement of Ethics

Our study was approved by the Ethical Committee of Zhejiang Chinese Medical University on Nov 16, 2021 (No. AF-20211116-1). Because the project was mainly based on statistical analyses of publicly accessible databases and published studies, in which informed consents were obtained and ethical review were completed separately in each study, we have received ethical waiver for the research project.

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Disclosure

The authors declare that they have no competing interests.

References

1. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: an overview. *Int J Cancer*. 2021;149(4):778–789. doi:10.1002/ijc.33588
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Schaid DJ. The complex genetic epidemiology of prostate cancer. *Hum Mol Genet*. 2004;13(90001):R103–R121. doi:10.1093/hmg/ddh072
4. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. 2000;343(2):78–85. doi:10.1056/NEJM200007133430201
5. Nakagawa H, Akamatsu S, Takata R, Takahashi A, Kubo M, Nakamura Y. Prostate cancer genomics, biology, and risk assessment through genome-wide association studies. *Cancer Sci*. 2012;103(4):607–613. doi:10.1111/j.1349-7006.2011.02193.x
6. Thapar R, Titus MA. Recent advances in metabolic profiling and imaging of prostate cancer. *Curr Metabolomics*. 2014;2(1):53–69. doi:10.2174/2213235X02666140301002510
7. Huang J, Mondul AM, Weinstein SJ, et al. Prospective serum metabolomic profiling of lethal prostate cancer. *Int J Cancer*. 2019;145(12):3231–3243. doi:10.1002/ijc.32218
8. Miyagi Y, Higashiyama M, Gochi A, et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. *PLoS One*. 2011;6(9):e24143. doi:10.1371/journal.pone.0024143
9. Qader G, Aali M, Smail SW, et al. Cardiac, hepatic and renal dysfunction and IL-18 Polymorphism in breast, colorectal, and prostate cancer patients. *Asian Pac J Cancer Prev*. 2021;22(1):131–137. doi:10.31557/APJCP.2021.22.1.131
10. Zhou J, He Z, Ma S, Liu R. AST/ALT ratio as a significant predictor of the incidence risk of prostate cancer. *Cancer Med*. 2020;9(15):5672–5677. doi:10.1002/cam4.3086
11. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27(8):1133–1163. doi:10.1002/sim.3034
12. Schumacher FR, Al Olama AA, Berndt SI, et al. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. *Nat Genet*. 2018;50(7):928–936. doi:10.1038/s41588-018-0142-8
13. Lotta LA, Pietzner M, Stewart ID, et al. A cross-platform approach identifies genetic regulators of human metabolism and health. *Nat Genet*. 2021;53(1):54–64. doi:10.1038/s41588-020-00751-5

14. Pazoki R, Vujkovic M, Elliott J, et al. Genetic analysis in European ancestry individuals identifies 517 loci associated with liver enzymes. *Nat Commun*. 2021;12(1):2579. doi:10.1038/s41467-021-22338-2
15. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*. 2013;37(7):658–665. doi:10.1002/gepi.21758
16. Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. 2015;34(21):2926–2940. doi:10.1002/sim.6522
17. Greenland S. Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev*. 1987;9:1–30. doi:10.1093/oxfordjournals.epirev.a036298
18. Zhao Q, Bowden J. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. *Anna Statist*. .2019;48(3):1742–1769. doi:10.1214/19-aos1866
19. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50(5):693–698. doi:10.1038/s41588-018-0099-7
20. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44(2):512–525. doi:10.1093/ije/dyv080
21. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. 2011;40(3):755–764. doi:10.1093/ije/dyr036
22. van der Meij BS, Teleni L, Engelen M, Deutz NEP. Amino acid kinetics and the response to nutrition in patients with cancer. *Int J Radiat Biol*. 2019;95(4):480–492. doi:10.1080/09553002.2018.1466209
23. Manig F, Kuhne K, von Neubeck C, et al. The why and how of amino acid analytics in cancer diagnostics and therapy. *J Biotechnol*. 2017;242:30–54. doi:10.1016/j.jbiotec.2016.12.001
24. Shingyoji M, Iizasa T, Higashiyama M, et al. The significance and robustness of a plasma free amino acid (PFAA) profile-based multiplex function for detecting lung cancer. *BMC Cancer*. 2013;13:77. doi:10.1186/1471-2407-13-77
25. Plewa S, Horala A, Derezinski P, et al. Usefulness of amino acid profiling in ovarian cancer screening with special emphasis on their role in cancerogenesis. *Int J Mol Sci*. 2017;18(12):2727. doi:10.3390/ijms18122727
26. Gao P, Zhou C, Zhao L, Zhang G, Zhang Y. Tissue amino acid profile could be used to differentiate advanced adenoma from colorectal cancer. *J Pharm Biomed Anal*. 2016;118:349–355. doi:10.1016/j.jpba.2015.11.007
27. Siminska E, Koba M. Amino acid profiling as a method of discovering biomarkers for early diagnosis of cancer. *Amino Acids*. 2016;48(6):1339–1345. doi:10.1007/s00726-016-2215-2
28. Derezinski P, Klupczynska A, Sawicki W, Palka JA, Kokot ZJ. Amino acid profiles of serum and urine in search for prostate cancer biomarkers: a pilot study. *Int J Med Sci*. 2017;14(1):1–12. doi:10.7150/ijms.15783
29. Hammarsten J, Hogstedt B. Clinical, haemodynamic, anthropometric, metabolic and insulin profile of men with high-stage and high-grade clinical prostate cancer. *Blood Press*. 2004;13(1):47–55. doi:10.1080/08037050310025735
30. Liu Z, Que S, Xu J, Peng T. Alanine aminotransferase-old biomarker and new concept: a review. *Int J Med Sci*. 2014;11(9):925–935. doi:10.7150/ijms.8951
31. Lee MH, Yang HI, Lu SN, et al. Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. *J Clin Oncol*. 2010;28(30):4587–4593. doi:10.1200/JCO.2010.29.1500
32. Zhang C, Wang H, Ning Z, et al. Serum liver enzymes serve as prognostic factors in patients with intrahepatic cholangiocarcinoma. *Onco Targets Ther*. 2017;10:1441–1449. doi:10.2147/OTT.S124161
33. Aktas G, Kus T, Balkan A, Metin T, Gulsen MT, Abali H. Prognostic factors in patients with advanced extrahepatic cholangiocarcinoma: a single center experience. *Medicine*. 2019;98(8):e14556. doi:10.1097/MD.0000000000014556
34. Katzke V, Johnson T, Sookthai D, Husing A, Kuhn T, Kaaks R. Circulating liver enzymes and risks of chronic diseases and mortality in the prospective EPIC-Heidelberg case-cohort study. *BMJ Open*. 2020;10(3):e033532. doi:10.1136/bmjopen-2019-033532
35. Kunutsor SK, Apekey TA, Van Hemelrijck M, Calori G, Perseghin G. Gamma glutamyltransferase, alanine aminotransferase and risk of cancer: systematic review and meta-analysis. *Int J Cancer*. 2015;136(5):1162–1170. doi:10.1002/ijc.29084
36. Hodgson MJ. Alanine aminotransferase in clinical practice. *Arch Intern Med*. 1992;152(1):208. doi:10.1001/archinte.1992.00400130196033
37. Banez LL, Loftis RM, Freedland SJ, et al. The influence of hepatic function on prostate cancer outcomes after radical prostatectomy. *Prostate Cancer Prostatic Dis*. 2010;13(2):173–177. doi:10.1038/pcan.2010.3
38. Katan MB. Commentary: Mendelian Randomization, 18 years on. *Int J Epidemiol*. 2004;33(1):10–11. doi:10.1093/ije/dyh023
39. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi:10.1136/bmj.k601