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Blood metabolites mediate the causal relationship between circulating CX3CL1 levels and prostate cancer

A 2-step Mendelian randomization study

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Abstract

Chemokines influence the progression of prostate cancer (PCa) through multiple mechanisms. However, the effect of C-X3-C chemokine ligand 1 (CX3CL1) on PCa risk remains controversial. Our study aimed to investigate whether circulating CX3CL1 is causally associated with PCa and to identify metabolites that have mediating effects using the 2-step bidirectional Mendelian randomization (MR) analysis process. Inverse variance weighting (IVW) results were used as the primary observations, while additional sensitivity analyses were conducted. For each standard deviation increase exhibited by the circulating CX3CL1 levels, the risk of PCa was reduced by 0.4% (IVW: OR = 0.996, [95% CI = 0.994–0.998], $P < .001$), and blood alliin levels increased by 19% (IVW: OR = 1.185, [95% CI = 1.01–1.54], $P = .003$). For each standard deviation increase in the blood alliin levels, the risk of PCa was reduced by 0.1% (IVW: OR = 0.999, [95% CI = 0.997–0.999], $P = .03$). Therefore, the protective effect of circulating CX3CL1 on PCa may be mediated by blood alliin levels (mediated proportion = 6.7%). The results supported the notion that high levels of circulating CX3CL1 indicate a lower PCa risk and the idea that the food-derived antioxidant alliin may mediate this association. We emphasize that the use of CX3CL1 as a protective factor against PCa may provide new strategies for PCa prevention and care in the future.

Abbreviations: BWMR = Bayesian-weighted Mendelian randomization, CI = confidence interval, CX3CL1 = C-X3-C chemokine ligand 1, GWAS = genome-wide association studies, IVs = instrumental variables, IVW = inverse variance weighting, MR = Mendelian randomization, OR = odds ratio, PCa = prostate cancer, PSA = prostate-specific antigen, SNPs = single-nucleotide polymorphisms, TCGA = The Cancer Genome Atlas.

Keywords: alliin, blood metabolites, C-X3-C chemokine ligand 1 (CX3CL1), Mendelian randomization (MR), prostate cancer (PCa)

1. Introduction

Prostate cancer (PCa) is the most common cancer among men worldwide, with new cases estimated to account for one-third of all male cancer diagnoses in 2024.[1] Chemokines are involved in a variety of developmental processes related to PCa, playing an especially crucial role in directing the migration of immune cells, which is necessary for producing an effective antitumor immune response. The upregulation of the expressions of C-X3-C chemokine ligand 1 (CX3CL1), CXCL9, and CXCL14 has significant positive effects on patient prognoses and therapeutic responses.[2,3] More in-depth studies of chemokines are necessary to fully reveal their complexity in the PCa

YZ and ZC contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are publicly available.

The summary GWAS data used in this study are publicly available and no specific ethical approval was required.

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development process and provide potential targets for antitumor therapies.

CX3CL1, also known as fractalkine, is the only member of the CX3C chemokine family and has a paradoxical effect on PCa risk. Several previous experiments have suggested that the CX3CL1/CX3CR1 axis can stimulate the proliferation, migration, and invasion of PCa cells, especially spinal metastasis, via the Src/FAK pathway.[4,5] In addition, a heightened CX3CL1 expression leads to epithelial–mesenchymal transition through the activation of the TACE/TGF-α/EGFR signaling pathway, indicating a poor prognosis.[6] Conversely, higher CX3CL1 levels in the blood of PCa patients improve their prognoses,[7] and the membrane-bound form of CX3CL1 increases the adhesion between PCa cells and endothelial cells, which reduces their migration potential.[8] Given these discrepancies and the insufficiencies of the current literature, we propose that CX3CL1 might have a total risk-reducing effect on PCa through multiple mechanisms, requiring further investigation.

Since small changes in living systems can result in large changes in the levels of metabolites, metabolomics can be considered an amplified output of microscopic changes and is often used to research the potential regulatory mechanisms of cancer.[9] The advantages of metabolomics include its wide variety, high sensitivity, and high accuracy. Testing the features of specific metabolites in various body fluids or tissues is emerging as a crucial avenue in research on the development of PCa.[10] Recently, improved materials have increased the accuracy of metabolic urinary and serum profiling for early PCa screening, especially in patients who are negative for circulating prostate-specific antigens (PSAs).[11] Another study utilized prostate fluid to identify 6 metabolites to construct a model that provided good predictions for patients with high Gleason scores.[12] CX3CL1 can affect the metabolism of the body through a variety of pathways, such as regulating inflammation and inflammatory cells.[13] Consequently, blood metabolites may offer insights into their mediating role in the effects of CX3CL1 on PCa development. Unfortunately, the potential causal relationships between CX3CL1, its metabolites, and PCa remain largely undefined and require further investigation.

The interactions between the environment and genetics determine the phenotype and progression of PCa. Genome-wide association studies (GWASs) have significantly enhanced our genetic understanding of PCa and provided a database for conducting Mendelian randomization (MR) studies. MR analyses use single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to explore the causal relationships between exposure factors and diseases.[14] Given the random distribution of the genetic variants among individuals, MR is similar to implementing randomized controlled trials with reduced confounder effects and reverse causality, thereby providing a more robust basis for obtaining causal deductions than conventional observational studies.[14] Therefore, this study utilized MR to assess the associations between CX3CL1, circulating metabolites, and PCa. The utilized GWAS datasets were derived from large, published studies. A 2-step bidirectional MR analysis process was adopted to assess the mediating role of metabolites,[15] as well as to explore the associations of CX3CL1 with PCa, of CX3CL1 with circulating metabolites, and of circulating metabolites with PCa, enabling the quantification of the mediating effects exerted by metabolites.

2. Methods

2.1. Study design

The present study was subject to 3 basic assumptions. The IVs were strongly correlated with circulating CX3CL1 ($P < 10^{-6}$). The IVs were not correlated with PCa ($P > .05$). The IVs could only impact the PCa risk by cycling CX3CL1. To explore the relationships between circulating CX3CL1, PCa, and possible blood metabolite mediators, we performed 2-sample, 2-step MR analyses by using reliable large-scale GWAS data (Fig. 1). Two-sample MR analyses can be used to calculate the total effect of

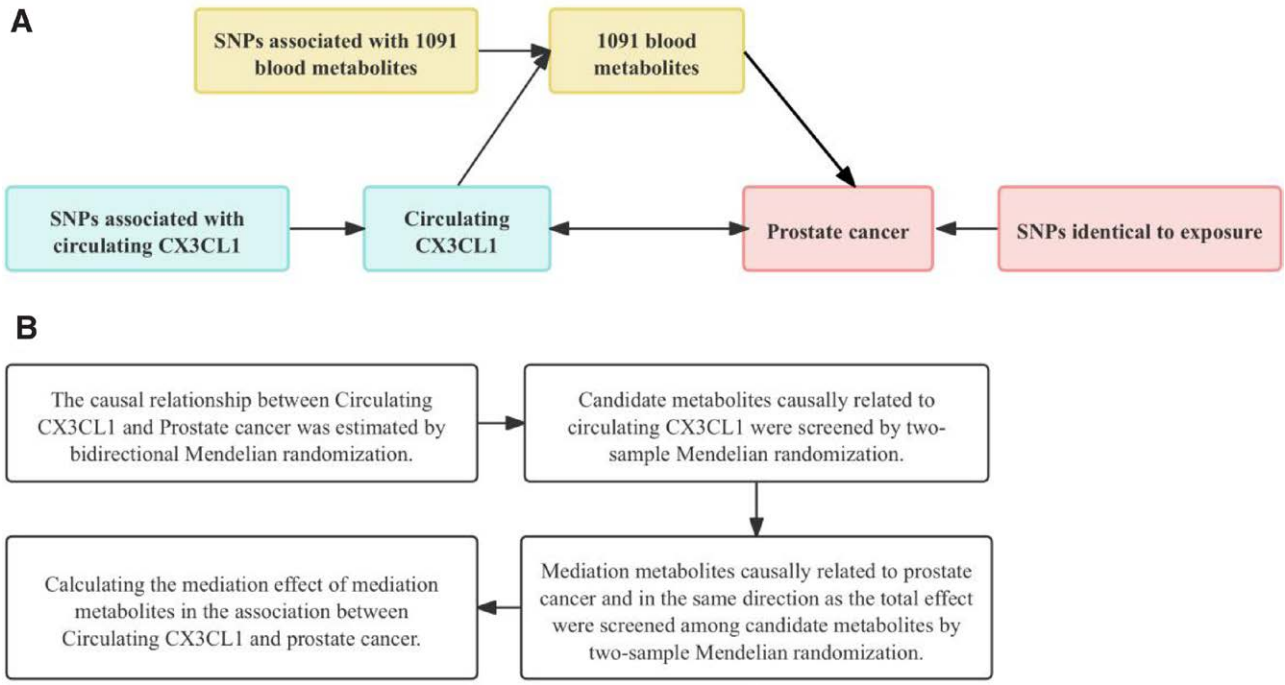

Figure 1. Overview of the study design (A) Schematic of the 2-step MR procedure. (B) Flowchart of the 2-step MR process, describing the sequence and analysis methods used for circulating CX3CL1, PCa, and blood metabolites. CX3CL1 = C-X3-C chemokine ligand 1, MR = Mendelian randomization, PCa = prostate cancer.

circulating CX3CL1 on PCa, and this total effect can be divided into mediated and direct effects. We required that the mediating effects of the metabolites aligned with the observed total effects to ensure the selection of circulating metabolites that could reflect the impact of CX3CL1 on PCa. The GWAS data used in this study were obtained from published literature or publicly available databases and were ethically approved by the corresponding authorities.

2.2. Data sources

The GWAS summary exposure data employed in this study were derived from a meta-analysis of quantitative trait loci for CX3CL1 levels in plasma involving 11 independent cohorts with 14,824 samples.[16] The PCa GWAS summary data used as outcomes were derived from the UK Biobank and included a total of 14,824 samples.[17] The source of the metabolites was a study in which the authors provided GWAS summary data for 1091 blood metabolites determined from 8299 samples.[18] We downloaded the GWAS summary total blood metabolite data from the study as an intermediate variable. The samples used for the GWAS summary data were all of European ancestry and were sourced from various research organizations to avoid sample overlap. See Table 1 for more specific details about the data sources.

2.3. IVs selection procedure

We used $P < 5 \times 10^{-6}$ as a threshold for identifying SNPs that were significantly different from the GWAS summary circulating CX3CL1 data. Next, to ensure independence between the SNPs and to avoid the bias caused by linkage disequilibrium, we used the PLINK clumping method to set $r^2 < .001$ and the window size = 10,000 kb.[19] Then, palindromic and ambiguous SNPs were excluded. The F statistic ($F = \beta^2/SE^2$) was calculated to exclude weak IVs with $F < 10$.[20] If the SNPs of IVs were absent in the outcome data, proxy SNPs with high linkage disequilibrium levels possessing r^2 values greater than 0.80 were selected as substitutes. Finally, we used the Phen Scanner website (http://www.phenoscanner.medschl.cam.ac.uk/acceded on February 7, 2024) to remove the SNPs associated with testosterone, obesity, smoking, and family history of PCa to limit the effect of confounders on the results.

2.4. MR analysis

Two-sample MR analyses were used to assess the causal relationship between CX3CL1 and PCa and to calculate the total effect value β_1 via inverse variance weighting (IVW),[21] MR–Egger,[22] and weighted median methods.[23] IVW was the main analysis method, which required all IVs to be meaningful. Then, the Wald ratios were calculated, higher weights were assigned to the IVs with less variance, and a meta-summarization of the effect values of each IV through was formed through weighted averaging to obtain an overall estimate of the effect of CX3CL1 on PCa. MR–Egger has the advantage of allowing for possible IV heterogeneity and providing a corrected estimate of the causal effect. Notably, the presence of an intercept term in the straight MR–Egger regression line was interpreted as an indicator of horizontal pleiotropy in the effect of the IVs on the outcome, rendering the associated IVW results unreliable.[24] The weighted median method ranked the effect estimates of the IVs, assigned weights to the median, and then calculates the causal effect estimates. This method ensures effectiveness even when up to 50% of the IVs are invalid. Causation between exposure and an outcome can be established if the results of other methods are significant and in the same direction as that of the IVW effect, even if the IVW effect is not statistically significant.

2.5. Sensitivity analysis

In addition to applying multiple methods to confirm the consistency of the conclusions, it was also necessary to utilize multiple methods to test their robustness. As the exposure and outcome were derived from 2 different samples, we assessed the heterogeneity between the IVs of the 2 samples using Cochrane Q test, which was interpreted as significant heterogeneity if $P < .05$.[25] The MR-PRESSO method was used to check for possible outliers in the IVs, which were recalculated after removing the IVs associated with the outliers.[26] The effect of horizontal pleiotropy on the results was assessed using triple tests involving the MR–Egger intercept method, the MR-PRESSO global test, and the cML-MA method.[27,28] A leave-one-out analysis eliminated each SNP in turn, and then MR analyses were performed to determine whether significant changes were exhibited by the results. Scatter plots, funnel plots, and forest plots were generated to visualize the results. Finally, we performed reverse MR analyses with PCa as the exposure and circulating CX3CL1 as the outcome. A lack of a statistically significant difference ($P > .05$) in any of the reverse analyses indicated the absence of reverse causality.

2.6. Mediation analysis

Based on the principle of the 2-step MR analysis process, we selected blood metabolites that were causally associated with both circulating CX3CL1 and PCa ($P < .05$) as candidate mediators. β_2 represents the effect of circulating CX3CL1 on the candidate mediators, while β3 represents the effect of each candidate mediator on PCa. We calculated the indirect effect imposed by CX3CL1 through the mediator (i.e., $\beta_2 \times \beta_3$) and the direct effect produced without the mediator (i.e., $\beta_1 - \beta_2 \times \beta_3$).[29] The candidate mediator whose indirect effect direction aligned with that of β1 served as the final mediator metabolite. Finally, the mediation proportion was obtained by dividing the indirect effect by the total effect.[15]

Table 1

Details of GWAS included in Mendelian randomization analyses.

Trait	Data sources	N. cases	N. controls	Sample size	Race	Yr	GWAS ID/PMID	Web resource
Malignant neoplasm of prostate	UK Biobank	6321	354873	361194	European	2018	ukb-d-C3_PROSTATE	https://gwas.mrcieu.ac.uk/datasets/ukb-d-C3_PROSTATE/
Blood metabolome	GWAS Catalog	NA	NA	8299	European	2023	PMID: 36635386	https://www.ebi.ac.uk/gwas/publications/36635386
Circulating CX3CL1	GWAS Catalog	NA	NA	14824	European	2023	PMID: 37563310	https://www.ebi.ac.uk/gwas/studies/GCST90274778

CX3CL1 = C-X3-C chemokine ligand 1, GWAS = genome-wide association studies, MR = Mendelian randomization.

2.7. Bayesian-weighted Mendelian randomization (BWMR) analysis

We applied a BWMR analysis to assess the results obtained from the IVW method. BWMR assigns weights to different IVs using Bayesian inference based on MR and corrects for measurement errors and pleiotropy to estimate causalities with greater accuracy.[30]

All the statistical analyses were performed using R software (version 4.3.1). The MR analyses were performed using the "Two-sample MR" package (version 0.5.7), the cML-MA method was performed using the "MRcML" package (version 0.0.0.9), the BWMR analyses were performed using the "BWMR" package (version 0.1.1), and visualizations were generated for the results using the "ggplot2" package (version 3.4.4).

3. Results

3.1. IVs selection

After ensuring quality control, we selected a total of 34 independent SNPs as the IVs of circulating CX3CL1 ($P < 5 \times 10^{-6}$, $r^2 < .001$, kb < 10,000), none of which were closely associated with any confounders. All IVs had F-statistics >20, suggesting that our analysis was free from weak instrument bias (Table S1, Supplemental Digital Content, http://links.lww.com/MD/M775, Supplemental Digital Content, showing IVs used for MR analysis of the association between circulating CX3CL1 and PCa).

3.2. Causal effects of circulating CX3CL1 on PCa

In the initial 2-sample MR analysis, an IVW analysis revealed that circulating CX3CL1 was associated with a reduced risk of PCa ($\beta_1 = -0.003$, OR = 0.996 [95% CI: 0.994–0.998], $P < .001$). The results of the MR–Egger and weighted median methods were consistent with those of IVW (Fig. 2). Cochrane Q test (IVW: Q = 34.584, $P = .30$; MR Egger: Q = 33.981, $P = .28$) suggested that there was no heterogeneity between the IVs, and the MR-PRESSO analyses did not reveal the presence of outliers. The MR–Egger intercept method ($P = .47$), MR-PRESSO global test ($P = .32$), and cML-MA test ($P < .001$) did not reveal evidence of horizontal pleiotropy (Table 2). The produced funnel plots were essentially symmetrical on both sides of the solid line (the effect values calculated by the IVW and MR–Egger analysis methods), and the forest plots yielded by the leave-one-out method showed that all error lines were to the left of 1, further supporting the results of the MR analyses (Figure S1, Supplemental Digital Content, http://links.lww.com/MD/M778, Supplemental Digital Content, which presents the scatter plots, funnel plots and leave-one-out analyses). In addition, the reverse MR analysis revealed no bidirectional causality between circulating CX3CL1 and PCa (Fig. 2; Table S2, Supplemental Digital Content, Supplemental Digital Content, http://links.lww.com/MD/M776, Supplemental Digital Content, showing IVs used for MR analysis of the association between PCa and circulating CX3CL1).

3.3. Two-step MR analysis for identifying the mediators between circulating CX3CL1 and PCa

Firstly, we performed 2-sample MR analyses for each of 1091 blood metabolite levels with CX3CL1. Only 46 metabolites levels showed a significant causal association with circulating CX3CL1. Among these metabolites, 5 had causal effects on PCa (Fig. 3). However, only blood alliin was found to be positively correlated with CX3CL1 (IVW: $\beta_2 = 0.170$, OR = 1.185 [95% CI: 1.057–1.328], $P = .003$) and negatively correlated with PCa (IVW: B3= –0.001, OR = 0.999 [95% CI: 0.997–0.999], $P = .03$). Therefore, only blood alliin levels were used as a mediating variable in this study. The MR-PRESSO and weighted median results were consistent with the IVW results (Fig. 3). The F-statistics produced for the IVs of blood alliin were all >20 (Table S3, Supplemental Digital Content, Supplemental Digital Content, http://links.lww.com/MD/M777, Supplemental Digital Content, showing IVs used for MR analysis of the association between blood metabolites and PCa). Cochrane Q test for the 2-step MR of blood alliin did not reveal heterogeneity (Q = 33.140, $P = .23$; Q = 11.357, $P = .88$). No horizontal pleiotropy was found by the MR–Egger intercept method ($P = .35$; $P = .62$), the MR-PRESSO global test ($P = .27$; $P = .90$), or the cML-MA test ($P = .02$; $P = .03$) (Table 2). Figure S1, Supplemental Digital Content, http://links.lww.com/MD/M778, Supplemental Digital Content, which presents the scatter plots, funnel plots, and leave-one-out analyses. After completing the BWMR analysis, we still detected positive results for circulating CX3CL1, blood alliin, and PCa (Fig. 4).

3.4. Mediating effect of blood alliin levels on the causal relationship between circulating CX3CL1 and PCa

After conducting a 2-step MR analysis, we observed the potential mediating role of blood alliin in the reduction of PCa risk via circulating CX3CL1: circulating CX3CL1 was associated with elevated blood alliin, which in turn was associated with a reduced risk of PCa. The results indicated that the indirect effect was –0.0002, the direct effect was –0.0028, and the proportion of the indirect effect to the total effect was 6.7% (Fig. 4).

4. Discussion

We used a 2-sample MR analysis to investigate the causal effect of CX3CL1 on PCa and found that increased circulating CX3CL1 levels significantly reduced the risk of PCa. We further

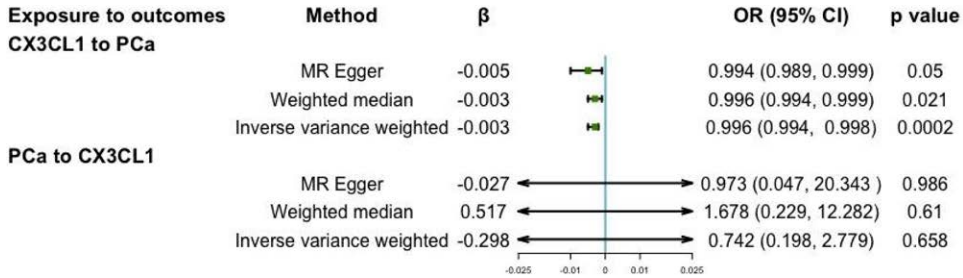

Figure 2. Bidirectional MR results obtained for circulating CX3CL1 levels in PCa patients. The green points indicate the effect value β. CI = confidence interval, CX3CL1 = C-X3-C chemokine ligand 1, MR = Mendelian randomization, OR = odds ratio, PCa = prostate cancer.

Table 2

Heterogeneity and pleiotropy analysis.

		Heterogeneity			Pleiotropy			
		Cochran Q			MR Egger		MR-PRESSO Global Test	cML-MA
Exposure to outcomes	Method	Q statistic	Q degree of freedom	*P*	Intercept value	*P*	*P*	*P*
CX3CL1 to PCa	MR Egger	33.981	30	.282			.323	**.0001***
	Inverse variance weighted	34.584	31	.3	0.0002	.471		
CX3CL1 to Allin	MR Egger	30.375	27	.298			.27	**.02***
	Inverse variance weighted	33.14	28	.231	-0.022	.129		
Alliin to PCa	MR Egger	11.332	17	.839			.902	**.03***
	Inverse variance weighted	11.357	18	.879	0	.876		

CX3CL1 = C-X3-C chemokine ligand 1, GWAS = genome-wide association studies, MR = Mendelian randomization.
*Statistically significant. The bold values mean statistical significance.

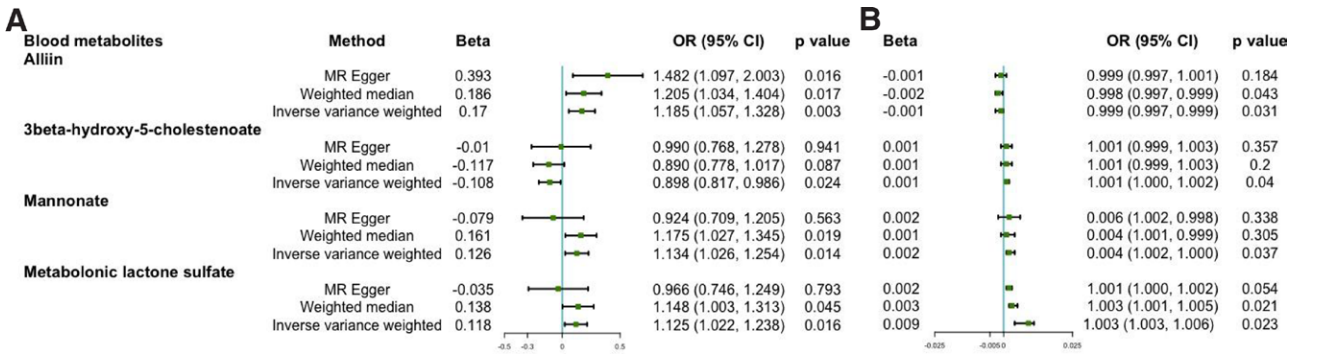

Figure 3. (A) Causal effect of circulating CX3CL1 on metabolites that acted as mediators. (B) Causal effects of metabolites acting as mediators on PCa. Green dots indicate the effect value β. CI = confidence interval, CX3CL1 = C-X3-C chemokine ligand 1, OR = odds ratio, PCa = prostate cancer.

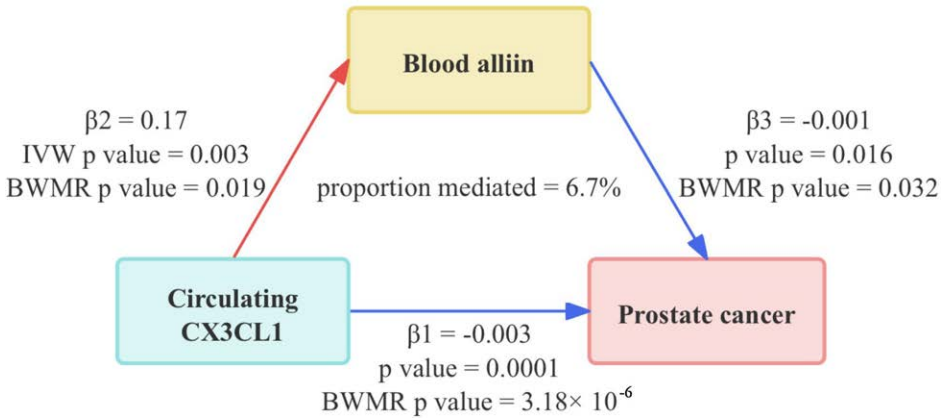

Figure 4. Summary plot of the causal associations among circulating CX3CL1, PCa, and blood alliin. CX3CL1 = C-X3-C chemokine ligand 1, BWMR = Bayesian-weighted Mendelian randomization, IVW = inverse variance weighting, PCa = prostate cancer.

identified potential mediators among 1400 circulating metabolites and found that circulating alliin levels formed a key mediator contributing to the negative association between CX3CL1 and PCa risk, with an indirect effect accounting for 6.7% of the total effect.

Few reports have been presented on the relationship between CX3CL1 and PCa risk, and consistent with previous clinical findings, we first noted that higher levels of CX3CL1 significantly reduced PCa risk. In patients who underwent radical prostatectomy, higher levels of CX3CL1 were associated with lower biochemical recurrence rates (log-rank test $P < .001$) and were shown to be prognostic biomarkers after performing radical prostatectomy.[31] A large-scale pathological study of the PCa transcriptome revealed that high CX3CL1 expression was associated with improved patient prognoses.[7] We further analyzed 499 PCa cases from The Cancer Genome Atlas (TCGA) database. A Kaplan–Meier curve analysis was used to compare PCa patients with different CX3CL1 expression levels, which revealed evidence suggesting the protective effect of CX3CL1 on PCa risk. Patients with higher CX3CL1 levels had longer overall survival (OS) ($P = .03$), disease-specific survival (DSS) ($P = .02$), and progression-free survival (PFS) times ($P = .006$) (Figure S2, http://links.lww.com/MD/M779, Supplemental Digital Content, showing differential CX3CL1 expression and Kaplan–Meier survival analysis obtained from the TCGA dataset).[3] However, related in vitro experiments

have produced opposite conclusions. CX3CL1 has been shown to play a key role in promoting PCa metastasis; in particular, osteoblasts with high CX3CL1 expression levels can attract PCa cells to migrate toward them and activate the PI3K/Akt signaling pathway to inhibit PCa cell apoptosis.[5] Compared with those in primary tumors, the overexpression of CX3CL1 in metastatic PCa tissues activates EGFR phosphorylation and the Src/FAK pathway, thus promoting the malignant progression of PCa cells.[4] It is not difficult to identify the involvement of CX3CL1 in multiple phases of PCa development, but the diverse views of its impact on PCa risk emphasize its research potential.

Considering that high CX3CL1 expression is associated with better prognoses, we used a 2-step MR analysis approach to explore the possible mediators of the effect of CX3CL1 on PCa risk reduction among 1091 circulating metabolites to reveal the metabolic mechanisms that affect PCa susceptibility. For the first time, we found a causal relationship between circulating CX3CL1 levels and circulating alliin levels. In humans, alliin is primarily absorbed in the small intestine, with the highest efficiency observed in the jejunum. It is mainly absorbed into the body through facilitated diffusion by specific carrier proteins.[32] The absorption of alliin is both concentration- and time-dependent, with rates ranging from approximately 20% to 70%.[33] Alliin is converted into various bioactive compounds through enzymatic reactions, and these compounds exhibit remarkable anticancer activities. Although the exact mechanism by which CX3CL1 increases alliin levels in the body is not known, in a double-blind trial, it was found that there was no significant difference between the amounts of alliin present in prostate tissue in participants who took alliin supplements for 4 consecutive weeks and those who took a placebo, but the alliin concentrations varied more among different samples within the same group.[34] Based on these experimental results, we proposed that the alliin levels in prostate tissue are not affected by immediate supplementation. Various biochemical molecules and genetic factors may regulate the accumulation of alliin and metabolism in the prostate. We subsequently used genetic evidence to demonstrate a link between alliin and PCa and found that higher alliin levels may reduce the risk of PCa. Several studies have suggested that alliin may contribute to reducing the incidence risk of PCa. In PCa cells, alliin was able to inhibit cancer cell proliferation and invasion by regulating the expression of E-cadherin and its suppressor (Snail), thereby inducing cell cycle arrest in the G2/M phase.[35] Alliin may cause BAD mitochondrial translocation through Akt inactivation, which in turn activates caspase-3 and caspase-9 to induce PCa cell apoptosis.[36] Clinical studies have shown that supplementation with alliin significantly reduces prostate mass and improves the symptoms of PCa patients while decreasing their circulating PSA levels.[37] Thus, alliin intake is potentially beneficial for the prevention and treatment of PCa.

Our study also had several limitations. First, this study was limited to a European population, which may affect the applicability of its conclusions to other ethnicities. Next, only 1 of the 1400 metabolites was validated as a mediator, and other potential mediators need to be further explored in future studies. In addition, although several studies have been conducted on the anti-PCa effects of alliin, comprehensive clinical studies are relatively limited, and the uncertain bioavailability of alliin may limit its use as a primary treatment in clinical settings. Finally, although the exclusionary assumptions of MR require genetic variations to affect outcomes only through exposure factors, unmeasured confounders remain unavoidable.

5. Conclusion

Our study revealed that blood alliin mediates the causal relationship between circulating CX3CL1 and PCa. These findings offer genetic evidence that CX3CL1 is a protective factor for PCa and suggest that the food-derived antioxidant alliin may be involved in the antitumor process through various mechanisms. However, more research is needed to determine how this connection works.

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Author contributions

Conceptualization: Zheng Chen, Yumin Zhuo.
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Writing – review & editing: Yinshu Zhou, Zheng Chen, Yumin Zhuo.

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