Original Article

Association of branched-chain amino acids and risk of three urologic cancers: a Mendelian randomization study

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Background: Multiple studies suggest a plausible connection between urologic cancers and branched-chain amino acids (BCAAs) breakdown metabolic enzymes. Nevertheless, there is scarce exploration into the variations in circulating BCAAs. In our research, we utilize bidirectional, two-sample Mendelian randomization (MR) analysis to predict the link between BCAAs levels and three distinct types of urological tumors.

Methods: The study examined data from the UK Biobank, including a comprehensive genome-wide association study (GWAS) of total BCAAs, leucine, isoleucine, and valine, alongside three urological system tumors [prostate cancer (PCa), kidney cancer, and bladder cancer] sourced from the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) and FinnGen Consortium databases. The primary analytical approach involved the use of the inverse variance weighted (IVW) method, complemented by MR-PRESSO global testing and MR-Egger regression to identify potential horizontal pleiotropy. Heterogeneity was evaluated using the Cochran Q test.

Results: The levels of circulating total BCAAs [odds ratio (OR) =1.002688, 95% confidence interval (CI): 1.000, 1.005, P=0.03], leucine (OR =1.0038, 95% CI: 1.001, 1.007, P=0.008), isoleucine (OR =1.003352, 95% CI: 1.000, 1.007, P=0.04), and valine (OR =1.00279, 95% CI: 1.001, 1.005, P=0.009) showed positive associations with PCa risk. However, there was inadequate evidence to establish a link between BCAAs and bladder or kidney cancer.

Conclusions: In summary, an association existed between elevated levels of circulating total BCAAs, leucine, isoleucine, and valine, and an increased risk of PCa. However, no correlation was detected between BCAAs and kidney or bladder cancer.

Keywords: Mendelian randomization (MR); branched-chain amino acids (BCAAs); kidney cancer; prostate cancer (PCa); bladder cancer

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Introduction

Tumor growth correlates significantly with changes in energy metabolism, a key biochemical feature of cancer cells commonly known as the "hallmark of cancer" (1). Cancer cells exhibit uncontrolled proliferation and sustained growth, depending on obtaining necessary nutrients from the tumor microenvironment to support their biomass and survival, even in low-nutrient and hypoxic settings (2-4).

Amino acids can be involved in the occurrence and development of tumors. They not only form the basic building blocks for protein production, but also serve as the suppliers of energy and metabolites for tumors (4). Tumors have a preference for absorbing branched-chain amino acids (BCAAs), in particular isoleucine, leucine, and valine. BCAAs make up a substantial part of the necessary amino acids, comprising 35% of the total. Previous studies have highlighted the crucial role of BCAAs in supporting cell survival, growth, proliferation, migration, and invasion (5-8). In addition to their direct contribution to protein synthesis, the breakdown of BCAAs generates various metabolites (e.g., glutamate) that participate in the metabolic processes of tumors. Furthermore, through mTORC1, BCAAs have the ability to function as signaling molecules that stimulate cell proliferation. Recent researches have shown that BCAAs play important roles in various malignant tumors (7,8), including glioblastoma (9,10), pancreatic ductal adenocarcinoma (11,12), leukemia (13,14), non-small cell lung cancer (15), ovarian cancer (16), clear cell renal cell carcinoma (ccRCC) (17), osteosarcoma (18), and hepatocellular carcinoma (HCC) (19).

The current emphasis of BCAAs and cancer research lies in fundamental investigation, particularly examining enzymes associated with breakdown (BCAA transferase, BCAT). Yet, limited attention has been given to exploring the influence of alterations in circulating BCAAs levels on cancer. A recent study by Xu *et al.* revealed a potential connection between circulating BCAAs levels and squamous cell lung cancer. However, that study did not extensively probe into the examination and analysis of urological tumors (20).

We employed Mendelian randomization (MR) methodology to substantiate the link between BCAAs and urologic cancers and to offer robust clinical evidence. MR is a sophisticated approach that leverages genetic variation to investigate connections between exposure factors and outcome phenotypes. It can address the constraints of observational studies and yield impartial estimates in the absence of randomized controlled trials (RCTs) (21). In our analysis, we utilized a two-sample bidirectional MR strategy to investigate the association between circulating BCAA concentration and the incidence of diverse cancers, such as prostate cancer (PCa), bladder cancer, and kidney cancer. We present this article in accordance with the STROBE-MR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1142/rc).

Highlight box

Key findings

- Some studies have found a certain relationship between branched-chain amino acids (BCAAs) and urologic cancers (prostate cancer, kidney cancer, and bladder cancer). Our research has discovered a relationship between BCAAs and prostate cancer, however, no relationship has been found between them and kidney or bladder cancers.

What is known and what is new?

- Some studies have explored the mechanisms between BCAA catabolic enzymes and urologic cancers, indicating that BCAA metabolic enzymes may affect urologic cancers through certain pathways.
- Few studies have explored the relationship between changes in circulating BCAAs and urologic cancers. Therefore, we conducted a bidirectional Mendelian randomization analysis with two samples to investigate the relationship between the two.

What is the implication, and what should change now?

- Our research results indicate a significant relationship between BCAAs and prostate cancer. Currently, there are few studies on the relationship between the two. Therefore, our research can provide opportunities for early and more effective therapeutic interventions for prostate cancer. In future clinical applications, BCAAs may become potential biomarkers and therapeutic targets for prostate cancer.

Methods

Study design

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study utilized public genome-wide association studies (GWAS) summary data and refrained from collecting new human data. With ethical approval and informed consent from patients obtained, no further approval was necessary. Bidirectional MR analysis was conducted to evaluate the relationship between BCAAs (total BCAAs, valine, leucine, and isoleucine) and urologic cancers, comprising prostate,

Table 1 The characteristics of GWAS studies on the exposures and outcomes

Trait	Consortium	Population	Case (n)	Control (n)	Sample size	SNPs (n)	GWAS ID
Total-BCAA	UK biobank	European	NA	NA	115,051	11,590,399	ebi-a-GCST90092984
Valine	UK biobank	European	NA	NA	115,052	11,590,399	ebi-a-GCST90092995
Leucine	UK biobank	European	NA	NA	115,078	11,590,399	ebi-a-GCST90092891
Isoleucine	UK biobank	European	NA	NA	115,079	11,590,399	ebi-a-GCST90092843
Prostate cancer	MRC-IEU	European	3,269	459,664	462,933	9,851,867	ukb-b-13348
Bladder cancer	MRC-IEU	European	1,101	461,832	462,933	9,851,867	ukb-b-8193
Kidney cancer	FinnGen	European	971	217,821	218,793	16,380,466	finn-b-C3_KIDNEY_NOTRENALPELVIS

GWAS, genome-wide association study; SNP, single-nucleotide polymorphisms; BCAA, branched-chain amino acid; MRC-IEU, Medical Research Council Integrative Epidemiology Unit; NA, not applicable.

bladder, and kidney tumors. Five MR methods were utilized to assess causal effects. Additionally, reverse MR analysis was conducted to scrutinize the causal impact of urological tumor-related features on BCAAs as outcomes.

Data sources

The blood levels of BCAAs were gathered via a GWAS meta-analysis using UK Biobank data. The analysis encompassed the measurement of 249 metabolic biomarkers in a cohort of 121,584 participants selected at random, which included total BCAAs, valine, leucine, and isoleucine (22).

Summary statistics for PCa, involving 9,851,867 single-nucleotide polymorphisms (SNPs) and consisting of 3,269 cases and 459,664 controls, were obtained from the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) database. Likewise, the summary statistics for bladder cancer encompassed 9,851,867 SNPs, 1,101 cases, and 461,832 controls from the same database. The summary statistics for kidney cancer, covering 16,380,466 SNPs, 971 cases, and 217,821 controls, were sourced from the FinnGen Consortium database. These datasets can be accessed at https://gwas.mrcieu.ac.uk/. For additional GWAS dataset information, please see *Table 1*.

Genetic instrumental variants selection

To acquire qualified SNPs as instrumental variables (IVs), a series of rigorous screening steps was implemented. Three assumptions outlined for MR analysis (23) must be fulfilled: (I) the relevance presumption, demonstrating that IVs are closely linked to the exposure factor; (II) the independence assumption, ensuring that IVs are not affected by confounding factors; (III) the exclusion assumption, indicating that IVs solely act on the outcome through exposure. To guarantee methodological rigor, our analysis adhered to three essential steps.

In order to fulfill the relevance assumption, the following criteria had to be met: (I) establishing a significant association ($P<5\times10^{-8}$) of genome-wide SNPs with exposure. To prevent weak instrument bias, we adopted an F statistic >10 [F calculation formula: $R^2(N-2)/(1-R^2)$] (24,25); (II) we selected independent SNPs using linkage disequilibrium (LD) ($r^2<0.001$, window size =10,000 kb), and performed minor allele frequency (MAF) filtering (<0.05). To examine the independence and exclusion assumptions, PhenoScanner was utilized to assess each important SNP linked to exposure and to exclude any SNPs associated with potential confounding factors, in order to ensure they did not exert multiple effects on other phenotypes at the genome-wide significance level. Lastly, the MR-PRESSO test was conducted to identify outliers among the SNPs used in the MR analysis.

In the final analysis, our study distinguished 16 SNPs linked to BCAAs, 20 SNPs linked to leucine, 16 SNPs linked to isoleucine, and 9 SNPs linked to valine as crucial genetic tools in our MR investigation.

Statistical analyses

In our analysis, we utilized various methods to assess the relationship and effects between the exposure and the outcome. Primarily, the inverse variance weighted (IVW) method was employed, which is the most commonly utilized approach in MR analysis and encompasses fixed-effects and random-effects versions. As an approach that is

meta-analytical in nature, IVW obtains the overall estimate of the exposure's impact on the outcome by combining the Wald estimates for each IV (26). To address the potential influence of exposure-related genetic tools on the outcome, supplementary methods such as the weighted median, the weighted mode, the simple mode, and MR-Egger regression were also utilized to assess the relationship between the exposure and the outcome. If the results of the IVW method are significant (P<0.05), even if the results of other methods are not significant, and no pleiotropy and heterogeneity are detected, it can be considered as a positive result, provided that the β values of other methods are in the same direction (27,28).

To address potential horizontal pleiotropy, we employed the MR-PRESSO test and MR-Egger regression. The intercept term in MR-Egger regression can provide a useful indicator of directional horizontal pleiotropy influencing the results of MR analysis. MR-PRESSO was utilized as a means to detect and handle outliers. To assess the detected heterogeneity, Cochran's Q statistic was computed using the IVW and MR-Egger regression methods, and a significance threshold of P<0.05 was used for heterogeneity (29).

All statistical analyses and plots in this study were performed using Rstudio software based on R version 4.3.3. Data analysis predominantly relied on the MR-PRESSO and Two-Sample MR packages.

Results

Association of total BCAAs and urologic cancers in the bi-directional MR analysis

We discovered 16 SNPs associated with total BCAA, with detailed information provided in Table S1. All these IVs had F statistics exceeding 10. Following LD clustering and harmonization, we used the MR-PRESSO test to identify any anomalies in the SNPs, but none were found. Ultimately, we acquired the IVs meeting the criteria (11 SNPs for PCa, 5 SNPs for bladder cancer, and 12 SNPs for kidney cancer) for the MR analysis of BCAA and urological tumors.

The IVW results demonstrated a potential link between genetically predicted BCAAs levels and PCa risk [odds ratio (OR) =1.002688, 95% confidence interval (CI): 1.000, 1.005, P=0.03]. While the P values from the other four methods exceeded 0.05, their β values aligned with the IVW's, underlining the strength of our findings. Moreover, we observed no substantial correlation between BCAAs and bladder or kidney cancer (bladder cancer: OR =0.9998688, 95% CI: 0.998, 1.001, P=0.88; kidney cancer: OR =1.616809, 95% CI: 0.772, 3.385, P=0.20). Since the ORs was low, it indicated that the relationship between BCAA and PCa may be weak; therefore, readers should interpret our results with caution. *Table 2* contains detailed MR analysis. Regarding the link between BCAAs levels and urological tumors, both MR-Egger regression and MR-PRESSO tests did not detect significant directional pleiotropy. Furthermore, Cochran's Q test did not reveal notable heterogeneity. Further details can be available in the Table S2.

In the reverse MR analysis, we detected 18 SNPs linked to PCa (using a threshold of $P<5\times10^{-8}$), 7 SNPs linked to bladder cancer (using a threshold of $P<1\times10^{-5}$), and 21 SNPs linked to kidney cancer (using a threshold of $P<1\times10^{-5}$) to assess their relationship with BCAAs (see Tables S3-S5 for specific SNPs information). The F statistics for all SNPs exceeded 10, indicating no weak instrument bias. Our study ultimately revealed no link between PCa, bladder cancer, kidney cancer, and BCAAs. For comprehensive details, consult Table S6. Both the MR-Egger regression and MR-PRESSO test did not detect significant horizontal pleiotropy. Additionally, the Cochran's Q test found no evidence of heterogeneity (Table S7).

Association of leucine and urologic cancers in the bi-directional MR analysis

We discovered 16 SNPs associated with leucine, with detailed information provided in Table S8. All the IVs had F statistics exceeding 10. Following LD clustering and harmonization, we performed the MR-PRESSO test to identify any SNP outliers, and we did not find any. Ultimately, we acquired valid IVs (9 SNPs for PCa, 3 SNPs for bladder cancer, and 13 SNPs for kidney cancer) for the MR analysis of leucine and urological tumors.

The IVW results unveiled a potential link between leucine and the risk of PCa (OR =1.0038, 95% CI: 1.001, 1.007, P=0.008). Furthermore, no substantial association was found between leucine and bladder or kidney cancer (bladder cancer: OR =0.9995049, 95% CI: 0.997, 1.002, P=0.64; kidney cancer: OR =1.616809, 95% CI: 0.543, 3.270, P=0.53). Since the ORs was low, it indicated that the relationship between leucine and PCa may be weak; therefore, readers should interpret our results with caution. Detailed MR analysis information is provided in *Table 3*. Concerning the association between Leucine concentration

Table 2 Two-sample MR estimates for the effect of total BCAAs on urologic cancers

Outcome	No. of SNP	Method	OR	Beta	95% CI	P value
Prostate cancer	11	MR-Egger	1.002463	0.0024604184	0.997, 1.008	0.40
		Weighted median	1.002607	0.0026038709	1.000, 1.006	0.09
		Inverse variance weighted	1.002688	0.0026840934	1.000, 1.005	0.028
		Simple mode	1.000530	0.0005294807	0.994, 1.007	0.87
		Weighted mode	1.002743	0.0027392777	0.999, 1.006	0.16
Bladder cancer	5	MR-Egger	0.9985937	–0.001407264	0.995, 1.002	0.47
		Weighted median	0.9995339	–0.000466174	0.998, 1.001	0.61
		Inverse variance weighted	0.9998688	–0.000131252	0.998, 1.001	0.88
		Simple mode	0.9997364	–0.000263669	0.997, 1.002	0.85
		Weighted mode	0.9995995	–0.000400553	0.998, 1.001	0.70
Kidney cancer	12	MR-Egger	1.178389	0.1641481	0.161, 8.628	0.88
		Weighted median	2.529479	0.9280135	0.927, 6.898	0.07
		Inverse variance weighted	1.616809	0.4804548	0.772, 3.385	0.20
		Simple mode	3.048981	1.1148073	0.518, 17.954	0.24
		Weighted mode	3.3748	1.2163362	0.608, 18.736	0.19

MR, Mendelian randomization; BCAA, branched-chain amino acid; SNP, single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

Table 3 Two-sample MR estimates for the effect of leucine on urologic cancers

Outcome	No. of SNP	Method	OR	Beta	95% CI	P value
Prostate cancer	9	MR-Egger	1.004057	0.004048404	0.998, 1.010	0.23
		Weighted median	1.003102	0.003097458	1.000, 1.007	0.08
		Inverse variance weighted	1.0038	0.003792936	1.001, 1.007	0.008
		Simple mode	1.000171	0.000170762	0.994, 1.007	0.96
		Weighted mode	1.003033	0.003028131	0.999, 1.007	0.16
Bladder cancer	3	MR-Egger	0.9996542	–0.0003458973	0.996, 1.004	0.90
		Weighted median	0.9995505	–0.0004495682	0.997, 1.002	0.68
		Inverse variance weighted	0.9995049	–0.0004952117	0.997, 1.002	0.64
		Simple mode	0.9998785	–0.0001215099	0.997, 1.003	0.95
		Weighted mode	0.9995233	–0.0004768055	0.997, 1.002	0.71
Kidney cancer	13	MR-Egger	1.178389	–0.9157530	0.037, 4.306	0.47
		Weighted median	2.529479	0.2756015	0.354, 4.903	0.68
		Inverse variance weighted	1.616809	0.2873080	0.543, 3.270	0.53
		Simple mode	3.048981	0.4420410	0.157, 15.408	0.71
		Weighted mode	3.3748	0.4274800	0.140, 16.792	0.73

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

Table 4 Two-sample MR estimates for the effect of isoleucine on urologic cancers

Outcome	No. of SNP	Method	OR	Beta	95% CI	P value
Prostate cancer	6	MR-Egger	1.005971	0.005953240	0.996, 1.016	0.31
		Weighted median	1.003668	0.003661245	1.000, 1.008	0.07
		Inverse variance weighted	1.003352	0.003346275	1.000, 1.007	0.038
		Simple mode	1.001609	0.001607280	0.996, 1.008	0.61
		Weighted mode	1.003604	0.003597654	0.999, 1.008	0.17
Bladder cancer	3	MR-Egger	0.9988763	–0.0011243316	0.992, 1.006	0.81
		Weighted median	0.9991481	–0.0008522174	0.997, 1.002	0.51
		Inverse variance weighted	0.9991929	–0.0008073841	0.998, 1.002	0.52
		Simple mode	0.9990779	–0.0009225399	0.996, 1.003	0.67
		Weighted mode	0.9991811	–0.0008192720	0.999, 1.002	0.61
Kidney cancer	8	MR-Egger	0.05801068	–2.8471281	0.004, 0.766	0.07
		Weighted median	0.63303566	–0.4572285	0.160, 2.498	0.51
		Inverse variance weighted	0.76731134	–0.2648626	0.243, 2.421	0.65
		Simple mode	2.94204562	1.0791051	0.227, 38.125	0.44
		Weighted mode	0.23321268	–1.4558044	0.0321, 1.694	0.19

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

and urological tumors, neither the MR-Egger regression nor the MR-PRESSO test indicated significant directional pleiotropy. Additionally, in the MR analysis of isoleucine and bladder cancer, only 3 SNPs were available, leading to unattainable results of the MR-PRESSO global test. Furthermore, the Cochran's Q test did not reveal notable heterogeneity. Further details are available in Table S2.

The reverse MR analysis revealed no significant association between leucine and PCa, bladder cancer, or kidney cancer. Refer to Table S9 for specifics. No evidence of substantial horizontal pleiotropy was found by both the MR-Egger regression and the MR-PRESSO test. Additionally, the Cochran's Q test did not detect heterogeneity (Table S7).

Association of isoleucine and urologic cancers in the bi-directional MR analysis

We discovered 9 SNPs linked to isoleucine, with detailed data available in Table S10. The F statistics for all these IVs exceeded 10. Following LD clustering and harmonization, the MR-PRESSO test was conducted to identify SNP outliers, but none were detected. Ultimately, we secured eligible IVs for MR analysis of the association between isoleucine and urological tumors, including 6 SNPs for PCa, 3 SNPs for bladder cancer, and 8 SNPs for kidney cancer.

The IVW analysis showed a possible link between isoleucine and PCa risk (OR =1.003352, 95% CI: 1.000, 1.007, P=0.04). We did not detect any notable correlation between isoleucine and bladder or kidney cancer (bladder cancer: OR =0.9991929, 95% CI: 0.998, 1.002, P=0.52; kidney cancer: OR =0.76731134, 95% CI: 0.243, 2.421, P=0.65). Since the ORs was low, it indicated that the relationship between isoleucine and PCa may be weak; therefore, readers should interpret our results with caution. Additional MR analysis details are available in *Table 4*. Both MR-Egger regression and MR-PRESSO test did not show significant directional pleiotropy regarding the association between isoleucine concentration and urological tumors. Moreover, due to the limited availability of only 3 SNPs for the MR analysis of isoleucine and bladder cancer, the global MR-PRESSO test result was inconclusive. Furthermore, Cochran's Q test did not uncover significant heterogeneity. For more information, please see Table S2.

In our reverse MR analysis, we found no evidence of a link between isoleucine and PCa, bladder cancer, or kidney cancer. For more details, please consult Table S11.

Table 5 Two-sample MR estimates for the effect of valine on urologic cancers

Outcome	No. of SNP	Method	OR	Beta	95% CI	P value
Prostate cancer	13	MR-Egger	1.002433	0.002430076	0.998, 1.007	0.28
		Weighted median	1.00263	0.002626786	1.000, 1.006	0.07
		Inverse variance weighted	1.00279	0.002785813	1.001, 1.005	0.009
		Simple mode	1.003412	0.003405751	0.998, 1.008	0.20
		Weighted mode	1.002711	0.002707217	1.000, 1.006	0.10
Bladder cancer	5	MR-Egger	0.998876	−1.124614E−03	0.996, 1.002	0.53
		Weighted median	0.9995597	−4.404440E−04	0.998, 1.001	0.60
		Inverse variance weighted	0.9999136	−8.642125E−05	0.998, 1.001	0.91
		Simple mode	0.9997387	−2.612941E−04	0.997, 1.002	0.86
		Weighted mode	0.9996122	−3.878993E−04	0.998, 1.001	0.67
Kidney cancer	17	MR-Egger	0.8515512	−0.1606956688	0.247, 2.933	0.80
		Weighted median	0.9697176	−0.0307503852	0.401, 2.348	0.95
		Inverse variance weighted	1.0002258	0.0002258054	0.558, 1.793	>0.99
		Simple mode	0.652576	−0.4268277174	0.111, 3.837	0.64
		Weighted mode	1.8552205	0.6180035701	0.354, 9.727	0.48

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

Additionally, both the MR-Egger regression and MR-PRESSO test did not reveal significant horizontal pleiotropy. Furthermore, the Cochran's Q test did not identify any heterogeneity (refer to Table S7).

Association of valine and urologic cancers in the bi-directional MR analysis

We discovered 20 SNPs associated with valine, with detailed information provided in Table S12. All these IVs had F statistics greater than 10. Subsequently, following LD clustering and harmonization, we utilized the MR-PRESSO test to identify SNP outliers, of which none were found. As a result, we acquired suitable IVs (13 SNPs for PCa, 5 SNPs for bladder cancer, 17 SNPs for kidney cancer) for the MR analysis of the link between valine and urological tumors.

The IVW analysis revealed a potential link between valine and PCa risk (OR =1.00279, 95% CI: 1.001, 1.005, P=0.009). Additionally, no notable association was found between valine and bladder or kidney cancer (bladder cancer: OR =0.9999136, 95% CI: 0.998, 1.001, P=0.91; kidney cancer: OR =1.0002258, 95% CI: 0.558, 1.793, P>0.99). Since the ORs was low, it indicated that the relationship between valine and PCa may be weak; therefore, readers should interpret our results with caution. For specific MR analysis details, please see *Table 5*. Both the MR-Egger regression and MR-PRESSO tests did not indicate significant directional pleiotropy in the relationship between valine concentration and urological tumors. Moreover, the Cochran's Q test did not reveal significant heterogeneity. Detailed information is available in Table S2.

In our reverse MR analysis, we found no evidence of a link between valine and prostate, bladder, or kidney cancer. Detailed information can be found in Table S13. Notably, neither the MR-Egger regression nor the MR-PRESSO test revealed any significant horizontal pleiotropy. Furthermore, the Cochran's Q test did not identify any heterogeneity (see Table S7).

Discussion

This study is the first to attempt exploring the relationship between plasma BCAAs levels and urologic cancers using bidirectional MR methods. Associations between plasma levels of circulating total BCAAs, isoleucine, leucine, and valine, and the risk of PCa, kidney cancer, and bladder cancer were examined in our investigation. Our study

uncovered a relationship only between circulating total BCAAs, isoleucine, leucine, and valine levels, and PCa, without any correlation with the other cancer risks.

The essential amino acids for human nutrition, leucine, isoleucine, and valine, are known as BCAAs. Not only do they act as fundamental elements of proteins, but they also have critical roles in cell signaling, molecular regulation, carbohydrate and lipid metabolism, apoptosis, and autophagy (7). Recent research has established a close association between abnormal BCAAs metabolism and the development of diseases such as insulin resistance (30), diabetes (31), atherosclerosis (32), and tumors (19). The accumulation of BCAAs in the plasma and various tissues due to irregular BCAAs metabolism is considered a significant risk factor for these diseases. To sustain their enhanced biosynthetic and nutritional needs, cancer cells require metabolic pathway alterations to accommodate features like enhanced proliferation and invasion. Consequently, it is hypothesized that the metabolism of BCAAs might satisfy the metabolic demands of tumor cells.

The MR research findings demonstrated no definitive relationship between BCAAs and kidney cancer. Kidney cancer represents only 2% of worldwide cancer instances, and its prevalence is steadily increasing (33). Currently, there are limited reports on the correlation between circulating BCAAs levels and kidney cancer, with research primarily focusing on enzymes involved in BCAAs breakdown metabolism. Existing studies have revealed that the regulation of physiological and pathological processes such as tumor growth, metastasis, cell cycle, apoptosis, necrosis, and angiogenesis is mediated by BCAA transaminase 1 (BCAT1), a key enzyme in BCAAs metabolism (16,34). As a critical enzyme in BCAAs metabolism, BCAT1 is involved in multiple processes. Zheng *et al.*'s results indicated a positive correlation between BCAT1 expression levels and poor prognosis in kidney cancer, indirectly suggesting a potential association between elevated plasma BCAAs levels and kidney cancer risk (35). In addition, recent studies have also found that BCAT1 was closely related to mTOR signaling activity in other cancers, such as breast cancer (36) and gastric cancer (37). Therefore, Zheng's research believed that BCAAs may activate the PI3K/Akt/mTOR pathway through overexpression of BCAT1, thereby promoting the occurrence and development of kidney cancer (35). Conversely, Yang *et al.*'s study suggested that BCAAs could also enhance tumor proliferation and progression through branched-chain ketoacid dehydrogenase kinase (BCKDK) (38). In conclusion, the analysis indicated a potential link between BCAAs metabolism and the detrimental effects of kidney cancer. However, our MR research results indicated no definitive relationship between BCAAs and kidney cancer. This suggests that the current research direction on the relationship between BCAAs and kidney cancer may be insufficient, and our findings may serve as the theoretical foundation for future studies. Furthermore, the current lack of related clinical studies to validate the reliability of the findings is a limitation of our research. Subsequent researchers must conduct more rigorous research to further explore the relationship between BCAAs and kidney cancer.

Our MR findings did not uncover a direct link between BCAAs and the risk of bladder cancer. Bladder cancer, prevalent among women and the fourth most common in men (39), has relatively limited research on the BCAAs connection. Comparable to kidney cancer, the focus of bladder cancer research centers on the enzymes associated with BCAAs breakdown and metabolism. In their study, Chang *et al.* observed a significant association of BCAT1 protein overexpression with adverse clinical-pathological features of bladder cancer, including advanced pT staging, lymph node metastasis, and high pathological grade (40). Their findings suggested the potential of BCAT1 as a prognostic biomarker and a new therapeutic target for bladder cancer. Yet, in-depth mechanistic studies have not been pursued. Our MR study results indicate a lack of direct relationship between BCAA and bladder cancer risk, laying a theoretical foundation for further research. Confirmation of high-quality future research is awaited.

Our findings suggest a potential relationship between BCAAs and PCa risk. PCa is prevalent among men, particularly in the US (41), significantly impacting public health. PCa comprises approximately 27% of new male cases and ranks second in male cancer mortality rates (42). Currently, no direct evidence supports the clinical association between BCAAs and PCa. However, extensive research indicates a link between the consumption of milk and dairy products and an increased risk of PCa (43-47), a point confirmed by meta-analyses and systematic reviews (48-50). Milk and dairy products are high in BCAAs, providing a plausible explanation for the potential link between BCAAs and PCa.

It is well known that BCAAs are essential amino acids that play a critical role in cell growth and survival. These amino acids can produce glutamate through transamination reactions, thereby promoting the metabolic processes of various tumor cells. On one hand, glutamate can act

as an indirect nitrogen donor through the glutamate-glutamine axis, providing the necessary substrates for cell growth and proliferation (51-54). On the other hand, the carbon skeleton of glutamate, α-ketoglutarate, serves as an intermediate in the tricarboxylic acid (TCA) cycle, supplying the main carbon flux for energy production, thereby meeting the energy demands of tumor cells (53,54). Additionally, BCAA can function as signaling molecules to stimulate biosynthesis, with a key role in promoting cell growth and development via mTORC1 (mTORC1 is a central factor involved in cell metabolism, growth, proliferation, and migration) (55-58). Research by Ericksen *et al.* indicates that the absence of BCAA metabolism enhances the activity of mTORC1, promoting tumor formation and progression (19). Furthermore, studies have found that BCAAs are closely related to hormone secretion, gene transcription, and cellular transformations, which may also contribute to tumor development (59). Additional research shows that leucine and isoleucine can stimulate insulin secretion, thereby affecting glucose metabolism (60,61). Valine may promote α-oxidation by activating peroxisome proliferator-activated receptor α, thereby facilitating fat production (62). All of these substances are essential for the growth of tumor cells. Overall, the discussions suggest that BCAA act as a risk factor, playing a promoting role in the occurrence and development of tumor cells.

Studies have recently uncovered unusual BCAT expression in PCa tissues (8,63). BCAT, targeted by the oncoprotein c-Myc, often triggers tumor proliferation and invasion. This occurs through the activation of the PI3K/PKB/mTOR pathway and the Wnt/β-catenin signaling pathway (63). Billingsley *et al.*'s research suggests that BCAT expression is low in PCa tissues, resulting in a decrease in hyperpolarized [1-(13)C]-α-ketoisocaproate (KIC) metabolite and disrupting the hyperpolarized [1-(13)C]-KIC pathway (63). Furthermore, it was observed that the gene *BCAT1*, a target of miR-218, is linked to various processes in cancer cells such as proliferation, invasion, spread, and resistance to drugs. The suppression of BCAT1 expression is believed to hinder tumor progression, hinting at its potential as a therapeutic target in PCa treatment. In addition, research by Melnik revealed that BCAAs possess the ability to enhance tumor cell growth and proliferation via the mTORC1 pathway and to impede their autophagy, thereby fostering cancer development. The signaling cascade of mTORC1 is responsible for triggering gene activity, protein synthesis, insulin production, cell expansion, and lipid generation while restraining autophagy (64-66). The activation of mTORC1 hinges largely on the presence of adequate amino acids, with a particular focus on leucine, an essential BCAA (67-69). Leucine is capable of prompting the relocation of inactive mTORC1 to the lysosomal membrane, rich in activated Rheb, via Rag GTPase stimulation, leading to mTORC1 activation. This assessment underscores the role of BCAAs in fueling the progression of PCa. The results were in line with our study findings, suggesting potential research directions. Currently, there is limited research on this relationship. Therefore, it is crucial to gain a better understanding of PCa risk and related mechanisms, as it may lead to early and more effective therapeutic interventions. However, further in-depth investigation of these aspects is needed by future researchers. In future clinical use, BCAAs could possibly serve as a biomarker and therapeutic target for PCa, pending extensive systematic validation.

Our MR study has several advantages. First, this study is the first to explore the genetic determinants of circulating BCAAs levels and their association with the risk of urological tumors. Second, we utilized the latest and most extensive GWAS database, which helped to more effectively estimate the relationship between circulating BCAA and urological tumors. Third, we performed multiple control steps to select eligible SNPs. Fourth, the data were derived from two reliable genetic databases and the European population to avoid ethnic bias. Finally, the robustness and reliability of our conclusions were enhanced through sensitivity analysis.

However, there are some inevitable limitations in this study. (I) Due to the limitations of the GWAS summary statistics, this study only included participants of European descent. (II) IVs found in the European population cannot be directly generalized to non-European populations, therefore more MR studies targeting other populations are needed to elucidate the relationship. Additionally, the results of MR studies can only determine the relationship between exposure and outcome, and cannot deeply investigate the biological mechanism of BCAA and urological tumors. (III) Our study did not conduct an in-depth analysis of the specific classifications of PCa, kidney cancer, and bladder cancer, which represents a limitation of our research. (IV) MR studies are positioned between interventional studies and observational studies in terms of evidence. Therefore, they cannot provide evidence as strong as randomized clinical trials or their systematic reviews (70).

Conclusions

In summary, this study observed that elevated levels of circulating total BCAAs, leucine, isoleucine, and valine may increase the risk of PCa. No correlation was detected between BCAAs and kidney or bladder cancer. Nevertheless, given the absence of clinical validation for this observation, additional research is imperative for verification.

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Footnote

Reporting Checklist: The authors have completed the STROBE-MR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1142/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1142/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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