No Genetic Causality between Branched-Chain Amino Acids and Diabetic Nephropathy: A Two-Sample Mendelian Randomization Study

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Research Article

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Abstract

Background

Numerous studies have reported the close relationship between branched-chain amino acids (BCAA) and diabetic nephropathy (DN). Nevertheless, whether there is a genetically causal association between them remains profoundly elusive.

Methods

A two-sample Mendelian randomization (MR) analysis was performed using the large genome-wide association studies of the European population. The causal association was primarily evaluated by the inverse variance weighted (IVW) method. In addition, MR-Egger regression, weighted median, simple mode, and MR-weighted mode were also conducted as the supplemented methods. For sensitivity, Cochrane's Q test, MR-Egger regression, and MR-PRESSO were employed to evaluate the heterogeneity and pleiotropy, respectively.

Results

According to the IVW method, no significant causal effect was measured between three BCAA and DN (valine: OR: 1.202, 95% CI: 0.714–2.023, $P = 0.488$; isoleucine: OR: 0.878, 95% CI: 0.400–1.924, $P = 0.744$; leucine: OR: 1.395, 95% CI: 0.686–2.839, $P = 0.358$; total BCAA: OR: 1.374, 95% CI: 0.703–2.685, $P = 0.352$). For reverse MR analysis, DN as an exposure factor also had no causal effect on BCAA (valine: OR: 1.004, 95% CI: 0.994–1.014, $P = 0.412$; isoleucine: OR: 0.999, 95% CI: 0.990–1.009, $P = 0.910$; leucine: OR: 1.001, 95% CI: 0.992–1.011, $P = 0.802$; total BCAA: OR: 1.002, 95% CI: 0.993–1.012, $P = 0.628$).

Conclusion

Our results first demonstrated no significant causal association between BCAA and DN at the genetic level.

Introduction

Diabetic nephropathy (DN), one of the most common microvascular complications of diabetes, has been the leading cause of chronic kidney disease worldwide [1]. The pathogenesis of DN involves complex pathophysiological processes, including insulin resistance, glucolipid metabolism disorders, long-term chronic inflammation, oxidative stress, and endothelial dysfunction [2]. At present, controlling blood glucose and blood pressure plus the usage of the blockade of the renin-angiotensin-aldosterone system are the main approaches to treat DN [3], however, the risk of disease progression to end-stage renal

disease remains very high. Therefore, early identification and discovery of the risk factors of DN is of great significance for delaying the occurrence and development of the disease.

Branched-chain amino acids (BCAA), including valine, isoleucine, and leucine, are indispensable amino acids that cannot be synthesized in higher organisms and are important nutrition for humans [4], playing significant physiological roles in the regulation of protein synthesis, metabolism, food intake, and aging [5]. The previous cross-sectional study reported that serum BCAA concentration increased in diabetic patients while decreasing with the occurrence and development of DN [6]. On the contrary, Zhu et al detected increased levels of valine and isoleucine in DN patients, and might be risk factors for the development of DN [7]. Nevertheless, whether there is a causal relationship between BCAA and DN at the genetic level remains profoundly elusive.

Mendelian randomization (MR) employs genetic variants as instrumental variables to determine whether there is a causal association between exposure and outcome [8]. The alleles of the exposure-related genetic variant are assigned randomly and not subject to reverse causation [9], therefore, MR could decrease the confounding variables, indicating better evidence of causal inference [10]. In this MR study, we extracted valid genetic variants from the published genome-wide association study (GWAS) summary date of three BCAA to evaluate their causal effect on DN, subsequently, the direction of causation was assessed through reversing the exposure and outcome.

Methods and Materials

Study design

The diagrammatic flow diagram of this MR analysis to clarify the causal association between BCAA and DN was shown in Fig. 1. There were three significant assumptions that should be met [11]. First, the genetic variants were closely related to the exposure factors. Second, the genetic variants were independent of known or potential confounders. Last, the genetic variants affected the outcomes only via the exposure factors rather than the other pathways.

Data sources

Genetic variants associated with the levels of BCAA were obtained from the large-scale GWAS, which included metabolic biomarkers in the UK Biobank [12]. The genetic variants of DN were acquired from the large GWAS including 1,032 cases and 451,248 control of 452,280 European ancestry [13], which was available at https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018832/.

Selection of instrumental variables

To filter valid SNPs for MR analysis, a series of methods were conducted. We used $P < 5 \times 10^{-8}$ to select SNPs that were significantly associated with BCAA [14]. Moreover, $r^2 < 0.001$ and clumping distance = 10,000 kb were adopted to avoid linkage disequilibrium (LD) [15]. For reverse MR analysis, we relaxed the $P < 5 \times 10^{-6}$ to acquire enough SNPs that related to DN [16]. Further, we only considered IVs with F

statistics > 10 to avoid weak instrument bias. The *F* statistics for the SNPs were calculated by the equation: $F = R^2 \times (N-2)/(1-R^2)$ [15]. R^2 was the proportion of variance. N was the sample size. In addition, we ruled out the SNPs associated with the outcome ($P < 5 \times 10^{-8}$). Finally, we harmonized the exposure and outcome SNPs to ensure that the effect estimated were aligned for the same effect allele.

Statistical analysis

We employed inverse variance weighted (IVW), weighted median, MR-Egger, weighted mode, and simple mode to explore the causal association between BCAA and DN, of which IVW was considered as the primary method in this MR analysis. Causal effects were described as odds ratio with 95% confidence intervals (CIs). For sensitivity analysis, we applied Cochran's Q test to estimate residual heterogeneity for the IVW method [17]. MR-Egger intercept and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) test were performed to evaluate the potential pleiotropy on causal estimates [18]. In addition, the leave-one-out analysis was conducted to find out whether an SNP could drive the bias of causal estimate [19].

All statistical analysis were performed by the Two-Sample MR package (version 0.5.7) of R software (version 4.3.1). $P < 0.05$ was regarded as statistically significant.

Results

No causal effect of BCAA on DN

The causal effect of BCAA on DN were summarized in Fig. 2, Figure S1, and Supplementary Table S1. The IVW method indicated that genetically determined BCAA was not causally related to DN. Specifically, valine: OR: 1.202, 95% CI: 0.714–2.023, $P = 0.488$; isoleucine: OR: 0.878, 95% CI: 0.400–1.924, $P = 0.744$; leucine: OR: 1.395, 95% CI: 0.686–2.839, $P = 0.358$; total BCAA: OR: 1.374, 95% CI: 0.703–2.685, $P = 0.352$. In addition, other methods also did not suggest the causal effect of BCAA on DN. The forest plot and funnel plot were summarized in Figure S2 and Figure S3. We showed the details of the relevant SNPs in Supplementary Table S2.

As shown in Table 1, Cochran's Q test indicated that no significant evidence of heterogeneity was observed by IVW method (P all > 0.05). Moreover, MR-Egger intercept test and MR-PRESSO test did not measure the pleiotropy (P all > 0.05). In addition, no SNP resulted in the pleiotropic bias in our causal estimates by leave-one-out analysis (Figure S4).

Table 1
Heterogeneity and horizontal pleiotropy tests of three BCAA and DN.

	Heterogeneity		Pleiotropy		MR-PRESSO global test
	Q	*P*	Egger intercept	*P*	
Exposures					
Valine	21.842	0.292	0.014	0.632	0.379
Isoleucine	10.202	0.334	0.006	0.910	0.417
Leucine	22.302	0.173	0.016	0.668	0.233
Total BCAA	21.253	0.169	0.034	0.373	0.206
Outcomes					
Valine	23.760	0.033	0.006	0.073	NA
Isoleucine	23.177	0.040	0.005	0.128	0.053
Leucine	23.531	0.036	0.004	0.269	0.052
Total BCAA	24.318	0.028	0.006	0.114	NA

No causal effect of DN on BCAA

To further clarify whether there was a reverse causality between BCAA and DN, we conducted a reverse MR analysis. As shown in Fig. 3, Figure S5, and Supplementary Table S3, no significantly causal effect of DN on BCAA by IVW method (valine: OR: 1.004, 95% CI: 0.994–1.014, $P = 0.412$; isoleucine: OR: 0.999, 95% CI: 0.990–1.009, $P = 0.910$; leucine: OR: 1.001, 95% CI: 0.992–1.011, $P = 0.802$; total BCAA: OR: 1.002, 95% CI: 0.993–1.012, $P = 0.628$). Similarly, other methods did not find a causal effect of DN on BCAA. The forest plot and funnel plot were summarized in Figure S6 and Figure S7. Supplementary Table S4 described the detailed information on the SNPs.

The results of the sensitivity analysis were summarized in Table 1. There was no significant pleiotropy by MR-Egger intercept test and MR-PRESSO test (P all > 0.05). Moreover, the leave-one-out analysis revealed that none of the SNPs could individually affect the causal estimate (Figure S8). Although the results of Cochran's Q test indicated heterogeneity, the IVW method could decrease the effect of heterogeneity on the causal effect if there was remarkable heterogeneity [20], therefore, our results remain reliable.

Discussion

This is the first MR study to evaluate the association when BCAA, including valine, isoleucine, and leucine, were regarded as exposures and DN as the outcome. Nevertheless, our results did not support the causal effect of BCAA on DN. Furthermore, if DN was seen as the exposure variable in this MR analysis and three BCAA were taken as outcomes, similarly, no significant causality was observed. Moreover, there was also

no significant causal correlation between total concentration of BCAA and DN. In other words, our findings did not reveal the causal association between BCAA and DN at the genetic level.

Recently, BCAA was observed indispensable biological effects in many diseases. It has been reported that BCAA could cause insulin resistance and promote diabetes progression [21]. A clinical study found that the level of BCAA increased in T2DM patients while gradually decreasing with the occurrence and development of kidney injury [6], with the possible reason that decreased appetite, the disorder of acid-base metabolism, and the inflammatory response affected the concentration of amino acids in DN patients. Recently, Liu et al recently reported targeting BCAA metabolism might help prevent the progression of the renal dysfunction in diabetes patients [22]. Nevertheless, our results did not support the genetically causal effect between BCAA and DN.

This study employed MR to establish causal inferences while minimizing confounding bias, giving it a significant advantage over conventional observational studies. Moreover, we used two database populations to explore the relationship between BCAA and DN respectively, the results of which were consistent, making our results more reliable. In addition, there were several limitations that deserved to be taken into account. First, the genetic instruments of BCAA and DN were all from European ancestry, therefore it is not clear whether our results can be extrapolated to other races. Second, the use of GWAS analysis made it impossible to explore any potential nonlinear relationships or stratification effects across subgroups such as age, gender, health status, or the types of diabetes. Lastly, although our results did not reveal the genetically causal relationship between BCAA and DN, current clinical studies have shown a correlation between them. Given these controversial results, future larger clinical studies are needed to clarify the relationship between BCAA and DN.

Conclusion

This was the first study to evaluate the causal association between BCAA and DN at the genetic level, which indicated no causal relationship between them. Further well-powered clinical studies and mechanism research are needed to confirm our findings.

Declarations

Competing interest

Tere are no conflicts to report.

Author contributions

Q. M. conducted conception, design, data collection, funding and manuscript writing. S.S. performed conception, design, data collection, G. X. was responsible for the funds and paper revision. All authors approved the final version of the manuscript.

Data availability statement

The datasets presented in this study could be found in online site: https://gwas.mrcieu.ac.uk/ and Supplementary Material.

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Figures

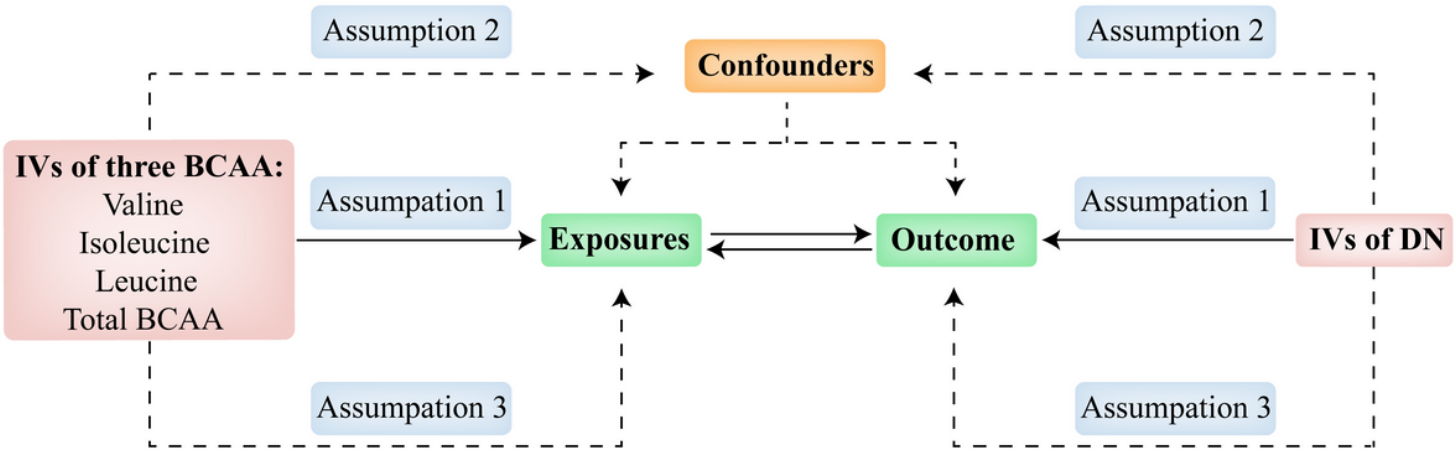

Figure 1

Overview of this bidirectional Mendelian randomization (MR) study. Instrumental variables were extracted for three branched-chain amino acids and diabetic nephropathy.

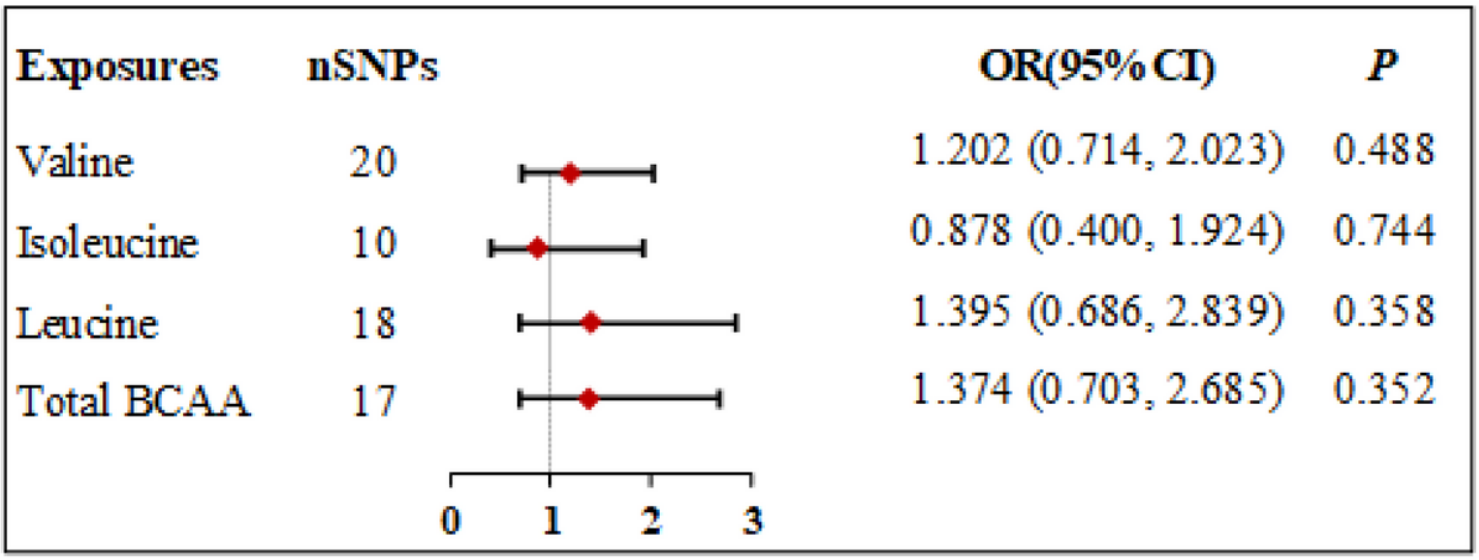

Figure 2

Forest plot of the causal effect of three branched-chain amino acidson diabetic nephropathy evaluated by inverse variance weighted.

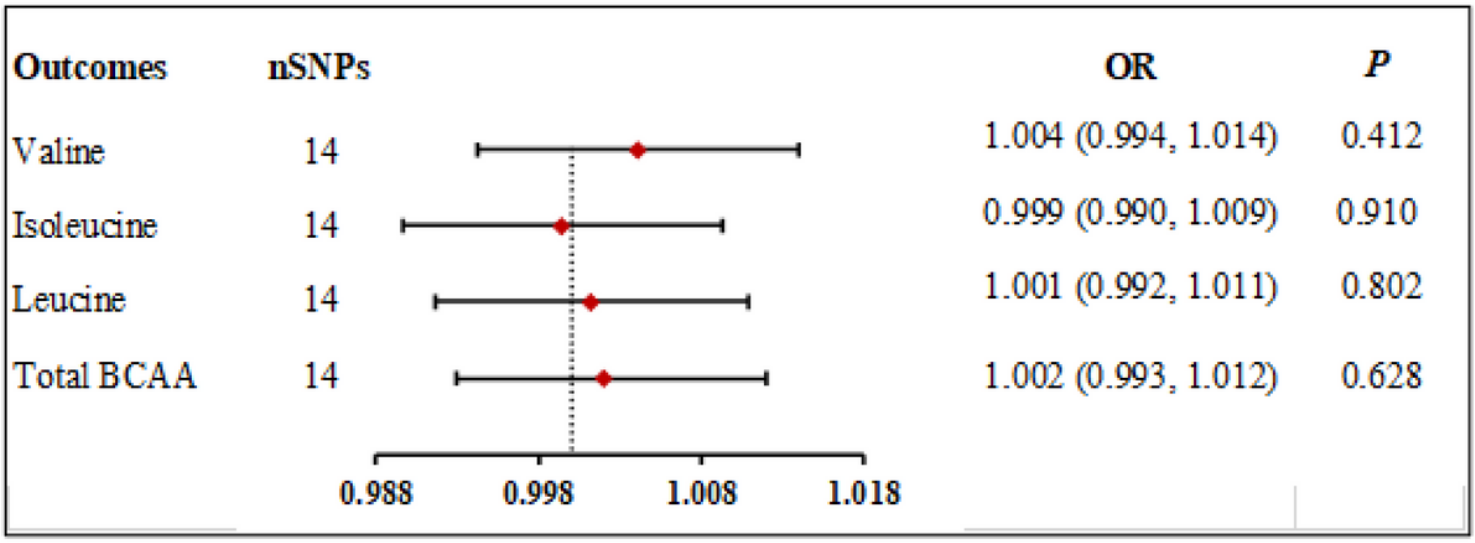

Figure 3

Forest plot of the causal effect of diabetic nephropathy on three branched-chain amino acids evaluated by inverse variance weighted.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1S8.docx
- SupplementaryTableS1S4.xlsx