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ARTICLE

# Causal Relationship between Branched-Chain Amino Acids and Inflammatory Bowel Disease: A Bidirectional and Multivariable Mendelian Randomization Study

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## Abstract

**Aims/Background** The relationship between dysregulated branched-chain amino acid (BCAA) and inflammatory bowel disease (IBD) is not fully understood. This study applied a bidirectional, two-sample Mendelian randomization (MR) approach to explore the potential causal relationship between circulating BCAA levels and IBD.

**Methods** Genome-wide association studies (GWAS) data on total BCAA levels, comprising leucine, valine, and isoleucine, were utilized. Data on IBD and its subtypes were sourced from the FinnGen study. The primary analytical method was the inverse-variance weighted (IVW) MR. To determine the direct causal effect of BCAA levels on IBD risk while accounting for confounders, we employed multivariable Mendelian randomization (MVMR).

**Results** IVW analysis revealed a positive correlation between circulating total BCAA levels, including valine, leucine, and isoleucine, and an increased risk of Crohn's disease (CD). No causal link was detected between BCAA levels and overall IBD or ulcerative colitis (UC). In the MVMR analysis, adjusting for common risk factors further validated a direct causal effect of elevated BCAA levels on CD risk.

**Conclusion** Our findings suggest that elevated circulating BCAA levels are associated with an increased risk of CD. Further research is warranted to explore the potential implications of these findings for CD risk management.

**Key words: amino acids, branched-chain; inflammatory bowel diseases; Mendelian randomization analysis; risk factors; causality**

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## Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder that affects the intestines, characterized by dysregulated immune responses and marked by recurrent episodes throughout the lifespan of an individual. The main subtypes of IBD include ulcerative colitis (UC) and Crohn's disease (CD) (Zhang and Li, 2014). Globally, the incidence of IBD is rising globally, particularly in newly industrialized countries, highlighting the urgent need for novel therapeutic strategies

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(Danpanichkul et al, 2024; Larsen et al, 2023). Although the etiology of IBD remains elusive, it is generally understood as a multifaceted disease influenced by genetic, environmental, and immune-related factors. Research suggests that IBD is associated with immune dysregulation within the gastrointestinal tract, an imbalance in gut microbiota, shifts between pro- and anti-inflammatory cytokine, and oxidative stress (Hausmann et al, 2024; Hua et al, 2023).

Branched-chain amino acids (BCAAs), such as leucine, valine, and isoleucine, are essential nutrients that play pivotal roles in proteolytic metabolism (Torres et al, 2023). Since the body cannot synthesize BCAAs, dietary intake is essential for the maintenance of metabolic functions (McGarrah and White, 2023; Reifenberg and Zimmer, 2024). Notably, the small intestinal mucosa expresses high levels of BCAA transaminase, demonstrating efficient BCAA catabolism in this region (Loveday, 2023; Wu, 1998). Given the importance of anabolic processes and bioenergetic metabolism (Blair et al, 2021; Le Couteur et al, 2020), BCAAs may influence the persistent immune activation observed in IBD and may also be affected by dietary factors.

Observational studies have suggested an association between elevated serum BCAA levels and an increased risk of IBD. Metabolomic analyses of serum from IBD patients during active disease phases, compared to healthy controls, have revealed heightened concentrations of leucine and isoleucine (Dawiskiba et al, 2014). Notably, increased serum leucine levels have been observed in CD patients, while elevated isoleucine levels have been noted in UC patients (Schicho et al, 2012; Sun et al, 2019). However, findings are inconsistent across studies; for instance, a population-based study of 67 participants (24 with CD, 20 with UC) reported lower isoleucine levels in CD patients (Williams et al, 2012). These inconsistencies may arise from factors that affect metabolites, such as lifestyle, diet, immune response, genetic predisposition, and the gut microbiome (Schirmer et al, 2019). These complex influences complicate the differentiation between disease manifestations and underlying causes, emphasizing the need for rigorous research methodologies to clarify causal relationships between BCAA levels and IBD.

Mendelian randomization (MR) is an advanced research method increasingly used to examine causal relationships between exposures and outcomes. By leveraging the random allocation of genetic variants, MR can infer causality while minimizing confounding factors common in an observational study (Skrivankova et al, 2021). Genetic variants associated with unique risk factors remain stable throughout life and are unaffected by external environmental influences or disease progression, thus circumventing issues of reverse causality (Skrivankova et al, 2021). The Two-Sample MR, a variant of the MR methodology, derives genetic correlations with exposure and outcome from separate populations. In this study, we employed univariable Mendelian randomization (UVMR) and multivariable Mendelian randomization (MVMR) analyses to rigorously explore the potential causal relationships between BCAA concentrations and IBD risk.

# Methods

## Data Sources

To ensure robustness in our Mendelian randomization (MR) analysis for assessing causal relationships and to enhance reproducibility, data were sourced from two extensive, publicly accessible genome-wide association studies (GWAS) datasets: the UK Biobank and the FinnGen study.

GWAS data for BCAAs, including total BCAA levels and individual leucine, valine, and isoleucine measurements, were sourced from the UK Biobank database (https://www.ukbiobank.ac.uk/). This dataset includes 115,047 participants for total BCAAs, 115,048 for valine, 115,074 for leucine, and 115,075 for isoleucine, all of European ancestry.

For IBD and its subtypes, GWAS summary data were obtained from the FinnGen datasets. These data include 9083 IBD patients, 2033 CD patients, and 5931 UC patients, all of European ancestry. Comprehensive information about case definitions, genotyping platforms, and statistical protocols is available on the FinnGen website (https://www.finngen.fi/en/). Notably, there was no overlap between participants in the exposure and outcome datasets.

## Selection of Instrumental Variables (IVs)

Instrumental variables (IVs) were selected as follows: (1) Single-nucleotide polymorphisms (SNPs) significantly associated with BCAA levels were identified using a $p$-value threshold of $p < 5 \times 10^{-8}$. (2) To ensure the independence of SNPs and reduce linkage disequilibrium (LD) bias, LD-based clumping was applied with parameters set at $r^2 < 0.001$ and a clumping window >10,000 kb. (3) The strength of IVs was evaluated using the F-statistic, where an F-value >10 indicated strong instruments, minimizing bias due to weak instruments. (4) During harmonization, SNPs with ambiguous allele alignment or palindromic SNPs with ambiguous strand orientation were either adjusted or removed. Additionally, the LDtrait tool (https://ldlink.nih.gov/?tab=ldtrait) was employed to exclude SNPs previously linked to the outcome or known confounders (Lin et al, 2020).

## Univariable MR Analyses

Our MR analyses were based on three core assumptions: firstly, the selected genetic variants were strongly associated with BCAA levels; secondly, these variants were not confounded by external factors; and thirdly, there was no horizontal pleiotropy, suggesting that the variants affected the outcome exclusively through their effect on BCAA levels. The study design is shown in Fig. 1.

In this study, the inverse-variance weighted (IVW) method was the primary approach to derive our principal analytical findings. Additionally, we utilized MR-Egger and weighted median (WM) methods as complementary analyses to evaluate the causal relationship between serum BCAA levels and IBD risk. These methods allowed for comparison odds ratios (ORs) across analyses, verifying consistency with the IVW results. The IVW method, commonly employed in Mendelian randomization (MR) studies, enables effective data aggregation without the need for

individual-level data and facilitates the immediate evaluation of causal effects. The IVW approach has been recognized for its effectiveness in identifying causal relationships within MR frameworks (Hartwig et al, 2017).

The MR-Egger regression analysis was applied to detect and account for potential pleiotropy, and reliable causal estimates were provided even in the presence of some invalid IVs (Bowden et al, 2015). The weighted median approach, in turn, aggregates various SNPs into a single causal estimate but requires fewer than 50% of the IVs to be invalid for consistent results (Bowden et al, 2016). To further investigate causal relationships, we performed a reverse MR analysis to evaluate the possible causal influence of IBD on circulating BCAA levels.

To correct for potential Type I errors due to multiple comparisons, we applied Bonferroni's correction (Curtin and Schulz, 1998), resulting in a significance threshold as 0.05/[4 (BCAAs levels, leucine, isoleucine, valine) × 3 (IBD, CD, UC)] = $4.167 \times 10^{-3}$. IVW method *p*-values below $4.167 \times 10^{-3}$ indicated a significant causal effect, while *p*-values between $4.167 \times 10^{-3}$ and 0.05 suggested a causal association. The findings from the three MR analyses are visualized in scatter plots.

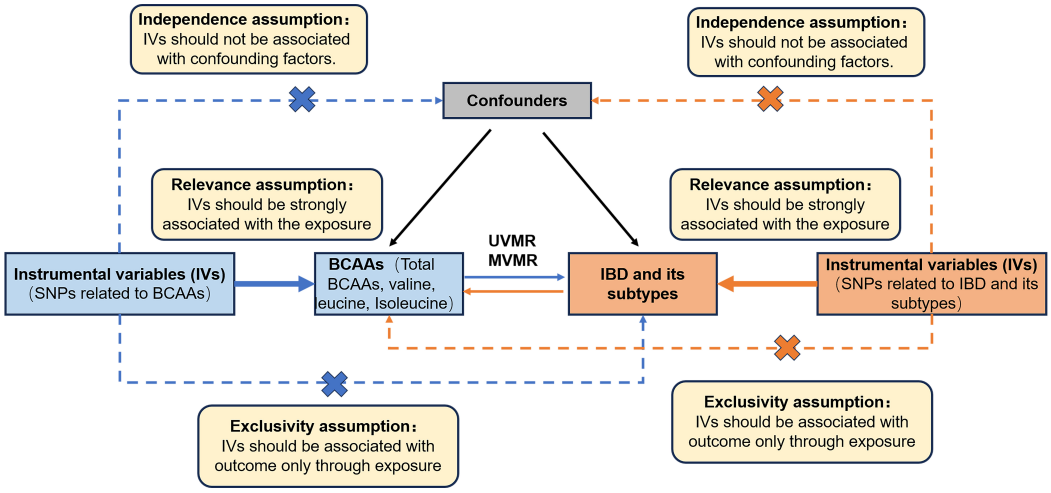


**Fig. 1. Schematic overview of the MR study on the association between BCAAs and IBD risk.** This schematic illustrates the MR study design assessing the association between circulating BCAA levels and the risk of IBD. Arrows indicate the association of instrumental variables with exposure, while dashed lines represent the independence of the instrumental variables from potential confounders. The figure was created using PowerPoint software (version 2016, Microsoft Inc., Redmond, WA, USA). MR, Mendelian randomization; SNP, single-nucleotide polymorphism; BCAAs, branched-chain amino acids; IBD, inflammatory bowel disease; UVMR, univariable Mendelian randomization; MVMR, multivariate Mendelian randomization; IVs, instrumental variables.

## Sensitivity Analyses

To validate the robustness of our MR results, we conducted sensitivity analyses. MR-Egger regression and IVW analyses were employed to assess heterogeneity, with Cochran's Q test used to quantify heterogeneity, where a *p*-value of less than 0.05 indicated significant heterogeneity (Cohen et al, 2015). Pleiotropy was further evaluated using MR-Egger regression and the MR-PRESSO analysis (Verbanck et

al, 2018). Additionally, a leave-one-out analysis was performed to mitigate the impact of pleiotropy (Hemani et al, 2018). **Supplementary Figs. 1,2** illustrate the results of these analyses and include funnel plots for the MR analysis on the impact of BCAA levels on IBD and its subtypes.

### MVMR Analyses

To address potential confounding variables, we performed MVMR, an extension of the standard univariable MR analysis. Smoking was included as a covariate due to its established association with IBD risk (Guillo et al, 2023; Wang et al, 2024a; Zhang et al, 2024a). Additionally, type 2 diabetes mellitus (T2DM) was incorporated as a covariate, given emerging evidence linking elevated circulating BCAAs with increased T2DM risk (Torki et al, 2023; Wang et al, 2024b; Zhang et al, 2024b). Other metabolites of BCAAs, specifically glutamine, and 3-hydroxybutyrate, were also considered covariates due to their potential association with IBD, as noted in previous studies (Suzuki et al, 2023; Xie et al, 2024). The MVMR analyses aimed to evaluate the potential causal relationships between BCAA levels and Crohn's disease (CD), independently of smoking, T2DM, glutamine, and 3-hydroxybutyrate levels. IVW served as the primary method for MVMR analysis, with MR-Egger regression and MR-PRESSO employed for sensitivity analysis. The conditional F statistic was calculated for each BCAA phenotype to evaluate the strength of the instrumental variables in MVMR analyses.

### Statistical Analyses

All MR analyses were conducted using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria), specifically with the "TwoSampleMR", "MendelianRandomization", and "MR-PRESSO" packages. The "forestploter" package in R was utilized for figure creation. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). **Supplementary Table 1** outlines the calculation methods for $R^2$ and MR power. A $p$-value $< 0.05$ was considered statistically significant for all analyses unless otherwise specified.

## Results

### Selection of IVs

After removal of palindromic SNPs, execution of clumping, and the harmonization of data, we identified 15 SNPs associated with circulating total BCAAs, 20 SNPs related to valine, 16 associated with leucine, and 9 with isoleucine levels. Detailed information on these SNPs, including $R^2$ and F-statistic values for each exposure, is provided in **Supplementary Tables 2–5**. All exposures exhibited F-statistic values greater than 10, indicating sufficient strength.

### UVMR Analysis

#### *Causal Effects of Circulating Total BCAA Levels on IBD*

Our findings suggest that higher serum BCAAs were positively associated with an increased risk of CD, with an OR of 2.247 (95% CI 1.346–3.752, $p = 0.0020$), as determined by the IVW analysis (Fig. 2). Additionally, the weighted median anal-

ysis indicated a suggestive association between genetically predicted total BCAA levels and increased CD risk (OR 2.094, 95% CI 1.205–3.638, $p = 0.0088$). However, not all analyses confirmed this association, as the MR-Egger analysis did not show a significant relationship ($p = 0.0933$). Scatter plots illustrating these associations are shown in Fig. 3B. MR-PRESSO ($p = 0.089$) and MR-Egger regression ($p = 0.693$) analyses did not indicate significant directional pleiotropy. Cochran's Q test showed no evidence of heterogeneity ($p = 0.055$). Furthermore, neither primary nor supplementary analyses identified causal relationships between total BCAA concentrations and IBD or UC (Fig. 2 and Fig. 3A,C). **Supplementary Fig. 1A–C** illustrate leave-one-out analyses, and the funnel plots are provided in **Supplementary Fig. 2A–C**.

| Exposure | Outcome | Method | nSNP | p.value | OR(95%CI) | Heterogeneity | Pleiotropy.MR.Egger. | MR.PRESSO.Global.Test |
|---|---|---|---|---|---|---|---|---|
| Total BCAA | IBD | MR Egger | 15 | 0.8610 | 0.945(0.508 to 1.757) | 0.002 | | |
| | | Weighted median | 15 | 0.8594 | 1.025(0.780 to 1.348) | | | |
| | | Inverse variance weighted | 15 | 0.8554 | 1.028(0.766 to 1.379) | 0.003 | 0.766 | 0.003 |
| | CD | MR Egger | 15 | 0.0933 | 2.728(0.927 to 8.023) | 0.041 | | |
| | | Weighted median | 15 | 0.0088 | 2.094(1.205 to 3.638) | | | |
| | | Inverse variance weighted | 15 | 0.0020 | 2.247(1.346 to 3.752) | 0.055 | 0.693 | 0.089 |
| | UC | MR Egger | 15 | 0.3602 | 0.696(0.330 to 1.469) | 0.003 | | |
| | | Weighted median | 15 | 0.6932 | 0.935(0.671 to 1.303) | | | |
| | | Inverse variance weighted | 15 | 0.4795 | 0.878(0.613 to 1.259) | 0.003 | 0.497 | 0.003 |
| Valine | IBD | MR Egger | 20 | 0.8966 | 1.033(0.635 to 1.680) | 0.001 | | |
| | | Weighted median | 20 | 0.8798 | 1.020(0.790 to 1.316) | | | |
| | | Inverse variance weighted | 20 | 0.6486 | 1.060(0.825 to 1.363) | 0.002 | 0.904 | 0.002 |
| | CD | MR Egger | 20 | 0.0545 | 2.202(1.041 to 4.657) | 0.186 | | |
| | | Weighted median | 20 | 0.0050 | 1.992(1.231 to 3.225) | | | |
| | | Inverse variance weighted | 20 | 0.0004 | 2.015(1.368 to 2.968) | 0.229 | 0.787 | 0.284 |
| | UC | MR Egger | 20 | 0.5903 | 0.845(0.464 to 1.540) | 0.001 | | |
| | | Weighted median | 20 | 0.7051 | 0.943(0.698 to 1.275) | | | |
| | | Inverse variance weighted | 20 | 0.6233 | 0.925(0.678 to 1.262) | 0.002 | 0.733 | <0.001 |
| Leucine | IBD | MR Egger | 16 | 0.6512 | 0.835(0.388 to 1.794) | <0.001 | | |
| | | Weighted median | 16 | 0.8011 | 1.040(0.768 to 1.407) | | | |
| | | Inverse variance weighted | 16 | 0.5780 | 1.105(0.777 to 1.570) | <0.001 | 0.432 | <0.001 |
| | CD | MR Egger | 16 | 0.3930 | 1.704(0.524 to 5.533) | 0.052 | | |
| | | Weighted median | 16 | 0.0071 | 2.287(1.253 to 4.174) | | | |
| | | Inverse variance weighted | 16 | 0.0036 | 2.291(1.310 to 4.006) | 0.065 | 0.583 | 0.146(outlier corrected, 1 outlier) |
| | UC | MR Egger | 16 | 0.2158 | 0.640(0.327 to 1.253) | 0.069 | | |
| | | Weighted median | 16 | 0.6869 | 0.926(0.639 to 1.343) | | | |
| | | Inverse variance weighted | 16 | 0.4840 | 0.894(0.652 to 1.225) | 0.057 | | |
| Isoleucine | IBD | MR Egger | 9 | 0.8748 | 0.904(0.269 to 3.033) | 0.006 | 0.292 | 0.031 |
| | | Weighted median | 9 | 0.6594 | 1.081(0.765 to 1.528) | | | |
| | | Inverse variance weighted | 9 | 0.8877 | 1.029(0.693 to 1.528) | 0.011 | 0.829 | 0.016 |
| | CD | MR Egger | 9 | 0.2500 | 4.233(0.443 to 40.412) | 0.027 | | |
| | | Weighted median | 9 | 0.0097 | 2.665(1.268 to 5.603) | | | |
| | | Inverse variance weighted | 9 | 0.0014 | 3.314(1.586 to 6.925) | 0.043 | 0.827 | 0.064 |
| | UC | MR Egger | 9 | 0.2797 | 0.640(0.327 to 1.253) | 0.069 | | |
| | | Weighted median | 9 | 0.6869 | 0.926(0.639 to 1.343) | | | |
| | | Inverse variance weighted | 9 | 0.4840 | 0.894(0.652 to 1.225) | 0.057 | 0.292 | 0.031 |

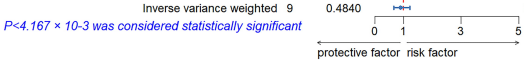


**Fig. 2. MR analysis of the association between circulating BCAA levels and IBD and its subtypes.** This figure presents MR analysis results examining the association between circulating BCAA levels and IBD, as well as its subtypes (CD and UC). Outcomes were derived using random-effects models of IVW, MR-Egger, and WM approaches. The final three columns display results from the sensitivity test. BCAA, branched-chain amino acid; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; IVW, inverse-variance weighted; WM, weighted median; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

### *Causal Effects of Circulating Valine Levels on IBD*

Using the IVW method, our analysis observed strong evidence that elevated valine levels are associated with a heightened risk of CD (OR: 2.015, 95% CI 1.368–2.968, $p = 0.0004$), as illustrated in Fig. 3E. However, MR-Egger did not confirm this association as statistically significant ($p = 0.0545$) (Fig. 2). The MR-PRESSO test ($p = 0.284$) and MR-Egger regression ($p = 0.787$) indicated no significant pleiotropy. Additionally, no heterogeneity was detected ($p = 0.229$). No

associations were observed between valine levels and overall IBD or UC (Fig. 2 and Fig. 3D,F). Leave-one-out analysis results are presented in **Supplementary Fig. 1D–F**, with funnel plots shown in **Supplementary Fig. 2D–F**.

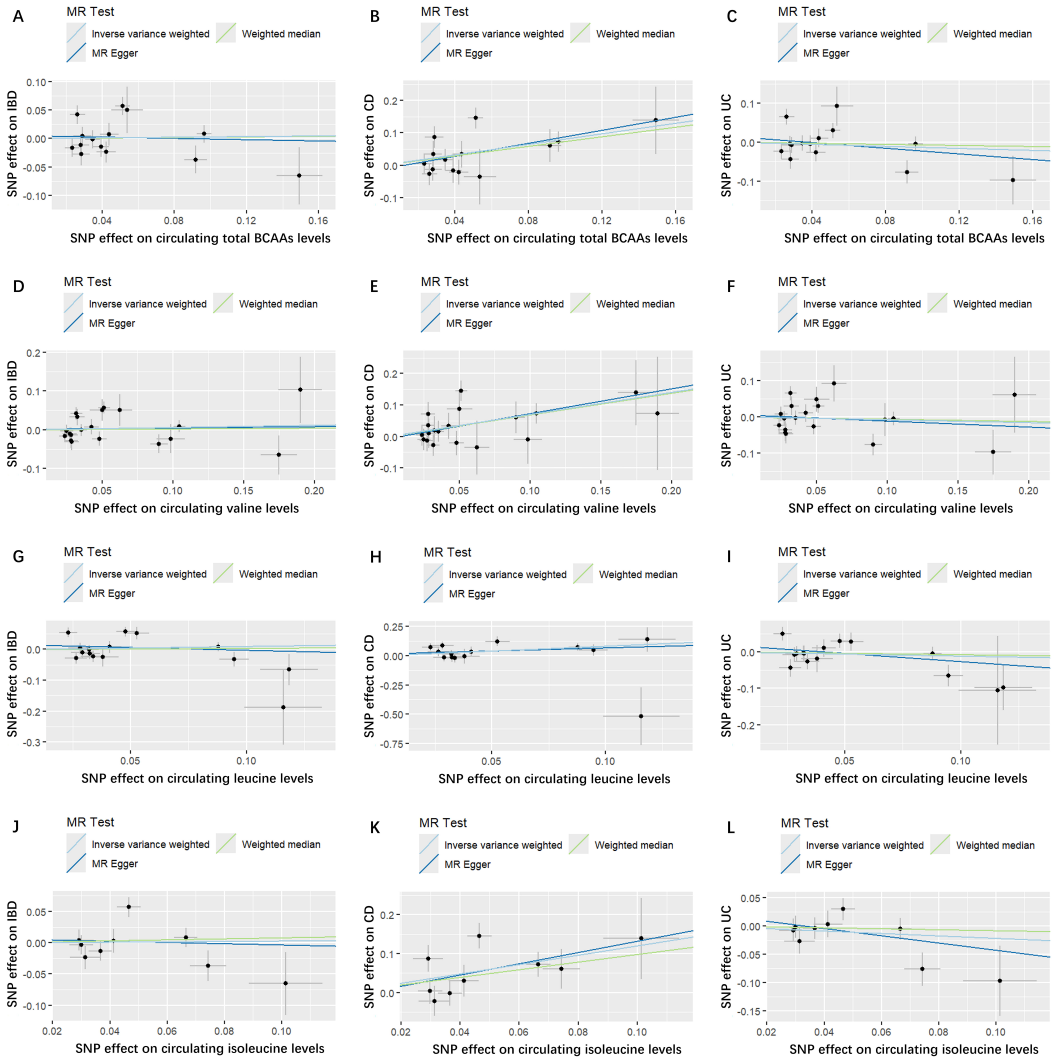


**Fig. 3. Scatter plots of SNPs associated with circulating BCAA levels and IBD risk.** Scatter plots illustrate the causal estimates of circulating BCAA levels on IBD risk, derived from MR regression slopes using IVW, MR-Egger, and WM methods. Each subplot represents a specific association: (A) Total BCAA levels with IBD, (B) Total BCAA levels with CD, (C) Total BCAA levels with UC, (D) Valine levels with IBD, (E) Valine levels with CD, (F) Valine levels with UC, (G) Leucine levels with IBD, (H) Leucine levels with CD, (I) Leucine levels with UC, (J) Isoleucine levels with IBD, (K) Isoleucine levels with CD, (L) Isoleucine levels with UC. BCAAs, branched-chain amino acids; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; IVW, inverse-variance weighted; WM, weighted median; SNP, single-nucleotide polymorphism.

## *Causal Effects of Circulating Leucine Levels on IBD*

Genetically predicted higher levels of leucine were significantly associated with an increased risk of CD in the IVW analysis (OR 2.291, 95% CI 1.310–4.006, $p = 0.0036$). The WM method similarly indicated a suggestive association between

leucine levels and CD risk (OR 2.287, 95% CI 1.253–4.174, $p = 0.0071$) (Fig. 2). However, the MR-Egger analysis showed no significant association (OR 1.704, 95% CI 0.524–5.533, $p = 0.3930$). MR-Egger regression revealed no evidence of significant pleiotropy ($p = 0.583$), while MR-PRESSO identified one outlier with potential pleiotropic effects (rs1260326, $p = 0.019$). After reanalysis to account for this outlier, results remained consistent ($p = 0.146$), and no heterogeneity was detected ($p = 0.065$). The funnel plot indicated a symmetrical distribution of effect estimates, suggesting minimal risk bias when a single SNP was used as an IV (**Supplementary Fig. 2H**). No causal relationship was observed between leucine levels and either IBD or UC in the primary or supplementary analyses. Scatter plots are shown in Fig. 3G–I. Detailed leave-one-out and funnel plots are shown in **Supplementary Fig. 1G–I** and **Supplementary Fig. 2G–I,** respectively.

### *Causal Effects of Circulating Isoleucine Levels on IBD*

The IVW method provided strong evidence that isoleucine levels are associated with an elevated risk of CD (OR 3.314, 95% CI 1.586–6.925, $p = 0.0014$). The weighted median analysis also indicated a suggestive association, with genetically predicted isoleucine levels increasing CD risk (OR 2.665, 95% CI 1.268–5.603, $p = 0.0097$). However, MR-Egger analysis did not demonstrate a causal impact (OR 4.233, 95% CI 0.443–40.412, $p = 0.2500$) (Fig. 2 and Fig. 3K). The MR-Egger regression test ($p = 0.827$) and Cochran's Q test ($p = 0.043$) showed no evidence of pleiotropy or heterogeneity, and the MR-PRESSO analysis identified no outliers ($p = 0.064$). The IVW analysis revealed no causal effects of isoleucine on IBD ($p = 0.8877$) or UC ($p = 0.4840$) (Fig. 2 and Fig. 3J,L). These findings were consistent with those from MR-Egger and the WM analyses. Leave-one-out plots are presented in **Supplementary Fig. 1J–L**, and funnel plots in **Supplementary Fig. 2J–L**.

## Reverse MR Analysis

The Mendelian randomization analysis includes a total of 52 SNPs associated with IBD, 9 SNPs specific to CD, and 43 SNPs linked to UC. Detailed genetic variation information is provided in **Supplementary Tables 6–8**. Across the three analyses, including the IVW method, no significant causal link was observed between IBD or its subtypes as exposure variables and circulating BCAA levels (Fig. 4). Scatter plots and leave-one-out analyses are presented in **Supplementary Figs. 3,4**, while funnel plots are shown in **Supplementary Fig. 5**.

## MVMR Analysis

After adjusting for confounders, we employed MVMR analyses to assess the significant causal associations identified in the UVMR analyses, including smoking, type 2 diabetes mellitus (T2DM), glutamine levels, and 3-hydroxybutyrate levels. Genetic IVs for daily cigarette consumption were extracted from a GWAS (ID: ieu-b-25). IVs for T2DM were sourced from a GWAS meta-analysis, which included 12,931 T2DM cases and 57,196 healthy controls (GWAS ID: ebi-a-GCST005413). SNPs associated with glutamine and 3-hydroxybutyrate levels were sourced

| Exposure | Outcome | Method | nSNP | p.value | OR(95%CI) | P.hete | Pleiotropy.MR.Egger | P.Global |
|---|---|---|---|---|---|---|---|---|
| IBD | Total BCAA | MR Egger | 48 | 0.4251 | 0.990(0.965 to 1.015) | 0.391 | | |
| | | Weighted median | 48 | 0.6331 | 1.004(0.989 to 1.019) | | | |
| | | Inverse variance weighted | 48 | 0.9258 | 1.000(0.991 to 1.010) | 0.398 | 0.368 | 0.315 |
| | Valine | MR Egger | 48 | 0.6598 | 0.994(0.969 to 1.020) | 0.292 | | |
| | | Weighted median | 48 | 0.5148 | 1.005(0.990 to 1.020) | | | |
| | | Inverse variance weighted | 48 | 0.8823 | 1.001(0.991 to 1.011) | 0.316 | 0.591 | 0.216 |
| | Leucine | MR Egger | 48 | 0.4080 | 0.989(0.964 to 1.015) | 0.279 | | |
| | | Weighted median | 48 | 0.9151 | 0.999(0.985 to 1.014) | | | |
| | | Inverse variance weighted | 48 | 0.9015 | 1.001(0.991 to 1.011) | 0.281 | 0.344 | 0.239 |
| | Isoleucine | MR Egger | 48 | 0.1394 | 0.981(0.957 to 1.006) | 0.59 | | |
| | | Weighted median | 48 | 0.6832 | 0.997(0.983 to 1.011) | | | |
| | | Inverse variance weighted | 48 | 0.8384 | 0.999(0.989 to 1.009) | 0.533 | 0.13 | 0.475 |
| CD | Total BCAA | MR Egger | 9 | 0.6207 | 0.990(0.953 to 1.029) | 0.424 | | |
| | | Weighted median | 9 | 0.9181 | 0.999(0.982 to 1.017) | | | |
| | | Inverse variance weighted | 9 | 0.9067 | 0.999(0.987 to 1.012) | 0.504 | 0.627 | 0.513 |
| | Valine | MR Egger | 9 | 0.6075 | 0.989(0.952 to 1.028) | 0.522 | | |
| | | Weighted median | 9 | 0.6539 | 0.996(0.979 to 1.013) | | | |
| | | Inverse variance weighted | 9 | 0.8972 | 0.999(0.986 to 1.012) | 0.599 | 0.616 | 0.606 |
| | Leucine | MR Egger | 9 | 0.6539 | 0.991(0.954 to 1.029) | 0.444 | | |
| | | Weighted median | 9 | 0.9647 | 1.000(0.983 to 1.018) | | | |
| | | Inverse variance weighted | 9 | 0.9477 | 1.000(0.987 to 1.012) | 0.528 | 0.65 | 0.496 |
| | Isoleucine | MR Egger | 9 | 0.7815 | 0.993(0.947 to 1.041) | 0.168 | | |
| | | Weighted median | 9 | 0.9712 | 1.000(0.981 to 1.019) | | | |
| | | Inverse variance weighted | 9 | 0.9541 | 1.000(0.986 to 1.015) | 0.229 | 0.755 | 0.177 |
| UC | Total BCAA | MR Egger | 42 | 0.5916 | 0.992(0.964 to 1.021) | 0.286 | | |
| | | Weighted median | 42 | 0.3665 | 1.006(0.993 to 1.020) | | | |
| | | Inverse variance weighted | 42 | 0.3473 | 1.004(0.995 to 1.014) | 0.292 | 0.378 | 0.216 |
| | Valine | MR Egger | 42 | 0.6642 | 0.994(0.966 to 1.022) | 0.324 | | |
| | | Weighted median | 42 | 0.5217 | 1.004(0.991 to 1.018) | | | |
| | | Inverse variance weighted | 42 | 0.3190 | 1.005(0.996 to 1.014) | 0.337 | 0.428 | 0.269 |
| | Leucine | MR Egger | 42 | 0.5733 | 0.991(0.962 to 1.021) | 0.148 | | |
| | | Weighted median | 42 | 0.7258 | 1.002(0.989 to 1.016) | | | |
| | | Inverse variance weighted | 42 | 0.3740 | 1.004(0.995 to 1.014) | 0.15 | 0.372 | 0.127 |
| | Isoleucine | MR Egger | 42 | 0.4672 | 0.989(0.961 to 1.018) | 0.295 | | |
| | | Weighted median | 42 | 0.4361 | 1.005(0.992 to 1.019) | | | |
| | | Inverse variance weighted | 42 | 0.5457 | 1.003(0.994 to 1.012) | 0.295 | 0.332 | 0.22 |

*P<4.167 × 10-3 was considered statistically significant*

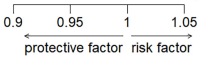


**Fig. 4. Mendelian randomization associations between IBD and its subtypes and circulating BCAA levels.** Outcomes were calculated using random-effects models of the IVW, MR-Egger, and WM methods. The final three columns display results from sensitivity analyses. BCAA, branched-chain amino acid; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; IVW, inverse-variance weighted; WM, weighted median; SNP, single-nucleotide polymorphism; OR, odds ratio.

from the MRC Integrative Epidemiology Unit's (IEU) OpenGWAS database (GWAS ID ebi-a-GCST90026170, n = 291; ebi-a-GCST90092811, n = 113,594). Detailed IV information is provided in **Supplementary Table 9**.

Fig. 5 summarizes the outcomes of the MVMR analyses. Consistent with previous findings, multivariable IVW analysis indicated that elevated circulating total BCAAs levels were causally associated with an increased risk of CD, even after adjusting for smoking ($OR_{IVW}$ = 2.246, 95% CI = 1.443–3.497, $p$ = 0.0003), T2DM ($OR_{IVW}$ = 2.470, 95% CI = 1.560–3.910, $p$ = 0.0001), glutamine levels ($OR_{IVW}$ = 2.124, 95% CI = 1.355–3.327, $p$ = 0.0010), and 3-hydroxybutyrate levels ($OR_{IVW}$ = 1.876, 95% CI = 1.183–2.973, $p$ = 0.0074). Valine was similarly identified as an independent risk factor for CD after adjusting for smoking ($OR_{IVW}$ = 1.997, 95% CI = 1.371–2.909; $p$ = 0.0003), T2DM ($OR_{IVW}$ = 2.206, 95% CI = 1.433–3.395, $p$ = 0.003), glutamine levels ($OR_{IVW}$ = 2.064, 95% CI = 1.397–3.048, $p$ = 0.0003), and 3-hydroxybutyrate levels ($OR_{IVW}$ = 1.680, 95% CI = 1.146–2.465, $p$ = 0.0079).

Further associations between higher plasma leucine levels and CD risk persisted after adjusting for smoking ($OR_{IVW}$ = 2.580, 95% CI = 1.480–4.500, $p$ = 0.0008), T2DM ($OR_{IVW}$ = 2.654, 95% CI = 1.561–4.512, $p$ = 0.0003), glutamine

levels ($OR_{IVW}$ = 2.274, 95% CI = 1.321–3.915, $p$ = 0.003), and 3-hydroxybutyrate levels ($OR_{IVW}$ = 2.108, 95% CI = 1.297–3.426, $p$ = 0.0026). Circulating isoleucine levels were also causally linked to increased CD risk, adjusting for smoking ($OR_{IVW}$ = 3.112, 95% CI = 1.624–5.965; $p$ = 0.0006), type 2 diabetes ($OR_{IVW}$ = 3.900, 95% CI = 2.080–7.315, $p < 0.0001$), glutamine levels ($OR_{IVW}$ = 2.935, 95% CI = 1.579–5.454, $p$ = 0.0007), and 3-hydroxybutyrate levels ($OR_{IVW}$ = 2.611, 95% CI = 1.251–5.449, $p$ = 0.0106).

To validate the causal estimates from each MR analysis, we performed sensitivity analyses. These analyses included the MR-Egger intercept test, the MR-PRESSO global test to detect pleiotropy, and Cochran's Q test to assess heterogeneity. The $p$-values from the MR-Egger intercept and MR-PRESSO global tests exceeded 0.05, indicating no significant horizontal pleiotropy. However, heterogeneity was observed in specific analyses: total BCAAs adjusted for T2DM ($p$ = 0.048), leucine adjusted for T2DM ($p$ = 0.031), and isoleucine adjusted for T2DM ($p$ = 0.049) and 3-hydroxybutyrate levels ($p$ = 0.034) (Fig. 5). These findings were further examined using random-effects models to ensure robust causal inferences.

## Discussion

In our two-sample MR analysis, we identified a potential causal association between genetically elevated circulating levels of total BCAAs, including leucine, valine, and isoleucine levels, and an elevated risk of CD. However, this causal relationship was not observed for IBD as a whole or for UC. After adjusting for potential confounders, such as smoking, T2DM, glutamine levels, and 3-hydroxybutyrate levels, and applying the Bonferroni corrections, the causal effects of BCAA levels on CD remained statistically significant. This study represents the first population-based analysis to explore the causal link between BCAA concentrations and CD risk.

Amino acid metabolism disturbances are commonly observed in IBD and are linked with disease severity. However, previous findings on this association have been inconsistent. For instance, a 2021 meta-analysis identified consistent alterations in BCAAs, including isoleucine, leucine, and valine, in serum and plasma metabolomics studies of IBD patients. Notably, elevated isoleucine levels were observed in CD (Dawiskiba et al, 2014; Fathi et al, 2014; Schicho et al, 2012) and UC (Dawiskiba et al, 2014; Sun et al, 2019) patients, while valine levels were lower in UC (Diab et al, 2019; Tews et al, 2023) and CD (Murgia et al, 2018; Scoville et al, 2018; Tews et al, 2023) patients, as were leucine levels (Diab et al, 2019; Tews et al, 2023). Contradictory results have also been reported. For example, Sun et al (2019) reported elevated valine levels in UC patients compared to healthy controls. Using metabolomic serum analysis, Tews et al (2023) observed downregulated isoleucine in IBD patients. A recent study of 103 CD patients demonstrated that leucine and valine levels were lower in active CD cases than those in remission, with a negative correlation to the Crohn's Disease Activity Index (CDAI) (Cioffi et al, 2023). 3-hydroxybutyrate, a byproduct of BCAA catabolism, has been reported at elevated levels in UC patients compared to healthy individuals (Keshteli et al, 2017; Zhang

| Exposure | Method | nSNP | p.value | OR(95%CI) | P.hete | MR.Egger.intercept | P.Global | F |
|---|---|---|---|---|---|---|---|---|
| Total BCAA | MVMR_IVW | | | | | | | |
| | Adjusted for Smoking | 37 | 0.0003 | 2.246(1.443 to 3.497) | 0.081 | | 0.098 | 44.173 |
| | Adjusted for T2DM | 29 | 0.0001 | 2.470(1.560 to 3.910) | 0.048 | | 0.072 | 49.106 |
| | Adjusted for Glutamine levels | 34 | 0.0010 | 2.124(1.355 to 3.327) | 0.122 | | 0.18 | 45.827 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0074 | 1.876(1.183 to 2.973) | 0.083 | | 0.126 | 50.281 |
| | MVMR_Egger | | | | | | | |
| | Adjusted for Smoking | 37 | 0.0010 | 2.677(1.490 to 4.809) | | 0.368 | | 44.173 |
| | Adjusted for T2DM | 29 | 0.0904 | 1.836(0.909 to 3.710) | | 0.277 | | 49.106 |
| | Adjusted for Glutamine levels | 34 | 0.0443 | 2.299(1.021 to 5.174) | | 0.817 | | 45.827 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0629 | 1.845(0.968 to 3.520) | | 0.942 | | 50.281 |
| | MVMR_Median | | | | | | | |
| | Adjusted for Smoking | 37 | 0.0155 | 1.944(1.135 to 3.330) | | | | 44.173 |
| | Adjusted for T2DM | 29 | 0.0061 | 2.180(1.249 to 3.804) | | | | 49.106 |
| | Adjusted for Glutamine levels | 34 | 0.0151 | 2.015(1.145 to 3.546) | | | | 45.827 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0149 | 1.972(1.141 to 3.409) | | | | 50.281 |
| Valine | MVMR_IVW | | | | | | | |
| | Adjusted for Smoking | 41 | 0.0003 | 1.997(1.371 to 2.909) | 0.156 | | 0.171 | 49.672 |
| | Adjusted for T2DM | 125 | 0.0003 | 2.206(1.433 to 3.395) | 0.086 | | 0.089 | 11.187 |
| | Adjusted for Glutamine levels | 39 | 0.0003 | 2.064(1.397 to 3.048) | 0.168 | | 0.223 | 48.921 |
| | Adjusted for 3-Hydroxybutyrate levels | 33 | 0.0079 | 1.680(1.146 to 2.465) | 0.221 | | 0.244 | 58.146 |
| | MVMR_Egger | | | | | | | |
| | Adjusted for Smoking | 41 | 0.0009 | 2.365(1.423 to 3.930) | | 0.331 | | 49.672 |
| | Adjusted for T2DM | 125 | 0.0009 | 2.351(1.420 to 3.892) | | 0.63 | | 11.187 |
| | Adjusted for Glutamine levels | 39 | 0.0452 | 2.020(1.015 to 4.019) | | 0.94 | | 48.921 |
| | Adjusted for 3-Hydroxybutyrate levels | 33 | 0.0069 | 2.019(1.212 to 3.361) | | 0.287 | | 58.146 |
| | MVMR_Median | | | | | | | |
| | Adjusted for Smoking | 41 | 0.0174 | 1.801(1.109 to 2.926) | | | | 49.672 |
| | Adjusted for T2DM | 125 | 0.0131 | 2.026(1.160 to 3.538) | | | | 11.187 |
| | Adjusted for Glutamine levels | 39 | 0.0068 | 2.031(1.216 to 3.390) | | | | 48.921 |
| | Adjusted for 3-Hydroxybutyrate levels | 33 | 0.0588 | 1.627(0.982 to 2.696) | | | | 58.146 |
| Leucine | MVMR_IVW | | | | | | | |
| | Adjusted for Smoking | 35 | 0.0008 | 2.580(1.480 to 4.500) | 0.076 | | 0.155 | 31.039 |
| | Adjusted for T2DM | 31 | 0.0003 | 2.654(1.561 to 4.512) | 0.031 | | 0.07 | 38.763 |
| | Adjusted for Glutamine levels | 33 | 0.0030 | 2.274(1.321 to 3.915) | 0.129 | | 0.213 | 32.929 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0026 | 2.108(1.297 to 3.426) | 0.104 | | 0.17 | 43 |
| | MVMR_Egger | | | | | | | |
| | Adjusted for Smoking | 35 | 0.0139 | 2.532(1.208 to 5.307) | | 0.938 | | 31.039 |
| | Adjusted for T2DM | 31 | 0.0949 | 1.881(0.896 to 3.948) | | 0.198 | | 38.763 |
| | Adjusted for Glutamine levels | 33 | 0.2487 | 1.812(0.660 to 4.975) | | 0.599 | | 32.929 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0076 | 2.521(1.279 to 4.967) | | 0.456 | | 43 |
| | MVMR_Median | | | | | | | |
| | Adjusted for Smoking | 35 | 0.0137 | 2.297(1.186 to 4.451) | | | | 31.039 |
| | Adjusted for T2DM | 31 | 0.0082 | 2.342(1.247 to 4.401) | | | | 38.763 |
| | Adjusted for Glutamine levels | 33 | 0.0121 | 2.348(1.205 to 4.576) | | | | 32.929 |
| | Adjusted for 3-Hydroxybutyrate levels | 30 | 0.0055 | 2.313(1.279 to 4.184) | | | | 43 |
| Isoleucine | MVMR_IVW | | | | | | | |
| | Adjusted for Smoking | 30 | 0.0006 | 3.112(1.624 to 5.965) | 0.086 | | 0.137 | 25.592 |
| | Adjusted for T2DM | 25 | <0.0001 | 3.900(2.080 to 7.315) | 0.049 | | 0.096 | 33.638 |
| | Adjusted for Glutamine levels | 29 | 0.0007 | 2.935(1.579 to 5.454) | 0.098 | | 0.145 | 28.869 |
| | Adjusted for 3-Hydroxybutyrate levels | 24 | 0.0106 | 2.611(1.251 to 5.449) | 0.034 | | 0.074 | 28.37 |
| | MVMR_Egger | | | | | | | |
| | Adjusted for Smoking | 30 | 0.0057 | 3.167(1.398 to 7.175) | | 0.943 | | 25.592 |
| | Adjusted for T2DM | 25 | 0.0171 | 3.197(1.230 to 8.306) | | 0.583 | | 33.638 |
| | Adjusted for Glutamine levels | 29 | 0.0128 | 4.892(1.401 to 17.085) | | 0.356 | | 28.869 |
| | Adjusted for 3-Hydroxybutyrate levels | 24 | 0.1003 | 2.258(0.855 to 5.962) | | 0.644 | | 28.37 |
| | MVMR_Median | | | | | | | |
| | Adjusted for Smoking | 30 | 0.0498 | 2.271(1.001 to 5.153) | | | | 25.592 |
| | Adjusted for T2DM | 25 | 0.0047 | 3.105(1.414 to 6.817) | | | | 33.638 |
| | Adjusted for Glutamine levels | 29 | 0.0294 | 2.380(1.091 to 5.194) | | | | 28.869 |
| | Adjusted for 3-Hydroxybutyrate levels | 24 | 0.0418 | 2.316(1.032 to 5.198) | | | | 28.37 |

*P<0.05/4 was considered statistically significant*



**Fig. 5. Forest plots of MVMR analyses assessing the causal impact of BCAA levels on CD.** BCAA, branched-chain amino acid; CD, Crohn's disease; MVMR, multivariable Mendelian randomization; IVW, inverse-variance weighted; SNP, single-nucleotide polymorphism; OR, odds ratio; T2DM, type 2 diabetes mellitus.

et al, 2013) and may serve as a biomarker of disease activity. For instance, Keshteli et al (2017) noted elevated 3-hydroxybutyrate levels in relapsing patients compared to those in remission.

These inconsistencies with our findings on causal relationships may arise from differences in study design. Observational studies, whether prospective or retrospective, may be affected by confounders that affect the evaluation of exposure-outcome relationships. Such studies may report associations, but establishing definitive causal inferences remains limited due to unavoidable confounders that complicate the accurate determination of exposure-outcome relationships. These con-

founders may weaken the conclusions about causality. Thus, while prospective or retrospective observational studies may suggest an association, they cannot definitively establish a direct causal link. In contrast, the MR methodology mitigates potential confounding by integrating genetic variants, offering a more precise estimate of causal relationships.

The current body of research on the impact of BCAA on IBD is limited, and the mechanisms through which BCAAs might influence IBD progression remain undefined. An animal study suggest that BCAA supplementation may worsen disease severity, potentially through the phosphorylation of mammalian target of rapamycin (mTOR) and its downstream target, ribosomal protein S6 kinase beta-1 (p70S6K) (Huang et al, 2024). Other research has observed a link between BCAA supplementation and reduced abundance of beneficial gut bacteria (Genton et al, 2021), which could represent an additional mechanism by which BCAAs increase IBD risk. Macrophages, which play a pivotal role in immune homeostasis, also appear to be influenced by BCAAs. A prospective study found that BCAAs can modulate macrophage activation and impact human physiology. Notably, Wuggenig et al (2020) reported that conditional deletion of the BCAA transporter CD98hc in macrophages attenuated colitis in an animal model.

Our findings suggest elevated serum BCAA levels are associated with CD, not UC. While genetic similarities exist across IBD subtypes, there are notable differences in immune responses, specific genes, and genetic profiles between CD and UC (Annese, 2020; James et al, 2023; Kaser et al, 2010; Saez et al, 2023; Skok et al, 2021). Additionally, CD appears more susceptible to dietary influences than UC. A prospective epidemiological study have linked certain dietary patterns and nutrient intake with CD onset, although the associations are less clear for UC (Fitzpatrick et al, 2022). Future studies should investigate whether these differences are attributable to variations in immune response, environmental factors, genetic predispositions, or gut microbiota composition.

This study has several strengths. First, to our knowledge, it is the first to leverage large-scale GWAS data in a two-sample MR approach to assess the bidirectional causal relationship between serum BCAA levels and IBD, reducing the effect of confounders relative to observational studies. Second, we conducted a detailed subclassification of IBD subtypes, minimizing potential bias from mixed disease forms. Third, multiple sensitivity analyses were performed to confirm the robustness of our results.

However, this study also has limitations. MR analysis relies on three key assumptions for valid instrumental variables in causal inference: (1) variants must be strongly associated with the exposure, (2) variants should be independent of confounders, and (3) variants should influence the outcome only through the exposure. While these assumptions may limit the generalizability of our findings, they are essential to uphold the validity of IVs and to avoid bias from linkage disequilibrium. The exclusion of SNPs highly correlated with the exposure ensures the independence of those used in the analysis. The inclusion of all genetic variations, however, could introduce noise, potentially compromising results precision. Moreover, our study was limited to summary-level GWAS data, preventing us from

conducting more detailed subgroup analyses based on demographic characteristics, disease severity, clinical symptoms, and IBD subtypes.

Future research should include extensive randomized controlled trials (RCTs) or expanded MR analyses with larger GWAS sample sizes to further explore these associations. The integration of multi-omics approaches will also be crucial for elucidating the pathophysiological mechanisms underlying these diseases. Finally, our findings primarily apply to populations of European descent, which may restrict their generalizability to more diverse populations.

## Conclusion

In summary, our MR analysis provides robust evidence supporting a causal link between elevated circulating levels of total BCAAs, including leucine, isoleucine, and valine, and an increased risk of Crohn's disease. However, no association was observed between serum BCAA levels and risk of IBD or UC. These findings suggest that serum BCAA concentrations could serve as novel biomarkers for CD and may present new therapeutic targets. Nonetheless, further validation through large-scale randomized controlled trials is necessary to establish their prognostic utility. Additional research is also needed to elucidate the mechanisms by which BCAAs influence the pathophysiology of IBD.

### Key Points

- Elevated circulating levels of total BCAAs, including leucine, valine, and isoleucine, are causally associated with an increased risk of CD.
- No association was observed between BCAA levels and either IBD or UC risk.
- There is no causal relationship between IBD or its subtypes as exposure factors and BCAA levels.
- Circulating BCAA levels causally increased CD risk, even after adjusting for smoking, type 2 diabetes, glutamine levels, and 3-hydroxybutyrate levels in multivariable IVW analysis.
- Further research is warranted to assess the potential benefits of modulating serum BCAA levels as a therapeutic strategy for CD.

## Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

## Author Contributions

JZ and LS designed the research study. JZ performed the research. FZ and JZ analyzed the data. JZ and LS were involved in drafting the manuscript. All authors contributed to revising the manuscript critically for important intellectual content.

All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.magonlinelibrary.com/doi/suppl/10.12968/hmed.2024.0722.

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