Original Article

Investigating Causal Links between Metabolite Profiles and Ulcerative Colitis: A Bidirectional Mendelian Randomization Study

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Abstract

Background: While metabolic biomarkers are known to play a significant role in the development of ulcerative colitis (UC), the exact causal relationships between them remain uncertain and warrant further investigations. Here we report a bidirectional two-sample Mendelian randomization (MR) study to evaluate causal relationships between 503 blood metabolites and UC. **Methods:** We used genome-wide association study (GWAS) data on blood metabolite levels from two separate studies on European individuals (n = 8299 and 24,925). In addition, for UC, we utilized GWAS data from the same ancestry, including 417,932 participants, comprising 5371 UC cases and 412,561 controls. We employed the inverse variance weighted method for our discovery stage of MR analyses. Then, we used other methods, including MR-Egger, weighted median, weighted mode, simple mode, MR-pleiotropy residual sum and outlier, heterogeneity, and pleiotropy tests for sensitivity analyses to further validate our findings and assess the robustness of our results. **Results:** Our study suggests that total lipids in small high-density lipoprotein levels (S.HDL.L) are marginal significant positive associated with the development of UC (odds ratio = 1.167, 95% confidence interval: 0.998–1.364, P = 0.051). In addition, UC did not have a statistically significant effect on the metabolites. **Conclusions:** Total lipids in S.HDL.L may offer a potential trend as valuable circulating metabolic biomarkers for the screening and prevention of UC in clinical practice. In addition, they could serve as potential candidate molecules for elucidating the mechanisms underlying UC and for identifying suitable drug targets.

Keywords: *Biomarkers, causality, colitis, inflammatory bowel diseases, ulcerative*

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by inflammation and ulceration of the colonic mucosa, typically starting in the rectum and extending proximally in a continuous manner. UC can significantly impact a patient's daily life due to its long-term burdensome complications. Achieving rapid improvement of symptoms, healing of the disease at the mucosal level, and restoring quality of life are the main goals of treatment in UC patients.[1] By 2030, it is projected that the prevalence rate of UC will reach 1% among Western populations, which represents a significant burden on global health resources.[2] Disorders of the metabolism are known as one of the main factors effective in the development of UC.[3-5] Previous studies have demonstrated correlations between blood metabolites and UC; however, the issue of causality remains unresolved. More studies are needed to reveal whether abnormal metabolite profiles are a cause or a consequence of UC.[4-8] Detecting causal links between metabolites and UC risk could lead to a better understanding of disease mechanisms, the discovery of biomarkers, the identification of new therapeutic targets, the development of preventive measures, and the implementation of personalized treatment strategies for patients with UC.[9-11] Mendelian randomization (MR) can provide more reliable estimates of causality than observational studies due to its use of genetic variants associated with exposures as unconfounded instrumental variables (IVs).[12,13] Various MR evaluations have investigated the association between metabolite levels and IBD. For instance, Long *et al*.'s study found that metabolites such as mannose, arachidonate,

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5-anhydroglucitol, and 2-stearoylglycerophosphocholine have a causal association with UC and Crohn's disease (CD).[11] In addition, Yao *et al*.'s study found that metabolites such as low-density lipoprotein cholesterol and total cholesterol (TC) have a causal protective effect on UC.[14] In Yu *et al.*'s study, it was also reported that tryptophan has a protective causal effect on IBD and its subtypes, and kynurenine is known to be a causal risk factor for IBD and its subgroups.[15] The current study implemented a bidirectional Two-Sample MR (2SMR) approach to examine the causal relationships between 503 blood metabolites and UC. For this purpose, the latest and most comprehensive datasets have been utilized. These datasets contain a vast amount of information regarding various metabolites, which enhances the innovation and strength of our study. Our findings can serve as a foundation for larger and more comprehensive studies aimed at improving diagnosis and treatment outcomes for individuals affected by UC.

Methods

Study design

This research is based on the up-to-date and most comprehensive genome-wide association study (GWAS) results for blood metabolite levels and UC disease, and consists of two phases: In Phase l, metabolite levels are used as exposures, with UC serving as the outcome. In Phase II, UC is used as the exposure, while metabolite levels serve as the outcome. This study was conducted with the approval of the Vice Chancellor for Research of Isfahan University of Medical Sciences (Grant number 3400922).

Metabolite datasets

The datasets reported by Chen *et al*.[16] and Kettunen *et al.*[17] were used as metabolite data in this study. Chen *et al.*,[16] study involves a total of 8299 healthy adults of European ancestry, with a mean age of 62.4 years, from the Canadian Longitudinal Study on Aging cohort. The study population was composed of 56.5% females [Table 1]. The researchers tested 1091 plasma metabolites. The known metabolites include amino acids, lipids, xenobiotics, carbohydrates, peptides, cofactors, vitamins, and nucleotides. In the study by Kettunen *et al.,*[17] a GWAS was conducted on metabolites using data from 14 European cohorts, comprising up to 24,925 adults [Table 1]. These samples were used to quantify 123 serum metabolites, capturing a comprehensive molecular signature of systemic metabolism. The metabolites include amino acids, glycolysis precursors, fatty acids, and lipoprotein lipids and subclasses, which provide insight into various metabolic pathways.

Ulcerative colitis dataset

Summary statistics for UC were obtained from GWASs conducted in the UK Biobank (UKB) and the FinnGen cohorts. The GWAS meta-analysis included 3121 cases and 281,398 controls from the UKB and 2250 cases and 131,163 controls from FinnGen, totaling 417,932 participants with 5371 UC cases and 412,561 controls across both cohorts. At recruitment, the mean age of participants in the UKB cohort was 56.8 years, with 53.8% being female. In the FinnGen project, the mean age of participants at DNA sample collection was 51.8 years, with 56.3% being female. All participants were Europeans, and the patients' diagnoses conformed to the established criteria for clinical assessments.[18]

Table 1: Features of populations relevant to metabolites

	CLSA cohort[16]	Characteristics of the studied cohort[17]
n	Up to 8229	25,072
Age	62.4 (9.9)*	44.56 (15.25)‡
Sex (% female)	56.5	54.58†
Ancestry	European	European
BMI	28.0 (5.3)*	25.91 (4.61)‡

*Mean (SD); ‡Weighted mean according to the study size (SD); †Total percentage female. BMI – Body mass index; SD – Standard deviation; CLSA – Canadian longitudinal study on aging; SD – Standard deviation

Quality control measures

To ensure the validity of our analysis, we employed quality control measures for single-nucleotide polymorphisms (SNPs) implemented in GWASinspector[19] R package.

Instrumental variables selection

We based our selection of genetic instruments or IVs on three key criteria: (1) the SNPs related to exposures with significance level of $P < 5 \times 10^{-8}$, (2) SNPs with matching alleles between the exposure and outcome data, and (3) SNPs with nonpalindromic alleles (A/T or G/C). To retain independent variants, exposure SNPs were subjected to clumping procedure. The threshold for clumping SNPs based on the default package settings (linkage disequilibrium $r^2 < 0.001$ and >10 kb distance), using the European subset of the 1000 Genomes Projects reference panel. PLINK v1.9 software (National Institutes of Health (Bethesda, Maryland, USA) and Massachusetts general hospital (Boston, Massachusetts, USA)) was used for clumping, which prunes linked SNPs within a particular window and retains only the SNP with the lowest *P* value. We utilized the MR-pleiotropy residual sum and outlier (PRESSO) test to identify horizontal pleiotropy by excluding outliers in the final step. In addition, F-statistics were calculated as (beta/se),[2,20] and values <10[21] were classified as weak and therefore removed from the analyses.

Mendelian randomization analyses

In both phases of our analyses, we conducted inverse variance weighted (IVW) method to determine the causal associations. In cases where the IVW method was

impossible due to a lack of sufficient IVs, Wald's ratio was used. To identify the set of metabolites for MR analysis, we first examined the overlap between 1091 metabolites from Chen *et al*.,[16] and 123 metabolites from Kettunen *et al*.,[17] datasets, and removed duplicate metabolites from the dataset with the smaller sample size[16] totaling to 1196 unique metabolites. This approach confirmed that our subsequent analysis was founded on a comprehensive and nonredundant set of metabolites. Next, we selected metabolites related to European ancestry from the combined dataset that were associated with at least one significantly associated SNP. Hence, we ended up with a total of 503 metabolites with known structures and functions. These 503 metabolites were then screened to gain a more thorough understanding of the causal relationships between metabolites and UC (Phase l) using the selected IVs. The reverse causality between UC and metabolites (Phase Il) was also investigated to rule out possibly confounded results due to reverse causation. To account for multiple testing, we applied the Bonferroni (i.e., 0.05/503) correction to the *P* values and set the significance level at $P < 9.94 \times 10^{-5}$. This approach ensured that our results were robust and minimally susceptible to type I error. In our study, we aimed to identify the strongest and most valid relationships while minimizing the risk of false positives. Incorrectly identifying causal relationships can lead to erroneous subsequent research, wasting time, and resources. Therefore, we opted for a highly conservative method like the Bonferroni correction to ensure the reliability of any reported results.

Sensitivity analyses

We performed sensitivity analyses to avoid false positive results, reduce the impact of horizontal pleiotropy, and obtain robust MR estimates for the two phases. Sensitivity tests, including the weighted median (WM), simple, and weighted mode, were performed to examine the association between metabolites and multiple SNPs. We also applied Cochrane's Q test to IVW and MR-Egger regression to detect potential violations of the assumptions resulting from heterogeneity in the relationships between individual IVs. In addition, we evaluated potential horizontal pleiotropy using the MR-Egger intercept and MR PRESSO global test results. A leave-one-out (LOO) analysis was finally used to further enhance strength and reliability of our findings. R software (version 4.3.2) and 2SMR package were used for our analyses.

Figure 1 illustrates the study flow diagram.

Results

Phase I: Investigating causal effects of metabolites on ulcerative colitis

Table 1 provides the characteristics of the population for metabolites as described by Chen *et al*. and Kettunen *et al*.[16,17] In the first phase, serum/plasma metabolites from the two mentioned datasets[16,17] were screened using IVW-MR (see methods). Based on Bonferroni-correction *P* value ($P < 9.94 \times 10^{-5}$), we recognized no significant causal associations between metabolites and the risk of UC. However, a marginally positive significant association was found in the metabolite dataset of Kettunen *et al*.,[17] between total lipids in small high-density lipoprotein levels (S.HDL.L) and UC (odds ratio [OR] = 1.167, 95% confidence intervals [CI]: 0.998–1.364, $P = 0.051$) [Table 2]. No evidence of pleiotropic effects was detected using the MR-PRESSO global test, with a $P > 0.05$. Furthermore, all the minimum F-statistics exceeded 10 [Supplementary Table 1]. This demonstrates the absence of weak instrument bias.

Phase II: Causal effects of ulcerative colitis on metabolites

When we treated UC as the exposure variable and metabolites as the outcome, no statistically significant association was obtained.

Sensitivity analyses' results

In addition to the IVW method, the WM method also showed a marginally positive significant association between total lipids in S.HDL.L and UC (OR = 1.201, 95% CI: 0.994–1.451, $P = 0.052$) [Table 2], although this association did not reach statistical significance at a Bonferroni-corrected threshold ($P < 9.94 \times 10^{-5}$). Subsequent sensitivity analyses did not reveal any statistically significant results. Since we detected no significant evidence of horizontal pleiotropy ($P > 0.05$), the marginally significant positive association between total lipids in S.HDL.L and UC is less likely to be driven by pleiotropy or other major sources of bias.

To further investigate potential issues, scatter [Figure 2] and funnel plots [Figure 3] were used to identify the outliers and horizontal pleiotropy in the results. In addition, LOO analysis was performed, which showed that the estimated relationships between total lipids in S.HDL.L and UC were robust and not significantly affected by any individual IV [Supplementary Table 2].

Table 2: Marginally positive significant Mendelian randomization analysis results of total lipids in small high-density lipoprotein levels for ulcerative colitis

Methods	OR	95% CI	*P*	Number SNP
IVW	1.167	0.998–1.364	0.051	7
WM	1.201	0.994–1.451	0.052	7
Simple mode	1.196	0.914–1.565	0.239	7
Weighted mode	1.208	0.956–1.526	0.163	7
MR-egger	1.375	0.926–1.544	0.174	7

MR – Mendelian randomization; OR – Odd ratio; CI – Confidence interval; No.SNP – Number of single nucleotide polymorphisms; IVW – Inverse variance weighted; WM – Weighted median; MR-Egger – Mendelian randomization-Egger; HDL – High-density lipoprotein; UC – Ulcerative colitis

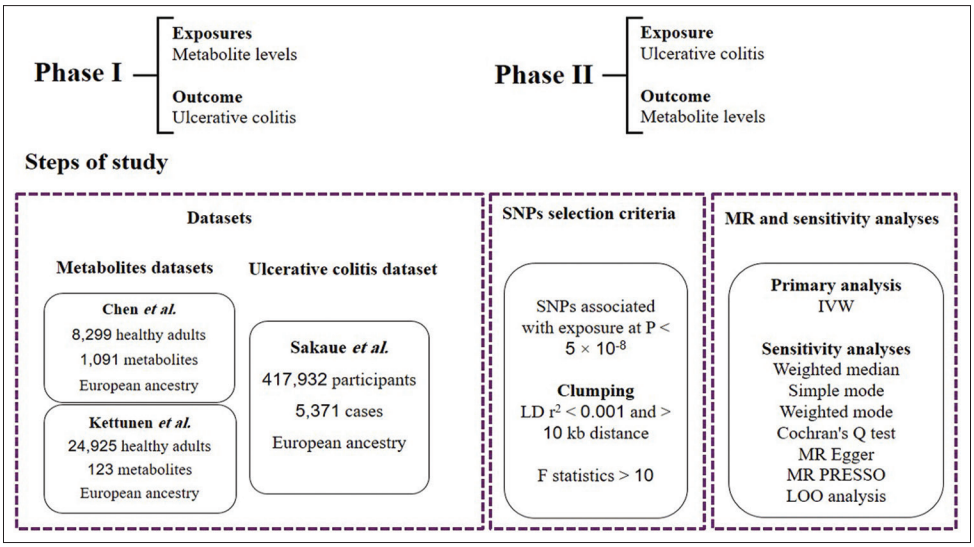

Figure 1: Flow diagram of the design of our study. SNP: Single-nucleotide polymorphism, LD: Linkage disequilibrium; kb: Kilobase, MR: Mendelian randomization, IVW: Inverse variance weighted, MR Egger: Mendelian randomization-Egger, MR PRESSO: MR pleiotropy residual sum and outlier, LOO: Leave-one-out

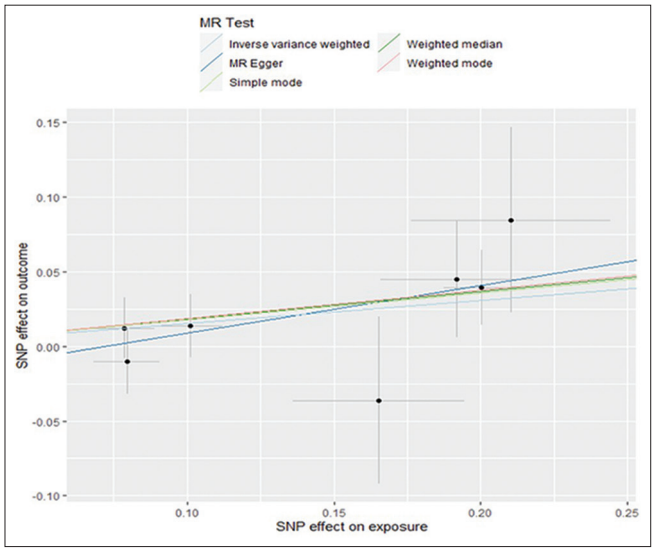

Figure 2: Scatter plot showing the genetic effects of the total lipids in small high-density lipoprotein levels on the risk of ulcerative colitis. HDL: High-density lipoprotein, UC: Ulcerative colitis

Discussion

In our study, a comprehensive 2SMR analysis was conducted using GWAS data to assess the causal relationships between metabolite profiles and the risk of UC. We detected a marginally positive significant association between total lipids in S.HDL.L and UC, which calls for further research and provides clues about the lipoprotein-related pathophysiology of the UC. Such relationships, if confirmed, can offer candidates for early diagnosis or treatment targets for the disease. The exact mechanisms underlying the development and progression of UC are complex, but there has been growing interest in the role of metabolites in understanding the pathophysiology of UC.[4] Metabolites can be viewed as a snapshot of the metabolic processes occurring in the gut during active inflammation. Metabolites disturbance have been observed in the perpetuation of inflammation, mucosal damage, and dysbiosis of gut microbiota in UC. Some metabolites are related to specific clinical features in patients with UC, such as severity of symptoms, response to treatment, and risk of complications. The study of metabolites in UC offers several advantages over traditional biomarkers, such as antibodies or inflammatory cytokines.[21,22] Metabolites are often more specific, sensitive, and readily accessible biomarkers of disease activity and can provide insight into the underlying biological processes driving disease pathogenesis.[23,24] Several recent MR studies have investigated the causal relationships between circulating metabolites and UC development. For instance, Long *et al.* demonstrated that specific metabolites, including arachidonate (20:4n6) ($P = 2.09 \times 10^{-11}$), 5-anhydroglucitol ($P = 1.50 \times 10^{-4}$), and 2-stearoylglycerophosphocholine ($P = 5.30 \times 10^{-4}$), are associated with UC. Their conclusion stemmed from the analysis of 486 blood metabolites in a cohort of 5034 UC patients and 371,530 controls.[11] Similarly, Li *et al.* identified myo-inositol, 1-arachidonoylglycerophosphocholine, mannitol, and 3-methylhistidine as having significant causal effects on UC in their analysis of 275 blood metabolites from 9487 patients and 358,040 controls.[9] To obtain more robust findings, a comprehensive MR analysis was conducted in the present study. This analysis utilized the most up-to-date and comprehensive GWAS summary data for both metabolites and UC, and employed a bidirectional approach to evaluate the causal association between serum/plasma metabolites and UC. MR reduces confounding bias by using genetic variants as proxies for exposures, making it less susceptible to confounding than traditional observational studies. Observational

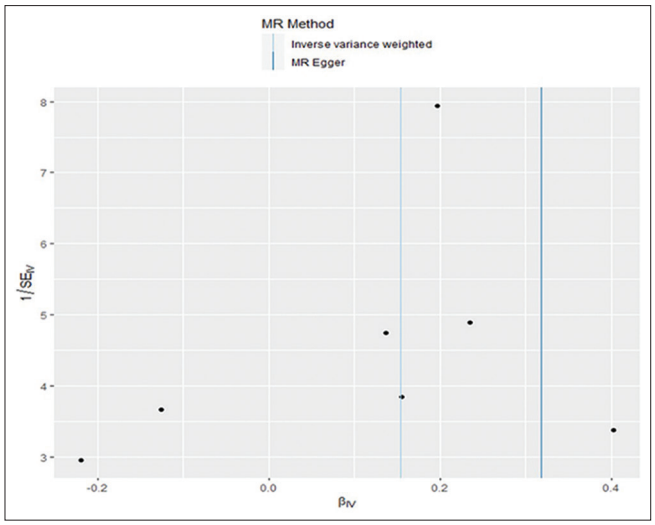

Figure 3: Funnel plot of instrumental variables for marginally positive significant association between total lipids in small high-density lipoprotein levels andulcerative colitis. IVs: Instrumental variables, HDL: High-density lipoprotein, UC: Ulcerative colitis

studies are often influenced by shared confounders, such as socioeconomic status, environmental exposures, and demographic variables, that affect both the exposure and outcome.[12] In addition, the MR framework mimics randomized clinical trials (RCT) under certain assumptions because of this random allocation of variants at the time of conception and enables stronger causal inference compared to traditional epidemiological techniques.[13] While the results of this 2SMR approach did not detect a causal association between metabolites and UC, the initial findings regarding the association between total lipids in S.HDL.L and UC should be replicated and confirmed with other (preferably larger) datasets. While lipoprotein changes in CD have been the primary focus of most studies, the other subtype of IBD, the available evidence for metabolite changes in UC is limited and controversial.[25-27] Therefore, to the best of our knowledge, we found the first evidence of underrecognized association between total lipids in S.HDL.L and UC. The first evidence from Yao *et al.*'s studies revealed that TC is causally related to a decreased risk of UC.[14] Furthermore, Liu *et al.* demonstrated that persistent dyslipidemia is linked to adverse consequences among patients with UC.[28] The results of our study showed an increase in total lipids in S.HDL.L is associated with an increased risk of UC. Although this is not in line with the effect direction of TC, previous studies demonstrate how different components of a single pathway can oppositely affect the overall outcome.[29] High-density lipoprotein (HDL) subtypes vary in their roles in lipid metabolism, cholesterol transport, and inflammation regulation. Small HDL (S-HDL) has a high capacity for adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) mediated cholesterol efflux. Cholesterol efflux is the first step of randomized clinical trials (RCT), a process mediated by this particle that accepts excess cholesterol from peripheral tissues.[30,31] Changes in cholesterol levels resulting from the elevation of S-HDL in the body due to excessive ATP consumption for cholesterol synthesis, alterations in the intestinal microbiota influenced by bile acids, and disturbances in the immune response caused by shifts in steroid hormone metabolism can contribute to the pathology of UC.[32,33] A very detailed explanation of the intermediate mechanisms involved in these relationships requires more knowledge of these metabolites. Although the statistical significance of our study's results is marginal, it seems promising to re-examine the precise direction and size of our estimated effect using larger sample sizes within other study populations. If the significance level of the obtained results in future studies is lower than the Bonferroni-corrected threshold, these metabolites could be considered as a therapeutic goal for UC. Considering targeted interventions in clinical practice may lead to improved diagnosis and management strategies for UC, ultimately enhancing patient outcomes.[14] The current study leverages the most advanced and comprehensive GWAS datasets on metabolic profile and UC to provide novel evidence into the potential of blood metabolite traits as biomarkers for screening and prevention of UC. While larger sample sizes are necessary to establish causality, our findings highlight the well-established relationship between blood metabolite traits and health outcomes. The application of this result may offer a cost-effective, accessible, and minimally invasive treatment approach in UC patient monitoring and management. Notably, when UC was used as an exposure in a 2SMR analysis (reverse causality), we did not observe significant associations. Therefore, it can be concluded that the causal effect of metabolites on the development of UC is significant. This study has some limitations. First, the association between metabolic profiles and UC subtypes could not be evaluated because this data was not available. Second, our study identified a suggestive association between total lipids in S.HDL. L and UC ($P = 0.051$), which requires further evaluation. Third, the generalizability of the results from our study may be limited due to the exclusive use of a population of European ancestry. One of the reasons for using only these datasets is the availability of their genetic data. Fourth, removing metabolites that had no associated SNPs may result in missing metabolites that are related to UC independently of SNPs. As a result, false-negative results may have been obtained in our study. To confirm these findings, larger sample sizes are essential to validate and generalize our results, thereby strengthening the overall scientific evidence.

Conclusions

In the present study, we investigated the causal association between blood metabolic profile and UC using forward and reverse MR analysis. Our results did not demonstrate

a causal relationship except for total lipids in S.HDL.L, which was found to be suggestively related to UC risk. However, due to the significance level being far from the Bonferroni-corrected threshold, we cannot draw any definitive conclusions about its effect on UC development. Confirmation of this result requires using larger datasets and will provide new insights into therapeutic strategies for the screening and prevention of UC, as well as future drug target selection and mechanisms exploration.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: The characteristics of single nucleotide polymorphisms and their genetic associations with total lipids in small high density lipoprotein levels and ulcerative colitis

Exposure	SNP	Outcome	Effect_ allele	Other_ allele	Beta. exposure	Beta. outcome	EAF. exposure	EAF. outcome	Palindromic
Total lipids in small HDL	rs10468017	Ulcerative colitis	T	C	−0.101011	−0.0138	0.313616	0.33320	False
Total lipids in small HDL	rs1260326	Ulcerative colitis	C	T	−0.078519	−0.0122	0.636562	0.64940	False
Total lipids in small HDL	rs144064722	Ulcerative colitis	G	A	0.210227	0.0847	0.025283	0.02929	False
Total lipids in small HDL	rs6073958	Ulcerative colitis	C	T	0.200125	0.0394	0.195862	0.17650	False
Total lipids in small HDL	rs633695	Ulcerative colitis	G	A	−0.079449	0.0100	0.293077	0.30520	False
Total lipids in small HDL	rs73424577	Ulcerative colitis	G	A	−0.165215	0.0363	0.036006	0.03674	False
Total lipids in small HDL	rs7412	Ulcerative colitis	T	C	−0.191894	−0.0450	0.055504	0.05263	False

Exposure	Ambiguous	*P*.outcome	SE.outcome	Sample size. outcome	SE.exposure	*P*.exposure	Sample size. exposure	mr_ keep	*F*
Total lipids in small HDL	False	0.5183	0.0213	417,932	0.011172	2.58e-19	24,925	True	81.74769
Total lipids in small HDL	False	0.5501	0.0204	417,932	0.010609	1.91e-13	24,925	True	54.77730
Total lipids in small HDL	False	0.1736	0.0622	417,932	0.034013	8.15e-10	24,925	True	38.20209
Total lipids in small HDL	False	0.1179	0.0252	417,932	0.012833	3.36e-54	24,925	True	243.19033
Total lipids in small HDL	False	0.6442	0.0217	417,932	0.011355	3.57e-12	24,925	True	48.95562
Total lipids in small HDL	False	0.5166	0.0560	417,932	0.029238	1.96e-08	24,925	True	31.93035
Total lipids in small HDL	False	0.2512	0.0392	417,932	0.026087	2.68e-13	24,925	True	54.10962

SE – Standard error; HDL – High-density lipoprotein; SNP – Single nucleotide polymorphism; EAF – Effect allele frequency

Supplementary Table 2: The results of a leave-one-out analysis with total lipids in small high-density lipoprotein levels and ulcerative colitis

Exposure	Outcome	ID.exposure	ID.outcome	SNP	Beta	SE	*P*
Exposure	Outcome	qlX8lh	Kn6cty	rs10468017	0.1576385	0.0859463	0.066
Exposure	Outcome	qlX8lh	Kn6cty	rs1260326	0.1545683	0.08360898	0.064
Exposure	Outcome	qlX8lh	Kn6cty	rs144064722	0.1352789	0.08263525	0.101
Exposure	Outcome	qlX8lh	Kn6cty	rs6073958	0.1265481	0.10270615	0.217
Exposure	Outcome	qlX8lh	Kn6cty	rs633695	0.1806729	0.08319999	0.059
Exposure	Outcome	qlX8lh	Kn6cty	rs73424577	0.176489	0.08187854	0.061
Exposure	Outcome	qlX8lh	Kn6cty	rs7412	0.1403522	0.08641809	0.104
Exposure	Outcome	qlX8lh	Kn6cty	All	0.1546441	0.07958932	0.051

SNP – Single nucleotide polymorphism; SE – Standard error