Dissecting the Impact of Appendectomy on Colorectal Cancer through Metabolites and Inflammatory Proteins: A Mendelian Randomization Study

Keywords
mendelian randomization, appendectomy□, colorectal cancer□, metabolites□, inflammatory proteins□

Abstract

Introduction
Traditional longitudinal epidemiological investigations have demonstrated an association between appendectomy and the cumulative incidence of Colorectal cancer(CRC). However, there is a paucity of evidence for a causal linkage between Appendectomy and CRC from alternative methodologies.

Material and methods
We extracted summary statistics for CRC, appendectomy, 1,400 metabolites, and 91 inflammatory proteins from the largest European ancestry genome-wide association studies (GWAS) and the FinnGen. Using two-sample bidirectional Mendelian randomization (MR), we explored the causal relationship between appendectomy and CRC. Moreover, we employed a two-step MR approach to identify metabolites and inflammatory proteins that mediatie the effects of appendectomy on CRC risk and analyzed their potential mediator variables. Furthermore, we utilized the significant single nucleotide polymorphisms (SNPs) associated with the risk of CRC due to appendectomy, searched for the nearest genes in the FinnGen, and explored the relationship between these genes and the prognosis of CRC patients by analyzing transcriptomic data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO).

Results
Two-sample MR analysis revealed a significant causal effect of appendectomy on the increased risk of CRC. Two-step MR analysis identified carnitine and interleukin-13 (IL-13) as key mediators in the positive causal relationship between appendectomy and CRC. Survival analysis showed that the upregulation of HLX and OSR1 expression is positively correlated with poor prognosis in CRC patients.

Conclusions
This MR investigation substantiates the causal impact of appendectomy on colorectal cancer, with carnitine and IL-13 acting as partial mediators of this effect.

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Conclusion: This MR investigation substantiates the causal impact of appendectomy on colorectal cancer, with carnitine and IL-13 acting as partial mediators of this effect.

Keywords： Appendectomy， Colorectal cancer， Metabolites， Inflammatory proteins， Mendelian randomization

Introduciton

Colorectal cancer (CRC) is one of the most common malignancies in the digestive system. According to global cancer statistics, CRC is the third most common cancer and the second leading cause of cancer-related deaths, with over 1.9 million new cases and approximately 935,000 deaths reported in 2020[1]. Although the efficacy of CRC treatment has improved to some extent with surgery-based comprehensive treatment, the overall efficacy for CRC patients has not fundamentally improved. The treatment landscape for colorectal cancer has shifted significantly in recent years. Initially, the focus was on radical tumor resection combined with postoperative adjuvant radiotherapy and chemotherapy. This has gradually evolved into a comprehensive treatment model that includes surgical resection following neoadjuvant therapy or conversion therapy. Moreover, there has been a transition from traditional systemic chemotherapy to precision therapy, utilizing biological targeted drugs or immunotherapy, based on

molecular biomarkers.). Furthermore, the emergence of multidisciplinary treatment models has significantly extended the survival of colorectal cancer patients and improved their quality of life[2].

Many factors influence the incidence of CRC and patient survival[3]. Research has shown that appendectomy can lead to gut microbiota dysbiosis and impaired gut barrier function, increasing the risk of CRC[4]. The appendix contains a high density of gut-associated lymphoid tissue, which plays an important role in gut immune regulation and microbial composition in synergy with the gut microbiome[5, 6]. The appendix is often considered a vestigial organ that can be removed without significant consequences. However, recent studies have found a rich presence of immune cells in the appendix, including various innate immune cells such as natural killer cells and intraepithelial CD8+ T cells[7]. Furthermore, studies have shown that patients who have undergone appendectomy have a significantly reduced severity of colitis, but transcriptomic analysis and animal experiments have suggested that the presence of the appendix may confer protective T cell immunity, potentially involving protection against tumor initiation rather than tumor progression[8]. Additionally, appendectomy has been closely associated with the occurrence of various diseases, including CRC, in the long term[9-12].

Mendelian randomization (MR) is a method that uses genetic variants, such as single nucleotide polymorphisms (SNPs), as instrumental variables (IVs) to assess causal relationships between exposures and outcomes[13]. Since associations between exposures and outcomes may be attributed to unmeasured confounders and reverse causality, using genetic determinants of exposure as IVs allows for the estimation of the causal component of observed associations. Additionally, IVs must satisfy three criteria: 1) associated with the exposure, 2) independent of confounders of the exposure-outcome association, and 3) independent of all unmeasured confounders of the exposure-outcome association[14]. The advantage of MR analysis lies in its ability to reduce the impact of confounding factors, providing more reliable evidence for etiological research of diseases. This method has been widely used to explore causal relationships between biological or medical variables[15-17]. However, the use of MR to study the causal relationship between appendectomy and CRC has yet to be explored.

In this study, we utilize data from genome-wide association studies (GWAS) and public datasets to explore the causal relationship between appendectomy and CRC risk using two-sample bidirectional MR. We employ two-step MR to identify metabolites and inflammatory proteins that may mediate the impact of appendectomy on CRC risk. Additionally, using SNPs significantly associated with CRC risk following appendectomy, we identify the five nearest genes from the FinnGen and employ transcriptomic analysis to further investigate the impact of these related genes on the survival prognosis of CRC patients.

Materials and methods

Study design

To comprehensively explore the causal relationship between appendectomy and CRC risk and potential mediating effects, we conducted an in-depth MR analysis. This approach utilized summary statistics from published GWAS. The MR framework employs SNPs as instrumental variables to rigorously quantify the

causal influence between exposure and outcome. The MR design adheres to three fundamental assumptions: 1) Relevance assumption: SNPs are closely associated with the exposure; 2) Independence assumption: SNPs are uncorrelated with confounding factors; 3) Exclusion restriction assumption: SNPs influence the outcome solely through the exposure. The second and third assumptions are collectively known as pleiotropy and independence, respectively, and can be tested using a series of statistical methods. Our study design is summarized in the figure below(Fig.1).

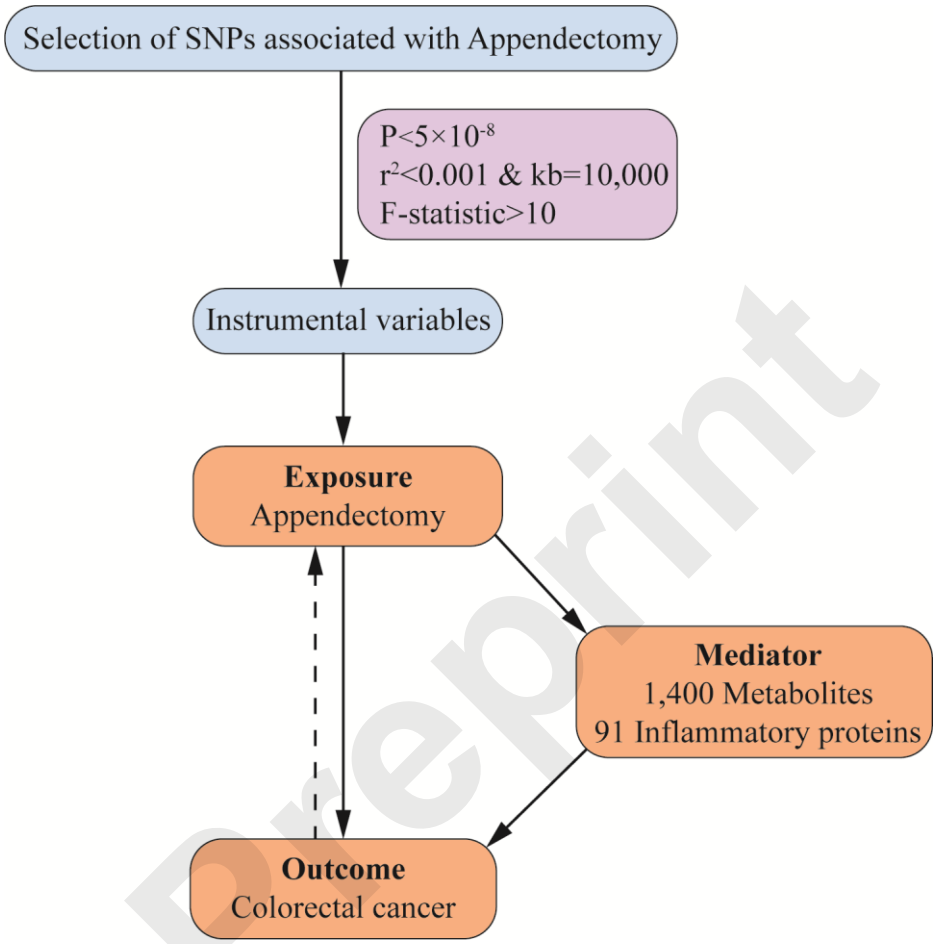

Fig.1 Study design of bidirectional and mediated Mendelian randomization analyses. *SNP*, single nucleotide polymorphism.

Data source

In this study, summary data on appendectomy were obtained from the FinnGen database (https://r10.finngen.fi/pheno/K11_APPENDECTOMY), included 28,601 cases and 383,580 controls. The summary data for CRC were obtained from the study by Huyghe JR et al.[18], which included 19,948 CRC patients, 12,124 healthy individuals, and 38,356,021 SNPs. These data are accessible at (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST012879/). The summary data for 1,400 metabolites were sourced from the study by Chen et al[19]. The data can be obtained from the GWAS Catalog database (GWAS Catalog) with catalog numbers GCST90199621-GCST90201020. The summary data for 91 inflammatory proteins were derived from the study by Zhao et al[20]. The GWAS Catalog catalog numbers

are GCST90274758-GCST90274848. Transcriptome sequencing data used to validate the nearest genes to SNPs and their association with colorectal cancer patient prognosis were obtained from the TCGA database (https://portal.gdc.cancer.gov/) and the GEO database (https://www.ncbi.nlm.nih.gov/geo/) with data summarized by Marisa et al[21].

Instrumental variable selection

In our MR analysis, we used SNPs as IVs to study the causal relationship between exposure and outcome at the genetic level. Significant SNPs for appendectomy, CRC, 1400 metabolites, and 91 inflammatory proteins were selected using a uniform criterion of $p < 5.0 \times 10^{-8}$. However, due to the limited availability of genome-wide significant SNPs for metabolites, we adopted a more lenient threshold for metabolites in the MR analysis, specifically $p < 1 \times 10^{-5}$[22]. This adjustment allowed us to obtain a broader range of SNPs associated with metabolites and CRC.

Simultaneously, we used a threshold of $r^2 < 0.001$ and kb = 10,000 to eliminate SNPs exhibiting linkage disequilibrium (LD) within a 10Mb region with the most significant SNPs ($r^2 > 0.001$)[23]. This strategy allowed us to exclude SNPs prone to LD effects. Additionally, to minimize bias from weak instruments, we calculated the F-statistic for each SNP. SNPs with F-statistic < 10 were considered weak instruments[24]. SNPs displaying palindromic or ambiguous characteristics were systematically excluded from the IVs used in the MR analysis[25].

MR analysis

We conducted a comprehensive two-sample bidirectional MR analysis using inverse variance weighted (IVW), MR-Egger, weighted median, weighted mode, and simple mode methods to explore the causal relationship between appendectomy and the outcome of CRC. IVW is one of the primary methods in MR studies, aggregating the Wald ratios of each SNP for a summary estimate[26]. The IVW method assumes that all SNPs are valid IVs. Due to its high statistical power and widespread use in MR analyses, the IVW method was chosen as the main analytical approach, with the other four methods serving as supplementary analyses[27, 28]. When the direction of the causal relationship remains consistent across these five methods, it is considered to be a relatively stable causal relationship. According to the results of the MR analysis, a p-value <0.05 indicates that there is a causal effect between the exposure and the outcome[29]. To assess the robustness and validity of the results, we conducted sensitivity analyses using Cochran's Q statistic to evaluate heterogeneity among the IVs, and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) to test for pleiotropy, with p-value <0.05 indicating significant heterogeneity and pleiotropy[30, 31]. The MR-PRESSO method can not only determine the degree of horizontal pleiotropy but also identify and exclude outlier genetic variants and estimate corrected results. Additionally, it evaluates whether there is a difference between the results before and after correction[32]. The "leave-one-out" approach is used to detect any instrumental variables that may influence the causal effect estimate, which involves sequentially removing individual SNPs and calculating the meta-effect of the remaining SNPs. We

observe whether the results change after removing each SNP to determine if the outcome is influenced by any specific SNP[33]. Horizontal pleiotropy is assessed using the MR-Egger intercept, and if p-value >0.05, it is considered that there is no horizontal pleiotropy[34]. Furthermore, we use two-step MR analysis to identify the mediating variables of metabolites and inflammatory proteins that regulate the causal relationship between appendectomy and CRC. In the two-step MR analysis, we calculate the causal effect of appendectomy on the mediator and the causal effect of the mediator on CRC. We then use the delta method to estimate the significance of the mediating effect and the proportion of the mediating effect in the total effect[35].

Transcriptomic analysis

Based on the SNPs obtained from the MR analysis that show a causal effect of appendectomy on CRC, we further explore the impact of the nearest genes to these SNPs, identified using the FinnGen database, on the survival prognosis of CRC patients. Survival analysis was conducted using the "survival" package in R, based on transcriptomic data from TCGA and GEO (GSE39582). A p-value <0.05 indicated statistical significance in survival time.

Results

MR analysis reveals a positive causal relationship between Appendectomy and CRC

In the two-sample MR analysis, SNPs were used as IVs, with appendectomy as the exposure and CRC as the outcome. GWAS data for appendectomy were used as the summary association statistics for exposure, and GWAS data for colorectal cancer were used as the summary association statistics for the outcome. Using the extracted 6 SNPs for appendectomy that met the selection criteria, we found a positive causal relationship between appendectomy and the risk of CRC occurrence (Table 1). Since the IVW method has high statistical power, it was used as the main analysis method. The MR analysis estimated based on the IVW method showed a significant causal relationship between appendectomy and an increased risk of CRC (OR = 1.328, 95% CI: 1.073-1.643, P = 0.009). Although the results of MR-Egger (OR = 1.575, 95% CI: 0.782-3.171, P = 0.272), simple mode (OR = 1.365, 95% CI: 0.957-1.949, P = 0.146), and weighted mode (OR = 1.341, 95% CI: 0.972-1.849, P = 0.133) did not reach statistical significance, as shown in the scatter plot and leave-one-out plot(Fig.2), the OR values of the other four methods are consistent with the direction of IVW, and no SNPs violate the general effect of appendectomy on the incidence of CRC. Furthermore, heterogeneity assessment shows that there is almost no evidence of heterogeneity in the association ($P_{heterogeneity}$ = 0.862). Additionally, two tests for horizontal pleiotropy indicate that there is little evidence of horizontal pleiotropy in this association ($P_{pleiotropy}$ = 0.642, P_{presso} = 0.909). The reverse MR analysis (Supplementary Table 1) showed that there is no significant causal relationship between genetic susceptibility to CRC and appendectomy (OR = 0.957, 95% CI: 0.898-1.020, P_{IVW} = 0.179). This suggests that the results of the MR analysis are robust.

To conclude, our study results indicate a positive causal relationship between appendectomy and the risk of CRC, suggesting that appendectomy is indeed a risk factor for the development of CRC.

Table 1 Six genome-wide significantly related SNPs were used as Ivs to study the causal relationship between Appendectomy and Colorectal cancer.

SNP	Gene	EA	OA	EAF	Beta.exposure	P.exposure	Beta.outcome	P.outcome	F
rs11931959	PITX2	G	A	0.307581	0.112966	5.01E-34	0.0320035	0.0855185	148
rs2044674	PITX2	T	C	0.389854	0.055885	3.40E-10	0.0139629	0.455235	39
rs2484697	HLX	G	A	0.497524	0.0558568	1.43E-10	0.0187648	0.273563	41
rs2584229	NR2F2	C	T	0.17521	-0.0734796	2.40E-10	-0.0362832	0.112237	40
rs3905887	C4orf32	T	C	0.646271	0.0498745	4.91E-08	-0.00684343	0.704644	30
rs6707044	OSR1	G	A	0.217896	0.0725584	3.00E-12	0.0260994	0.247414	49

**SNP* single nucleotide polymorphism, *EA* effect allele, *OA* other allele, *EAF* effect allele frequency, *F* F-statistic.

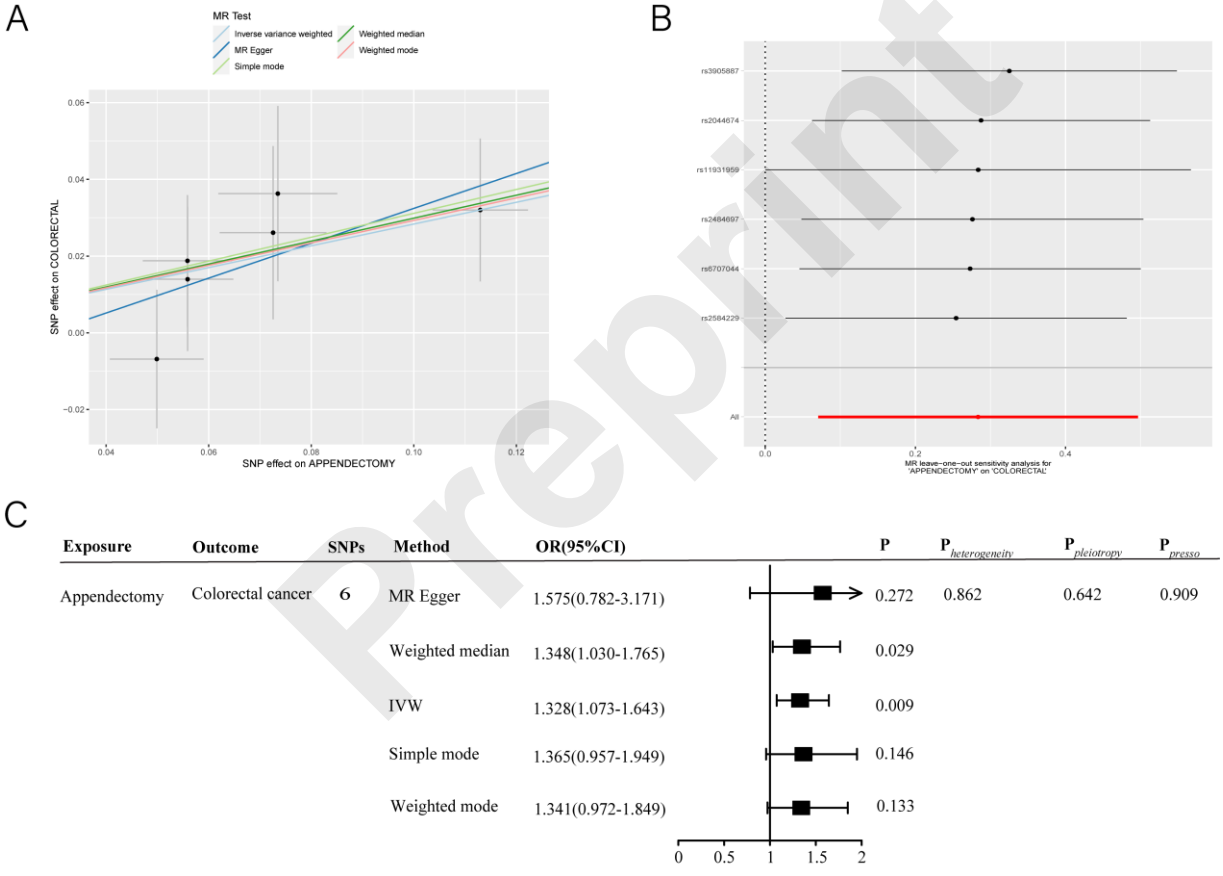

Fig.2 Mendelian randomization analyses show causal effects between Appendectomy and Colorectal cancer. (A) Scatter plots and causal estimation for five different methods. (B) Leave-one-out plot of SNPs associated with Appendectomy and Colorectal cancer. (C) MR analysis results of Appendectomy and Colorectal cancer based on IVW method. *SNP* single nucleotide polymorphism, *IVW* inverse variance weight, *OR* odds ratio, *CI* confidence interval.

Mediation analyses of potential metabolites and inflammatory proteins

To explore the potential mechanisms of the causal influence of appendectomy on CRC, we conducted two-step MR analyses with 1,400 metabolites and 91 inflammatory proteins as mediator variables. Firstly, in the MR analysis of 1,400 metabolites on CRC risk, based on $P_{IVW} < 0.05$, consistent OR values across five methods, and satisfying the requirements of the MR-PRESSO method[32] (excluding metabolites with less than 3 SNPs), we identified 82 metabolites associated with CRC risk (Supplementary Table 2, Supplementary Table 3). Some evidence of heterogeneity in SNP effects was observed (Supplementary Table 4), but the "leave-out-one" analysis did not indicate that the causal estimate was driven by specific SNPs (Supplementary Fig.1). Next, the MR-PRESSO method was used to test for horizontal pleiotropy in the 82 metabolites, and 14 metabolites were found to exhibit horizontal pleiotropy ($P_{pleiotropy} < 0.05$) (Supplementary Table 3). After re-analyzing the metabolites with horizontal pleiotropy using MR-PRESSO to exclude outlier SNPs and estimating the corrected results with MR analysis, 5 metabolites had $P_{IVW} < 0.05$ and consistent OR values across all testing methods. In summary, 73 metabolites were identified as having a causal effect on CRC risk (Fig. 3). Subsequently, MR analysis was performed between appendectomy and the 73 metabolites, and the results showed that carnitine levels (GCST90199621) played a role in the causal effect of appendectomy on CRC, with a mediated effect proportion of 4.47% (95% CI: -9.73%-18.7%).

Similarly, using the same method, we analyzed the mediating role of 91 inflammatory proteins in the causal effect between appendectomy and CRC risk. We identified 18 inflammatory proteins associated with CRC risk (Supplementary Table 5, Supplementary Table 6), with some SNPs showing heterogeneity (Supplementary Table 7). However, the leave-one-out analysis indicated that no single SNP drove the causal estimate (Supplementary Fig.2). The MR-PRESSO test for horizontal pleiotropy identified 3 inflammatory proteins with horizontal pleiotropy ($P_{pleiotropy} < 0.001$) (Supplementary Table 6). After re-analyzing these 3 proteins with MR-PRESSO to exclude outlier SNPs and estimating the corrected results with MR analysis, 1 inflammatory protein had $P_{IVW} < 0.05$ with consistent OR values across all testing methods. Therefore, we identified 16 inflammatory proteins associated with CRC risk (Fig. 4). Performing MR analysis with these 16 inflammatory proteins as outcomes and appendectomy as the exposure, we found that Interleukin-13 (IL-13,GCST90274799) played a mediating role in the causal effect of appendectomy on CRC, with a mediated effect proportion of 12.8% (95% CI: -2.76%-28.4%).

Taken together, our study results suggest that changes in the levels of carnitine and IL-13 play a mediating role in the causal effect of appendectomy on the risk of colorectal cancer.

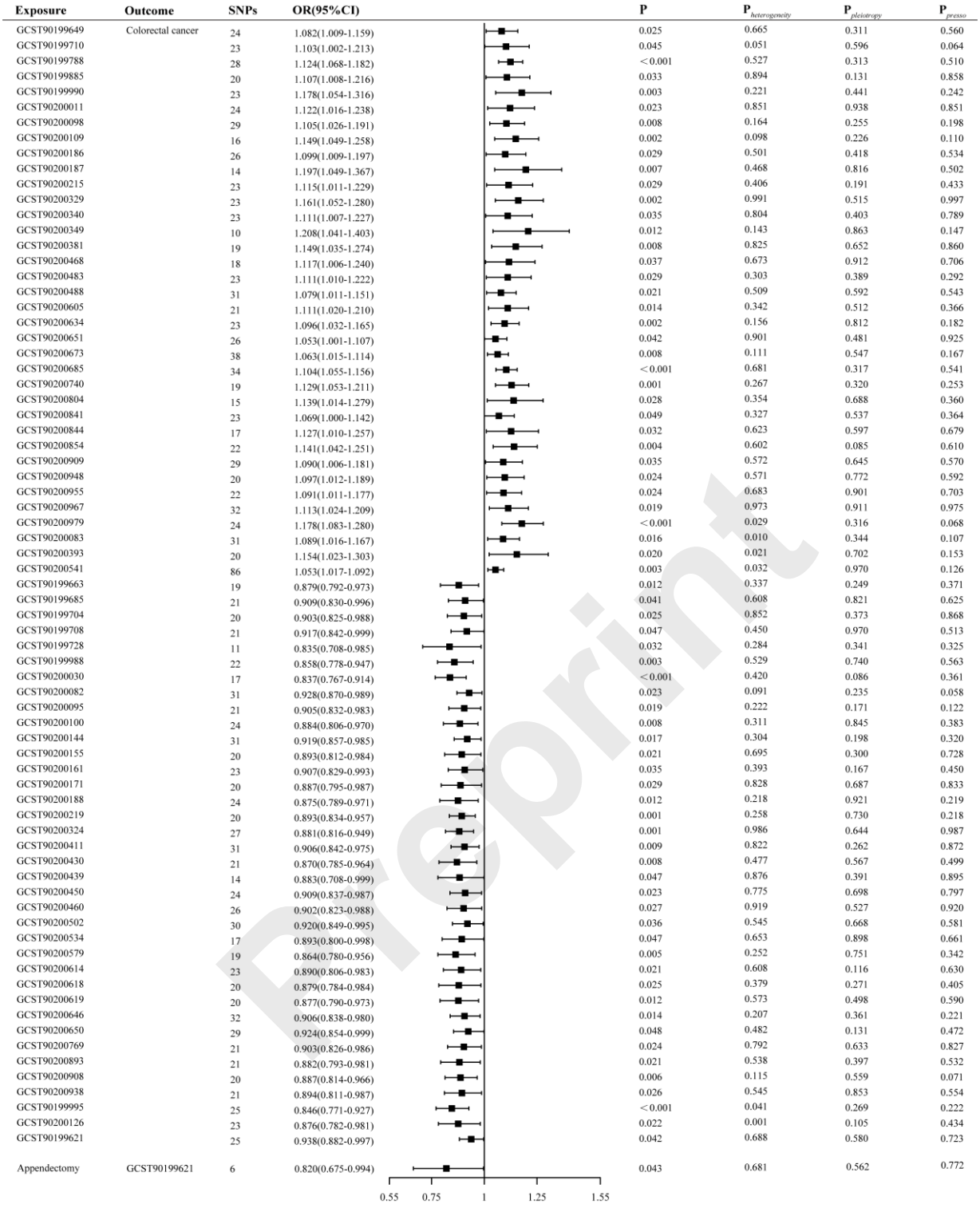

Fig.3 Two-step Mendelian randomization analysis revealed the causal relationship between metabolites and Colorectal cancer, Appendectomy and GCST90199621**.** *SNP* single nucleotide polymorphism, *IVW* inverse variance weight, *OR* odds ratio, *CI* confidence interval.

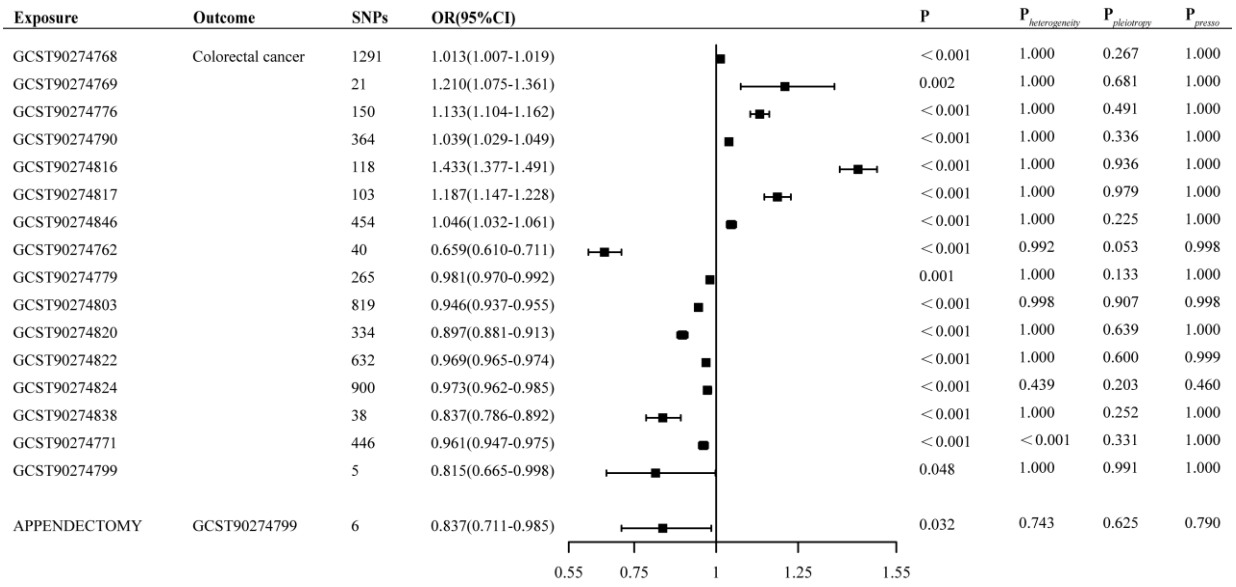

Fig.4 Two-step Mendelian randomization analysis revealed the causal relationship between inflammatory proteins and Colorectal cancer, Appendectomy and GCST90274799**.** *SNP* single nucleotide polymorphism, *IVW* inverse variance weight, *OR* odds ratio, *CI* confidence interval.

Appendectomy may influence the risk of CRC through HLX, OSR1, PITX2, C4orf32, and NR2F2

To explore whether appendectomy affects CRC risk at the genetic level, we identified the nearest genes to 6 significant SNPs showing a causal effect between appendectomy and CRC risk using the FinnGen database. The genes identified were HLX, OSR1, PITX2, C4orf32, and NR2F2, suggesting that appendectomy may influence CRC disease risk through these five genes.

To further investigate the role of HLX, OSR1, PITX2, C4orf32, and NR2F2 in CRC, we conducted survival analyses using transcriptomic sequencing data from CRC patients in the TCGA and GEO databases. The results showed that high expression of HLX and OSR1 was positively correlated with poor prognosis in CRC patients in both TCGA and GSE39582 datasets(Supplementary Fig.3). In summary, we believe that appendectomy may influence the prognosis of CRC patients by affecting the expression levels of HLX and OSR1.

Discussion

In this MR study, we explored the causal relationship between appendectomy and the risk of developing CRC. By using genetic variations as IVs for appendectomy, our analysis revealed a positive causal relationship between appendectomy and the risk of CRC. Additionally, we found that metabolites and inflammatory proteins played a mediating role in this relationship.

Appendectomy, a common surgical procedure, is primarily used to treat acute appendiciti[36]. The appendix is rich in lymphoid tissue and is a major producer of IgA in the large intestine[37, 38]. Appendectomy may impair gut immunity, leading to decreased surveillance and reduced ability to identify and kill tumor cells. The appendix's rich biofilm and secluded anatomical location make it a safe house for

gut microbiota; its removal inevitably alters its composition[39, 40], which may contribute to the development of colorectal cancer. Past studies have suggested a possible link between appendectomy and an increased risk of colorectal cancer[4], but the exact mechanisms of this relationship remain unclear. Our Mendelian randomization study provides further evidence supporting a positive causal relationship between appendectomy and an increased risk of colorectal cancer.

MR is an analytical method used to assess the causal relationship between variable exposures and clinically relevant outcomes[41]. As an emerging epidemiological method for causal research, MR is a powerful tool that complements traditional epidemiological studies by identifying risk factors for diseases[42]. In our study, genetic variations were used as instrumental variables for appendectomy, which helps to reduce the impact of confounding factors and provide more reliable causal inferences. Through this approach, we found a significant positive causal relationship between appendectomy and the risk of CRC. This finding is consistent with some previous epidemiological studies that also reported an association between appendectomy and an increased risk of CRC[43-47].

Furthermore, our study revealed the mediating role of metabolites and inflammatory proteins in the causal relationship between appendectomy and CRC risk. This finding suggests that appendectomy may increase the risk of CRC by affecting metabolic pathways and inflammatory responses. Specifically, appendectomy may lead to changes in the levels of the metabolite carnitine, which could trigger the onset of CRC. Additionally, appendectomy may cause alterations in the expression of the inflammatory protein IL-13, thereby promoting the progression of CRC.

Emerging evidence reveals a sophisticated immunomodulatory network involving vitamin D homeostasis, Th17 cell dynamics, the IL-31/IL-33 signaling axis, and gut microbial ecology in governing immune-mediated inflammatory responses. Th17 cells—a proinflammatory CD4+ T-cell subset—are pivotal drivers of chronic inflammatory microenvironment[48]. The alarmin cytokine IL-33 mediates dual activation of innate and adaptive immunity through ST2 receptor signaling, inducing Th2-polarized cytokine production (IL-5, IL-13)[49]. Murdaca et al. demonstrated that vitamin D insufficiency correlates with IL-31/IL-33 axis hyperactivation, disrupting the Th1/Th17–Treg equilibrium and facilitating bacterial translocation, thereby exacerbating intestinal immune dysfunction[50, 51]. Conversely, vitamin D sufficiency confers a significant protective effect against colorectal carcinogenesis versus placebo in clinical studies[52]. The gut microbiome’s compositional diversity is fundamental to immune homeostasis, aligning with the hygiene hypothesis. Physiological microbial colonization orchestrates the differentiation of Th1/Th2/Th17/Treg lineages, while commensal-derived metabolites expand peripheral Treg populations to maintain mucosal immune tolerance[53]. We propose that appendectomy-induced immunological perturbations—particularly the depletion of microbial reservoirs and aberrant IL-33/ST2 signaling—may disrupt this equilibrium. Surgical removal of the appendix could potentiate excessive release of mucosal alarmins, upregulating protumorigenic cytokines, and dysbiosis-mediated carnitine metabolic dysfunction, collectively fostering colorectal carcinogenesis.

Despite our study providing evidence for a causal relationship between appendectomy and an increased risk of CRC, there are some limitations to consider. First, MR study rely on genetic variations as IVs, so their results may be affected by genetic heterogeneity. Second, our analysis is based on specific genetic variations and may not cover all relevant biological pathways. Therefore, future research should aim to further explore the mechanisms of this causal relationship and validate our findings, considering more genetic variations and biomarkers, as well as factors such as individuals' genetic backgrounds and lifestyles, to better reveal the complex interactions between appendectomy and CRC.

In conclusion, our study revealed a positive association between appendectomy and CRC risk, as well as the potential mediating effects of metabolites and inflammatory proteins, providing important guidance for future research and clinical practice. These findings may help improve prevention strategies and risk assessment methods for CRC, thereby reducing its incidence and related mortality.

Declarations

Ethics approval and consent to participate

Ethical approval and participant consent were obtained in the original studies. Patients or the public were not involved in the design, or conduct, or re-porting, or dissemination plans of our research.

Availability of data and materials

All data and materials associated with this study are available within the paper.

Competing interests

All authors declare no conflicts of interest.

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Authors' contributions

QM, LML, LCYmade substantial contributions to conception and design, or analysis and interpretation of data; QM, LML carried out Article modifcation; QM, LML participated in the drafted the manuscript; LCY, XZY, WHT, WZZ, WJY participated in the acquisition of data and performed the statistical analysis. All authors read and approved the fnal manuscript.

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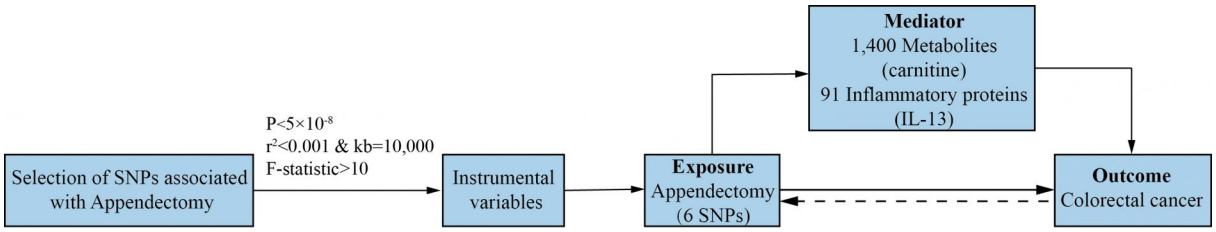
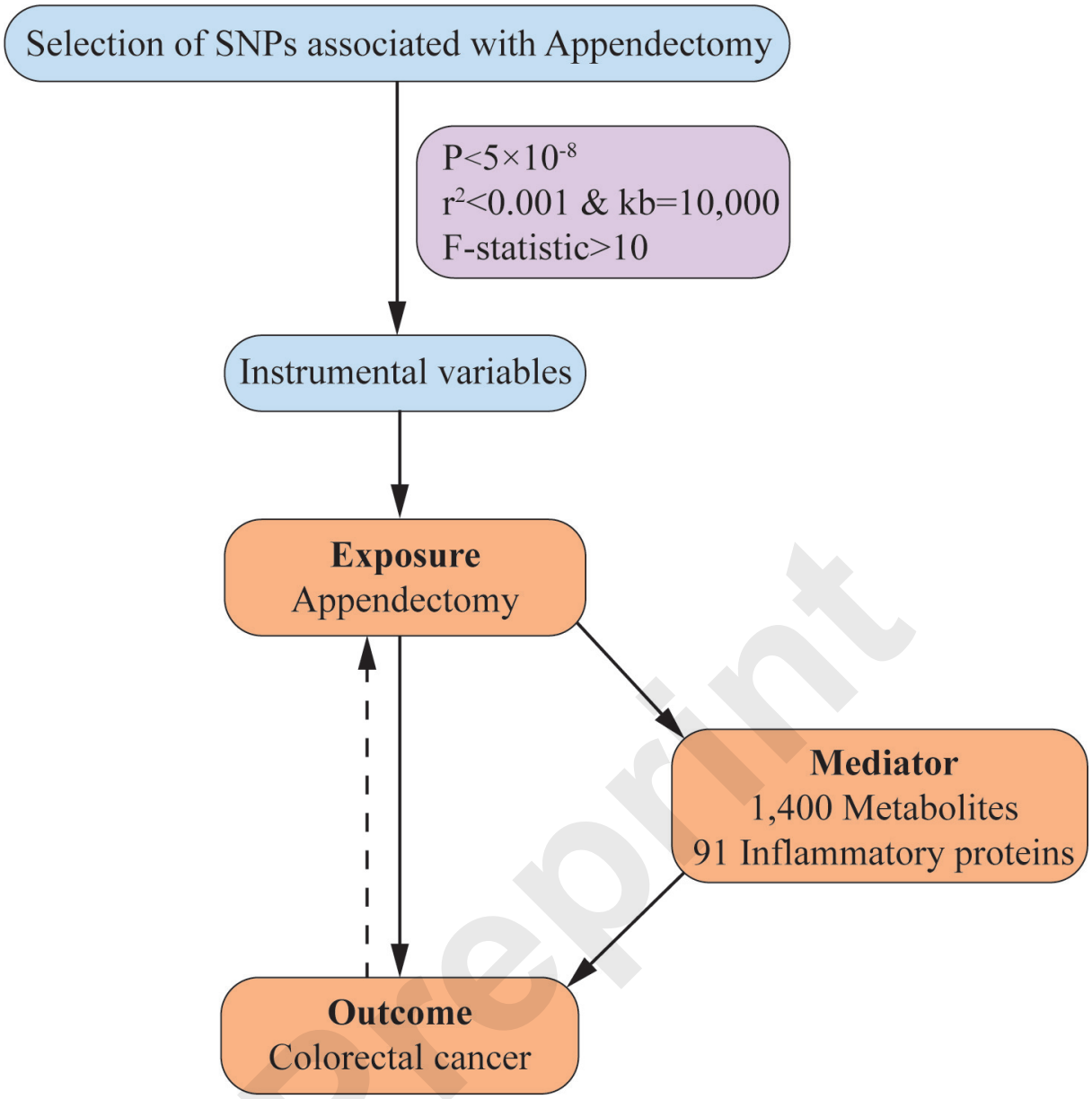

Mediator
1,400 Metabolites
(carnitine)
91 Inflammatory proteins
(IL-13)
P<5×10-8
r2<0.001 & kb=10,000
F-statistic>10
Selection of SNPs associated with Appendectomy
Instrumental variables
Exposure
Appendectomy
(6 SNPs)
Outcome
Colorectal cancer

Table 1 Six genome-wide significantly related SNPs were used as Ivs to study the causa
and CRC.

SNP	Gene	EA	OA	EAF	Beta.exposure	P.exposure
rs11931959	PITX2	G	A	0,307581	0,112966	5.01E-34
rs2044674	PITX2	T	C	0,389854	0,055885	3.40E- 10
rs2484697	HLX	G	A	0,497524	0,0558568	1.43E- 10
rs2584229	NR2F2	C	T	0,17521	-0,0734796	2.40E- 10
rs3905887	C4orf32	T	C	0,646271	0,0498745	4.91E-08
rs6707044	OSR1	G	A	0,217896	0,0725584	3.00E- 12

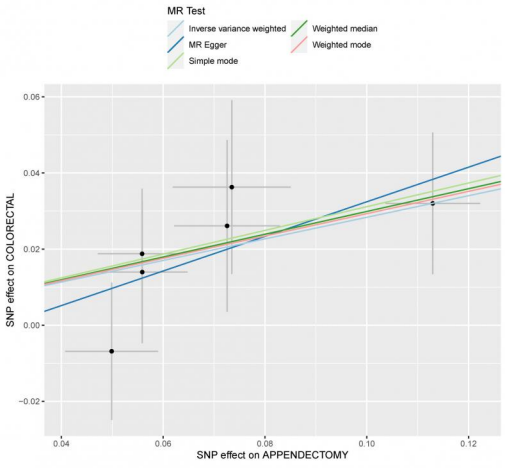
al relationship between Appendectomy

Beta.outcome	P.outcome	F
0,0320035	0,0855185	148
0,0139629	0,455235	39
0,0187648	0,273563	41
-0,0362832	0,112237	40
-0,00684343	0,704644	30
0,0260994	0,247414	49

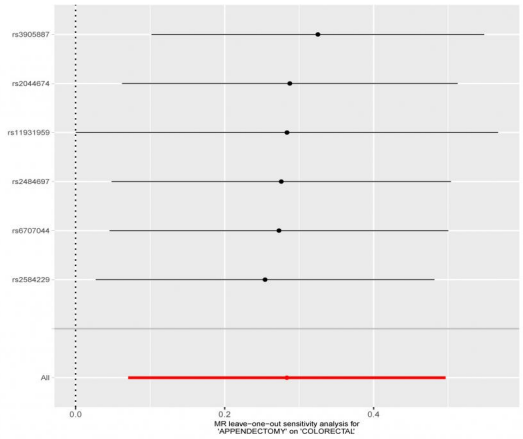


Study design of bidirectional and mediated Mendelian randomization analyses. SNP, single nucleotide polymorphism.

A



B

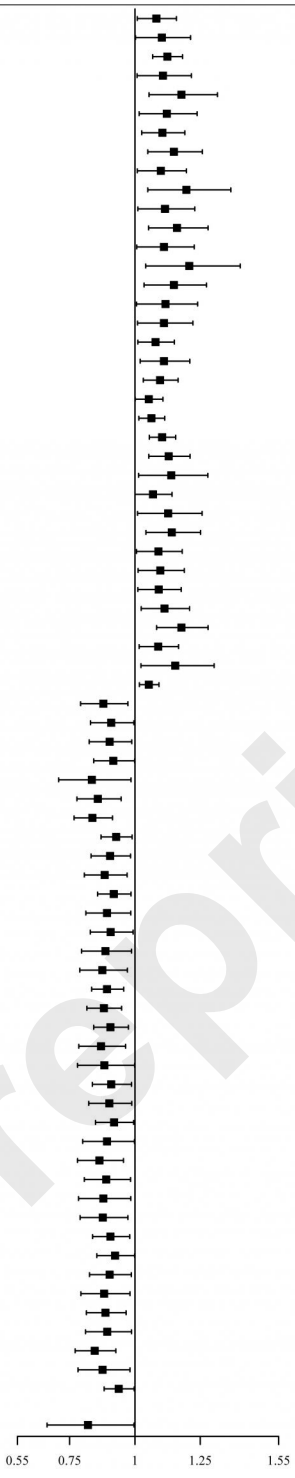


C

Exposure	Outcome	SNPs	Method	OR(95%CI)	P	$P_{heterogeneity}$	$P_{pleiotropy}$	P_{presso}
Appendectomy	Colorectal cancer	6	MR Egger	1.575(0.782-3.171)	0.272	0.862	0.642	0.909
			Weighted median	1.348(1.030-1.765)	0.029			
			IVW	1.328(1.073-1.643)	0.009			
			Simple mode	1.365(0.957-1.949)	0.146			
			Weighted mode	1.341(0.972-1.849)	0.133			

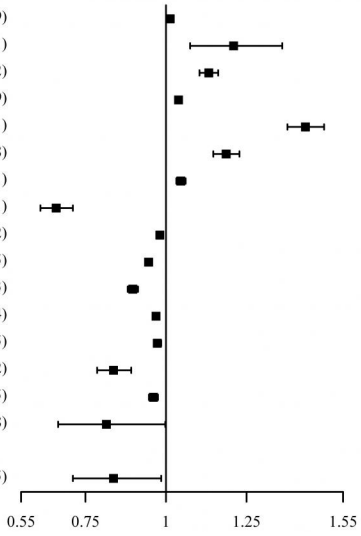
Mendelian randomization analyses show causal effects between Appendectomy and Colorectal cancer. (A) Scatter plots and causal estimation for five different methods. (B) Leave-one-out plot of SNPs associated with Appendectomy and Colorectal cancer. (C) MR analysis results of Appendectomy and Colorectal cancer based on IVW method. SNP single nucleotide polymorphism, IVW inverse variance weight, OR odds ratio, CI confidence interval.

Exposure	Outcome	SNPs	OR(95%CI)	P	$P_{heterogeneity}$	$P_{pleiotropy}$	P_{presso}
GCST90199649	Colorectal cancer	24	1.082(1.009-1.159)	0.025	0.665	0.311	0.560
GCST90199710		23	1.103(1.002-1.213)	0.045	0.051	0.596	0.064
GCST90199788		28	1.124(1.068-1.182)	< 0.001	0.527	0.313	0.510
GCST90199885		20	1.107(1.008-1.216)	0.033	0.894	0.131	0.858
GCST90199990		23	1.178(1.054-1.316)	0.003	0.221	0.441	0.242
GCST90200011		24	1.122(1.016-1.238)	0.023	0.851	0.938	0.851
GCST90200098		29	1.105(1.026-1.191)	0.008	0.164	0.255	0.198
GCST90200109		16	1.149(1.049-1.258)	0.002	0.098	0.226	0.110
GCST90200186		26	1.099(1.009-1.197)	0.029	0.501	0.418	0.534
GCST90200187		14	1.197(1.049-1.367)	0.007	0.468	0.816	0.502
GCST90200215		23	1.115(1.011-1.229)	0.029	0.406	0.191	0.433
GCST90200329		23	1.161(1.052-1.280)	0.002	0.991	0.515	0.997
GCST90200340		23	1.111(1.007-1.227)	0.035	0.804	0.403	0.789
GCST90200349		10	1.208(1.041-1.403)	0.012	0.143	0.863	0.147
GCST90200381		19	1.149(1.035-1.274)	0.008	0.825	0.652	0.860
GCST90200468		18	1.117(1.006-1.240)	0.037	0.673	0.912	0.706
GCST90200483		23	1.111(1.010-1.222)	0.029	0.303	0.389	0.292
GCST90200488		31	1.079(1.011-1.151)	0.021	0.509	0.592	0.543
GCST90200605		21	1.111(1.020-1.210)	0.014	0.342	0.512	0.366
GCST90200634		23	1.096(1.032-1.165)	0.002	0.156	0.812	0.182
GCST90200651		26	1.053(1.001-1.107)	0.042	0.901	0.481	0.925
GCST90200673		38	1.063(1.015-1.114)	0.008	0.111	0.547	0.167
GCST90200685		34	1.104(1.055-1.156)	< 0.001	0.681	0.317	0.541
GCST90200740		19	1.129(1.053-1.211)	0.001	0.267	0.320	0.253
GCST90200804		15	1.139(1.014-1.279)	0.028	0.354	0.688	0.360
GCST90200841		23	1.069(1.000-1.142)	0.049	0.327	0.537	0.364
GCST90200844		17	1.127(1.010-1.257)	0.032	0.623	0.597	0.679
GCST90200854		22	1.141(1.042-1.251)	0.004	0.602	0.085	0.610
GCST90200909		29	1.090(1.006-1.181)	0.035	0.572	0.645	0.570
GCST90200948		20	1.097(1.012-1.189)	0.024	0.571	0.772	0.592
GCST90200955		22	1.091(1.011-1.177)	0.024	0.683	0.901	0.703
GCST90200967		32	1.113(1.024-1.209)	0.019	0.973	0.911	0.975
GCST90200979		24	1.178(1.083-1.280)	< 0.001	0.029	0.316	0.068
GCST90200083		31	1.089(1.016-1.167)	0.016	0.010	0.344	0.107
GCST90200393		20	1.154(1.023-1.303)	0.020	0.021	0.702	0.153
GCST90200541		86	1.053(1.017-1.092)	0.003	0.032	0.970	0.126
GCST90199663		19	0.879(0.792-0.973)	0.012	0.337	0.249	0.371
GCST90199685		21	0.909(0.830-0.996)	0.041	0.608	0.821	0.625
GCST90199704		20	0.903(0.825-0.988)	0.025	0.852	0.373	0.868
GCST90199708		21	0.917(0.842-0.999)	0.047	0.450	0.970	0.513
GCST90199728		11	0.835(0.708-0.985)	0.032	0.284	0.341	0.325
GCST90199988		22	0.858(0.778-0.947)	0.003	0.529	0.740	0.563
GCST90200030		17	0.837(0.767-0.914)	< 0.001	0.420	0.086	0.361
GCST90200082		31	0.928(0.870-0.989)	0.023	0.091	0.235	0.058
GCST90200095		21	0.905(0.832-0.983)	0.019	0.222	0.171	0.122
GCST90200100		24	0.884(0.806-0.970)	0.008	0.311	0.845	0.383
GCST90200144		31	0.919(0.857-0.985)	0.017	0.304	0.198	0.320
GCST90200155		20	0.893(0.812-0.984)	0.021	0.695	0.300	0.728
GCST90200161		23	0.907(0.829-0.993)	0.035	0.393	0.167	0.450
GCST90200171		20	0.887(0.795-0.987)	0.029	0.828	0.687	0.833
GCST90200188		24	0.875(0.789-0.971)	0.012	0.218	0.921	0.219
GCST90200219		20	0.893(0.834-0.957)	0.001	0.258	0.730	0.218
GCST90200324		27	0.881(0.816-0.949)	0.001	0.986	0.644	0.987
GCST90200411		31	0.906(0.842-0.975)	0.009	0.822	0.262	0.872
GCST90200430		21	0.870(0.785-0.964)	0.008	0.477	0.567	0.499
GCST90200439		14	0.883(0.708-0.999)	0.047	0.876	0.391	0.895
GCST90200450		24	0.909(0.837-0.987)	0.023	0.775	0.698	0.797
GCST90200460		26	0.902(0.823-0.988)	0.027	0.919	0.527	0.920
GCST90200502		30	0.920(0.849-0.995)	0.036	0.545	0.668	0.581
GCST90200534		17	0.893(0.800-0.998)	0.047	0.653	0.898	0.661
GCST90200579		19	0.864(0.780-0.956)	0.005	0.252	0.751	0.342
GCST90200614		23	0.890(0.806-0.983)	0.021	0.608	0.116	0.630
GCST90200618		20	0.879(0.784-0.984)	0.025	0.379	0.271	0.405
GCST90200619		20	0.877(0.790-0.973)	0.012	0.573	0.498	0.590
GCST90200646		32	0.906(0.838-0.980)	0.014	0.207	0.361	0.221
GCST90200650		29	0.924(0.854-0.999)	0.048	0.482	0.131	0.472
GCST90200769		21	0.903(0.826-0.986)	0.024	0.792	0.633	0.827
GCST90200893		21	0.882(0.793-0.981)	0.021	0.538	0.397	0.532
GCST90200908		20	0.887(0.814-0.966)	0.006	0.115	0.559	0.071
GCST90200938		21	0.894(0.811-0.987)	0.026	0.545	0.853	0.554
GCST90199995		25	0.846(0.771-0.927)	< 0.001	0.041	0.269	0.222
GCST90200126		23	0.876(0.782-0.981)	0.022	0.001	0.105	0.434
GCST90199621		25	0.938(0.882-0.997)	0.042	0.688	0.580	0.723
Appendectomy	GCST90199621	6	0.820(0.675-0.994)	0.043	0.681	0.562	0.772



Two-step Mendelian randomization analysis revealed the causal relationship between metabolites and Colorectal cancer, Appendectomy and GCST90199621. SNP single nucleotide polymorphism, IVW inverse variance weight, OR odds ratio, CI confidence interval.

Exposure	Outcome	SNPs	OR(95%CI)	P	$P_{heterogeneity}$	$P_{pleiotropy}$	P_{presso}
GCST90274768	Colorectal cancer	1291	1.013(1.007-1.019)	<0.001	1.000	0.267	1.000
GCST90274769		21	1.210(1.075-1.361)	0.002	1.000	0.681	1.000
GCST90274776		150	1.133(1.104-1.162)	<0.001	1.000	0.491	1.000
GCST90274790		364	1.039(1.029-1.049)	<0.001	1.000	0.336	1.000
GCST90274816		118	1.433(1.377-1.491)	<0.001	1.000	0.936	1.000
GCST90274817		103	1.187(1.147-1.228)	<0.001	1.000	0.979	1.000
GCST90274846		454	1.046(1.032-1.061)	<0.001	1.000	0.225	1.000
GCST90274762		40	0.659(0.610-0.711)	<0.001	0.992	0.053	0.998
GCST90274779		265	0.981(0.970-0.992)	0.001	1.000	0.133	1.000
GCST90274803		819	0.946(0.937-0.955)	<0.001	0.998	0.907	0.998
GCST90274820		334	0.897(0.881-0.913)	<0.001	1.000	0.639	1.000
GCST90274822		632	0.969(0.965-0.974)	<0.001	1.000	0.600	0.999
GCST90274824		900	0.973(0.962-0.985)	<0.001	0.439	0.203	0.460
GCST90274838		38	0.837(0.786-0.892)	<0.001	1.000	0.252	1.000
GCST90274771		446	0.961(0.947-0.975)	<0.001	<0.001	0.331	1.000
GCST90274799		5	0.815(0.665-0.998)	0.048	1.000	0.991	1.000
APPENDECTOMY	GCST90274799	6	0.837(0.711-0.985)	0.032	0.743	0.625	0.790



Two-step Mendelian randomization analysis revealed the causal relationship between inflammatory proteins and Colorectal cancer, Appendectomy and GCST90274799. SNP single nucleotide polymorphism, IVW inverse variance weight, OR odds ratio, CI confidence interval.