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A Mendelian Randomization Analysis Investigates Causal Associations between Inflammatory Bowel Diseases and Variable Risk Factors

Mohamed J. Saadh [1,2], **Rashmi Saxena Pal** [3], **José Luis Arias-Gonzáles** [4], **Juan Carlos Orosco Gavilán** [5], **Darshan JC** [6], **Mohamed Mohany** [7], **Salim S. Al-Rejaie** [7], **Abolfazl Bahrami** [8,*], **Mustafa Jawad Kadham** [9], **Ali H. Amin** [10] **and Hrosti Georgia** [11,*]

1 Faculty of Pharmacy, Middle East University, Amman 11831, Jordan
2 Applied Science Research Center, Applied Science Private University, Amman 11152, Jordan
3 School of Pharmaceutical Sciences, Lovely Professional University, Phagwara 144001, Punjab, India
4 Department of Social Sciences, Faculty of Social Studies, Pontifical University of Peru, San Miguel 15088, Peru
5 Campus Virtual, Universidad Privada del Norte, Lima 15003, Peru
6 Department of Pharmacy Practice, Yenepoya Pharmacy College & Research Centre, Yenepoya Deemed to Be University, Mangalore 575018, Karnataka, India
7 Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 55760, Riyadh 1145, Saudi Arabia
8 Biomedical Center for Systems Biology Science Munich, Ludwig Maximilians University, 80333 Munich, Germany
9 College of Medical Techniques, Al-Farahidi University, Baghdad 10011, Iraq
10 Zoology Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt
11 Institute of Immunology, Hannover Medical School, 30625 Hannover, Germany
* Correspondence: a.bahrami@ut.ac.ir (A.B.); georgia.hrosti@mh-hannover.de (H.G.); Tel.: +49-5116729722

Abstract: The question of whether variable risk factors and various nutrients are causally related to inflammatory bowel diseases (IBDs) has remained unanswered so far. Thus, this study investigated whether genetically predicted risk factors and nutrients play a function in the occurrence of inflammatory bowel diseases, including ulcerative colitis (UC), non-infective colitis (NIC), and Crohn's disease (CD), using Mendelian randomization (MR) analysis. Utilizing the data of genome-wide association studies (GWASs) with 37 exposure factors, we ran Mendelian randomization analyses based on up to 458,109 participants. Univariable and multivariable MR analyses were conducted to determine causal risk factors for IBD diseases. Genetic predisposition to smoking and appendectomy as well as vegetable and fruit intake, breastfeeding, n-3 PUFAs, n-6 PUFAs, vitamin D, total cholesterol, whole-body fat mass, and physical activity were related to the risk of UC ($p < 0.05$). The effect of lifestyle behaviors on UC was attenuated after correcting for appendectomy. Genetically driven smoking, alcohol consumption, appendectomy, tonsillectomy, blood calcium, tea intake, autoimmune diseases, type 2 diabetes, cesarean delivery, vitamin D deficiency, and antibiotic exposure increased the risk of CD ($p < 0.05$), while vegetable and fruit intake, breastfeeding, physical activity, blood zinc, and n-3 PUFAs decreased the risk of CD ($p < 0.05$). Appendectomy, antibiotics, physical activity, blood zinc, n-3 PUFAs, and vegetable fruit intake remained significant predictors in multivariable MR ($p < 0.05$). Besides smoking, breastfeeding, alcoholic drinks, vegetable and fruit intake, vitamin D, appendectomy, and n-3 PUFAs were associated with NIC ($p < 0.05$). Smoking, alcoholic drinks, vegetable and fruit intake, vitamin D, appendectomy, and n-3 PUFAs remained significant predictors in multivariable MR ($p < 0.05$). Our results provide new and comprehensive evidence demonstrating that there are approving causal effects of various risk factors on IBDs. These findings also supply some suggestions for the treatment and prevention of these diseases.

Keywords: Mendelian randomization; ulcerative colitis; risk factor; Crohn's disease; non-infective colitis; inflammatory bowel disease

1. Introduction

Chronic, progressive inflammatory bowel disease (IBD) can cause bowel injury, the condition for hospitalization, disability, and a reduction in life quality. Although North America and Europe have the highest rates, developing nations such as Asia have seen rising incidence rates as a result of these regions' increased development [1]. Although the exact source of these phenomena is unknown, it can be connected with the intricate interaction between the environment and genetics [2]. Due to the statement that the incidence speeds of IBD are higher in developed countries, the latter has been linked to disease development [3]. In addition, the urbanization of societies is associated with changes in diet, antibiotic use, hygiene status, microbial exposure, and pollution, which have been implicated as potential environmental risk factors for IBD. Environmental risk factors for individual, familial, community-based, country-based and regionally based origin could all contribute to the pathogenesis of IBD [4–6]. Lending further support to the critical importance of environmental influences is the recognition of the central role of the gut microbiota in the development and propagation of inflammation in IBD [7]. Although host genetics might partly determine gut microbial structure, external environmental exposure from the time of birth to adulthood continue to alter the composition, structure, and function of the gut microbiome, thereby dynamically altering the risk and natural history of disease throughout life [8,9]. Discovering how environmental factors influence the onset of IBD and contribute to its pathogenesis could ultimately help to determine how individuals can reduce their risk of disease or have a milder clinical course. The search for pathogenic environmental factors is also important, as many unmet therapeutic needs and suboptimal outcomes in IBD remain. Mechanistic insights obtained from robustly defining environmental influences could also lead to the identification of new therapeutic targets and treatment strategies. The best treatment should be started early in disease progression to avoid problems, because IBD can be a progressive and chronic condition. To conduct clinical remission and mucosal healing, which can improve life quality, the current treatment paradigm for ulcerative colitis (UC), Crohn's disease (CD), and non-infective colitis (NIC) uses biological therapy [10]. However, measuring the causal impact of these variable factors on IBD can be challenging due to possible reverse causality and potential residual confounders that can induce spurious associations and mask the effects of real risk factors. Therefore, it is essential to clarify whether these possible risk factors recreate causal functions in the evolution of IBD or serve as transferred risk factor consequences. In addition, genetic and environmental variables work together to cause the disease, but the exact mechanisms are still poorly understood, particularly concerning the non-genetic hazards. Hundreds of variations have already been correlated to IBD by genome-wide association studies (GWASs) [11,12], but our understanding of the different risk variables and genetic relations that influence IBD is still restricted. Numerous factors that may affect the IBD risk have been specified by prior studies, but these investigations have not always come to the same results, in part because of the difficulties in establishing satisfactory statistical authority, avoiding bias, and correcting for confounding aspects [13,14]. Additionally, only a small number of research studies have examined the genetic–environmental interactions linked to IBD, including single-nucleotide polymorphisms (SNPs) [15,16]. Therefore, both environmental and genetic elements play a function in the complex etiology of IBD. Numerous modifiable factors have been investigated [12], but none are reliably detrimental nor protective. There is currently a lack of solid research to conduct IBD prevention. In addition, there has not been an organized action to compile and evaluate this evidence, despite the vast number of research studies that have looked at environmental factors and IBD, including Mendelian randomization (MR) analyses. MR is an instrumental variable analysis method for examining causal relationships between disease outcomes and risk factors [17]. It employs genetic variants that are closely connected with a risk factor as instrumental variables (IVs) and mimics a randomized controlled background in which all other factors excluding exposure are almost distributed over subgroups. Thus, MR analysis can avoid reversing causation and confounding biases that are common in observational studies.

In this study, two-sample Mendelian randomization analysis was performed to investigate the causal effects of 37 genetically predicted potential risk factors and nutrients, including related diseases, drugs, lifestyle, surgeries, lipid and glucose metabolism, blood parameters, and obesity traits, on IBD (including UC, CD, and NIC) in the European population. This study aimed to provide a comprehensive overview of putative variable risk factors for IBD and offer novel insights into the etiology of IBD or colitis disorders.

2. Materials and Methods

2.1. MR Design

MR was utilized to investigate the relationships between various risk factors and IBD or different types of colitis. A total of 37 primary risk factors were selected and classified into eight categories: exposure to drugs, lifestyle behaviors, surgeries, related diseases, blood parameters, lipid metabolism, glucose metabolism, and obesity traits. SNPs associated with these risk factors were utilized as the instrumental variables. The following three assumptions served as the foundation for the MR study: (1) the SNPs are closely connected with the risk factors; (2) the SNPs are irrelevant to various confounders; (3) the SNPs only influence the outcomes via the risk factors. This study was performed according to the STROBE-MR procedures (Figure 1).
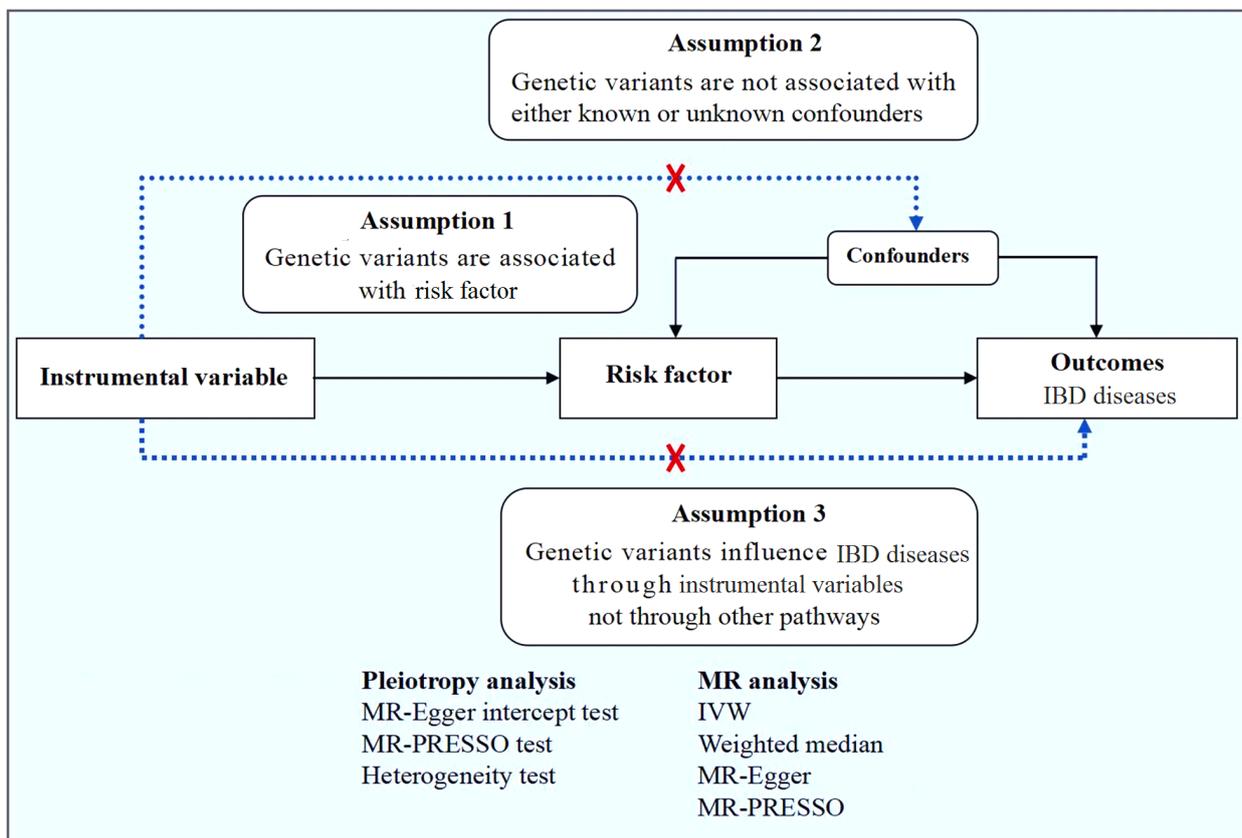
Figure 1. The entire flow chart of the study, the red X represents potential pleiotropic or direct causal effects between instrumental variables that would violate Mendelian randomization assumptions.

2.2. Selection of Genetic Instruments

GWASs of participants of European ancestry were selected as data sources for genetic agencies associated with the 37 risk factors. Genetic instruments of cigarettes per day, smoking, and alcoholic drinks were extracted from the GSCAN (GWAS and Sequencing Consortium of Alcohol and Nicotine use) consortium [11]. GWAS summary statistics for coffee, tea, fruit, and vegetable intake; vitamin D consumption; and breastfeeding were obtained from the MRC-IEU (MRC Integrative Epidemiology Unit) consortium. IVs for

education level and physical activity were chosen from SSGAC (Social Science Genetic Association Consortium) [18]. GWAS summary statistics for appendectomy, tonsillectomy, and autoimmune diseases were received from FinnGen [19]. The UK Biobank study was utilized as the data source for GWAS summary statistics for lipid metabolism traits, including *n*-3 PUFA, *n*-6 PUFA, triglycerides, apolipoprotein A-I, and total cholesterol [20]. Genetic instruments for whole-body fat mass and body mass index (BMI) were selected from Neale Lab (http://www.nealelab.is (accessed on 24 May 2022)). GWAS summary statistics for waist circumference, waist-to-hip ratio, and hip circumference were obtained from GIANT (Genetic Investigation of Anthropometric Traits) [21]. For CKD [22], cesarean delivery [23], celiac disease [24], SLE [25], blood calcium [26], blood lactose [27], zinc intake [28], T2D [29], fasting glucose, HbA1c, fasting insulin [30], antibiotics, and Isotretinoin [31,32] were selected from associated GWAS studies. Significance SNP levels ($p < 5 \times 10^{-8}$) were obtained, and those with a window ($\geq$10,000 kb) and smaller linkage disequilibrium probability ($R^2 > 0.001$) were included.

2.3. GWAS Summary Statistics for IBD Cohorts

GWAS summarization for UC, CD, and NIC was received from the FinnGen consortium. The R5 release of the FinnGen data was utilized [19]; this data set contains 1213 cases and 164,254 controls for CD, 2155 cases and 186,103 controls for UC, 411 cases, and 103,973 controls for NIC. All selected GWASs from the Biobank obtained ethical approval from FinnGen Steering Committee, and individuals provided informed consent.

2.4. Statistical Analysis

The F-statistic was utilized to evaluate genetic instrument strength. F-statistics ($F = beta^2/se^2$) were calculated for each SNP, and a general F-statistic was calculated for all SNPs for the corresponding exposure. F > 10 was considered to be sufficient strength. All F-statistics were over 10. The random-effect inverse-variance-weighted (IVW) technique was utilized as the main analysis method to estimate the association between genetic liability to modifiable risk factors and the risk of pancreatitis. Given that the analysis is sensitive to outliers and horizontal pleiotropy, three sensitivity analyses, including the weighted median, MR-Egger, and MR-PRESSO methods, were used to examine the consistency of the results. The weighted median model was used; this can produce unbiased estimates under the precondition that at least 50% of the selected IVs are valid [33]. MR-Egger regression was used to obtain cogent causal estimates under the influence of pleiotropy [34]. The MR-PRESSO method was conducted to specify outliers due to the existing pleiotropy; causal effect estimates were obtained with the IVW approach after removing these outliers [35]. In addition, a leave-one-out sensitivity test was applied to examine if the SNPs possessing effective horizontal pleiotropic effects could affect the causal estimates [36]. MR-PRESSO and Cochran's Q statistics were used to evaluate pleiotropy and heterogeneity, respectively. The multivariable MR analysis of the genetic associations between the instruments and UC was adjusted for appendectomy, while the multivariable MR analysis of the associations between the instruments and CD was adjusted for alcohol consumption and smoking. All statistical analyses were performed using R 4.2.1, (R Foundation for Statistical Computing, Vienna, Austria), with the R packages "TwoSampleMR" (https://github.com/MRCIEU/TwoSampleMR) and "MRPRESSO". (https://github.com/rondolab/MR-PRESSO (accessed on 24 July 2022)).

The results are reported as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). A Bonferroni-corrected significance level of $p < 1.67 \times 10^{-4}$ (0.05/30) was used and p-values ranging from 1.67×10^{-4} to 0.05 were classified as suggestive of causal associations.

3. Results

3.1. Baseline Characteristics of the 36 Candidate Risk Factors

Thirty-seven potential risk factors were included in the analyses. The risk factors were classified into eight categories: lifestyle behaviors, surgeries, exposure to drugs, related diseases, blood parameters, lipid metabolism, glucose metabolism, and obesity traits (Table 1). The lifestyle behaviors included smoking, alcohol consumption, coffee consumption, tea consumption, fruit intake, vegetable intake, breastfeeding, cesarean delivery, vitamin D intake, and physical activity. The surgeries included appendectomy and tonsillectomy, and the related diseases include T2D, CKD, and autoimmune diseases (including celiac disease and SLE). The blood parameters included calcium, lactose, CRP, and zinc. Exposure to drugs included antibiotics and isotretinoin. Additionally, three traits related to glucose metabolism, five traits related to lipid metabolism, and five obesity traits were analyzed. SNP numbers ranged from 11 to 523. Across the 37 variable potential risk factors that were explored, the F-statistics of their separate genetic instruments were all greater than the empirical threshold of 10, indicating no possible inadequate instrument bias.

Table 1. Characteristics of the GWAS summary data.

Exposure	SNPs	Ancestry	Unit	F	Source
Drugs					
Antibiotics	50	European	SD	97.99	PMID
Isotretinoin	17	European	SD	72.22	PMID
Lifestyle					
Smoking	102	European	SD	39.98	GSCAN
Cigarettes per day	33	European	SD	87.24	GSCAN
Breastfeeding	42	European	SD	70.52	GSCAN
Alcoholic drinks	42	European	SD	68.99	GSCAN
Fruit intake	71	European	SD	98.13	MRC-IEU
Vegetable intake	32	European	SD	102	MRC-IEU
Coffee intake	33	European	SD	123.14	MRC-IEU
Tea intake	23	European	SD	111.19	MRC-IEU
Vitamin D level	87	European	SD	76.17	MRC-IEU
Cesarean delivery	46	European	NA	66.57	PMID
Physical activity	56	European	SD	43.6	SSGAC
Surgeries					
Appendectomy	65	European	NA	97.99	FinnGen
Tonsillectomy	50	European	NA	90.24	FinnGen
Related diseases					
Type 2 diabetes	88	European	logOR	72.02	FinnGen
Chronic kidney disease	14	European	NA	58.26	PMID
Autoimmune	81	European	NA	111.1	FinnGen
Celiac disease	14	European	logOR	323.75	PMID
Systemic lupus erythematosus	23	European	logOR	88.61	PMID
Blood parameters					
Calcium	11	European	SD	300.13	PMID
Lactose	12	European	SD	95.09	PMID
C-reactive protein	11	European	NA	160.09	PMID
Zinc	117	European	SD	79.24	PMID
Lipid metabolism					
n-3 PUFA	103	European	SD	82.52	PMID
Triglycerides	114	European	SD	114.05	UK Biobank
n-6 PUFA	84	European	SD	97.55	PMID
Total cholesterol	36	European	SD	114.03	UK Biobank
Apolipoprotein A-I	42	European	SD	125.4	UK Biobank

Table 1. *Cont.*

Exposure	SNPs	Ancestry	Unit	F	Source
Glucose metabolism					
HbA1c	42	European	SD	118.71	PMID
Fasting insulin	33	European	SD	95.93	PMID
Fasting glucose	54	European	SD	60.13	PMID
Obesity traits					
Whole-body fat mass	412	European	SD	55.36	GIANT
BMI	523	European	SD	48.78	GIANT
Waist-to-hip ratio	36	European	SD	49.15	Neale Lab
Waist circumference	67	European	SD	57.45	Neale Lab
Hip circumference	55	European	SD	52.89	GIANT

3.2. Causal Effects of Various Factors on UC

The univariable MR analyses revealed that genetically predicted appendectomy (OR = 1.368, $p < 0.001$) and vegetable intake (OR = 0.731, $p = 0.001$), fruit intake (OR = 0.726, $p = 0.001$), breastfeeding (OR = 0.791, $p = 0.002$), level of *n*-6 PUFAs (OR = 0.854, $p = 0.038$), and level of *n*-3 PUFAs (OR = 0.200, $p = 0.001$) were connected with increased risk of UC (Figure 2). Genetic predisposition to smoking, tea intake, fruit intake, higher triglycerides, and whole-body fat mass, as well as increased waist circumference, were suggestively associated with UC. The ORs were 1.342 ($p = 0.021$) for smoking initiation, 1.301 ($p = 0.015$) for tea intake, 0.475 ($p = 0.021$) for physical activity, 0.859 ($p = 0.017$) for total cholesterol, 1.363 ($p = 0.003$) for whole-body fat mass, and 1.392 ($p = 0.005$) for BMI.

Possible pleiotropy and heterogeneity were observed for *n*-3 PUFAs ($p_{pleiotropy} = 0.035$; $p_{heterogeneity} = 0.025$) and *n*-6 PUFAs ($p_{pleiotropy} = 0.002$; $p_{heterogeneity} = 0.005$). Thus, MR-PRESSO analysis was performed after eliminating the outliers. The association remained unchanging in the MRPRESSO-corrected outcomes (Supplementary Table S1).

3.3. Causal Effects of Various Factors on CD

Genetically predicted appendectomy, tonsillectomy, cesarean delivery, fruit intake, and vitamin D intake were significantly related to raised risk of CD, while genetically predicted antibiotic exposure, smoking, alcohol consumption, vegetable intake, physical activity, autoimmune diseases, and T2D, as well as blood calcium, blood lactose, blood zinc, *n*-3 PUFA levels, and waist-to-hip ratio (WHR), were significantly associated with CD (Figure 3). The odds of CD increased with the increase in smoking (OR = 1.595, $p = 0.005$), alcoholic drinks per week (OR = 1.728, $p = 0.020$), blood lactose (OR = 1.024, $p = 0.032$), cesarean delivery (OR = 1.301, $p = 0.002$), and WHR (OR = 1.281, $p = 0.023$) and the decrease in vegetable intake (OR = 0.660, $p = 0.011$), blood calcium (OR = 1.729, $p = 0.018$), blood zinc (OR = 0.538, $p = 0.017$), and *n*-3 PUFAs (OR = 0.222, $p = 0.021$). Genetically predicted autoimmune diseases and T2D were suggestively related to raised risk of CD (autoimmune: OR = 1.123, $p = 0.008$; T2D: OR = 1.121, $p = 0.029$). There was possible heterogeneity in alcoholic drinks per week ($p_{heterogeneity} = 0.05$) and appendectomy ($p_{heterogeneity} = 0.039$). Physical activity (OR = 0.536, $p = 0.006$) and breastfeeding (OR = 0.710, $p = 0.002$) were protective of CD; however, possible pleiotropy for physical activity was observed ($p_{pleiotropy} = 0.041$) (Supplementary Table S2).

Variable Exposure	SNPs		OR (95% CI)	*P*
Exposure to drugs				
Antibiotics	50		1.068 (0.676–1.460)	0.055
Isotretinoin	17		1.447 (0.937–1.957)	0.107
Lifestyle behaviors				
Smoking	102		**1.342 (1.035–1.649)**	**0.021**
Cigarettes per day	33		0.974 (0.776–1.118)	0.446
Breastfeeding	42		**0.791 (0.671–0.912)**	**0.002**
Alcoholic drinks	42		1.596 (0.706–2.487)	0.380
Fruit intake	71		**0.726 (0.492–0.961)**	**0.001**
Vegetables intake	32		**0.731 (0.581–0.882)**	**0.001**
Coffee intake	33		1.351 (0.613–2.089)	0.693
Tea intake	23		**1.301 (1.081–1.522)**	**0.015**
High vitamin D level	87		**0.490 (0.225–0.755)**	**0.001**
Cesarean delivery	46		1.121 (0.893-1.218)	0.414
Physical activity	56		**0.475 (0.250–0.701)**	**0.001**
Surgeries				
Appendectomy	65		**1.368 (1.276–1.460)**	**0.000**
Tonsillectomy	50		0.915 (0.705–1.189)	0.508
Related diseases				
Chronic kidney disease	14		1.210 (0.987–1.484)	0.067
Autoimmune	81		0.985 (0.915–1.060)	0.682
Celiac disease	14		0.999 (0.971–1.028)	0.937
Systemic lupus erythematosus	23		1.008 (0.980–1.036)	0.990
Blood parameters				
Serum calcium	11		1.092 (0.720–1.657)	0.679
Serum Lactose	12		1.080 (0.988–1.181)	0.091
C-reactive protein	11		1.002 (0.869–1.155)	0.981
Serum zinc	117		1.082 (0.725–1.614)	0.699
Lipid metabolism				
n-3 PUFA	103		**0.200 (0.033–0.368)**	**0.001**
Triglycerides	114		0.951 (0.840–1.078)	0.435
n-6 PUFA	84		**0.854 (0.718–0.991)**	**0.038**
Apolipoprotein A-I	36		1.013 (0.872–1.177)	0.867
Total cholesterol	42		**0.859 (0.752–0.966)**	**0.017**
Glucose metabolism				
Type 2 diabetes	88		1.042 (0.963–1.128)	0.304
Fasting glucose	42		1.283 (0.943–1.745)	0.113
HbA1c	33		1.103 (0.649–1.876)	0.716
Fasting insulin	54		1.925 (0.961–2.858)	0.165
Obesity traits				
Body mass index	523		**1.392 (1.144–1.641)**	**0.005**
Whole body fat mass	412		**1.363 (1.106–1.621)**	**0.003**
Waist circumference	67		2.466 (2.093–2.968)	0.111
Hip circumference	55		1.280 (0.631–1.863)	0.121
Waist-to-hip ratio	36		1.354 (0.937–1.957)	0.107

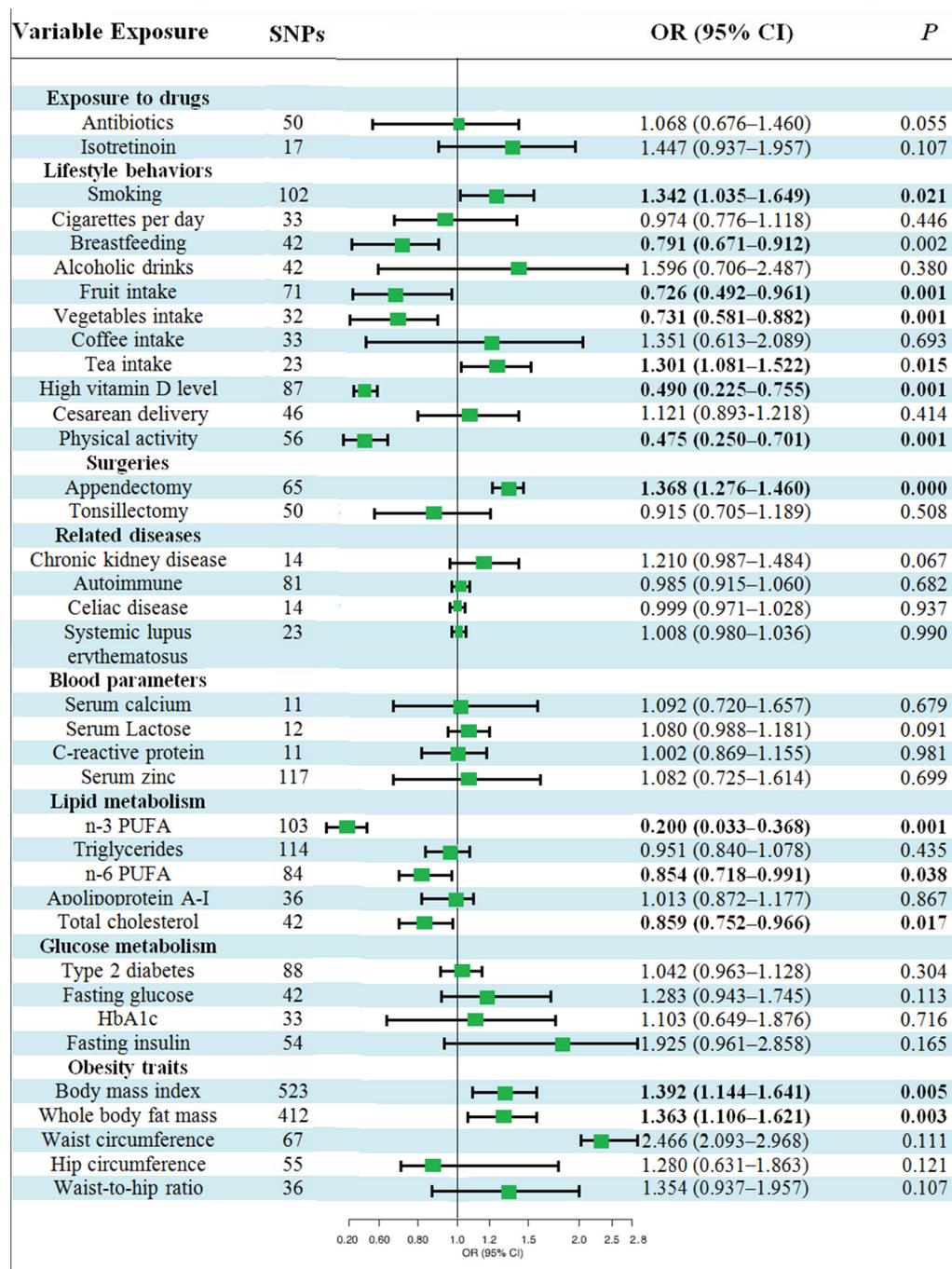0.20 0.60 0.80 1.0 1.2 1.5 2.0 2.5 2.8
OR (95% CI)

Figure 2. Forest plot of odds ratio and 95% confidence intervals (lines). Overview impact calculations of MR indicating relationships between UC and risk factors; significant impacts are exhibited in bold.

3.4. Causal Effects of Various Factors on NIC

Next, the causal relationships between risk factors and NIC were examined (Figure 4). Notably, genetic liability to alcohol consumption (OR = 1.545, *p* = 0.001) and fruit intake (OR = 0.497, *p* = 0.001) was strongly associated with increased odds of NIC. Genetic liability to smoking and appendectomy, as well as decreased vegetable intake, *n*-3 PUFA intake, and vitamin D deficiency, was suggestively related to raised risk of NIC.

Variable Exposure	SNPs		OR (95% CI)	*P*
Exposure to drugs				
Antibiotics	50		**1.745 (1.356–2.230)**	**0.005**
Isotretinoin	17		1.354 (0.937–1.957)	0.107
Lifestyle behaviors				
Smoking	102		**1.595 (1.155–2.204)**	**0.005**
Cigarettes per day	33		1.249 (0.972–1.604)	0.082
Breastfeeding	42		**0.710 (0.591–0.839)**	**0.002**
Alcoholic drinks	42		**1.728 (1.195–2.261)**	**0.020**
Fruit intake	71		**0.571 (0.441–0.739)**	**0.001**
Vegetables intake	32		**0.660 (0.401–0.898)**	**0.011**
Coffee intake	33		1.052 (0.482–2.295)	0.898
Tea intake	23		1.281 (1.081–1.521)	0.115
High vitamin D level	87		**0.620 (0.465–0.835)**	**0.001**
Cesarean delivery	46		**1.301 (1.093–1.521)**	**0.002**
Physical activity	56		**0.536 (0.397–0.723)**	**0.006**
Surgeries				
Appendectomy	65		**1.180 (1.075–1.296)**	**0.001**
Tonsillectomy	50		**1.370 (1.159–1.621)**	**0.041**
Related diseases				
Chronic kidney disease	14		1.110 (0.849–1.450)	0.446
Autoimmune	81		**1.123 (1.031–1.224)**	**0.008**
Celiac disease	14		0.972 (0.938–1.007)	0.117
Systemic lupus erythematosus	23		0.998 (0.958–1.041)	0.942
Blood parameters				
Serum calcium	11		**1.729 (1.119–2.339)**	**0.018**
Serum Lactose	12		**1.024 (1.011–1.151)**	**0.032**
C-reactive protein	11		1.048 (0.880–1.248)	0.601
Serum zinc	117		**0.538 (0.324–0.893)**	**0.017**
Lipid metabolism				
n-3 PUFA	103		**0.222 (0.130–0.450)**	**0.021**
Triglycerides	114		0.907 (0.771–1.066)	0.235
n-6 PUFA	84		0.994 (0.819–1.205)	0.051
Apolipoprotein A-I	36		0.977 (0.814–1.172)	0.802
Total cholesterol	42		1.030 (0.839–1.264)	0.776
Glucose metabolism				
Type 2 diabetes	88		**1.121 (1.012–1.241)**	**0.029**
Fasting glucose	42		1.328 (0.872–2.023)	0.186
HbA1c	33		1.430 (0.915–1.945)	0.097
Fasting insulin	54		1.310 (0.568–2.052)	0.559
Obesity traits				
Body mass index	523		1.103 (0.906–1.341)	0.328
Whole body fat mass	412		1.101 (0.891–1.362)	0.374
Waist circumference	67		1.365 (0.959–1.943)	0.084
Hip circumference	55		0.813 (0.616–1.074)	0.144
Waist-to-hip ratio	36		**1.281 (1.069–1.493)**	**0.023**

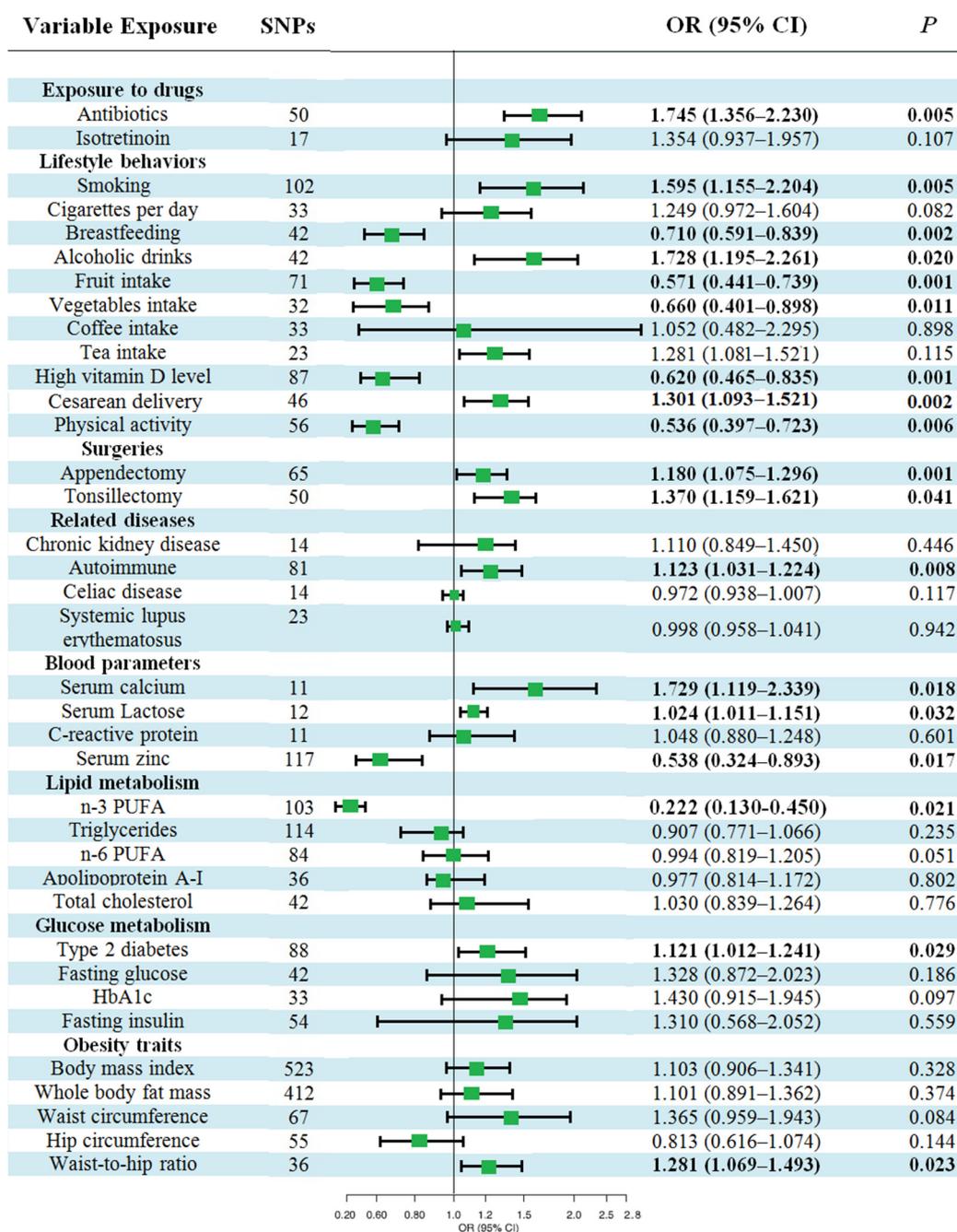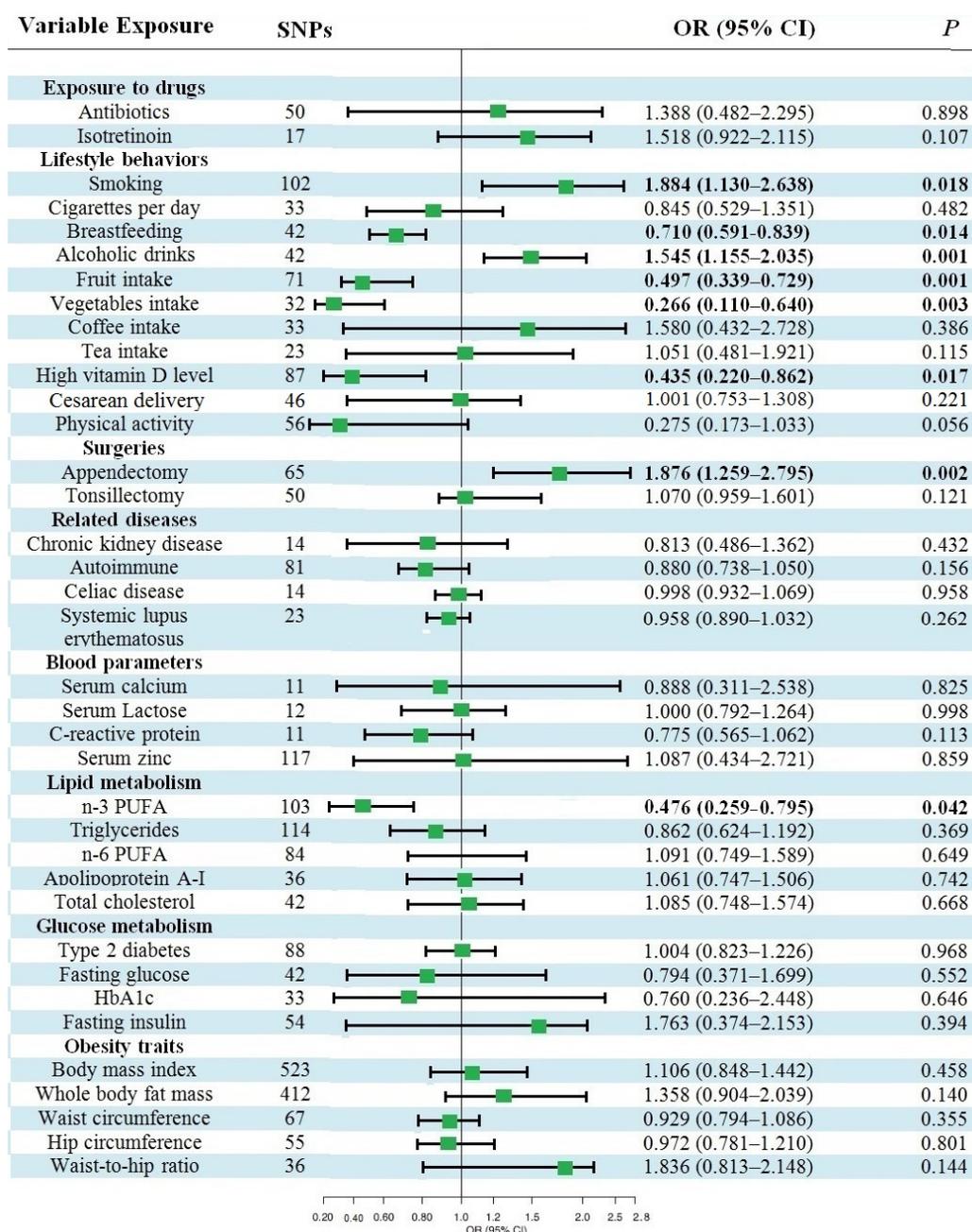OR (95% CI): 0.20 0.60 0.80 1.0 1.2 1.5 2.0 2.5 2.8

Figure 3. Forest plot of odds ratio and 95% confidence intervals (lines). Overview impact calculations of MR indicating relationships between CD and risk factors; significant impacts are exhibited in bold.

The odds of NIC increased with the increase in smoking (OR = 1.884, *p* = 0.018), appendectomy (OR = 1.876, *p* = 0.002), breastfeeding (OR = 0.710, *p* = 0.014), vegetable intake (OR = 0.266, *p* = 0.003), *n*-3 PUFA intake (OR = 0.476, *p* = 0.042), and vitamin D deficiency (OR = 0.435, *p* = 0.017). There was possible heterogeneity in appendectomy ($p_{heterogeneity}$ = 0.036) and *n*-3 PUFAs ($p_{heterogeneity}$ = 0.002) (Supplementary Table S3).

Variable Exposure	SNPs		OR (95% CI)	*P*
Exposure to drugs				
Antibiotics	50		1.388 (0.482–2.295)	0.898
Isotretinoin	17		1.518 (0.922–2.115)	0.107
Lifestyle behaviors				
Smoking	102		**1.884 (1.130–2.638)**	**0.018**
Cigarettes per day	33		0.845 (0.529–1.351)	0.482
Breastfeeding	42		**0.710 (0.591–0.839)**	**0.014**
Alcoholic drinks	42		**1.545 (1.155–2.035)**	**0.001**
Fruit intake	71		**0.497 (0.339–0.729)**	**0.001**
Vegetables intake	32		**0.266 (0.110–0.640)**	**0.003**
Coffee intake	33		1.580 (0.432–2.728)	0.386
Tea intake	23		1.051 (0.481–1.921)	0.115
High vitamin D level	87		**0.435 (0.220–0.862)**	**0.017**
Cesarean delivery	46		1.001 (0.753–1.308)	0.221
Physical activity	56		0.275 (0.173–1.033)	0.056
Surgeries				
Appendectomy	65		**1.876 (1.259–2.795)**	**0.002**
Tonsillectomy	50		1.070 (0.959–1.601)	0.121
Related diseases				
Chronic kidney disease	14		0.813 (0.486–1.362)	0.432
Autoimmune	81		0.880 (0.738–1.050)	0.156
Celiac disease	14		0.998 (0.932–1.069)	0.958
Systemic lupus erythematosus	23		0.958 (0.890–1.032)	0.262
Blood parameters				
Serum calcium	11		0.888 (0.311–2.538)	0.825
Serum Lactose	12		1.000 (0.792–1.264)	0.998
C-reactive protein	11		0.775 (0.565–1.062)	0.113
Serum zinc	117		1.087 (0.434–2.721)	0.859
Lipid metabolism				
n-3 PUFA	103		**0.476 (0.259–0.795)**	**0.042**
Triglycerides	114		0.862 (0.624–1.192)	0.369
n-6 PUFA	84		1.091 (0.749–1.589)	0.649
Apolipoprotein A-I	36		1.061 (0.747–1.506)	0.742
Total cholesterol	42		1.085 (0.748–1.574)	0.668
Glucose metabolism				
Type 2 diabetes	88		1.004 (0.823–1.226)	0.968
Fasting glucose	42		0.794 (0.371–1.699)	0.552
HbA1c	33		0.760 (0.236–2.448)	0.646
Fasting insulin	54		1.763 (0.374–2.153)	0.394
Obesity traits				
Body mass index	523		1.106 (0.848–1.442)	0.458
Whole body fat mass	412		1.358 (0.904–2.039)	0.140
Waist circumference	67		0.929 (0.794–1.086)	0.355
Hip circumference	55		0.972 (0.781–1.210)	0.801
Waist-to-hip ratio	36		1.836 (0.813–2.148)	0.144

0.20　0.40　0.60　0.80　1.0　1.2　1.5　2.0　2.5　2.8
OR (95% CI)

Figure 4. Forest plot of odds ratio and 95% confidence intervals (lines). Overview impact calculations of MR indicating relationships between NIC and risk factors; significant impact are exhibited in bold.

3.5. Multivariable MR Analysis of IBD

In the multivariable MR model, smoking (OR = 1.415, *p* = 0.022), physical activity (OR = 0.519, *p* = 0.002), breastfeeding (OR = 0.466, *p* = 0.001), *n*-3 PUFAs (OR = 0.290, *p* = 0.017), *n*-6 PUFAs (OR = 0.785, *p* = 0.017), fruit intake (OR = 0.164, *p* = 0.009), vegetable intake (OR = 0.661, *p* = 0.017), and vitamin D (OR = 0.312, *p* = 0.047) had similar significant causal effects on UC after adjusting for genetically predicted appendectomy, whereas apolipoprotein A-I, total cholesterol, BMI, whole-body fat mass, hip circumference, and waist circumference did not reach statistical significance (Figure 5A). This suggests that these latter associations could be affected by appendectomy. Adjusting for the genetic risk of alcohol consumption and smoking did not change the associations between CD and antibiotic exposure (OR = 1.368, *p* = 0.003), physical activity (OR = 0.694, *p* = 0.013), appendectomy (OR = 1.281, *p* = 0.003), blood zinc (OR = 0.637, p = 0.019), fruit intake

(OR = 0.164, p = 0.001), vegetable intake (OR = 0.630, p = 0.002), *n*-3 PUFAs (OR = 0.316, p = 0.023), and vitamin D (OR = 0.304, p = 0.021), while no significant associations remained between CD and autoimmune diseases, and T2D (Figure 5B). Finally, multivariable MR models of NIC were examined (Figure 5C). Smoking (OR = 1.884, p = 0.008), alcoholic drinks (OR = 1.186, p = 0.002), breastfeeding (OR = 0.344, p = 0.001), *n*-3 PUFAs (OR = 0.639, p = 0.002), fruit intake (OR = 0.598, p = 0.001), vegetable intake (OR = 0.367, p = 0.002), and vitamin D (OR = 0.636, p = 0.007) had similar significant causal effects on NIC after adjusting for genetically predicted appendectomy. Genetically predicted exposure to drugs, related diseases, blood parameters, glucose metabolism, and obesity traits were no longer significant risk factors for NIC in the multivariable MR model.

A — Ulcerative Colitis

Exposure	SNPs	OR (95% CI)	P
Smoking	102	1.415 (1.144–1.758)	0.022
Breastfeeding	42	0.466 (0.377–0.561)	0.001
Fruit intake	71	0.164 (0.117–0.212)	0.009
Vegetables intake	32	0.661 (0.519–0.851)	0.001
Physical activity	56	0.519 (0.351–0.802)	0.002
Appendectomy	65	0.451 (0.365–0.557)	0.001
n-3 PUFA	103	0.290 (0.134–0.469)	0.017
n-6 PUFA	84	0.785 (0.626–0.944)	0.017
High vitamin D level	87	0.312 (0.209–0.450)	0.047

B — Crohn's Disease

Exposure	SNPs	OR (95% CI)	P
Antibiotics	50	1.368 (1.276–1.460)	0.003
Fruit intake	71	0.164 (0.117–0.221)	0.001
Vegetables intake	32	0.630 (0.418–0.842)	0.002
Physical activity	56	0.694 (0.528–0.860)	0.013
Appendectomy	65	1.281 (1.176–1.397)	0.003
Blood zinc	117	0.637 (0.294–0.983)	0.019
n-3 PUFA	103	0.316 (0.110–0.523)	0.023
High vitamin D level	87	0.304 (0.025–0.614)	0.021

C — Non-Infective Colitis

Exposure	SNPs	OR (95% CI)	P
Smoking	102	1.884 (1.130–2.638)	0.008
Breastfeeding	42	0.344 (0.232–0.457)	0.001
Alcoholic drink	42	1.786 (1.236–2.336)	0.002
Fruit intake	71	0.598 (0.440–0.830)	0.001
Vegetables intake	32	0.367 (0.211–0.741)	0.002
High vitamin D level	87	0.636 (0.321–0.963)	0.007
Appendectomy	65	1.939 (1.340–2.826)	0.001
n-3 PUFA	103	0.639 (0.340–0.826)	0.002

OR (95% CI): 0.20 0.60 0.80 1.0 1.2 1.5 2.0 2.5 2.8

Figure 5. Forest plot of odds ratio and 95% confidence intervals (lines). Summary effect estimates of multivariable MR reporting associations between (**A**) UC, (**B**) CD, and (**C**) NIC, and risk factors; significant estimates are shown in bold.

4. Discussion

Genetic susceptibility factors play crucial functions in IBD development [37]. The development and progression of IBD are multidimensional, with interactions between environmental and genetic elements [38]. Therefore, a critical evaluation of the environmental and genetic factors related to IBD is offered in this study, which encompasses MR analyses of observational research. A total of 37 variables, such as lifestyle behavior, dietary intake, blood parameters, obesity traits, related diseases, drug exposure, and glucose and lipid metabolism were examined. Among them, we found 10 risk variables and 10 defensive factors with epidemiological evidence of moderate-to-high strength. The methodological standards among the MR analyses greatly differed. Several variables were connected with CD, UC, or NIC. The found specificity might represent various pathogeneses and traits of these diseases [39]. Subgroup analyses for smoking [40–45], breastfeeding [46], cesarean delivery [23,47], and high vitamin D levels have found significant differential relationships

among groups [48]. These variations could be explained by unique genetic predispositions and environmental exposures happening in particular geographical regions. The investigation by publication year revealed more conventional calculations for fiber intake and breastfeeding in analyses disseminated after the year 2000 [12,49,50].

Cigarette smoking and alcohol use are two well-recognized lifestyle risk factors for colitis. Smoking promotes the progression from US to CD and accelerates the development of IBD [43,51]. MR analysis confirmed that smoking was related to a higher risk of IBD. The impacts of smoking on NIC were partially attenuated after adjusting for alcohol consumption, suggesting that this association is not robust enough in NIC. Alcohol consumption contributes to the progression or initiation of IBD and amplifies the relationship between the risk factors of genetics and CD in a dose-dependent manner [52]. Our results verified the causal associations between CD or NIC and alcohol consumption. Notably, the risk of CD due to genetically predicted alcohol consumption was higher than that of NIC. However, there was no proof of a positive connection between UC and alcohol consumption. Furthermore, alcohol accounts for 40–70% of CD etiologies and only 20% of NIC etiologies; thus, this causal relationship may be statistically attenuated by other NIC risk factors. The associations between coffee or tea consumption, and IBD are controversial. Some studies have reported that tea and coffee reduce the risk of colitis, while another prospective cohort analysis discovered no relationship between tea consumption and the risk of colitis [53,54]. Our MR study found no associations between genetically predicted coffee consumption and colitis risk. Moreover, appendectomy, autoimmune diseases, and CKD were associated with increased risk of IBD events [55–60]. Autoimmune diseases, including celiac disease, and SLE have been reported to be associated with IBD in previous studies. The current MR analysis supports a suggestive association between autoimmune diseases and CD; however, this association did not persist when corrected for smoking and alcohol consumption [61–64]. No effect was seen for causal connections between IBD and celiac disease, SLE, nor CKD in this study. Additionally, appendectomy was associated with IBD. However, the risk was reduced if more than five years had expired between colitis diagnosis and appendectomy, proposing that the connection may be biased by unneeded appendectomies operated in individuals with developing colitis [56,57]. Tonsillectomy was linked with CD but not NIC or UC. However, patients undergoing tonsillectomy have often been exposed to previous antibiotics [65].

Furthermore, the current results indicate that physiologically normally higher blood calcium levels raised CD risk, while there were no connections between IBD and genetic predisposition to higher lactose and CRP.

Retrospective case-control studies were used to determine the pre-illness diet in order to infer the significance of nutrition in CD and UC development. According to pediatric case-control research study by Amre et al. [66], there is a negative correlation between vegetable or fruit intake and the development of CD. The impacts of dietary micronutrients and macronutrients on the risk of disease have also been more robustly estimated by previous research in North America and Europe. Women in the most elevated percentile of dietary fiber consumption had significantly decreased risk of incident CD than those in the lowest quintile in a considerable cohort of 170,776 females followed for 28 years [67]. In contrast, dietary fiber from bran, cereal, and whole grains was not linked to modified risk of disease. The highest percentage of dietary fiber intake from fruits was related to decreased CD risk [67]. Consuming fruit lowered the risk of developing CD and UC [68–71]. In addition, eating vegetables reduced the risk of UC. These results have some biological plausibility. Fibers can impact the function of the intestine, which is compromised in inflammatory bowel disease (IBD), and plant ingredients can influence the microorganism translocation across the intestine mucosa [72]. CD was also linked to lactose level but did not impact UC, which is consistent with a previous study [73]. Dietary *n*-3 PUFA consumption has been also shown, in two prospective cohort studies, to be inversely related to UC risk, but dietary *n*-6 PUFA consumption is positively connected with incident UC risk [74,75]. *n*-3 PUFA consumption reduced the clinical colitis severity in an investigation

of generated colitis in mice [76]. These results are consistent with other studies that showed the preventive effect of *n*-3 PUFAs on the risk of IBD [77–79]. In addition, the current results suggested that high vitamin D levels were related to diminished risk of UC, CD, and NIC, and these associations persisted in the CD cohort after adjusting for alcohol consumption [80,81].

Furthermore, high zinc intake was found to be negatively correlated with women's chance of improving CD in some cohort investigations [77,82]. With 16 mg daily zinc consumption, or double the suggested everyday dose, the risk was detected to be reduced. Low blood zinc was linked to raised risk of surgeries, hospitalizations, and illness-related complications in colitis patients [83]. Additionally, improvement in results was linked to the normalization of zinc levels. Zinc supplementation was linked to a decline in intestinal permeability, as specified by the mannitol:lactulose ratio, in small interventional research [84]. In addition, apolipoprotein A-I was previously reported to be related to the severity of UC [85]. However, these results provided no effects of any associations between IBD risk and genetically predicted apolipoprotein A-I. Notably, genetically predicted total cholesterol was suggestively connected with a lower odds ratio of UC, whereas these associations were not significant after adjustment for appendectomy. There were no relationships between total cholesterol and IBD, in agreement with a previous investigation [86]. A previous meta-analysis documented a positive link between CD risk and T2D [87,88]. However, there were no associations between UC nor NIC and T2D in the findings of our study. Despite this, we observed a suggestive relationship between CD risk and T2D; however, this relationship was not significant after adjusting for alcohol consumption.

Causal associations between IBD and fasting glucose, fasting insulin, or HbA1c were not observed in this study. Furthermore, suggestive associations between obesity traits and UC were observed, which is consistent with previous research [89,90]. However, these associations did not persist after adjusting for appendectomy, suggesting that elevated risk of appendectomy due to higher BMI or whole-body fat mass may explain this relationship.

It is worth mentioning that some protective factors for colitis were also identified in the present MR study. Relationships between colitis, and both physical activity and breastfeeding have not been reported in previous studies. Genetically predicted breastfeeding was also associated with lower risk of UC, CD, and NIC. Lack of breastfeeding has been linked to immune-mediated illnesses and clostridium difficile colonization [91]. The protective effect might be mediated by increased innate mucosal immunity development as a result of microbiome interaction [92,93]. Physical activity may modulate colitis risk by affecting multiple pathways, including individuals' health behaviors, living environments, and lifestyles [94]. Our results also verified the causal associations between CD and cesarean delivery. Notably, the risk of CD due to genetically predicted cesarean delivery was higher than that of UC and NIC, which is consistent with previous research [23,47]. However, there was no proof of a positive connection between UC or NIC and cesarean delivery.

Furthermore, antibiotics raised the likelihood of eventual CD but not UC or NIC. In initial experiments, a dose–response association was found; therefore, IBD risk was elevated by all antibiotic classes [33]. Antibiotics can change the taxonomic richness and diversity of the human gut microbiota while impairing its metabolic condition, altering the composition of the microbiome [26,95]. Antibiotics have been observed to exacerbate the dysbiosis prevalent in CD patients, and a more changed microbiota has been detected in CD than in NIC or UC [34,96]. Isotretinoin was not linked to UC, CD, or NIC, according to the results.

There are several strengths of the attending investigation related to the data source and research design. First, the MR design promoted the computation of the causal links between heritable complex traits, which avoids the biases inherent in conventional observational epidemiological studies. We applied multiple sensitivity computations to verify the plausibility of the instrumental variable assumptions and interpreted the outcomes after viewing horizontal pleiotropy and outliers. Second, this study systematically analyzed the most extensive number of variable causal factors for IBD to date. In this regard, no MR

studies have analyzed the causal consequences of genetic liability on potential risk factors for IBD or colitis. Third, the GWAS data utilized in this analysis were primarily taken from participants of European ancestry, which can decrease the bias of population stratification. Aside from autoimmune diseases, this study avoided sample overlap between most exposure types and outcomes, thereby controlling the type 1 error to be as low as possible. Nonetheless, there are some limitations of the current study that also need to be considered. First, as in all MR studies, it is difficult to confirm a lack of bias for horizontal pleiotropy. Thus, the MR-PRESSO global analysis and MR-Egger regression were operated to detect widespread horizontal pleiotropy [29,30]. Importantly, the results of this study remained robust after the discarding of outlier variants identified using the MR-PRESSO outlier test. Second, the sample size for NIC was rather small, which could limit the power of statistics to detect true causal relationships.

5. Conclusions

In this study, an MR investigation comprehensively reveals the causal relationship between IBD and a variety of lifestyle factors, associated disorders, drug exposure, surgeries, blood markers, lipid metabolism, glucose metabolism, nutrients, and obesity. Additionally, this study lists the precise genera of variable risk factors implicated in UC, CD, NIC, or IBD pathogenesis. Our discovery may potentially provide fresh perspectives on the design of targeted IBD, UC, NIC, and CD prevention and therapy strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15051202/s1, Table S1: Variable risk factors for UC, Table S2: Variable risk factors for CD, Table S3: Variable risk factors for NIC.

Author Contributions: Conceptualization, M.J.S., R.S.P. and A.B.; methodology, M.J.S., J.L.A.-G., J.C.O.G., D.J.C. and A.B.; formal analysis, M.J.S., A.B., J.L.A.-G. and J.C.O.G.; investigation, M.J.S., R.S.P., J.L.A.-G., J.C.O.G., D.J.C., M.M., S.S.A.-R., A.B., M.J.K., A.H.A. and H.G.; writing—original draft preparation, M.J.S. and R.S.P.; writing—review and editing, A.B., M.J.K., A.H.A. and H.G.; visualization, M.J.S., R.S.P. and A.B.; project administration, A.H.A. and H.G. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: GWAS summary data of IBD, colitis, and autoimmune diseases were downloaded from UKB, SSGAC, IIBDGC, GIANT, FinnGen, GSCAN, and MRC-IEU consortium. For CKD, celiac disease, SLE, serum calcium, serum amylase, CRP, T2D, fasting glucose, HbA1c, and fasting insulin, the GWAS summary data were selected from associated GWAS studies.

Conflicts of Interest: The authors declare no conflict of interest.

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