Clinical and Experimental Gastroenterology

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HYPOTHESIS

Bowel Habits, Obesity, Intestinal Microbiota and Their Influence on Hemorrhoidal Disease: a Mendelian Randomization Study

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Purpose: Hemorrhoids (HEM) are the most common perianal disease, but current observational studies have yielded inconsistent results in investigating the risk factors. Our further exploration of the risk factors will help prevent the disease.

Patients and Methods: We conducted a two-sample bidirectional Mendelian randomization (MR) analysis using publicly available genome-wide association studies (GWAS) statistics from multiple consortia. The inverse-variance weighted (IVW) method was used for the primary analysis. We applied four complementary methods, including weighted median, weighted mode, MR-Egger regression, and Cochrane's Q value, to detect and correct the effects of horizontal pleiotropy.

Results: Genetically determined constipation (OR = 0.97, 95% CI: 0.91–1.03, P = 0.28) and diarrhea (OR = 1.00, 95% CI: 0.99–1.01, P = 0.90) did not have a causal effect on HEM but stool frequency (OR = 1.28, 95% CI: 1.05–1.55, P = 0.01), waist-to-hip ratio adjusted for BMI (OR = 1.11, 95% CI: 1.06–1.64, P = 1.59×10-5), and order Burkholderiales (OR = 1.09, 95% CI = 1.04–1.14, p = 1.63×10-4) had a causal effect on. Furthermore, we found a significant causal effect of constipation on HEM in the reverse MR analysis (OR = 1.21, 95% CI: 1.13–1.28, P = 3.72×10-9). The results of MR-Egger regression, Weighted Median, and Weighted Mode methods were consistent with those of the IVW method. Horizontal pleiotropy was unlikely to distort the causal estimates, as indicated by the sensitivity analysis.

Conclusion: Our MR analysis reveals a causal association between stool frequency and waist-to-hip ratio with HEM, despite variations in results reported by observational studies. Unexpectedly, we found a relationship between the order Burkholderiales in the gut flora and HEM, although the mechanism is unclear.

Keywords: hemorrhoids, HEM, gut microbiota, GM, constipation, diarrhea, stool frequency, waist-to-Hip ratio

Introduction

According to the 2010 data from the National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey, hemorrhoids (HEM) were the third most common outpatient gastrointestinal diagnosis, resulting in over 4 million office and emergency department visits.[1] HEM were the most common reason for a visit, surpassing colon cancer, diverticular disease, irritable bowel syndrome, and inflammatory bowel disease.[2] The most common symptoms associated with HEM include bleeding, pain, itching, and mucosal prolapse.[3] HEM are normal structures composed of clusters of vascular tissue, smooth muscle, and connective tissue. They are distributed in three columns along the anal canal.[4,5] Since Burkitt's research in the 1970s, it has been believed that HEM are caused by a low-fiber diet and constipation.[6] However, the data from the 1990s show differences in the epidemiological behavior of HEM and constipation, which raises doubts about the assumption of a causal relationship between constipation and HEM.[7] And Johanson conducted a case-control investigation on HEM. Using national VA data, the study found that the comorbidities associated with HEM were conditions related to diarrhea (such as colitis, malabsorption, intestinal bypass, and chronic pancreatitis), rather than constipation.[8] According to a 2014 study published in the JAMA journal, HEM can be caused by various factors, including straining during bowel movements, prolonged sitting on the toilet, constipation or diarrhea,

Received: 30 November 2023
Accepted: 12 April 2024
Published: 10 May 2024

being overweight, pregnancy, and the natural weakening of tissues with age.[9] In recent years, the search for the risk factors of HEM has been distinguished by inconsistent results from several observational and prospective cohort studies.[10,11] The risk factors for HEM have not been thoroughly researched. Further research is necessary before providing evidence-based recommendations to patients.

The gut microbiome has been the focus of research in recent years, findings from animal models have revealed diverse and context-specific roles of the gut microbiota in health and disease, ranging from protective to pro-inflammatory actions.[12] A consensus exists that multiple digestive system diseases are associated with compositional and metabolic changes in the intestinal microbiota (dysbiosis), such as inflammatory bowel disease and colorectal cancer.[13,14] However, the causal effect of gut microbiota on HEM is unknown.

Mendelian randomization is a method used to determine if there is a consistent relationship between a risk factor and an outcome by analyzing genetic variants.[15] Individuals have the option of inheriting a genetic variant that affects a risk factor or not inheriting it.[15] Because these genetic variants are often unassociated with confounding factors, differences in outcomes between individuals who carry the variant and those who do not can be attributed to the difference in the risk factor.[15] Here, we included four predominant risk factors (Figure 1), including both definite and controversial ones, and the summary statistics from the genome-wide association study (GWAS) MiBioGen to investigate the causal relationship between these risk factors and HEM using Mendelian randomization (MR).

Method

There are Three Major Assumptions in MR Analysis (Figure 2)

The first assumption is that the genetic variants used as instrumental variables should have a strong association with the exposure. The second assumption is that these genetic variants should not be associated with any confounding factors. The third assumption is that the selected genetic variants should only affect the risk of the outcome solely through the risk factors and not through alternative pathways.[16]

To infer the direction of the causal relationship between the risk factors and HEM, bidirectional and multivariable two-sample MR analyses were performed. First, we performed univariable MR (UVMR) analyses to determine the causality between the risk factors and HEM in the forward direction. Second, we proceeded to identify the causality between HEM and the risk factors in the reverse direction. In addition, we conducted multivariable MR (MVMR) analyses on positive results to estimate whether they are associated with HEM independently.

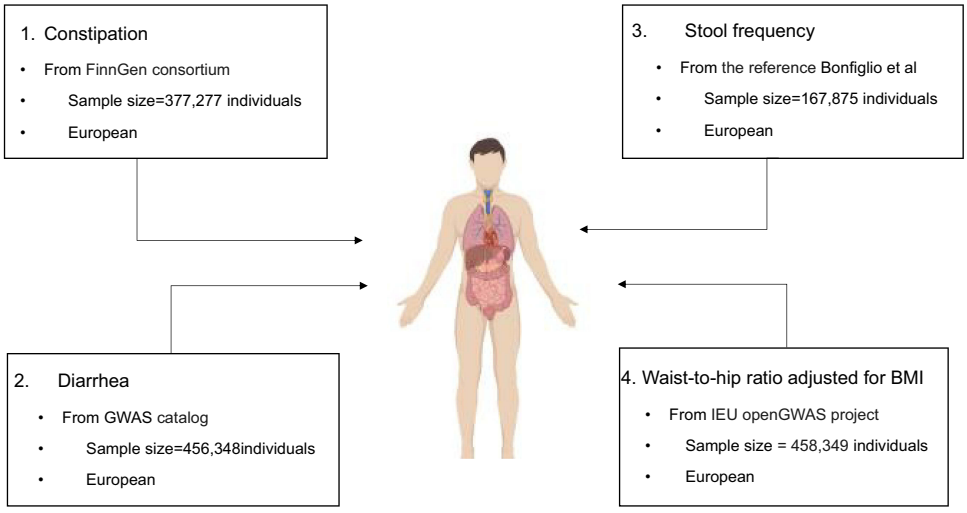

Figure 1 List of risk factors for Hemorrhoidal disease.

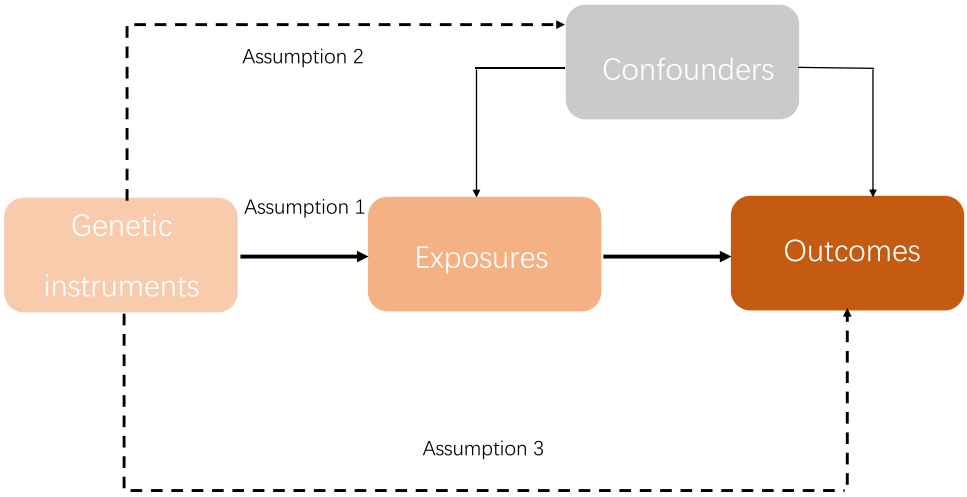

Figure 2 Study design overview.

Data Source

We selected the SNPs intensely related to exposures at the genome-wide significance level ($p < 5\times10^{-8}$, clumping window > 10,000 kb, and the linkage disequilibrium level (r2 < 0.001). Genetic instruments for constipation were obtained from the FinnGen consortium R9 release data (36,022 cases and 341,255 controls. Genetic instruments for diarrhea were directly downloaded from the GWAS Catalog. The analysis included 356,348 patients of European ancestry. Bonfiglio et al investigate the genetics of human gut motility using genotype and questionnaire data on stool frequency in 167,875 individuals from the UK Biobank and four other cohorts.[17] They identified 14 loci associated with stool frequency. Genetic instruments for waist-to-hip ratio adjusted for BMI were identified from the IEU Open GWAS project, including 458,349 European patients. Zheng et al reported the first genome-wide association study (GWAS) to identify genetic risk factors for HEM. Genetic variants for investigating the causal effects of HEM (Ncase=218,920, Ncontrol=725,213) were screened from the GWAS summary statistics of individuals of European descent.[18] All GWAS were adjusted for age, sex, and population structure.

SNPs related to the composition of the human gut microbiome were chosen as instrumental variables (IVs) from a GWAS dataset provided by the international consortium MiBioGen.[19] This was a multi-ethnic large-scale GWAS that coordinated 16S ribosomal RNA gene sequencing profiles and genotyping data from 18,340 participants from 24 cohorts from the USA, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the UK to explore the association between autosomal human genetic variants and the gut microbiome. A total of 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) were included.

MR Statistical Analyses and Sensitivity Diagnostics

The primary statistical model utilized was the inverse-variance weighted (IVW) method. We used the inverse variance weighted (IVW) method with random effects to estimate the relationships between exposures and outcomes. Additionally, weighted-mode and weighted-median methods were used as supplements to the IVW method. We used the MR Steiger directionality test[20] to examine whether exposure had a directional causal effect on the outcome. The Cochrane's Q value was used to assess heterogeneity. The MR-Egger regression can detect and correct for possible pleiotropy, and the fact that the P value of the intercept is > 0.05 indicates the absence of horizontal pleiotropy.[21] All statistical analyses and data visualization were conducted using R software version 3.4.0 (https://www.r-project.org/).

Ethics Approval

All studies included in the GWAS cited here were approved by a relevant review board and our study have been approved by the local ethics committee (Ethics Review Committee of Xinhua Hospital affiliated to Dalian University).

Result

The Mr Estimates from Various Methods of Assessing the Causal Effect of the Factors on Hem Are Presented in Table 1 andFigure 3

Genetically determined constipation (OR = 0.97, 95% CI: 0.91–1.03, P = 0.28) and diarrhea (OR = 1.00, 95% CI: 0.99–1.01, P = 0.90) did not have causal effects on HEM. Genetically predicted stool frequency (OR = 1.28, 95% CI: 1.05–1.55, P = 0.01) and waist-to-hip ratio adjusted for BMI (OR =1.11, 95% CI: 1.06–1.64, P = 1.56×10-5) have causal effects on HEM. The results were identified with a mixed population from the study of Lindsay Fernández-Rhodes about the latest genetic instruments on waist-to-hip ratio adjusted for BMI (OR =1.16, 95% CI: 1.07–1.24, P = 1.165×10-4) (Figure 4). [22] Furthermore, we found a significant causal effect of constipation on HEM in the reverse MR analysis (OR = 1.21, 95% CI: 1.13–1.28, P =3.725×10-9) (Figure 5). Based on the FDR test, we discovered that the order Burkholderiales was causally associated with HEM (OR = 1.09, 95% CI = 1.04–1.14, p = 1.63×10-4) (Figure 6). Besides, the weighted-mode and weighted-median techniques produced causal estimates that were identical in magnitude and direction. Subsequently, the low intercepts from the MR-Egger regression indicated that directional genetic pleiotropy was unlikely to introduce bias in the results. In addition, the Cochrane Q statistics revealed no significant heterogeneity ($p > 0.05$).

However, there were heterogeneity and outliers in the waist-to-hip ratio adjusted for BMI. We conducted MR-PRESSO-corrected analysis when outliers were detected. Although there was horizontal pleiotropy, the results from MR-Egger indicated that the causal link still exists (MR-Egger p =0.00011/0.000367).

Notably, after conducting multivariable MR (MVMR) analyses on positive results, we discovered that stool frequency and the order Burkholderiales remained significantly and independently causally related. However, it should be noted that the waist-to-hip ratio adjusted for BMI was corrected for the fact that it may have a weaker direct effect on HEM compared to other risk factors. (Table 2)

Discussion

To the best of our knowledge, this is the first MR study to investigate whether the risk factors and gut microbiota are causally associated with HEM. The current study found that, instead of constipation or diarrhea, HEM are associated with frequent bowel movements and the waist-to-hip ratio. Furthermore, based on comprehensive genetic data from over 450,000 European individuals, we discovered genetic liability to some gut microbiota causally associated with HEM. Surprisingly, the genetic liability to the order Burkholderiales was causally associated with HEM.

The central role of multivariate Mendelian randomization is to analyze the respective effects of multiple exposures contained in the total population. Our results show that significantly positive risk factors according to the UVMR study

Table 1 Mendelian Randomization of Estimates of the Associations Between the Exposures and Hemorrhoidal Disease

EXPOSURES	IVW Method		Weighted Median		Weighted Mode		Cochrane's Qvalue	MR-Egger Intercept
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	Q-pval	P-value
1. Constipation	0.97 (0.91,1.03)	0.28	0.91 (0.85,0.99)	0.04	0.90 (0.82,1.00)	0.09	0.32	0.34
2. Stool frequency	1.28 (1.05,1.55)	0.01	1.24 (1.00,1.53)	0.05	1.31 (0.98,1.76)	0.11	0.10	0.47
3. Diarrhea	1.00 (0.99,1.01)	0.90	1.00 (0.98,1.00)	0.55	1.00 (0.98,1.00)	0.60	0.16	0.58
4.Waist-to-hip ratio Adjusted for BMI	1.11 (1.06,1.64)	1.59×10-5	1.10 (1.04,1.16)	0.00006	1.15 (1.02,1.31)	0.028	5.93×10-27	0.28

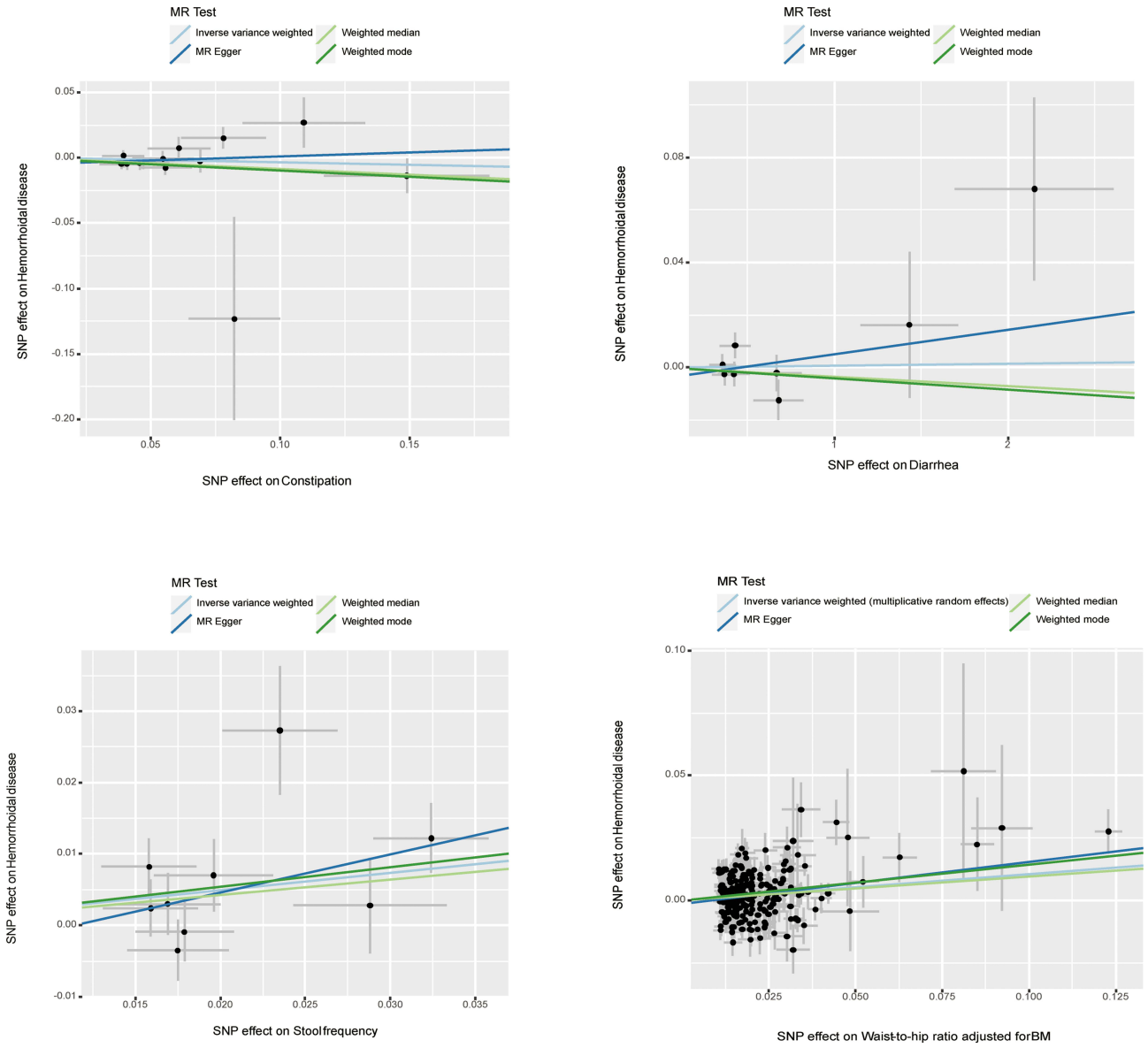

Figure 3 The results of MR.

Exposure	No.of SNP	Method	OR(95% CI)	or	P
Waist−to−hip ratio adjusted for BMI	46	IVW	1.16 (1.07 to 1.24)	1.155550	0.000
		MR Egger	1.21 (0.90 to 1.62)	1.206038	0.220
		Weighted median	1.17 (1.08 to 1.27)	1.173321	0.000
		Weighted mode	1.18 (1.01 to 1.36)	1.175306	0.039

Figure 4 The results of mixed population.

do not show the same proportion of correlation when the MVMR analysis is included. In the work of Stephen Burgess, they introduced multivariable Mendelian randomization, an important and practically relevant extension of the Mendelian randomization paradigm for estimating causal effects using genetic variants associated with more than one risk factor.[23] For a valid analysis, the variants must meet a set of assumptions similar to those for an instrumental variable in conventional Mendelian randomization, but modified to account for the multiple risk factors. A multivariable analysis of Mendelian randomization may be advantageous when genetic variants are associated with multiple related risk factors.

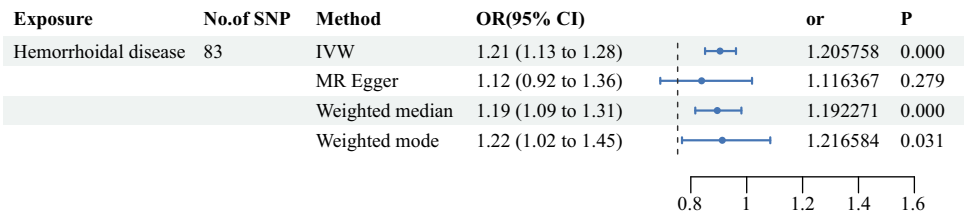

Figure 5 The result of the reverse MR analysis.

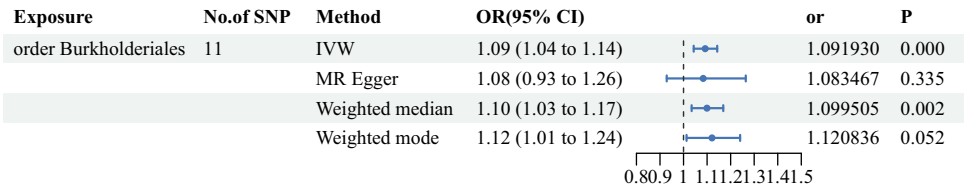

Figure 6 Mendelian randomization of estimates of the associations between the order Burkholderiales and Hemorrhoidal disease.

This indicates that different risk factors have varying degrees of influence on the occurrence of diseases. For the occurrence of hemorrhoidal disease, stool frequency and the order Burkholderiales have a higher degree of influence. After MVMR analysis, stool frequency and the order Burkholderiales remained significantly positive, which further supports our findings.

HEM are collections of submucosal, fibrovascular, and arteriovenous sinusoids that are a normal part of the anorectum. These "vascular cushions" support anal closure, facilitate continence, and protect the anal sphincter from injury during defecation.[22] The increased frequency of defecation is the cause of these injuries, rather than hard, dry feces or irregular stools. HEM can result from straining during bowel movements because they can disrupt the stromal scaffolding, increase the vascular component, elevate anal pressure, and cause rectal redundancy.[24] HEM can cause pain, incomplete bowel movements, and a sensation of anorectal obstruction. The initial difficulty with bowel movements caused by HEM, which leads to fear and reluctance to use the toilet or bathroom, often progresses into a pattern of increasing fecal retention as the rectum enlarges and the frequent passage of large, hard stools becomes common.[25]

This study shows that individuals with a larger waist-to-hip ratio are at an increased risk of developing HEM. The findings of the MR study are consistent with the findings of Kibret's investigation.[26] Body mass index (BMI) has traditionally been the most extensively used metric for determining the prevalence of obesity. However, Haufs argues that the waist-to-hip ratio is more appropriate than BMI for diagnosing obesity.[27] Ayush Giri et al discovered that obesity, especially central obesity, leads to a decrease in pelvic floor muscle strength, resulting in pelvic organ prolapse. It can be concluded that there is a causal link between obesity and hemorrhoidal prolapse.[28]

Since the late 1970s, the order Burkholderiales has become more prevalent in clinical settings.[29] It is particularly dangerous for patients in intensive care units and those with chronic pulmonary issues.[30] Although our findings show that the incidence of HEM increases with an increasing intestinal order Burkholderiales, the specific mechanism remains unknown. A mechanistic examination of our findings is required for further exploration.

Table 2 The Results of the Multivariable MR Analysis

	UVMR	MVMR
EXPOSURES	IVW- P-vALUE	IVW- P-vALUE
STOOL FREQUENCY	0.014	0.017
WAIST-TO-HIP RATIO ADJUSTED FOR BMI	1.59×10-5	0.74
ORDER BURKHOLDERIALES	1.64×10-3	1.03×10-3

A GWAS is unlikely to explain all the heritability of complex traits.[31] Given that linkage disequilibrium patterns differ among ethnic groups, it was previously unsuitable for non-European populations.[32] This challenge could potentially be addressed by utilizing the latest high-density arrays and by collecting more sequencing data from a wider range of populations.[33] Although causal relationships between variants and diseases can be found, identifying causal variations among multiple variants on the same haplotype is difficult.[34]

Both the environment, lifestyle, and genes can impact disease symptoms. Some lifestyles have been identified as contributing to an increased incidence of hemorrhoids: long-term alcohol consumption, a preference for spicy and stimulating foods, too much protein intake, and too little fiber intake.[35] In addition, the GWAS data on lifestyles is not yet perfect. In future studies, we will include more genetic instruments to improve the risk factors for hemorrhoids.

We obtained reliable results using a variety of statistical methods. Nonetheless, there were several limitations to our investigation. First, while the majority of patients in the GWAS summary data utilized in our analysis were of European descent, only a limited amount of gut microbiota data and SNPs were collected from individuals of other races. This limited representation may result in biased estimations and impact the generalizability of our findings. In addition, one major limitation is the potential for horizontal pleiotropy, which suggests that genetic instruments may increase the risk of HEM through pathways other than the ones we examined. Because we do not yet know the biological action of these SNPs, it is impossible to entirely rule out pleiotropic mechanisms without extensive functional follow-up of these loci. Although we performed the most recent array of sensitivity studies to rule out horizontal pleiotropy,[36] it is still necessary to conduct further investigations to fully understand the potential pleiotropic effects. Last but not least, we selected the genetic tool for HEM from the Zheng et al paper, which did not provide clear diagnostic criteria or grading for the condition.

Conclusion

This MR investigation offers genetic support for the causal relationships between HEM and the order Burkholderiales, waist-to-hip ratio, and stool frequency. Reducing these important risk factors could be a method of preventing HEM. We should raise awareness of HEM and encourage functional exercises, such as Kegel exercises, in populations that are at risk due to frequent bowel movements, obesity, and an increased presence of Burkholderiales in the intestinal flora.

Acknowledgments and Data Sharing Statement

The authors thank all investigators and participants for sharing genetic association estimates. The datasets generated and analyzed during the current study are available in the internet repository. The website addresses are as follows: IEU (https://gwas.mrcieu.ac.uk/);Finn Gen (https://www.finngen.fi/en);GWAS Catalog (https://www.ebi.ac.uk/gwas/);UK biobank (http://www.nealelab.is/uk-biobank);MiBioGen repository (https://mibiogen.gcc.rug.nl/);Original GWAS articles of Bonfiglio et al and Zheng et al reported.

Disclosure

The authors report no conflicts of interest in this work.

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