

Relationship Between Gut Microbiota and Intussusception: An Analysis Based on Mendelian Randomization

Cheng Feng, Luo Chun*, Shang KeLei, Tan XiaoGuang

ABSTRACT

Objective: To explore the relationship between intestinal flora and intussusception based on Mendelian randomization.

Methods: Genetic data of 211 intestinal microbiota collected by the MiBioGen consortium were used as instrumental variables, and data from a GWAS on intestinal condyloma susceptibility genes from FinnGen were used as outcome variables, which were analyzed by inverse variance weighting (IVW), MR-Egger, Weighted median, Weighted mode, and MR-PRESSO were used to analyze the causal relationship between intestinal flora and intussusception.

Results: The inverse variance weighted results showed that Bifidobacterium was inversely associated with the occurrence of intussusception (OR 0.376, 95% CI: 0.157-0.899). In addition, Ruminococcustorques group (OR 1.461, 95% CI: 1.058-3.807), Adlercreutzia (OR 1.893, 95% CI: 1.318-4.502), Allisonella (OR 1.715, 95% CI: 1.009-2.915), and Clostridiumsensustricto1 (OR 1.974, 95% CI: 1.046-4.451) suggested a positive association with the development of intussusception.

Conclusion: This two-sample Mendelian randomization study found that Bifidobacterium reduces the risk of intussusception and postoperative recurrent intussusception. Further randomized controlled trials are needed to elucidate the protective effects of probiotics on the development of intussusception and its recurrence, as well as their specific protective mechanisms.

INTRODUCTION

Intussusception is a prevalent acute abdominal disease in children, characterized by the telescoping of one part of the intestine into another. The primary form of intussusception can usually be successfully treated through non-invasive methods such as air or water enema. However, it is concerning that the disease tends to recur after surgery, leaving those with recurrent intussusception with no alternative but to undergo further surgical intervention. Despite its clinical significance, the specific underlying cause of intussusception remains unclear Sarin et al. (2023). This knowledge gap highlights the need for extensive research efforts to uncover the etiology of this disease. Investigating the causes is of utmost importance to not only improve the prevention and treatment strategies for intussusception but also to address its recurrence. The intestinal flora, comprising the vast array of microorganisms residing in the gut, plays a crucial role in the growth, development, and overall health of children. Numerous observational studies have already demonstrated alterations in the

structure and diversity of the intestinal flora following an episode of intussusception. However, it is worth noting that the evidence from randomized controlled trials (RCTs) examining the relationship between intussusception and intestinal flora is currently limited and requires further evaluation Saeed et al (2022), Ididrisu et al (2021). Moreover, conducting randomized controlled studies involving intestinal flora analysis poses additional challenges. Factors such as diet, mood, and lifestyle habits greatly influence the composition and activity of gut microbiota. Therefore, the implications of specific populations of gut flora in the development of intussusception and recurrent intussusception post-treatment remain ambiguous. As a result, it is imperative to investigate the causal effect of gut microbiota on intussusception and ascertain the microbial populations that are associated with this condition. Such understanding will pave the way for targeted interventions and more effective management of intussusception and its recurrence. Due to constraints in medical ethics and the high costs involved, certain

Fuyang Women and Children's Hospital, China.

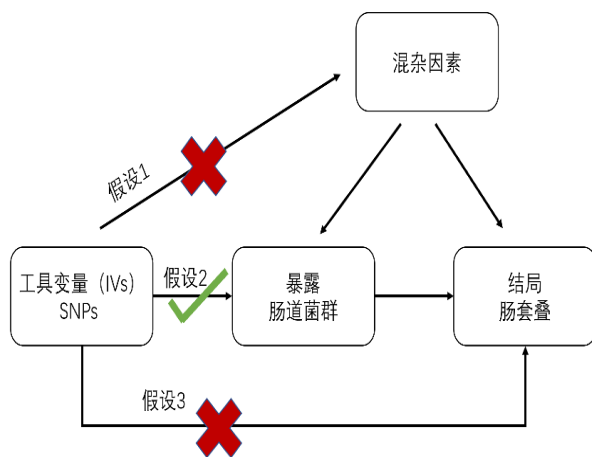
Correspondence to: Luo Chun, Fuyang Women and Children's Hospital, China.

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randomized controlled trials (RCT) are challenging to conduct in actual clinical practice. As a result, Mendelian randomization (MR) has emerged as a solution to infer the epidemiological causes of diseases Birney et al (2022), Zuber et al (2022). MR is a novel method used to investigate the causal relationship between intestinal flora and intestinal condylomata. It serves as a crucial approach for establishing the cause-and-effect link between exposures and disease outcomes by randomly assigning genes, thus allowing for the observation of phenotypic changes. Compared to randomized controlled studies, MR studies assess causality with regard to the outcomes by utilizing genetic variation instead of exposure Burgess et al (2023). This approach helps to minimize the potential influence of confounding factors, such as dietary habits. By examining phenotypic changes through the random assignment of genes, MR has become a central tool in determining the causal relationship between exposure and disease outcome. MR has gained widespread popularity for exploring the causal relationships between gut microbiota and various diseases, encompassing a range of metabolic and autoimmune conditions.

This method enables researchers to investigate the intricate connections between gut microbiota and disease development, providing valuable insights into the underlying mechanisms and potential therapeutic interventions XU et al (2022).

Figure 1: MR analysis model



In this study, the intestinal microbiota was selected as the exposure and intussusception as the result of MR analysis to explore the causal relationship and to provide a theoretical basis for further research on the mechanism of intussusception. In addition, by identifying the relationship between specific microorganisms and patients with intussusception, new biomarkers as well as diagnostic and therapeutic strategies can be provided.

MATERIALS AND METHODS

Methods

Mendelian randomization analysis was performed using intestinal flora as exposure, single nucleotide polymorphisms (SNPs) significantly associated with intestinal flora as instrumental variables (IVs), and intussusception as an outcome variable. The causal relationship between intestinal flora and intussusception was examined by inverse variance weighting (IVW), MR-Egger, weighted median, weighted mode, and MR-PRESSO methods.

Data sources and screening of instrumental variables

In this study, a two-sample MR analysis was performed using genome-wide association study (GWAS) summary statistics from the MiBioGen and FinnGen consortiums to assess the causal relationship between the gut microbiota and intestinal condylomata. The summary gut microbiota in the meta-analyses of genome-wide association studies (GWAS) conducted by the MiBioGen consortium (n = 13, 266) statistics included a total of 18,340 individuals from 24 cohorts, targeting the V3-V4 and V1-V2 variable regions of the 16S rRNA gene to categorize microbial composition into 211 genus-level taxa Kurilshikov et al (2021). Summary statistics for intussusception were obtained from GWAS data on intussusception susceptibility genes (232 cases and 182,423 controls) from the FinnGen consortium (<https://r5.finnngen.fi/>), with a total of 16,380,364 SNPs. The GWAS database (<https://www.ebi.ac.uk/gwas/>) was ethically approved and consented, and the pooled data are publicly available.

The selection criteria for instrumental variables (IVs) were as follows: (1) SNPs associated with each genus were selected as potential IVs at a gene-wide significance threshold ($P < 1.0 \times 10^{-5}$); (2) using data from the European samples of the 1000 Genomes Project as a reference panel, linkage disequilibrium (LD) between SNPs was calculated, and among those with an $R^2 < 0.001$ only those with the P-value were retained lowest SNPs;

Data analysis methods

All statistical analyses were performed using R 4.2.1 software. MR analysis was performed using the TwosampleMR, MR-PRESSO package.

The results of the MR analyses were examined one by one for the effect of the instrumental variables on the outcome using the leave-one-out method, and the reliability of the results and the presence of pleiotropy were analyzed using the MR-Egger regression analysis and the MR-PRESSO regression analysis.

RESULTS

Results of MR analysis of intestinal flora on intussusception

The final screening according to the criteria for selection of instrumental variables yielded 3631 instrumental variables from 211 bacterial groups, which were included in the analysis. A total of five intestinal bacteria were detected to be associated with intussusception by the IVW method, of which Bifidobacteriaceae were inversely correlated with the occurrence of intussusception (OR 0.376, 95% CI: 0.157-0.899), and Ruminococcustorques group (OR 1.461, 95% CI: 1.058-3.807), Adlercreutzia (OR 1.893, 95% CI: 1.318-4.502), Allisonella (OR 1.715, 95% CI: 1.009-2.915), and Clostridiumsensustricto1 (OR 1.974,

95% CI: 1.046-4.451) were positively associated with the development of intestinal volvulus (Figure 2, Table 1).

Reliability evaluation

MR Egger regression analysis was used to detect gene pleiotropy, and the results showed that all intercepts were close to zero ($P > 0.05$), and the results of the MR-PRESSO regression analysis were consistent with the MR Egger regression ($P > 0.05$), indicating that there was no gene pleiotropy in this result. The leave-one-out method was used to analyze the results of the IVW method, which showed that the results were consistent with the IVW method after removing each SNP individually, indicating that no single SNP affected the causal estimation results (Figures 3 and 4).

Table 1: Results of MR analysis in the sample

	Outcome	Method	SNP	β	SE	Oval	P_{PRESS}
Bifidobacteriaceae	intussusception	MR Egger	11	-0.171	1.448	0.909	0.783
		Weighted median	11	-0.833	0.629	0.185	
		IVW	11	-0.978	0.445	0.028	
		Weighted mode	11	-0.824	0.769	0.309	
Adlercreutzia	intussusception	MR Egger	8	0.338	2.58	0.9	0.253
		Weighted median	8	0.496	0.684	0.468	
		IVW	8	1.359	0.553	0.014	
		Weighted mode	8	0.212	1.114	0.855	
Allisonella	intussusception	MR Egger	8	1.125	1.83	0.561	0.7
		Weighted median	8	0.561	0.342	0.101	
		IVW	8	0.539	0.271	0.046	
		Weighted mode	8	0.589	0.532	0.305	
Clostridiumsensustricto1	intussusception	MR Egger	7	-0.771	1.398	0.605	0.757
		Weighted median	7	0.864	0.699	0.216	
		IVW	7	1.09	0.533	0.041	
		Weighted mode	7	0.65	0.918	0.506	
Ruminococcustorques group	intussusception	MR Egger	7	4.801	3.212	0.195	0.594
		Weighted median	7	1.403	0.989	0.156	
		IVW	7	1.495	0.734	0.042	
		Weighted mode	7	2.343	1.526	0.176	

Figure 2: Forest plot of a causal association between intestinal flora and intussusception

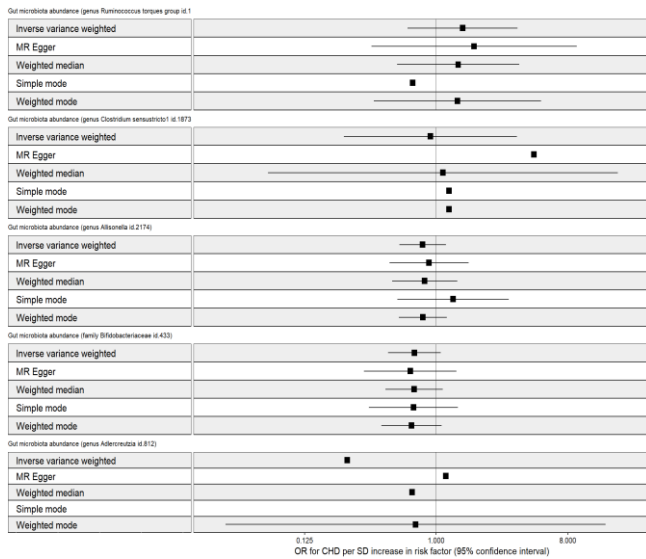


Figure 3: Scatterplot of the relationship between intestinal flora and intussusception analyzed by different MR methods

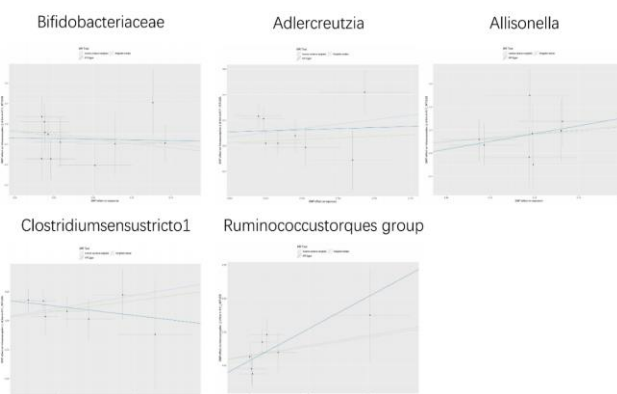
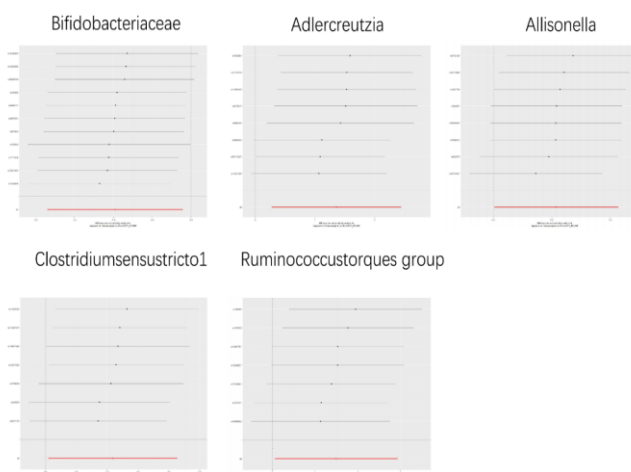


Figure 4: Results of the "leave-one-out" sensitivity analysis of the causal relationship between intestinal flora and intussusception



DISCUSSION

It is currently believed that the development of intussusception is closely associated with intestinal infections and intestinal motility disorders. A large number of observational studies have found that intestinal bacterial infections (*Salmonella*, *Escherichia coli*, *Shigella*, etc.) are significantly associated with the development of intussusception, which often occurs within one month after infection Nylund et al (2010), Jain et al (2023), Wu et al (2019). In this study, MR analysis based on the GWAS database showed that intestinal microorganisms play an important role in the progression of intestinal volvulus, and rumenococci, *Adenococcus aureus*, *Alcaligenes*, and *Clostridium difficile* increase the risk of intestinal volvulus, and *Bifidobacterium bifidum* reduces the risk of intestinal volvulus and recurrent volvulus.

The gut is the largest immune organ in the human body, and the intestinal microbiota not only plays an important role in inflammatory diseases in the gut but is also closely related to immune responses. As the "second human genome", the intestinal microbiota can participate in the regulation of multiple metabolic pathways, such as human gene expression, nutritional development, and adaptive immunity Kc et al (2020). The balance of intestinal flora plays an indispensable role in regulating the development of the host immune system and maintaining normal immune function of the body Zheng et al (2020). *Bifidobacterium bifidum* is the most common probiotic, and intake of probiotics can effectively restore the balance of intestinal microbiota and improve gastrointestinal function. A large number of studies have found that bifidobacteria have a protective effect on intussusception, which is consistent with the analysis results of this study. 1. Anti-inflammatory effect: bifidobacteria have an anti-inflammatory effect, which can reduce intestinal inflammation. Intussusception is usually accompanied by intestinal inflammation, and the anti-inflammatory properties of *Bifidobacterium* may help to reduce the inflammatory response and thus have a protective effect on intussusception. 2. Regulation of the immune system: *Bifidobacterium* can regulate the function of the intestinal immune system, enhance the activity of the immune cells, increase the immune response, maintain the intestinal immune balance, and reduce the incidence of intussusception Gao et al (2018), De et al (2022). 3. Protecting the intestinal mucosal barrier: *Bifidobacterium bifidum* increases the production of the colonic luminal metabolite short-chain fatty acids (SCFA) and modulates inflammation in mice and humans. The transcription factor aryl hydrocarbon receptor (AHR) in enteric neural circuits is a cytoplasmic receptor and transcription factor that is activated primarily through homologous ligand binding. It is an important factor in immune and tissue homeostasis, which is closely related to the intestinal microenvironment. Studies have shown that intestinal

microorganisms can bind to AHR through metabolites to maintain intestinal epithelial barrier function and microbial diversity Shinde et al (2018), which helps to maintain the integrity of the intestinal mucosa and reduces the incidence of intestinal intussusception.4. Promote intestinal peristalsis: Bifidobacteria can regulate the signaling pathways in the enteric neurons to improve intestinal microecology Promote intestinal peristalsis and improve the frequency and strength of intestinal motility to reduce the risk of intussusception Ding et al (2016), Obata et al (2020). In addition, this study found that rumenococci, Adler's Crohnobacterium, Garlicobacterium, and Clostridium were positively associated with the development of intussusception, which suggests that the maintenance of intestinal function depends on the balance of pathogenic and probiotic bacteria and that an increase in the abundance of harmful bacteria, such as Clostridium, leads to the development of the disease Allam-Ndoul et al (2020)], while there are no relevant and targeted studies on the other bacterial flora.

In this study, several methods were employed to ensure the reliability of the results. Firstly, confounding factors were carefully excluded through Mendelian randomization (MR) analysis. This approach allowed us to establish a causal relationship between intestinal trocars and the composition of the intestinal flora. Additionally, bias was taken into account and minimized by using MR-PRESSO and MR-Egger regression techniques. As a result of this rigorous analysis, we were able to identify specific bacteria species that can serve as novel biomarkers for intestinal trocars. These biomarkers, namely Bifidobacterium and rumenococci, Adler Croceae, Alcaligenes, and Clostridium, hold the potential to revolutionize the prevention and management of intestinal trocars. By detecting these bacteria in patients, we may be able to predict the occurrence of intestinal trocars and their postoperative recurrence, allowing for early interventions and improved outcomes. However, it is important to acknowledge that there are limitations to our study. The majority of the data on intestinal flora used in our analysis came from European populations. This raises the possibility of ethnographic differences that could influence the relationship between intestinal trocars and the identified biomarkers. Therefore, for future research, it would be valuable to gather data from diverse populations to further validate and refine our findings on a larger scale. This would ensure that our conclusions can be more widely applicable and relevant across different ethnic backgrounds.

DECLARATIONS

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Authors Contributions

All authors actively contributed to the conceptual development of this article.

Conflicts of interest

We report no conflicts of interest.

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