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The Causal Effects of Appendicectomy on Immune Bowel Disease and Digestive Cancers: A Two-sample Mendelian Randomization Study

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ABSTRACT

Background: Previous observational studies have reported that appendicectomy is associated with IBD and digestive cancers. Using a two-sample mendelian randomization (MR) analysis, we aimed to investigate whether appendicectomy is causally associated with IBD and digestive cancers.

Methods: The instrumental variables (IVs) were obtained from public genome-wide association studies (GWAS) data. We used the inverse-variance weighted (IVW) method as the primary statistical method complemented with weighted median and MR-Egger approaches.

Results: The IVW method revealed that genetically determined appendicectomy had a causal effect on pancreatic cancer (OR 202.61; 95% CI 1.39, 29563.10; P = 0.037), but did not have causal effects on IBD (P > 0.05) and other digestive cancers (P > 0.05).

Conclusion: This study revealed that genetically determined appendicectomy had a causal effect on pancreatic cancer and patients with appendectomy should be screened for pancreatic cancer.

INTRODUCTION

The appendix was originally regarded as a dispensable organ which located at the end of the cecum. Since then, appendicectomy has been widely adopted as the predominant treatment of appendiceal diseases, especially in acute appendicitis Snyder et al. (2018). In some special cases, prophylactic appendicectomy is performed to eliminate the potential for future appendicitis Koc et al. (2020), Choksuwattanasakul et al. (2017), Thakkar et al. (2019). A systematic review showed that appendicectomy had a low prevalence of long-term surgical complications Rasmussen et al. (2018).

However, there are new insights into the function of the appendix recently. Studies have found that the appendix contains a large amount of lymphoid tissue with macrophages, B lymphocytes, and T lymphocytes, which plays a crucial role in mucosal immunity and microbial ecology in the intestines Koc et al. (2020), Sehgal et al. (2002), Liu et al. (2017). Several scholars believe that appendicectomy raise the risk of inflammatory bowel diseases (IBD) and digestive cancers because of the change of intestinal environment Babakhanov et al. (2021), but the evidences to date show that the effects of appendicectomy on IBD and digestive cancers are still highly controversial, and notably, these data are largely from observational studies, which are usually susceptible to residual confounding bias and reverse causation Thiese et al. (2014). Therefore, new approaches to research are needed to confirm these associations.

Mendelian randomization (MR) analysis, which overcomes the bias due to confounder and reverse causation mentioned above, has been widely used for etiological inference in epidemiology by using genetic variants as instrumental variables for assessing causal relationships from observational data Sekula et al. (2016).

Here, we performed a two-sample MR analysis to investigate the causal effects of appendicectomy on IBD and digestive cancers.

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MATERIALS AND METHODS

In a two-sample MR, single-nucleotide polymorphisms (SNPs) were chosen as instrumental variables, which follow three key assumptions: firstly, instrumental variables are associated with the exposure; secondly, instrumental variables are independent of all confounders; and thirdly, instrumental variables affect the outcome only via the exposure Davies et al. (2018). (Figure 1).

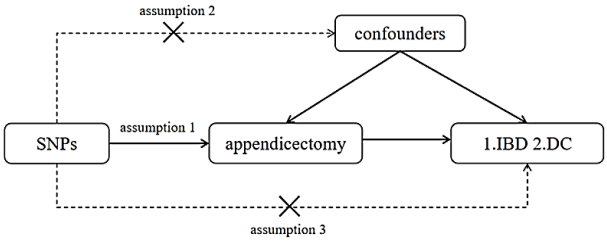

Figure 1
Plot of key assumptions for MR analysis. SNPs, single-nucleotide polymorphisms; IBD, inflammatory bowel disease; DC, digestive cancers.

Data sources

All the data were extracted from the public domain and thus no ethical approval was required for this study. The IEU openGWAS database (https://gwas.mrcieu.ac.uk/datasets/) was used to identify genetic risk variants for appendicectomy, which was accurately defined as "Operation code: appendicectomy". Similarly, the FinnGen data release 9 (https://r9.finngen.fi/) was used to obtain summary-level data including crohn's disease, ulcerative colitis, esophagus cancer, gastric cancer, pancreatic cancer, liver cancer, biliary tract cancer and colorectal cancer. The diagnosis of crohn's disease and ulcerative colitis was according to the ICD-10 (International Classification of Diseases) criteria, while the diagnosis of digestive cancers was according to the ICD-O-3 (International Classification of Disease for Oncology) criteria. All data were obtained for European ancestry to reduce the resulting bias caused by ethnically related confounding factors (Table 1).

SNPs Selection

The SNPs were filtered according to the following criteria. Firstly, a genome-wide significance level of $p < 5 \cdot 10\text{-}8$ and a clumping algorithm with a cutoff of r2 = 0.001 and kb = 10000 were used to avoid linkage disequilibrium (LD). Secondly, we measured instrument strength of each SNPs using F statistic, and the SNPs would be removed if its F statistic was less than 10(13). Thirdly, we harmonized the exposure and outcome data to ensure alleles were aligned, with ambiguous or palindromic SNPs being removed. After these filtering steps, the rigorously selected SNPs were used as the final instrumental SNPs for the subsequent MR analysis.

Statistical Analysis

In this study, we used the inverse variance-weighted (IVW) method, including the fixed-effects and the random-effects versions, as main analysis method, which provides accurate estimates when there is no horizontal pleiotropy between the exposure and outcome variable Burgess et al. (2017), and we used the weighted median and MR-Egger methods for complement IVW estimates Bowden et al. (2016). In addition, we used the Cochran's Q test to test for the heterogeneity of selected SNPs and the intercept of MR-Egger regression to test for horizontal pleiotropy, and if a P-value less than 0.05 was considered heterogeneous, a random-effect model was applied for subsequent analyses, otherwise a fixed-effect model was used Verbanck et al. (2018). We also performed a leave-one-out sensitivity analysis to assess the impact of each SNP Zheng et al. (2017).

All analyses were performed using the packages Two Sample MR (version 0.5.7) in R (version 4.3.1; http://www.rproject.org) packages, and p values were 2-sided, and evidence of association was declared at $p < 0.05$.

RESULTS

In total, we choosed 9 SNPs as genetic instrumental variables for appendicectomy (Supplementary Table S1). The IVW method was our primary MR method in the absence of horizontal pleiotropy, and we found a causal effect of appendicectomy on pancreatic cancer (OR 202.61; 95% CI 1.39, 29563.10; P = 0.037), but not found causal effects of appendicectomy on IBD (P > 0.05) and other digestive cancers (P > 0.05) (Table 2). Additionally, only the weighted median method show a causal effect of appendicectomy on crohn's disease (OR 635.98; 95% CI 1.32, 305379.40; P = 0.040) in the complemented MR methods (Supplementary Table S2). The leave-one-out sensitivity analysis demonstrated that only a few single SNPs may driven the results (Supplementary Figures S1–S8b).

DISCUSSION

In our study, we had found that genetically determined appendicectomy had a causal effect on pancreatic cancer, but not on IBD and other digestive cancers. In recent years, it has been believed that the appendix has abundant gut-associated lymphoid tissue producing a large number of SIgA Vitetta et al. (2019), Mörbe et al. (2021), Masahata et al. (2014), which is the major immunoglobulin of the

Table1: Characteristics of exposure dataset and outcome datasets.

Phenotype	Consortium	Year	Cases	Controls	Population
Exposure appendicectomy Outcome	MRC-IEU	2018	55398	407535	European
Crohn's disease	FinnGen	2023	1665	375445	European
Ulcerative colitis	FinnGen	2023	5034	371530	European
Esophagus cancer	FinnGen	2023	566	287137	European
Gastric cancer	FinnGen	2023	1307	287137	European
Pancreatic cancer	FinnGen	2023	1416	287137	European
Liver cancer	FinnGen	2023	453	287137	European
Biliary tract cancer	FinnGen	2023	1081	287137	European
Colorectal cancer	FinnGen	2023	6509	287137	European

Table 2: MR analysis of the causality of appendicectomy on IBD and digestive cancers

Outcome	OR (95%confidence interval), P-value	Cochran's Q P-value	MR-Egger intercept P-value
	IVW		
CD	4.66(0.0004-44176.28), 0.742a	<0.001	0.11
UC	0.19(0.01-2.77), 0.228b	0.196	0.896
EC	0.40(0.000005-27692.57), 0.871a	0.04	0.454
GC	0.37(0.002-66.59), 0.706b	0.671	0.642
PC	202.61(1.39-29563.10), 0.037b	0.748	0.66
LC	11.57(0.002-77897.39), 0.586b	0.726	0.569
BTC	4.93(0.02-1494.73), 0.584b	0.609	0.419
CRC	0.61(0.01-25.95), 0.799a	0.037	0.389

CD, crohn's disease; UC, ulcerative colitis; EC, esophagus cancer; GC, gastric cancer; PC, pancreatic cancer; LC, liver cancer; BTC, biliary tract cancer; CRC, colorectal cancer; IVW, inverse variance weighted; MR, mendelian randomization, [a] IVW random-effect model; [b] IVW fixed-effect model.

intestinal mucosa, playing an important role inprotection against bacterial invasion and the maintenance of intestinal homeostasis Pietrzak et al. (2020). Moreover, the appendix contains a robust and varied microbiota including members of 15 phyla Guinane et al. (2013). Study found disrupted gut barrier function and altered gut microbiota composition after appendicectomy in human Cai et al. (2021), and several epidemiological studies have noted the link between appendicectomy and digestive diseases Chung et al. (2021), Zhang et al. (2023), Deng et al. (2016), Lee et al. (2018), Lee et al. (2021), Shi et al. (2023), Park et al. (2020), Cope et al. (2003), Song et al. (2016), van den Boom et al. (2022). IBD is a group of chronic non-specific intestinal inflammatory diseases mainly comprising CD and UC, resulted from a variety of factors including the gut microbiota, environment, and host genetics Dowdell et al. (2021), Ananthakrishnan et al. (2018). A large retrospective cohort study including 246562 cases and 246562 controls suggested that appendicectomy increases the risk of CD (HR = 3.48, 95% CI = 2.42-4.99) and UC (HR = 2.23, 95% CI = 1.59-3.12) regardless of age, sex, and comorbidity in Chinese Taiwan population Chung et al. (2021). A meta-analyse by zhang et al including 28 observational studies (22 case-control and 6 cohort studies) with 2748387 study participants showed that patients with appendicectomy had increased the odds (OR = 1.59, 95% CI = 1.22-2.08) and the risk ratio (RR = 2.28, 95% CI= 1.66-3.14) of CD Zhang et al. (2023). Conversely, Deng et al. analyzed 19 studies and indicated that appendicectomy can reduce the risk of UC (OR = 0.44, 95% CI=0.30- 0.64) Deng et al. (2016). However, unlike most other studies, we did not draw causal effect of appendicectomy on IBD through our MR study. Evidence is increasing that appendicectomy has been associated with an increased risk for colon cancer Lee et al. (2018), Lee et al. (2021), Shi et al. (2023), and the mice

model experiment indicated the microbial dysbiosis induced by appendicectomy plays a key role Shi et al. (2023). Besides, some studys indicated similar results in other parts of the digestive tract Park et al. (2020), Cope et al. (2003), Song et al. (2016). Yet, a recent prospective cohort study showed that appendicectomy had a reduced risk of digestive cancers (HR 0.75, 95% CI 0.56-0.99) van den Boom et al. (2022). In our study, we had found that genetically determined appendicectomy had a causal effect on pancreatic cancer, but not on other digestive cancers.

Our study has several strengths including the use of the MR analysis, which can effectively avoid both reverse causation bias and potential confounding bias in epidemiological studies. Besides, our study population was restricted to individuals of European ancestry that minimizes heterogeneity commonly observed when individuals of different ancestry populations are used in genetic studies. However, there are also limitations. Firstly, the subjects in this study are all of the European ancestry, so it should be prudent to apply the results of this study to other races. Secondly, the proportions of cases for some outcomes were low, and it might result in a low precision of the estimates. Thirdly, some confounding factors like age, gender, and other environmental confounding factors also have a certain impact on MR analysis.

CONCLUSIONS

In conclusion, our study revealed that genetically determined appendicectomy had a causal effect on pancreatic cancer, but not on IBD and other digestive cancers. We suggested that patients with appendectomy should be screened for pancreatic cancer. However, future researches based on higher quality GWAS data and more advanced methods are needed to confirm our results.

DECLARATIONS

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author Contributions

ZL conceived the study, participated in its design and coordination, and critically revised the manuscript. HJ finished data collection and analysis and drafted the manuscript. Both authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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