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Serum urate is associated with an increased risk of inflammatory bowel disease: A bidirectional Mendelian randomization study

MR for urate levels and IBD

Song Zhang, Sheng-Bing Zhao, Xue Fang, Le Kang, Xiangyu Sui, Miao Liu, Yujia Luo, Shuo Fu, Zhao-Shen Li, Yu Bai

Abstract

BACKGROUND

Previous studies have indicated bidirectional associations between urate levels and inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). However, it remains unclear whether the observations are causal because of confounding factors.

AIM

To investigate the causal associations between urate levels and IBD using bidirectional Mendelian randomization (MR).

METHODS

Independent genetic variants for urate levels and IBD were selected as instrumental variables from published genome-wide association studies (GWASs). Summary statistics for instrument-outcome associations were retrieved from three separate databases for IBD (UK Biobank, FinnGen database and a large GWAS meta-analysis) and one for urate levels (a large GWAS meta-analysis). MR analyses included the inverse-variance-weighted method, weighted-median estimator, MR–Egger and sensitivity analyses (MR-PRESSO). Meta-analysis was conducted to merge the data from separate outcome databases using a fixed-effects model.

RESULTS

Genetically higher serum urate levels were associated with a strongly increased risk of UC (odds ratio (OR): 1.95, 95% confidence interval (CI) = 1.86-2.05) after outlier correction, and the ORs (95%CI) for IBD and CD were 0.94 (95%CI=0.86-1.03) and 0.91 (95%CI=0.80-1.04), respectively. Animal studies confirmed the positive association between urate levels and UC. Moreover, genetically predicted IBD was inversely related to urate levels (OR: 0.97, 95%CI = 0.94-0.99). However, no association was observed between genetically influenced UC or CD and urate levels.

CONCLUSION

Genetically higher serum urate levels were associated with a strongly increased risk of UC (odds ratio (OR): 1.95, 95% confidence interval (CI) = 1.86-2.05) after outlier correction, and the ORs (95%CI) for IBD and CD were 0.94 (95%CI=0.86-1.03) and 0.91 (95%CI=0.80-1.04), respectively. Animal studies confirmed the positive association between urate levels and UC. Moreover, genetically predicted IBD was inversely related to urate levels (OR: 0.97, 95%CI = 0.94-0.99). However, no association was observed between genetically influenced UC or CD and urate levels.

Key Words: Inflammatory bowel disease; urate levels; antioxidant; Mendelian randomization; single nucleotide polymorphism

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Core Tip: Previous observational studies indicated the association between urate levels and inflammatory bowel disease (IBD) (including ulcerative colitis (UC) and Crohn's disease (CD)). To overcome the limitation of conventional observational studies and investigate the causal association between urate levels and IBD, we conducted the bidirectional mendelian randomization (MR) study. The MR analysis concluded that higher urate levels may be risk factors of UC and genetically predicted IBD was inversely associated with urate levels.

INTRODUCTION

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic inflammation and prolonged duration of the gastrointestinal tract[1]. Epidemiological studies have confirmed that the incidence of

IBD in developing countries was over 0.3% with the rapid adoption of the Western lifestyle[1, 2]. Specifically, there are 322 and 214 per 100,000 for CD and 505 and 214 per 100,000 for UC in Europe and the USA, respectively. The long course of IBD lasts throughout the patient's life, and the risk of colorectal cancer is much higher than that in the general population[3, 4]. The pathogenesis of IBD is an interplay between environmental risk factors (not limited to smoking, unfavorable lifestyles and diets) and genetic variants, resulting in inadequate intestinal immune activation and dysbiosis of the gut microbiota[5, 6]. Previous studies demonstrated that the depleted mucosal antioxidant defense was common in IBD and thus may impede mucosal repair and compromise the inflamed mucosa[7]. Over the past decade, the association between antioxidants and IBD has gained considerable interest[7-10] in light of the strong association between antioxidant capacity and the severity and disease activity of IBD.

Urate is vital as an antioxidant in neutralizing hydroxyl, superoxide and peroxynitrite radicals, which can decrease oxidative stress in vivo[11, 12]. Previous studies indicated that the serum uric acid-to-creatinine ratio was positively correlated with the disease activity of CD[13]. Increased urate levels were positively correlated with an increased risk of UC[14]. Moreover, the use of a clinical drug (allopurinol) improved the severity of colitis by reducing urate levels[15]. An animal study by Rahimian *et al*[9] further demonstrated that uric acid mediated the protective effects of inosine against colitis. Collectively, the relationship between urate levels and IBD (including UC and CD) is not well established. A recent Mendelian randomization (MR) study by Chen *et al*[16] did not support the causal effect of serum urate on UC and CD. However, the causal effect remains elusive because of the limited number of single-nucleotide polymorphisms (SNPs) used as instrumental variables (IVs). The causal effect of IBD (including UC and CD) on urate levels remains unclear.

Using genetic variants identified through genome-wide association studies (GWAS), MR is a popular approach to investigate the causal relationship between exposures and outcomes[17]. Therefore, to overcome the limitations of conventional observational studies, we aimed to examine the potential bidirectional relationship

between IBD (including UC and CD) and serum urate levels in the current MR study. In addition, we conducted *in vivo* animal studies to verify the association between urate levels and IBD. This study provides reliable insight into the causal associations between urate levels and IBD.

MATERIALS AND METHODS

Study design

A bidirectional two-sample MR analysis was performed to assess the causal relationship between IBD and urate levels (Figure 1). SNPs associated with risk factors were selected as IVs. The MR study was based on three assumptions: (i) the SNPs used as IVs are robustly associated with exposure (urate level or IBD), (ii) the SNPs are not associated with any confounder of exposure-outcome associations, and (iii) the SNPs exert effects through exposure only. In combination with the three principles mentioned above, palindromic SNPs would be identified and excluded in IV selection. All data used in the current study were publicly available GWAS summary statistics; therefore, no additional ethical approval or informed consent was needed. GWAS summary statistics were searched to extract leading SNPs related to urate levels and IBD (including UC and CD) as IVs. Gene-outcome associations were retrieved from three databases: (i) a large-scale GWAS meta-analysis, (ii) Finngen database (version 7, https://r7.finngen.fi/) and (iii) UK Biobank (UKB).

Selection of the instrumental genetic variables

SNPs related to urate levels were selected as instrumental variables from a GWAS (Köttgen A *et al*), which contained a total sample size of 110,347 European individuals with various serum urate levels[18]. SNPs that were significantly associated with urate levels ($p < 5 \times 10^{-8}$) were extracted. A linkage disequilibrium (LD)-based clumping procedure was performed using the 1000 Genomes EUR reference panel ($r^2 < 0.01$ and clump distance > 10,000 Kb) to ensure that each IV was independent. When SNPs related to exposure were absent in the outcome GWAS statistics, the proxy SNPs significantly associated with the variants of interest were selected ($r^2 > 0.8$).

Summary statistics for IBD were obtained from the GWAS meta-analysis (Liu JZ *et al*)[19], which contained a total of 34,652 participants of European ancestry (case/controls for IBD: 12,882/21,770; UC: 6968/20,464; CD: 5956/14,927). Nearly 12 million SNPs were included in all three GWAS summary statistics. SNPs ($p < 5 \times 10^{-8}$) were selected and used for LD-based clumping. The proxy SNPs were extracted when SNPs related to exposure were absent. The IV selection procedure for IBD was the same as that for urate levels (described in the previous paragraph).

F-statistics, calculated as $(beta/SE)^2$, were used to quantify the strength of each IV, and a value > 10 was considered sufficient[20]. In the current study, all F-statistics were higher than 10, indicating that there is little possibility of weak instrument bias based on summary statistics.

SNP-outcome data sources

Summary-level data for urate levels were available from the GWAS statistics (Köttgen A *et al*)[18], as described in section 2.2. Gene-outcome associations for IBD were obtained from three separate databases: (i) the GWAS meta-analysis from Liu JZ *et al*[19]; (ii) the Finngen database; and (iii) the UKB (for UC data only). The Liu JZ *et al* study has been described previously. In the Finngen study, CD and UC were defined by their ICD codes, while IBD was a term consisting of CD, UC and indeterminate colitis. The cases and controls were 8966/312,336 for IBD, 3243/318,059 for CD, and 6803/314,499 for UC, respectively. The UKB data for UC were extracted from a GWAS meta-analysis by Jiang L *et al*[21], which consisted of 2569 cases and 453,779 controls. GWAS on IBD and CD was not available in the UKB.

Statistical analysis of primary MR

The primary analysis method employed was the inverse-variance weighted (IVW) method, which assumes all single nucleotide polymorphisms (SNPs) as valid instruments and yields the most precise estimates[22]. In the presence of a sufficient sample size and absence of the pleiotropic effect of IVs, the IVW estimate is robust to confounding factors and approximates the true value[23]. A multiplicative random effect IVW model was applied when the heterogeneity significantly differed ($p < 0.05$).

Supplementary and sensitivity analysis

In addition to the IVW method, other robust methods (weighted median, MR–Egger and MR-PRESSO) were used to ensure the consistency and efficiency of the MR results. The weighted-median method could provide consistent causal estimates even when more than half of IVs were invalid[23]. The MR–Egger estimates allowed the included IVs to demonstrate unbalanced pleiotropy[24]. The MR-PRESSO approach was used to detect horizontal pleiotropic outliers[25], and IVW estimates were performed to further investigate the causal relationship between exposure and outcome through outlier removal. Cochran's Q test was applied to further examine the heterogeneity among all SNPs within each database. Leave-one-out analyses and scatter plots describing the causal relationship between serum urate levels and IBD were also provided.

Animal studies

All animal experimental procedures were approved and conducted in accordance with the guidelines of the Animal Care Committee of Navy Medical University. Co-housed, seven-week-old male C57BL/6 mice were administrated 2% dextran sulfate sodium (DSS) (36–50 kDa; MP Biomedicals) in their drinking water ad libitum for 7 consecutive days, following by 2 days of normal water.

Disease activity score and histological analysis in mice

Body weight, the presence of occult per rectum, stool consistency, and colon length were documented. A scoring system was used to assess diarrhea and the presence of occult or overt blood in the stool. Changes in body weight are reported as percentage loss of baseline body weight[26]. The ring of the rectum was harvested postmortem, fixed in 4% buffered formalin, and embedded in paraffin for following H&E staining procedure.

Enzyme-linked immunosorbent assay (ELISA)

IL-6, IL-1β, TNF-α and urate levels in serum were quantified using commercial ELISA kits in accordance to the manufacturer's instructions (Multi Sciences LTD, Hangzhou, China).

MR results were presented as odds ratios (OR) with 95% confidence intervals (CIs) of the outcome risk of a unit change in exposure. A two-sided p value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed mainly in R software (version 4.2.0, The R Foundation for Statistical Computing; TwosampleMR and MR-PRESSO package) and SPSS 26.0.

RESULTS

Urate levels to IBD

Twenty-seven independent SNPs were identified as genetic IVs for urate levels, and the median (minimum, maximum) F statistic was 63.4 (35.4-1406.3) (Supplementary Table 1). Detailed information for urate-related SNPs is listed in Supplementary Table 2.

In the meta-analysis of IVW estimates, the pooled ORs for IBD, UC and CD of genetically predicted per log-OR increase in urate levels were 0.94 (95%CI = 0.86-1.03), 0.97 (95%CI = 0.89-1.07) and 0.91 (95%CI = 0.80-1.04), respectively (Figure 2).

In the sensitivity analysis (Supplementary Table 3), the three results were similar in the weighted-median estimator (Supplementary Figure 1). No pleiotropic effect was detected in each database by MR–Egger estimate. Different outliers were identified by MR-PRESSO for IBD (n = 4), UC (n = 5) and CD (n = 5) in the GWAS meta-analysis by Liu JZ *et al* and UC (n = 3) in the UKB database, which resulted in potential pleiotropy assessed by global testing. Most of the results remained similar after outlier exclusion correction except for IVs of urate levels on UC (UKB database) (before correction: OR: 0.93, 95%CI: 0.75-1.17; after correction: OR: 2.70, 95%CI: 2.54-2.87). Cochran's Q test was performed after outlier exclusion to test heterogeneity. In urate level-related genetic IVs on IBD and CD by Liu JZ *et al* and UC (UKB database), a multiplicative random effect IVW model was used to evaluate the genetic estimate after heterogeneity was detected. A strongly positive causal relationship was detected between urate levels and UC after outlier exclusion and the application of a multiplicative random-effect IVW estimate (OR: 1.95, 95%CI = 1.86-2.05). A scatter plot was used to visualize the effect size of each MR method (Figure 3). Leave-one out analysis indicated that the associations between

urate levels and IBD were unlikely to be driven by certain specific SNPs (Supplementary Figure 2).

IBD to urate levels

A total of 117, 87, and 60 SNPs reached a genome-wide level of significance with IBD, UC and CD, respectively. A summary and detailed description of the variants are presented in Supplementary Tables 1 and 4.

The results of IVW analysis demonstrated that IBD was negatively correlated with urate levels (OR: 0.97, 95%CI = 0.94-0.99) (Figure 4). However, no association between UC (or CD) and urate levels was observed. The combined ORs of UC and CD on urate levels were 0.99 (95%CI = 0.97-1.01) and 1.00 (95%CI = 0.99-1.02), respectively.

In sensitivity analysis, the weighted-median estimator showed comparable results to the estimates from IVW analysis (Supplementary Figure 3). The MR–Egger analysis demonstrated no evidence of pleiotropy, while the MR-PRESSO global test indicated there were 7 outliers from the association between IBD and urate levels (P = 0.02) and 2 statistically nonsignificant outliers from the association between CD and urate levels (P = 0.006). Heterogeneity was detected from the association between IBD and urate levels after outlier correction by Cochran's Q statistics. However, the results remained similar after the correction for outliers and the application of the multiplicative random-effect IVW estimate (OR: 0.97, 95%CI = 0.94-0.99). A scatter plot was used to visualize the effect size of each MR method (Figure 5). The results remained consistent in leave-one-out analysis (Supplementary Figure 4), indicating that the results of the current analyses were stable and reliable.

Results of animal studies, HE and ELISA

To validate the positive association between serum urate levels and UC, 2% DSS was used to induce experimental colitis. The effect of this treatment included loss of body weight (Figure 6A), increased disease activity index score (Figure 6B), shortened colon length (Figure 6C), and increased inflammatory infiltration by HE (Figure 6D). Expression levels of proinflammatory factors in the serum, including IL-6, IL-1β and TNF-α, were significantly elevated in IBD mice (Figure 6E). Additionally, serum urate

level was also increased in IBD mice (Figure 6F). Together, these results provided evidence that there existed a positive association between urate levels and IBD.

DISCUSSION

In the current study, we evaluated the causal relationship between IBD and urate levels. We found evidence that genetic liability to urate levels was strongly associated with a higher risk of UC after outlier correction, and the genetic liability to IBD was slightly anti-correlated to urate levels. Animal studies confirmed the association between high urate levels and IBD. However, our study did not observe a causal relationship between CD and urate levels.

Previous observational studies have suggested that urate levels might be a risk factor for IBD. Zhu *et al*[13] included over four hundred IBD patients and 51 non-IBD controls and reported that urate levels were significantly higher in IBD patients. Similarly, Tian *et al*[14] suggested that increased urate levels were associated with UC in a retrospective case–control study. Meanwhile, IBD patients had an increased incidence of nephrolithiasis as well as urolithiasis[27]. To date, the only MR analysis conducted to investigate the causal relationship of urate levels on IBD demonstrated that genetically predicted urate levels were not associated with the risk of CD or UC. In part, our study was consistent with previous reports in that we found a strong positive association of urate levels with UC but not with CD or IBD. Animal studies further demonstrated a positive association between urate levels and colitis. In addition, IBD but not UC or CD was inversely correlated with urate levels.

The biological connection between IBD and urate levels has not been fully elucidated. Current studies suggested that intestinal inflammation (including oxidative stress) and dysbiosis of the gut microbiota are the main etiologies of IBD[6]. Increased urate levels mediate the exacerbation of mucosal colitis induced by DSS by enhancing intestinal permeability[15]. Treatment with allopurinol gavage alleviated the pathogenic increase in pro-inflammatory cytokines and reduced oxidative stress biomarkers in subjects with colitis[15, 28]. A recent study reported that rhein significantly alleviated

DSS-induced colitis and led to decreased urate levels, while the probiotic Lactobacillus was involved in regulating host metabolism[29]. These results support the idea of the relationship between serum urate levels and intestinal inflammation, suggesting that urate levels might be a therapeutic target for IBD. Our results supported previous results that urate levels were positively associated with an increased risk of UC rather than IBD or CD. One of the reasons could be the lack of association between urate levels and CD (a major subtype of IBD). Our results also confirmed that there was no bidirectional causal relationship between urate levels and CD. Furthermore, we only considered the dichotomous IBD diagnosis rather than IBD course or severity, which greatly influenced patients' clinical manifestations. Further MR analysis should be conducted to investigate the causal relationship between urate levels and disease activity and course of IBD as the relevant GWAS data are available. Meanwhile, we found a slightly inverse association between IBD and urate levels. One possible explanation could be that including summary statistics from only one GWAS increased the heterogeneity and reduced the credibility of our results. A meta-analysis should be conducted once multiple data sources for urate levels are available.

There are three major strengths in the current study. First, the MR design is suitable for causal inference. As an alternative to randomized controlled trials, the MR method can partly avoid bias from confounding factors and reverse causation, which might increase the reliability of the results compared with those of observational studies. To our knowledge, this is the first bidirectional MR analysis investigating the causal relationship between IBD (and its subtypes) and urate levels. Second, we obtained summary-level data from large genetic consortia and GWASs, which included large sample sizes, with 110,347 participants for urate levels, 355,952 (21,846 patients) for IBD, 805,082 (16,340 patients) for UC and 342,185 (9199 patients) for CD. Third, the population stratification bias was minimized because all GWAS summary statistics data in the current study were generated from the European population.

Nevertheless, potential limitations in our MR study should be considered. First, MR design can be biased by pleiotropic effects. The current study involved the

implementation of various sensitivity analyses, which were performed based on distinct assumptions regarding the fundamental characteristics of pleiotropy, and most of the analyses showed stable results. Moreover, MR– Egger tests and MR-PRESSO analyses were conducted to explore horizontal pleiotropy[24, 25]. After removing potential outlier SNPs, we observed a strong positive causal relationship between urate levels and UC, and most of the results were robust. Second, all participants included in the current study were European populations, which may limit the generalizability of our findings to other populations. Further MR analyses should be conducted to verify our findings in individuals of non-European descent. Third, in our present research, summary statistics for IBD were obtained from three databases, while data on urate levels were sourced solely from one large GWAS meta-analysis (Köttgen A *et al*). The utilization of data from a single source may compromise the reliability of the results. Therefore, once GWAS summary statistics from diverse sources become available, meta-analyses should be conducted to further verify our findings on the inverse association between IBD and urate levels.

The findings that serum urate levels increase the risk of UC add to the evidence from another MR analysis demonstrating a new risk factor for IBD. Recently, a meta-analysis based on large-scale cohorts demonstrated that the consumption of several types of food and drinks, for example, beer, wine, and beef, were associated with increased serum urate levels[30], while we are unaware of the risk related to the foods mentioned above. Meanwhile, many dietary approaches have been developed to reduce inflammation, prevent relapse, and manage the disease severity of IBD[31]. Our current study indicated that monitoring and managing urate levels in patients with IBD and accounting for diets that are associated with elevated urate levels in dietary therapy may provide additional benefits.

CONCLUSION

In summary, we systemically evaluated the potential causal relationship between IBD and urate levels. Our current MR analysis demonstrated that genetically predicted

urate levels are causally associated with an elevated risk of UC, while IBD was inversely correlated with urate levels. Considering the tight relationship between diet and urate levels, our study sheds crucial new insight into treating and preventing IBD. This indicates that IBD patients may benefit from monitoring and reducing serum urate levels.

ARTICLE HIGHLIGHTS

Research background

Inflammatory bowel disease (IBD), mainly consisted of Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disease. As a vital antioxidant, urate can decrease oxidative stress in vivo, which may be associated with IBD state. However, the causality between IBD and urate levels has not been investigated.

Research motivation

Previous studies indicated uric acid-to-creatinine ratio and urate were positively correlated with the disease activity of CD and UC. Despite the existing findings demonstrated the bidirectional associations between urate levels and inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), the causality association between them remains unclear. This study seeks to investigate the causal association between IBD and urate through Mendelian randomization (MR) study, which may shed crucial new insight into treating and preventing IBD. In specific, IBD patients may benefit from monitoring and reducing serum urate levels.

Research objectives

The study aims to investigate the bidirectional causal relationship between urate levels and IBD by performing MR analysis, to better understand the gene susceptibility of urate levels and IBD.

Research methods

Single nucleotide polymorphisms retrieved from genome-wide association studies (GWASs) was selected as instrument variants. Summary GWAS statistics for instrument-outcome associations were retrieved from three separate databases for IBD (UK Biobank, FinnGen database and a large GWAS meta-analysis) and one for urate levels (a large GWAS meta-analysis). Inverse-variance-weighted was performed to investigate the bidirectional causal relationship, and other sensitivity analysis were conducted to strengthen the results. Meta-analysis was conducted to merge the data from separate outcome databases using a fixed-effects model.

Research results

The current study found that the genetic susceptibility to urate levels was associated with increased UC risk (odds ratio (OR): 1.95, 95% confidence interval (CI) = 1.86-2.05), and animal studies confirmed the positive association between urate levels and UC. Additionally, genetically predicted IBD was inversely related to urate levels (OR: 0.97, 95%CI = 0.94-0.99). However, no association was observed between genetically influenced UC or CD and urate levels.

Research conclusions

This study identified urate levels might be risk factors for UC, whereas genetically predicted IBD was inversely associated with urate levels. The current results shed new insight into prevention and treatment of IBD.

Research perspectives

Although the current study investigated the causal relationship between urate levels and UC, which was further verified by animal studies, the precise mechanism by which high urate levels affects the development of UC remains unknown. More basic and clinical studies should be conducted for identification of key regulators and molecules during the process.

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