• Clinical Research •

Inflammatory bowel disease and risk of ophthalmic inflammation-related diseases: a two-sample Mendelian randomization study

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Abstract

● **AIM:** To investigate the causal effect of inflammatory bowel disease (IBD) on ocular inflammation using Mendelian randomization (MR) analysis.

● **METHODS:** Genetic instruments associated with inflammatory bowel disease (IBD), ulcerative colitis (UC), and Crohn's disease (CD) were derived from the largest genome-wide association studies (GWAS) published to date. The FinnGen research project was utilized to identify genetic risk variants associated with conjunctivitis, keratitis, iridocyclitis, chorioretinitis, episcleritis, and optic neuritis. All participants were of European ancestry. Three methods which included inverse variance weighting (IVW), weighted median (WM), and MR-Egger regression were performed to estimate the causal association in this study. IVW took the inverse variance of each study as the weight to calculate the weighted average of effect sizes, to summarize the effect sizes of multiple independent studies, which could provide the most precise estimated results. IVW was used as the primary outcome, while WM and MR-Egger were used to improve the estimation of IVW.

● **RESULTS:** A nominal causal effect of genetically predicted IBD on risk of non-infectious conjunctivitis, keratitis, iridocyclitis, and optic neuritis, but not on chorioretinitis or episcleritis. After Bonferroni correction, the results showed that genetically predicted UC was significantly associated with an increased risk of iridocyclitis (IVW: OR, 1.17; 95%CI, 1.10-1.24, $P=2.54\times10^{-7}$). CD was significantly associated with conjunctivitis (IVW: OR, 1.05; 95%CI, 1.03-1.08, $P=3.20\times10^{-5}$), keratitis (IVW: OR, 1.06; 95%CI, 1.02-1.09; $P=1.13\times10^{-3}$), and iridocyclitis (IVW: OR, 1.09; 95%CI, 1.04-1.14; $P=1.43\times10^{-4}$).

● **CONCLUSION:** IBD causally poses a risk of inflammation of conjunctiva, cornea, Iris-ciliary body complex, and optic neuritis. CD is more closely associated with the eye inflammation than UC. These impliy that the relationship of IBD and different parts of the eye structure are different, and provide novel evidence linking based on the association of the gut-eye axis.

● **KEYWORDS:** inflammatory bowel disease; ulcerative colitis; Crohn's disease; ocular inflammation; Mendelian randomization

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INTRODUCTION

Since the concept of the gut-retina axis was first proposed, considerable interest has been garnered by exploring the link between the gut and eye in recent years[1]. Increasing evidence from research indicated that gut dysbiosis played a pivotal role in the onset and progression of multiple ocular diseases, such as uveitis, diabetic retinopathy[2]. Furthermore, some studies have offered insights into potential mechanisms underlying the gut dysbiosis-ocular surface-lacrimal gland axis[3]. The link between bowel disease and eye disease requires further investigation.

Inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is a recurrent, immune-mediated inflammatory condition characterized by chronic diarrhea, abdominal pain, and perianal bleeding[4-5]. In

recent years, given the natural growth in patients (Western populations), assuming a 1% prevalence, the projected number of IBD in 2030 is anticipated to surpass 10 million, marking the highest ever recorded number of IBD patients[6]. In addition, the symptoms of IBD primarily manifest in the gut, but they are not limited to it. Extraintestinal manifestations (EIM) can significantly impact the quality of life for patients with IBD[7]. The existing epidemiological observational studies have suggested a link between IBD and ocular inflammation, which may result in irreversible visual impairment and sequelae[8]. Conjunctivitis and corneal infiltration, manifesting as ocular EIM, were first reported in two IBD patients as early as 1925[9]. The reported incidence of ocular complications in IBD patients ranges from 3.5% to 11.8%, with the majority being inflammatory in nature[10]. The published observational study further indicated that conjunctivitis and episcleritis were the most prevalent ocular EIMs associated with IBD, with the overall incidence of ocular fundus manifestations remaining low, at less than 1% among IBD patients, including chorioretinitis and optic neuritis[11-12]. However, observational studies are non-causal and susceptible to confounding factors, thus making it challenging to definitively establish whether this is indeed the case. It is hoped that further clarification of the causal relationship between IBD and ocular inflammation will enhance awareness of ocular EIM beyond a mere observational association.

Mendelian randomization (MR) study is a method for causal inference, leveraging genetic variants. This approach hinges on the natural and random allocation of genetic variation during meiosis, allowing for the identification of disparities in outcomes between carriers of the variant and those who are not, which can be attributed to differences in risk factors[13]. Due to the non-causal nature of observational studies and the challenges associated with randomized controlled trials[14], MR has emerged as a popular and convenient analytical technique, particularly in epidemiological etiology inference in recent years. MR effectively mimics randomized controlled trials[15-16], providing a valuable tool for causal inference. In the field of ophthalmology, MR Analysis is mainly used for etiology research, including but not limited to the impact of environment, traits and microorganisms on the occurrence, development and outcome of specific eye diseases. It provides new ideas for the treatment and prognosis of eye diseases, such as allergic conjunctivitis, iridocyclitis, cataracts, glaucoma, uveal diseases, retinopathy and myopia[17-18]. However, our study is the first to systematically analyze the causal relationship between IBD and multiple ocular inflammatory diseases using MR Study.

In this study, we performed a two-sample MR analysis to investigate the causal relationship between IBD and ocular inflammation, using the summary statistics from genome-wide association studies (GWAS) of IBD (including UC and CD) and ocular inflammation (conjunctivitis, keratitis, iridocyclitis, chorioretinitis, episcleritis, and optic neuritis), which were common ocular EIM in previous IBD observational studies.

MATERIALS AND METHODS

Ethical Approval Ethical approval was not sought for this specific project because all data came from the summary statistics of published GWAS, and no individual-level data were used. The study adheres to the principles outlined in the Declaration of Helsinki (2013).

Study Design According to the three key assumptions of MR: 1) The instrumental variables (IVs) is associated with risk factors; 2) IVs is not associated with confounding factors; 3) IVs affects results only through risk factors[19], single nucleotide polymorphisms (SNPs) representing IVs after screening were selected. Figure 1 showed the flowchart of the two-sample MR study between IBD and ocular inflammation. A nominal causal effect of genetics (P<0.05) was firstly applied to predict IBD on risk of conjunctivitis, keratitis, iridocyclitis, chorioretinitis and episcleritis. Then, significant causal effects of genetic correlation between UC and CD and increased risk of ocular inflammation were obtained through Bonferroni correction. In addition, our findings were reported in accordance with MR-STROBE guidelines.

Data Sources IBD-associated SNPs were derived from the largest GWAS published to date for IBD, UC and CD in the European Genome-phenome Archive[20]. The statistics came from an extended cohort of 86 640 European individuals and 9846 non-Europeans. Although studies showed that majority of the genetic risk were shared across diverse populations, a few loci that exhibit heterogeneity effects between populations can be detected[20-21]. In order to reduce the resulting racial bias among the elderly, our study population's genetic background is limited to European ancestry. The summary statistics for IBD (n=12 882 cases, 21 770 controls), UC (n=6968 cases, 20 464 controls), and CD (n=5956 cases, 14 927 controls).

FinnGen research project (https://r5.finngen.fi/) was used to identify genetic risk variants for conjunctivitis, keratitis, iridocyclitis, episcleritis, chorioretinitis, and optic neuritis. The summary statistics for conjunctivitis (n=13 655 cases, 203 517 controls), keratitis (n=5 561 cases, 209 287 controls), iridocyclitis (n=3 622 cases, 209 287 controls), episcleritis (n=660 cases, 209 287 controls), chorioretinitis (n=384 cases, 203 018 controls), and optic neuritis (n=582 cases, 217 491 controls). All participants were of european ancestry.

Single Nucleotide Polymorphisms Selection First, SNPs closely related to IBD were screened from the GWAS data ($P<5\times10^{-8}$) at the genome-wide significance level. To further eliminate linkage disequilibrium, we take clump steps with the

TwoSampleMR package of the R software, the parameter is set to R^2<0.001, and <10000 from the index variant[22]. Second, the SNPs associated with outcome (P<5×10^{-6}) were excluded from retrieving each SNP from outcome GWAS. Meanwhile, palindromic SNPs and SNPs with non-concordant alleles were excluded from the process of harmonizing the IBD and outcome datasets[23-24]. Third, MR Pleiotropy REsidual Sum and outlier (MR-PRESSO) is used to get rid of potential outliers before each MR Analysis[25]. Figure 1 showed the selection criteria and process of the above SNPs.

Mendelian Randomization Estimates Three methods which included IVW, WM, and MR-Egger regression were performed in this study to estimate the causal association of exposures (IBD, UC, and CD) on risk of outcomes (ocular inflammation). IVW takes the inverse variance of each study as the weight to calculate the weighted average of effect sizes, to summarize the effect sizes of multiple independent studies, which can provide the most precise estimated results when all selected SNPs are valid IVs[26]. In this study, IVW was used as the primary outcome, while WM and MR-Egger were used to improve the estimation of IVW as they could provide more reliable, albeit less efficient estimates over a wider set of scenarios[27-30].

Sensitivity Analysis The MR-Egger intercept test was performed to assess the potential pleiotropic effects of the SNPs used as IVs[31]. If the MR-Egger intercept was statistically significant (P<0.05), the MR analysis was considered to be unreliable. Additionally, to identify potentially influential SNPs, we performed a "leave-one-out" sensitivity analysis to where the MR is performed again but leaving out each SNP in turn. Heterogeneity of IVs was assessed by Cochrane's Q-statistic. A P value of <0.05 would be regarded as significant heterogeneity. Causal estimates are presented as odds ratios (ORs) with 95% confidence intervals (CI).

Statistical Analysis Before MR analysis, F statistics of these IVs were calculated to determine whether there was a weak IV bias. Respectively, for all IVs, the F>10, the impact of weak IV bias is small, so the selected SNPs can be further used in MR studies[32].

TwoSampleMR was used in all statistical analysis software package (https://github.com/mrcieu/TwoSampleMR) and MR-presSO package (statistical computing internal resistance project) 4.2.0 version in R (version 3.6.1) packages. For a global-level test, a nominally significant two-sided P-value was set as 0.05. For region-level analyses, given the 12 MR estimates, a Bonferroni-corrected P-value was set as 0.05/12 (4.17×10^{-3}).

RESULTS

Main Results After excluding outlier SNPs through the MR-PRESSO global test and PhenoSacnner, we used the selected SNPs to explore the causal effects of genetically predicted IBD on ocular inflammation. Using these SNPs, we performed a comprehensive MR study and identified nominal and significant ocular inflammation influenced by IBD (Figure 2).

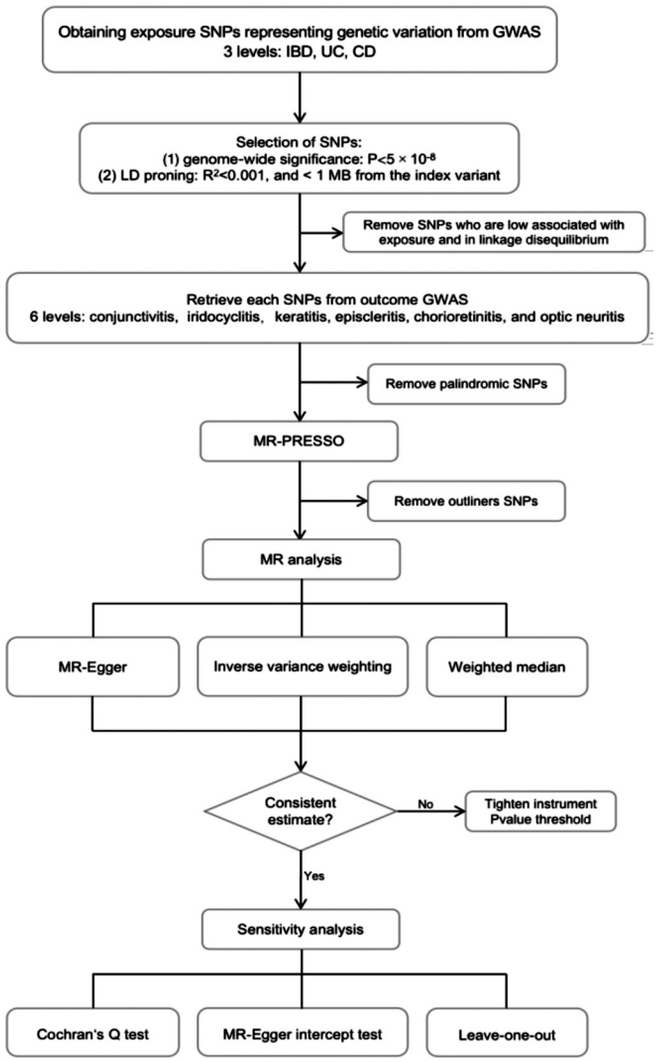

Figure 1 Study flame chart of the MR study revealing the causal relationship of IBD on the risk of ocular inflammation SNP: Single-nucleotide polymorphisms; GWAS: Genome-wide association studies; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; LD: Linkage disequilibrium; MR-PRESSO: MR Pleiotropy REsidual Sum and outlier; MR: Mendelian randomization.

Causal Effects of IBD on Ocular Inflammation The results of MR showed that genetically predicted IBD was associated with an increased risk of conjunctivitis (IVW: OR,1.05; 95%CI, 0.98-1.12; P=1.94×10^{-3}), keratitis (IVW: OR, 1.05; 95%CI, 1.01-1.10; P=6.42×10^{-3}; MR Egger: OR, 1.05; 95%CI, 0.99-1.12; P=7.20×10^{-2}; WM: OR, 1.05; 95%CI, 1.02-1.10; P=9.38×10^{-2}), iridocyclitis (IVW: OR, 1.18; 95%CI, 1.12-1.24; P=6.83×10^{-11}; MR Egger: OR, 1.19; 95%CI, 1.14-1.45; P=9.97×10^{-5}; WM: OR, 1.19; 95%CI, 1.11-1.29; P=5.95×10^{-6}), optic neuritis (IVW: OR, 1.14; 95%CI, 1.02-1.28; P=2.93×10^{-}

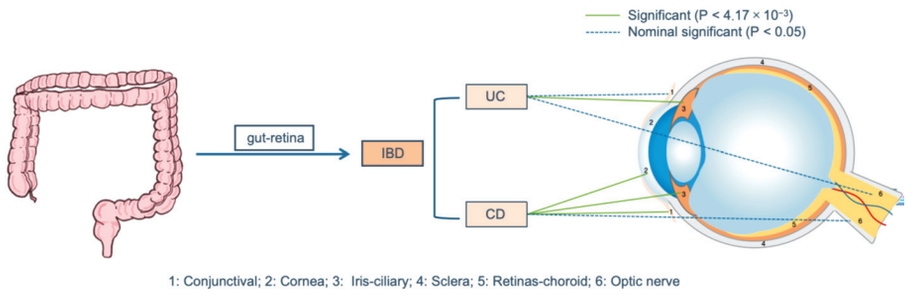

Figure 2 Using two-sample MR framework, we reveal that IBD causally influences ocular inflammation, supporting the existence of eye-brain axis IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; MR: Mendelian randomization.

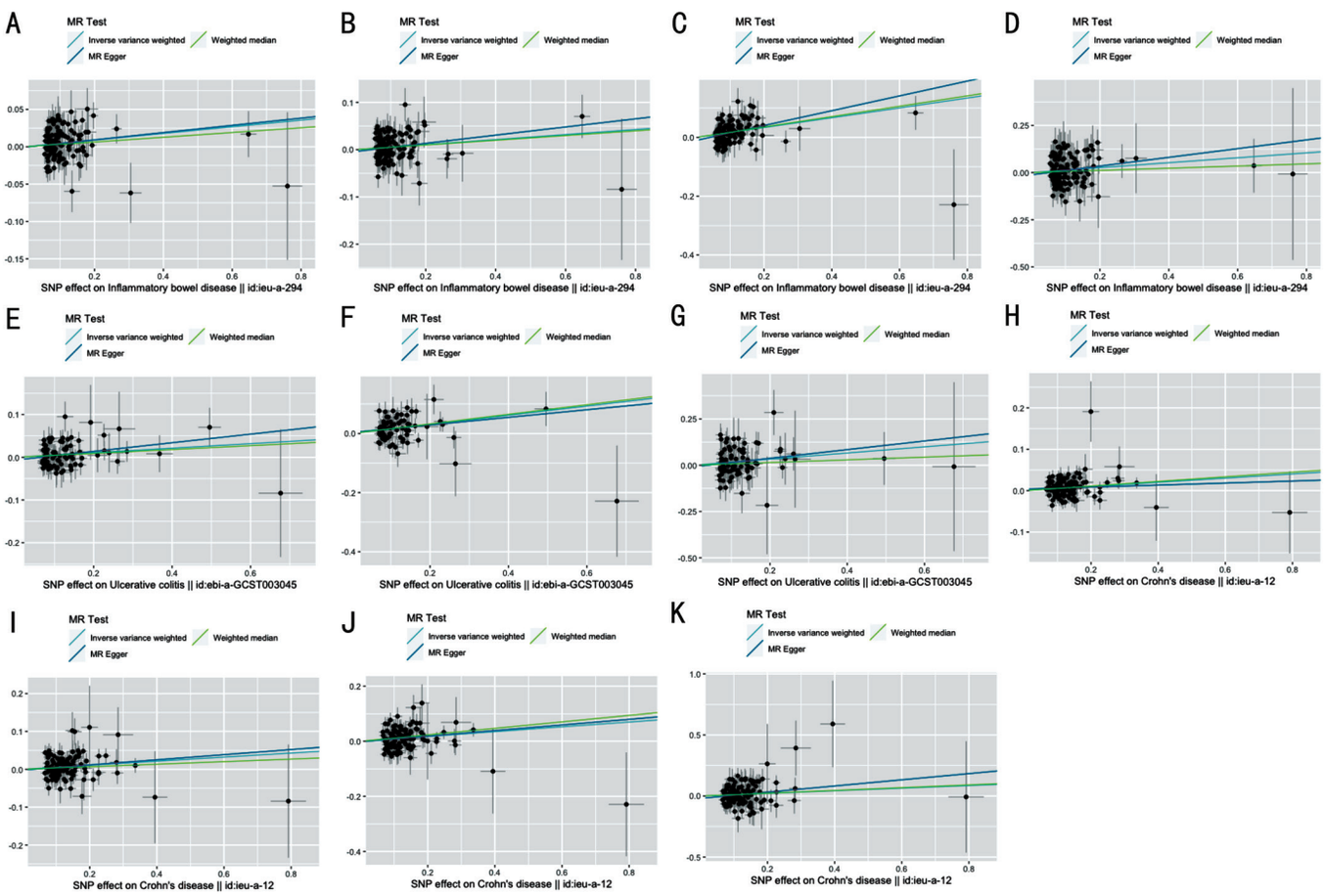

Figure 3 Scatter plots for MR analyses of the causal effect of IBD (including UC and CD) on ocular inflammation A: IBD-conjunctivitis; B: IBD-keratitis; C: IBD-iridocyclitis; D: IBD-optic neuritis; E: UC-keratitis; F: UC-iridocyclitis; G: UC-optic neuritis; H: CD-conjunctivitis; I: CD-keratitis; J: CD-iridocyclitis; K: CD-optic neuritis. MR: Mendelian randomization; SNP: Single-nucleotide polymorphisms; MR-PRESSO: MR Pleiotropy REsidual Sum and outlier; UC: Ulcerative colitis; CD: Crohn's disease; LD: Linkage disequilibrium; IBD: Inflammatory bowel disease.

^{2}). It can be seen from the scatter plot (Figure 3A-3D) that the causal effect among the three methods is consistent. However, IVW, WM and MR-Egger methods showed no significant association of genetically predicted IBD on episcleritis and chorioretinitis (all P>0.05). More details were shown in Figure 4.

Causal Effects of UC on Ocular Inflammation The results of the IVW methods showed that genetically predicted UC was associated with an increased risk of keratitis (IVW: OR, 1.05; 95%CI, 1.01-1.10; P=1.10×10^{-2}; MR Egger: OR, 1.11; 95%CI, 1.00-1.22; P=5.95×10^{-6}), iridocyclitis (IVW: OR, 1.17; 95%CI, 1.10-1.24; P=2.54×10^{-7}; WM: OR, 1.18; 95%CI, 1.08-1.28; P=2.11×10^{-4}), optic neuritis (IVW: OR, 1.18; 95%CI, 1.04-1.34; P=9.82×10^{-3}). It can be seen from the scatter plot (Figure 3E-3G) that the causal effect among the three methods is consistent. However, IVW, WM and MR-Egger methods showed no significant association of genetically predicted UC on conjunctivitis, episcleritis, or chorioretinitis (all P>0.05). More details were shown in Figure 4.

Causal Effects of CD on Ocular Inflammation The results of the IVW methods showed that genetically predicted UC was associated with an increased risk of conjunctivitis (IVW: OR, 1.05; 95%CI, 1.03-1.08; P=3.20×10^{-5}; WM: OR, 1.06;

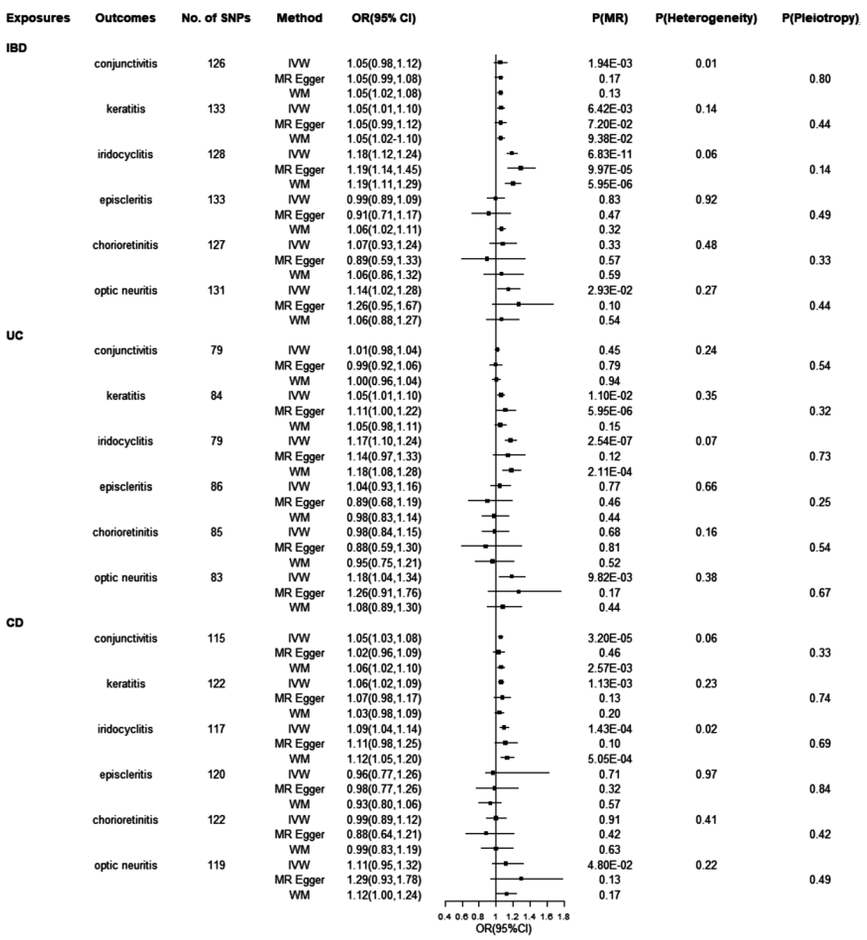

Figure 4 Association of IBD (including UC and CD) on ocular inflammation risk using MR SNP: Single-nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval; MR: Mendelian randomization; *P*(heterogeneity): *P* value for heterogeneity using Cochran *Q* test; *P* (pleiotropy): *P* value for MR-Egger intercept; IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease.

95%CI, 1.02-1.10; $P=2.57\times10^{-3}$), keratitis (IVW: OR, 1.06; 95%CI, 1.02-1.09; $P=1.13\times10^{-3}$), iridocyclitis (IVW: OR, 1.09; 95%CI, 1.04-1.14; $P=1.43\times10^{-4}$; WM: OR, 1.12; 95%CI, 1.05-1.20; $P=5.05\times10^{-4}$), optic neuritis (IVW: OR, 1.11; 95%CI, 0.95-1.32; $P=4.80\times10^{-2}$). It can be seen from the scatter plot (Figure 3H-3K) that the causal effect among the three methods is consistent. However, IVW, WM and MR-Egger methods showed no significant association of genetically predicted UC on episcleritis, and chorioretinitis (all $P>0.05$). More details were shown in Figure 4.

Sensitivity Analysis To further verify the reliability of the above results, we performed pleiotropy, heterogeneity, and sensitivity analysis. No directional pleiotropy was found by MR-Egger regression analysis (Figure 4). Actually, as we used the random-effects IVW as main result, heterogeneity is acceptable[33-34].

DISCUSSION

Main Findings The primary finding of our study is that UC causally elevates the risk of iridocyclitis, whereas CD similarly elevates the risk of conjunctivitis, keratitis, and iridocyclitis. These estimated effects have passed the Bonferroni correction threshold for statistical significance. Furthermore, the estimated impact of UC on conjunctivitis and optic neuritis, as well as CD on optic neuritis, is nominally significant. Given that the IVW results showed a *P*-value of less than 0.05 without Bonferroni correction, these estimates should be interpreted with caution. On the other hand, UC did not notably increase the risk of keratitis, and IBD (including UC and CD) did not notably increase the risk of episcleritis or chorioretinitis. This means that the causal effects of IBD on ocular inflammation in different parts of the eye structure are different.

Results in Context with the Published Literature Approximately 50% of IBD patients experience at least one EIM, whose pathogenic mechanisms remain poorly understood. Elucidating these pathogenic pathways has the potential to not only deepen our comprehension of EIMs but also advance our understanding of IBD in general[33-34]. Researchers reported that most patients had been diagnosed with IBD prior to the development of ocular EIM, while in a small number of instances, ocular disorders preceded the diagnosis of IBD[11,35]. IBD is often active in a majority of patients when ophthalmic inflammation occurs, and the

incidence of ocular EIM rates among these patients ranges from 3.5% to 11.8%[10,36]. Therefore, the occurrence of ocular inflammation may serve as an indicator of the early onset of IBD, aiding in the clinical diagnosis and targeted treatment of IBD and related EIM.

Our study showed that IBD was indeed genetically associated with ocular inflammation, and we believe that this may be due to the imbalance of intestinal flora caused by IBD, which induces the development of ocular inflammatory diseases through the entero-retinal axis. Reports indicate that the ecological dysregulation of gut microbiota in the human body could trigger the development of inflammatory, metabolic, mental, and immune diseases[37]. Furthermore, studies have reported a relationship between this dysregulation of gut microbiota and the development of eye diseases[38]. Another study postulated that dysbacteriosis or significant alterations in the healthy gut microbiome might be the determining factor in the onset and progression of IBD[39]. In a comprehensive Meta-analysis examining the gut microbiome in over 3000 individuals with CD and UC, Aldars-García *et al*[40] identified *Christensenellaceae* as one of five taxaindicative of a healthy gut microbiota. In an MR study conducted by Liu *et al*[41] on gut microbiota in patients with diabetic retinopathy, they hypothesized that *Christensenellaceae* and *Peptococcaceae* might reduce inflammatory damage to the retina *via* the intestinal-retinal axis, thereby influencing disease progression in diabetic retinopathy. Indeed, *Christensenellaceae* were consistently depleted in individuals with CD and UC, the two major sub-types of IBD[42-44].

Elucidating the causal relationships and pathogenic mechanisms underlying EIMs is challenging, owing to the absence of standardized diagnostic criteria and the challenge in differentiating drug-induced extraintestinal pathologies from EIMs, including ocular inflammation[45]. In this study, we conducted an MR study to disentangle the causal relationships between IBD and ocular inflammation by excluding confounding factors. Additionally, the MR study utilized openly available GWAS data, which can save on research costs and time. All data banks were sourced from European populations, thus minimizing potential population bias[46]. Compared with traditional experimental studies, MR simulates a more realistic random assignment process. The design of the study is relatively straightforward, and its implementation adheres to ethical standards[47]. Furthermore, MR analysis effectively prevents confusion and offers a novel method to explore the mechanisms of the gut-retina axis. Most importantly, it examines the etiological strength of the causal association between IBD and ocular inflammation risk.

Difference Between the Anterior and Posterior Segments of eye The summary of previous observational studies indicateed that the most prevalent eye EIM was episcleritis, affecting up to 29% of patients with IBD. In contrast, uveitis is less frequent, occurring in 0.5% to 5.3% of patients, and primarily manifests as anterior uveitis. More severe forms of uveitis, including scleritis and posterior or intermediate uveitis, were also observed[48-51]. In our study, we found that IBD (including UC and UD) can cause iridocyclitis (the main inflammation involved in anterior uveitis), but neither can cause the important inflammation of posterior uveitis, chorioretinitis, which partially supports previous observational studies. However, our study did not demonstrate a causal relationship between either UC or CD and episcleritis, which contradicts previous observational studies. This discrepancy primarily arises from the influence of other pertinent factors, aside from the disease itself, on the outcomes of observational epidemiological studies. One of the advantages of MR lies in its ability to exclude such confounding effects. An alternative explanation for EIMs is that they manifest as independent inflammatory events that share common genetic or environmental risk factors with IBD[51-52]. This study suggested that episcleritis observed in the context of IBD may be an etiology worthy of further investigation. Given the significant prevalence of episcleritis among IBD patients, external scleritis remains an important aspect to consider, despite the absence of a direct causal link between IBD and external scleritis.

Castellano *et al*[12] reported that the overall incidence of posterior segment manifestations in patients with IBD was low, with a prevalence of less than 1%. Similar trends were observed in our study, where we found that IBD (both UC and CD) had a nominal causal relationship with optic neuritis and no causal relationship with chorioretinitis. Lee *et al*[53] speculated that the low incidence of posterior segment manifestations may also be attributed to the use of systemic steroids in the treatment of IBD, as these medications can lead to rapid resolution of these manifestations. Therefore, IBD may be somewhat associated with posterior segment inflammation of the eye, albeit to a lesser degree than its association with anterior segment inflammation, given the causal relationship established in this study between IBD and conjunctivitis, keratitis, and iridocyclitis.

Difference Between UC and CD Usually, it is challenging to definitively diagnose whether a patient has UC or CD, as these diseases share numerous similarities such as abdominal pain, diarrhea, and rectal bleeding. Moreover, it may take several years for the clinical manifestations to evolve sufficiently to allow for a confident diagnosis[11]. Greenstein *et al*[54], who conducted a study involving 700 patients with IBD to assess the relative incidence and distinguishing characteristics of EIM, reported that ocular EIM occured more frequently in patients with CD compared to UC. This finding aligns with the

observations made in other studies[55-57]. In this MR analysis, we aimed to assess the causal relationship between IBD, including UC and CD, and ocular inflammation. Our findings indicated a significant causal effect of genetically predicted CD on conjunctivitis, keratitis, and iridocyclitis. Conversely, UC demonstrated a significant causal association only with iridocyclitis. Although UC nominally influenced conjunctivitis, it did not exert a definitive effect on keratitis. Hence, CD may have a stronger association with ocular inflammatory diseases than UC, making it more prone to causing multiple inflammatory eye diseases. CD, being a systemic condition with a prolonged course, differs significantly from UC, which is typically an acute mucosal disorder confined to the distal colon. Furthermore, CD exhibits crucial immunological disparities compared to UC[57-58]. These differences between the two diseases may be the cause.

Limitations The study possesses several limitations that are worthy of acknowledgment. First, it's important to mention that the GWAS data utilized in this study were exclusively derived from a Meta-analysis that had undergone adjustments for age and sex. Additionally, all the ocular inflammation data were sourced exclusively from the Finnish database[59]. IVs selected in our study were robust, the potential for sample overlap to introduce bias cannot be overlooked. Second, our MR study indicated a causal relationship between genetically predicted IBD and ocular inflammation; however, the findings from the MR analysis solely constitute genetic evidence. The putative causal relationship and its underlying mechanisms require further exploration and confirmation through animal experiments or population-based observational studies. Third, despite the fact that symptoms of ocular inflammation were primarily confined to the eye, with minimal or no impact on the gut, our inability to identify a potential mutual causal relationship between ocular inflammation and IBD was attributed to the insufficient number of IVs available for reverse MR analysis.

In conclusion, our estimates demonstrate that IBD is causally associated with an increased risk of inflammation in the conjunctiva, cornea, and iris-ciliary body complex, rather than the posterior segment of the eye. This finding suggests that IBD may exert distinct effects on different ocular structures, offering novel insights into the gut-eye axis association. Furthermore, our findings indicate no causal association between IBD and episcleritis, contradicting previous observational studies. This discrepancy may be attributed to the influence of confounding factors on the results of observational epidemiological investigations. CD exhibits a stronger association with ocular inflammation than UC, implying that UC is more prone to inducing multifocal ocular inflammation. Our study, a comprehensive MR analysis, sheds light on the links between IBD and ocular inflammation, thereby facilitating the diagnosis and differentiation of UC and CD. The mechanisms of the association between them should be studied further.

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