• Clinical Research •

Does uveitis increase the risk of age-related wet macular degeneration? A Mendelian randomization study

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Abstract

● **AIM:** To use two-sample Mendelian randomization (MR) method to study uveitis causal association with wet age-related macular degeneration (wAMD) risk from the genetic level.

● **METHODS:** Two-sample MR analysis was used to assess the causal role of uveitis on wAMD risk, using the 8 genetic variants associated strongly with uveitis as instrumental variables. Besides, eight MR methods [inverse variance weighted (IVW), weighted median, MR-Egger regression, weighted mode, simple mode, robust adjusted profile score (RAPS), contamination inverse-variance weighted method, and debiased inverse-variance weighted method] were used to get the whole causal estimate for multiple instrumental single nucleotide polymorphism (SNPs). The MR analysis was based on Europeans.

● **RESULTS:** Uveitis was related to a higher risk of wAMD [odds ratio (OR): 1.08, 95% confidence interval (CI) 1.03–1.12; P=1.03×10^{-3}] with the IVW method. No heterogeneity and directional pleiotropy were detected. On the contrary, no significant results were detected in reverse MR analysis.

● **CONCLUSION:** Uveitis is related to an increased risk of wAMD. Due to the high blindness rate of wAMD, understanding and controlling the risk factors of AMD is of great significance for reducing its incidence and early diagnosis and treatment.

● **KEYWORDS:** uveitis; wet age-related macular degeneration; Mendelian randomization

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INTRODUCTION

Age-related macular degeneration (AMD) is a prevalent, chronic, and progressive macular disorder predominantly affecting older adults. It is characterized by pathological alterations in the photoreceptors, retinal pigment epithelium, Bruch's membrane, and the choroidal complex, ultimately leading to a deterioration of central vision and significant visual impairment. AMD can be broadly divided into two types: dry and wet. Dry AMD presents with geographic atrophy. Wet AMD (wAMD) is the cause of most severe vision loss and can occur within weeks to months due to neovascularization. AMD affects approximately 196 million people worldwide and is expected to affect approximately 288 million people worldwide by 2040[1].

Chronic inflammation, lipid deposition, oxidative stress, and compromised extracellular matrix maintenance are all intricately intertwined and strongly implicated in the pathogenesis of AMD. These factors collectively contribute to the progressive deterioration of the macula, leading to the characteristic visual impairment associated with this condition[2]. It is reported that increased AMD incidence was observed in osteoarthritis, rheumatoid arthritis (RA)[3], inflammatory bowel disease (IBD), and IgA glomerulonephritis[4] patients. Recent research hints at immune-mediated inflammatory diseases (IMID) promoted AMD emerge, including IBD which encompasses Crohn's

disease (CD) and ulcerative colitis (UC), RA, ankylosing spondylitis (AS), multiple sclerosis (MS), and asthma, among others[5]. Increasing evidence in uveitis reveals the involvement of the Janus Kinase (JAK) signalling pathway[6], and JAK inhibitors usage is associated with a reduced risk of AMD incidence[7]. The pathogenesis of AMD that follows RA may be orchestrated by the intricate interplay of the complement pathway within the immune response, along with the pivotal role played by the inflammatory cytokine, tumour necrosis factor alpha (TNF-α). These mechanisms may jointly contribute to the development and progression of AMD in individuals with RA[8].

Uveitis are classified as infectious or non-infectious. Non-infectious uveitis, presumed to be immune-mediated in nature, can be further classified into two distinct categories: those that are associated with a well-established systemic disease and those that are confined to the eye, with no systemic manifestations. This distinction aids in the targeted management and understanding of the underlying pathophysiology of these conditions[9]. Uveitis-related choroidal neovascularization (CNV), known as inflammatory CNV (iCNV), is an uncommon complication of uveitis[10]. The eye is recognized as a unique, locally immune-privileged organ, with the retina residing within the choroid, a structure that provides essential blood supply to the retina. Consequently, the choroid and retina engage in a dynamic interplay, where they mutually influence and support each other's functions. This intricate relationship underscores the importance of maintaining their health and integrity for optimal visual function. So, it is imperative to find a novel research strategy for exploring the causal relationship between uveitis and AMD.

Mendelian randomization (MR) is a powerful analytical approach employed to elucidate causal relationships between various exposures and their corresponding outcomes. By leveraging naturally occurring genetic variations as instrumental variables, MR provides a robust framework for disentangling the complex web of associations and confounding factors, ultimately offering insights into the causal mechanisms underlying exposure-outcome relationships[11]. This methodology is firmly grounded in the fundamental principles of instrumental variable analysis within the statistical domain. At its core, the single nucleotide polymorphism (SNP) serves as the pivotal instrumental variable, acting as a proxy for the exposure factor of interest. By leveraging this natural genetic variation, MR offers a rigorous and unbiased means of estimating causal effects, minimizing the influence of confounding factors and potential biases. An ever-growing body of MR studies is shedding new light on various research questions, opening up exciting avenues for further exploration and discovery. By harnessing the power of genetic variants as instrumental variables, these studies are unravelling the intricate causal relationships between exposures and outcomes, offering invaluable insights that can inform clinical practice and guide future research directions. To date, our review has uncovered a notable absence of MR studies or randomized controlled trials (RCT) that have delved into the intricate relationship between uveitis and wAMD. This gap in the literature highlights an unmet need for research that can elucidate the potential causal links between these two conditions, providing crucial insights for the development of targeted therapies and improved patient outcomes. Therefore, we embarked on a rigorous forward and reverse MR analysis, aimed at elucidating the causal relationship between uveitis and wAMD. This endeavour contributes significantly to the advancement of AMD diagnosis and prevention strategies.

MATERIALS AND METHODS

Ethical Approval This study used public AMD and uveitis GWAS data without additional participant consent and ethical approval. The original GWAS studies were approved by the respective ethics committees. All methodologies employed in this study were rigorously adhered to and fully compliant with the ethical principles outlined in the Declaration of Helsinki.

Study Design In our meticulous study, we meticulously curated SNPs from reputable GWAS data, leveraging them as instrumental variables to delve into the elusive causal relationship between uveitis and wet macular degeneration. This study adheres rigorously to the three fundamental assumptions that underpin the validity and reliability of MR studies: 1) all instrumental variable (IVs) are strongly correlated with exposure factors; 2) all of the selected IVs were rigorously screened to ensure their independence from any confounding factors that might potentially bias the relationship between the exposure and the outcome; 3) all IVs employed in this study exert their influence solely through the exposure of exposure, without any confounding influence through alternative pathways. Analogous steps were followed in the reverse MR analysis, ensuring a comprehensive and symmetrical approach. The study design is concisely illustrated in Figure 1, providing a clear visual representation of the methodology employed.

Data Sources for MR Analysis The summary dataset of uveitis GWAS was found by searching GWAS ID: ebi-a-GCST90018938 on the web https://gwas.mrcieu.ac.uk/datasets/. This GWAS consisting of 2616 cases and 478 126 controls was identified in 2021.The summary dataset of wAMD GWAS was also found by searching GWAS ID: Finn-b-WET_AMD on the web https://gwas.mrcieu.ac.uk/datasets/. This GWAS consisting of 2114 AMD cases and 206 601 controls was identified in 2021. All individuals are from European ancestry.

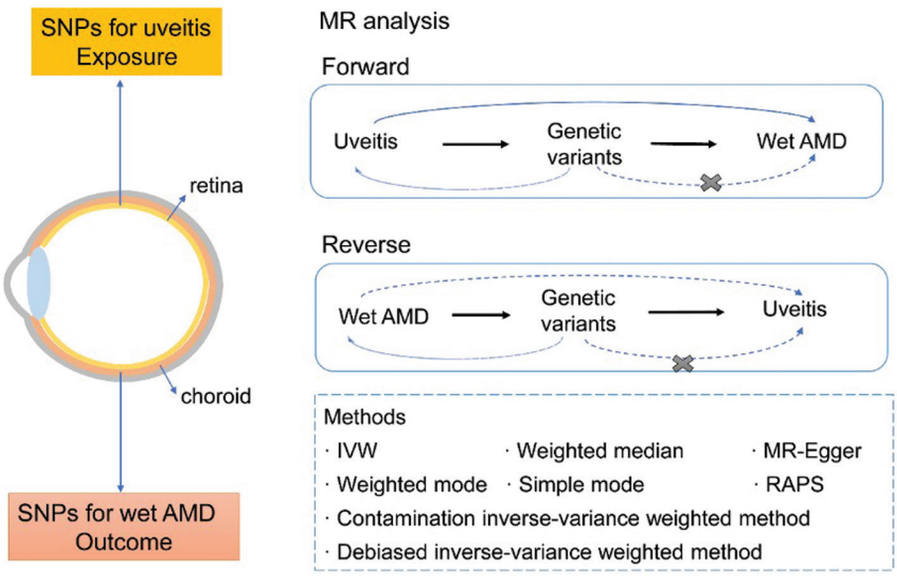

Figure 1 The study design MR: Mendelian randomization; AMD: Age-related macular degeneration; IVW: Inverse variance weighted; RAPS: Robust adjusted profile score; SNPs: Single nucleotide polymorphism.

Selection and Validation of Instrumental Variables Three stringent criteria were meticulously applied in the selection of appropriate SNPs. First, SNPs linked to uveitis were chosen based on genome-wide significance with a *P*-value threshold of $<5\times10^{-8}$. Second, the independence of the selected SNPs was assessed through pairwise linkage disequilibrium analysis. SNPs with an $r^2>0.001$ within a clumping window of 10 000 kb were removed if they were correlated with multiple SNPs or had a higher *P*-value. Lastly, the F-statistic was computed to confirm the efficacy of individual SNPs. When the F-statistics surpassed a rigorous threshold of ten, the SNPs were deemed sufficiently robust to effectively mitigate potential biases, thus reinforcing the credibility and reliability of our findings. Before embarking on the MR analysis, we implemented meticulous data harmonization procedures to guarantee the congruence of the allele effects of each SNP on both the exposure and the outcome.

MR Analysis To uphold the highest standards of accuracy in our findings, we employed an extensive array of eight distinct statistical methods to rigorously verify the potential causal relationship between uveitis and AMD. Among them, the IVW method was used as the primary statistical method[12-13], and others were used as supplement methods, including the following: contamination mixture method, Robust adjusted profile score (RAPS), Debiased inverse-variance weighted method, MR Egger, weighted median, weighted mode, simple mode[14-16]. The "TwoSampleMR" R package (version 0.5.8, Stephen Burgess, Chicago, IL, USA) was used for two-sample MR analysis between exposures and outcomes and the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) package (version 1.0). $P<0.05$ was considered statistically significant.

Sensitivity Analysis Sensitivity analysis is an essential method employed to assess potential bias in MR studies. It's involves conducting heterogeneity and pleiotropy tests. Heterogeneity is assessed using Cochran's *Q* test within the IVW approach, while horizontal pleiotropy is indicated by an intercept with a *P*-value less than 0.05 in MR-Egger regression[12,16]. Furthermore, SNPs exhibiting pleiotropic outliers are eliminated using MR-PRESSO with a significance threshold of $P<0.05$[17]. Upon the identification of potential outliers, they were promptly excluded from the analysis, and subsequently, the IVW estimate was recalculated. Upon conducting a leave-one-out analysis, we further delved into examining whether the MR estimate was unduly influenced by any individual SNP This process entailed meticulously removing each SNP from the analysis, one at a time, and subsequently reassessing the MR estimate. This systematic approach aimed to discern whether the causal estimate was unduly influenced by any specific individual SNP, thereby strengthening the credibility and robustness of our findings.

RESULTS

Selection of IVs After filtration of SNPs significantly related to exposure in outcome data (the relationship between these SNPs and outcomes $P<5\times10^{-5}$). A total of 8 SNPs were selected to genetically analyse in the forward MR analysis. In addition, F statistics for all SNPs were higher than 10 in this study, indicating a small chance of weak IV bias.

Causal Relationship Between Uveitis and wAMD The forward two-sample MR result between uveitis and AMD is listed in Figure 2. The causal estimates from the inverse variance weighted method showed that genetically predicted uveitis was positively associated with the risk of AMD [OR: 1.08, 95% confidence interval (CI): 1.03 to 1.12, $P=1.03\times10^{-3}$]. The

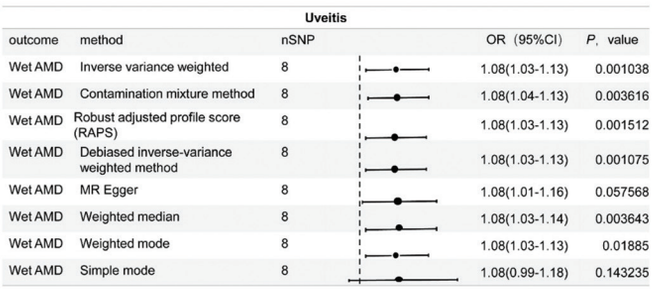

Figure 2 Forest plot shows causal relationship between uveitis and AMD Forest plot the dashed vertical line represents the ineffective line (OR=1), the horizontal coordinate corresponding to each square represents the OR value calculated by different methods, and each horizontal solid line represents the 95%CI of the corresponding OR value. AMD: Age-related macular degeneration; MR: Mendelian randomization; SNP: Single-nucleotide polymorphisms; OR: Odds ratio; CI: Confidence interval.

individual MR estimates revealed that uveitis was statistically significantly associated with the increasing risk of wAMD. The forest plot showed the causal effects of uveitis on wAMD were influenced by every single variant. On the contrary, reverse MR analysis showed that AMD had no significant association with uveitis.

Sensitivity Analysis We conducted sensitivity analysis to clarify our inferred causalities. First, the leave-one-out cross validation showed that there is no single SNP drove the causal estimates. Second, the results of IVW and MR-Egger method showed that no heterogeneity detected between uveitis and wAMD (P>0.05). Third, there is no significant difference detected in pleiotropy test between uveitis and wAMD (P>0.05). Additionally, the funnel plot also shows no signs of heterogeneity since the plot is symmetric.

DISCUSSION

Previous observational studies have found that large drusen is associated with uveitis, but the causality of uveitis and wAMD is not clear. In this study, we first performed the bidirectional two-sample MR analysis to investigate the relationship of uveitis and wAMD. Our results strongly indicated that uveitis had a causal effect on wAMD by various methods, strongly proposing that uveitis is a risk factor of wAMD.

It is well known that uveitis is an immune disease which breaks the balance between regulatory mechanisms of the immune system and mechanisms of inflammation by polygenic and environment[18]. It occurs by not only adaptive immunity but also innate immunity. Meanwhile, Th1 and Th17 cells are both observed to participate in immune response in animal models[19]. Hsu *et al*[20] found that autoimmune uveitis can be suppressed by inhibiting oxygen species reaction. Several studies showed that males, old people, people who live in the city, smoking, hypovitaminosis D, gout and air pollution may be the increased risks of uveitis[18,21-23].

Owing to the limited relevant studies at present, the specific pathogenesis of AMD is still unknown, involving the multiple genetic and environment factors. Brandli *et al*[24] found that pattern recognition receptors are essential factors leading to activation of the innate immune system and inflammation. In addition, autoantibodies were found to play a role in inflammation and immune response in AMD[25]. What's more, oxidative stress is also a main factor in pathogenesis[26]. Importantly, with the emergence of vascular endothelial growth factor (VEGF), increased angiogenesis and vascular permeability cause photoreceptor destruction and macular atrophy[27]. The increased risks probably involved age, air pollution, smoking, genetic cholesteryl ester transfer protein deficiency and several genetic background[28-31].

Our reverse MR analysis of wAMD and uveitis showed no significant result. Previous studies have also suggested that patients with uveitis are less likely to develop AMD[9]. The purinergic receptor P2X7 was found plays a role in both AMD and uveitis, but how it connects the two diseases deserves further research[32]. There is more and more evidence showed that low choroidal perfusion is relevant to disease progress of AMD[33]. It seems uveitis may influence AMD and no reverse affect. But several questions remain to be answered by further studies.

Our research possesses several notable strengths. First, the application of MR in our study underscores the inherent advantage of utilizing naturally occurring genetic variants that are randomly allocated during meiosis. This methodology adeptly sidesteps potential confounding factors and reverses causation, thereby bolstering the credibility and robustness of our results. Second, our research harnesses the extensive pool of GWAS data, which enables us to achieve a heightened level of precision in our analyses. This comprehensive approach sets our study apart from other observational studies, underscoring the reliability and depth of our findings. Lastly, given the scarcity of comprehensive and long-term RCT data within this field, our findings serve as invaluable insights that can inform and advance scientific understanding. These results fill a crucial gap in knowledge, highlighting the potential implications and directions for future research.

Our research also has some limitations. First, we can't rule out the potential heterogeneity although we used the MR-PRESSO test to screen and discard SNPs. Second, since the enrolled participants were mainly of European ancestry, our results may not be repeated in other races for we only used Europeans. Third, the exact molecular pathophysiological mechanisms between uveitis and wAMD are still unclear and require further studies. In the last, the causal association we investigated between uveitis and wAMD might be partially

mediated by other intermediate factors unknown. However, this does not change the finding that uveitis is a risk factor for wAMD.

In conclusion, we first thoroughly estimated the causal relationship between uveitis and AMD. Our findings revealed genetic evidence for causal links of uveitis on wAMD, but not wAMD on uveitis, contributing to the pathogenesis deciphering of AMD.

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Data Availability Statement: The summary-level GWAS data for both uveitis and age-related macular degeneration were obtained from https://gwas.mrcieu.ac.uk/datasets/, corresponding to the GWAS IDs: ebi-a-GCST90018938 and Finn-b-WET_AMD, respectively.

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