• Review Article •

Research progress of Mendelian randomization analysis for glaucoma etiology

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Abstract

● In recent years, Mendelian randomization (MR) has been increasingly utilized, leveraging genetic variants as instrumental variables. This approach significantly mitigates confounder effects and reverse causation, precisely clarifying the causal links between exposures and outcomes. MR's unique advantages have made it instrumental in medicine, especially in elucidating glaucoma's etiology. It facilitates the identification of potential risk factors, laying the groundwork for developing preventative and therapeutic strategies against glaucoma. Recent MR research has delved into diverse potential glaucoma risk factors, including behavioral habits, metabolic profiles, and their causative linkage to the disease. This review encapsulates MR's analysis in glaucoma etiology, heralding new avenues for understanding underlying mechanisms and establishing causality.

● **KEYWORDS:** Mendelian randomization; glaucoma; etiology; risk factors
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INTRODUCTION

The causal association plays a crucial role in medical research. By establishing the cause-and-effect connection between a certain exposure or risk factors and the result in an environment, modulating the exposure factors can alter the outcomes, resulting in disease treatment or prevention. However, determining the causal association between two relative factors remains challenging in the real world, even when their correlation is observable. This challenge primarily stems from three limitations: 1) confounding factors: The real world comprises diverse confounding variables, possibly generating false correlations between two variables. Without proper control of these factors, the research findings could be misleading; 2) random occurrence: In observational studies, two variables might demonstrate notable correlation, merely originating from random happenstance; 3) reverse causation: The causality might also exhibit a backward relationship, leading to simultaneous changes in both dependent and independent variables, hence turning off any conclusions drawn from conventional statistical methods.

Mendelian randomization (MR), a burgeoning method for studying causal relationships, can be employed to assess causality between exposures and outcomes. This approach utilizes genetic variations strongly linked to exposures as instrumental variables, facilitating their substitution into regression models for analysis, consequently determining whether a causal link exists between the exposures and outcomes[1]. Genetic variations, randomly distributed during conception, are minimally influenced by external factors post-birth and display a unidirectional relationship with exposure factors. Moreover, MR analysis must adhere to its three principal assumptions: relevance, signifying that the instrumental variable should showcase significant association with exposure factors; exclusion restriction, indicating the instrumental variable should not associate with confounders present amidst exposure factors and the outcome; and instrument strength, implying the instrumental variable cannot directly instigate the outcome (Figure 1). Thus, MR strives

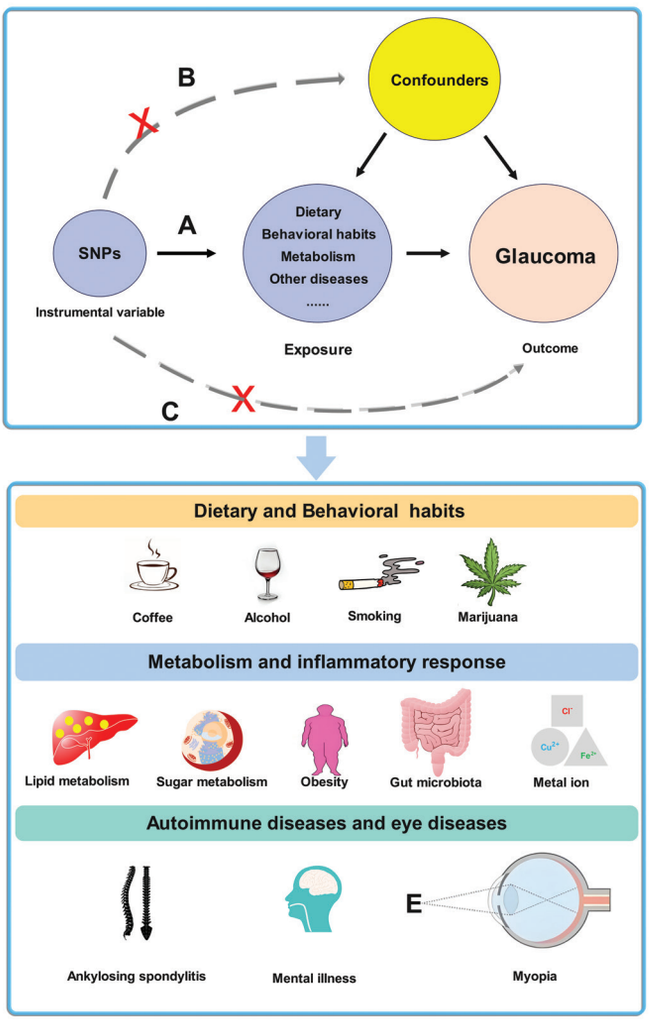

Figure 1 The three central assumptions of Mendelian randomization

A: The instrumental variable is required to showcase a significant correlation with the exposure factor; B: Instrumental variable should not correlate with confounding factors, implying an absence of heterogeneity; C: The instrumental variable should exhibit no direct correlation with the outcome factors, indicating it does not demonstrate horizontal pleiotropy. SNPs: Single nucleotide polymorphisms.

to minimize confounding variables and reverse causality, meanwhile, as shown in Figure 1, MR analysis identified many risk factors associated with glaucoma.

Glaucoma encompasses a range of neurodegenerative diseases, typified by the loss of retinal ganglion cells, optic disc atrophy, visual fields and visual acuity[2]. Reports suggest that the worldwide prevalence of glaucoma in adults is around 3.54%, with primary open angle glaucoma (POAG) prevailing at 3.05%[3]. The number of global glaucoma patients is projected to surpass a hundred million by 2040. Glaucoma serves as a leading cause of blindness, potentially resulting in irreversible vision loss if adequate treatment isn't timely administered[4]. Even though elevated pathological intraocular pressure (IOP) is recognized as a substantial risk factor for glaucoma, it doesn't fully account for all symptoms related to the disease[2].

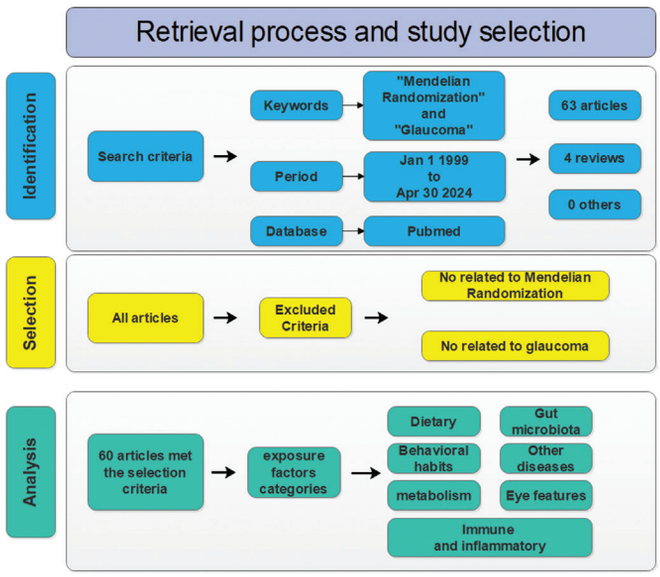

Figure 2 Retrieval strategy and research process diagram.

Thus, exploring other potential risk factors offers significant implications for prevention and recurrence diagnosis of glaucoma.

Given the long natural course of glaucoma and the prevalence of numerous confounding factors, instigating randomized controlled trials in real-world settings typically poses significant challenges. MR, however, proffers a method to bypass the constraints of conventional observational studies, underscoring its utility in examining glaucoma's influencing attributes. In glaucoma-related research, MR analysis primarily aids in etiological investigations. This encompasses but isn't restricted to, biological traits, serum metabolites, microbial abundance, and the influences exerted by other diseases on glaucoma's occurrence, evolution, and prognosis. This paper presents a literature review on applying MR analysis within the context of glaucoma and its exposure factors. The strategy for literature retrieval is depicted in Figure 2.

MENDELIAN RANDOMIZATION APPLIED IN GLAUCOMA ETIOLOGY

Relationship Between Diet, Behavioral Habits and Glaucoma Diet and behavioral habits may be exposure factors for glaucoma. Currently, relevant MR analysis has explored the causal relationship between various beverages, foods, and behavioral habits and glaucoma (Figure 3).

Common Beverages Coffee, a globally consumed beverage abundant in nourishing components, has been suggested to influence IOP. However, establishing its relationship with glaucoma remains a contentious issue[5-7]. A randomized controlled trial[8] conveyed that consuming a cup of coffee (approximately 182 mg of caffeine) bears no significant association with glaucoma. Conversely, a cross-sectional study by Bae *et al*[6] identified a significant correlation between coffee intake and POAG risk among Koreans. Utilizing two-

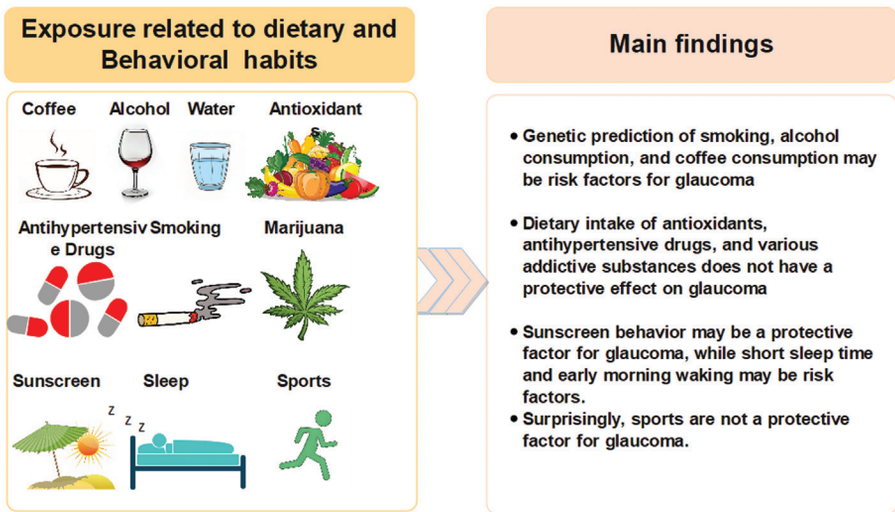

Figure 3 The causal relationship between dietary and behavioral habits and cyanosis.

sample MR analysis, Li *et al*[9] discovered a positive correlation between genetically predicted increased coffee intake and the incidence of POAG. Kim *et al*[10], through MR analysis, reported insignificance in the habitual consumption of coffee and glaucoma, though high caffeine intake in populations with a high genetic predisposition to IOP is associated with elevated glaucoma risk. However, this study did not analyze other subtypes of glaucoma, which may have led to biased results.

The role of alcohol consumption in relation to glaucoma remains indeterminate. Alcohol intake might trigger an escalation in optic nerve head blood flow, potentially thwarting glaucoma development. However, the association of persistent alcohol consumption with multiple neurodegenerative diseases indicates potential risk factors for glaucoma[11]. A recent Meta-analysis[12] hints at a negative correlation between habitual alcohol consumption and both IOP and POAG. Still, inherent biases and heterogeneity limit the clarity of these findings. Stuart *et al*[13], employing a cohort study, discovered that habitual drinkers exhibited higher IOP and decreased macular ganglion cell layer thickness. They further corroborated the causal link between alcohol consumption and the thinning of the macular ganglion cell layer through MR analysis, thereby enforcing the correlation between alcohol consumption and glaucoma.

Water plays a critical role in maintaining bodily stability. Prior studies have highlighted the significant implications of an increased water intake in forestalling ophthalmic diseases, positing dehydration as a potential intensifier of glaucoma risk[14-15]. According to MR analysis conducted by Mi *et al*[16], changes in water intake are seen to protect against age-related cataracts, age-related macular degeneration, and diabetic retinopathy. However, no causal connection with glaucoma was identified. After adjusting for confounding factors like tea, alcohol, coffee intake, smoking, and physical labor, the increased water intake still retained a causal association with decreased risk of age-related cataracts and diabetic retinopathy. Oxidative stress plays an intrinsic role in the onset of glaucoma. Yet, the conflicting views surrounding the potential protective influence of dietary antioxidants against glaucoma persist. Certain studies espouse that dietary antioxidant consumption correlates with a decreased glaucoma risk. At the same time, other research asserts that natural antioxidants obtained from fruits and vegetables do not offer protection against glaucoma[17-19]. Employing a two-sample MR approach, Xiong *et al*[20] examined six diet-related antioxidants (ascorbic acid, β-carotene, retinol, lycopene, γ-tocopherol, and α-tocopherol) as exposure factors. They established that these antioxidants lack a causal correlation with POAG at a genetic level. At the same time, there is no causal relationship at the genetic level between these antioxidants and glaucoma related factors such as IOP, macular retinal nerve fiber layer thickness, macular ganglion cell inner plexus layer thickness, and vertical cup to disc ratio. This means that antioxidants obtained from food do not have a protective effect on POAG.

In the prevailing MR research focused on the interplay between dietary factors and glaucoma, a strong consensus shows that alcohol intake can impact macular ganglion cells, with the remainder demonstrating little, if any, significant correlation to glaucoma. However, habitual coffee consumption could potentially influence IOP. Hence, individuals with a familial history of glaucoma or carriers of high IOP susceptibility genes should carefully consider their coffee intake and ensure regular IOP monitoring.

Antihypertensive and Statin Drugs There has been some controversy concerning the protective role of antihypertensive medications (β-blockers, calcium channel blockers, *etc*.) and statin drugs in glaucoma[21-25]. Plotnikov and Agliullina[26] reported no causal relationship between β-blockers, calcium

channel blockers, and POAG *via* MR. Liu *et al*[27] also concluded no causal relationship between antihypertensive drugs and glaucoma, using single-variable and multivariable MR analysis with blood pressure and twelve antihypertensive drugs as exposures. Furthermore, Kim *et al*[28] combined observational research with MR analysis to find that statins do not offer protection against glaucoma. In summary, the use of antihypertensive and statin drugs does not serve to protect against glaucoma.

Addictive Substances Smoking is considered a risk factor for various ophthalmic diseases, including age-related cataracts and age-related macular degeneration[29-30]. However, its role in glaucoma is unclear. Some research[31] reported a correlation between smoking intensity and retinal nerve fiber layer thinning. However, a recent study reported on two large cohorts[32] stated no correlation between smoking and glaucoma. Tran *et al*[33] noted in their MR analysis that the start of smoking might be associated with lower IOP; the intensity of smoking showed a negative correlation with POAG, but this correlation with POAG was not present after multiple adjustments. This would imply that smoking could be a potential risk factor for POAG. Cannabis has been found to offer protection against glaucoma potentially, but the conclusions remain unclear due to confounding factors from observational studies[34]. Katsimpris *et al*[35] concluded that the use of cannabis does not affect glaucoma after utilizing an MR approach on three large-scale Genome-Wide Association Studies (GWAS) datasets. In conclusion, genetically predictive addictive substances do not offer protection against POAG, but smoking could pose a risk factor; ophthalmologists should not use addictive substances in the treatment or prevention of glaucoma as it is quite evident that these substances could bring about other health implications, like lung cancer, cardiovascular disease, and mental disorders.

Impact of Behavioral Habits on Glaucoma

Sun protection behavior Ultraviolet (UV) radiation is typically associated with a variety of illnesses[36]. Some studies suggest that UV light is associated with the exfoliation of glaucoma[37]. Dai *et al*[38] used single nucleotide polymorphisms (SNPs) associated with sun protection and ease of tanning as instrumental variables to study the relation between sun protection and exfoliation glaucoma. MR analysis shows that sun protection and skin tanning are related to reducing the occurrence of exfoliation of glaucoma. However, due to the lack of GWAS data related to UV radiation, there currently need to be MR studies on the direct association between UV light and glaucoma.

Sleep Sleep disorders are considered to be associated with a variety of illnesses. Sleep may affect the body's nervous system and hormone levels and possibly influence IOP[39-40]. Some studies suggest that glaucoma patients are more likely to suffer from insomnia or obstructive sleep apnea, but other reports indicate no correlation between sleep duration and glaucoma. Zhang *et al*[41] performed bidirectional MR analysis on POAG using eight different sleep phenotypes or features, including self-reported chronotype (morning/evening person), ease of getting up in the morning, sleep duration, daytime napping, insomnia, daytime dozing/narcolepsy, snoring, and obstructive sleep apnea. The results showed a significant causal relationship between short sleep duration and early morning waking with an increased risk of POAG. No causal relationship was found between other sleep phenotypes and POAG. In addition, an MR study by Ingold *et al*[42] also confirmed no genetic evidence proving a causal link between sleep apnea and glaucoma.

Physical exercise Physical activity is thought to play a protective role in various chronic diseases[43-44]. While some studies suggest that exercise also protects against glaucoma, other reports suggest that intense activity may be a risk factor for glaucoma[45-47]. Madjedi *et al*[48] used two-sample MR analysis to find that although physical exercise is associated with the thickness of the macular ganglion cell-inner plexiform layer, both high levels of overall physical activity and duration of moderate and intense activities do not show a causal relationship with IOP or glaucoma.

MR analysis investigates the causal relationship between physical behavioral habits and glaucoma. Among them, it is found that both sun protection and sleep quality are closely related to glaucoma, but physical activity may be unrelated to glaucoma.

Metabolic Features Metabolic characteristics are also a hot topic in MR research. In glaucoma etiology, MR analysis is used to explore the association between metabolic characteristics such as lipid metabolism, glucose metabolism, metal metabolites, obesity, hormones, and gut microbiota with glaucoma (Figure 4).

Lipid metabolism Prior studies have indicated a relationship between hyperlipidemia and plasma lipid components with a heightened POAG risk[49-50]. However, some studies have proposed that hyperlipidemic patients have a lowered glaucoma risk[51]. Nusinovici *et al*[52] discovered in their metabolomic research that only high density lipoprotein 3(HDL3) cholesterol showed a significantly negative correlation amongst the 130 lipid components analyzed, implying all remaining lipid components, including total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, were irrelevant to glaucoma. A two-sample MR analysis verified the causative association of HDL3 cholesterol and POAG[53-54].

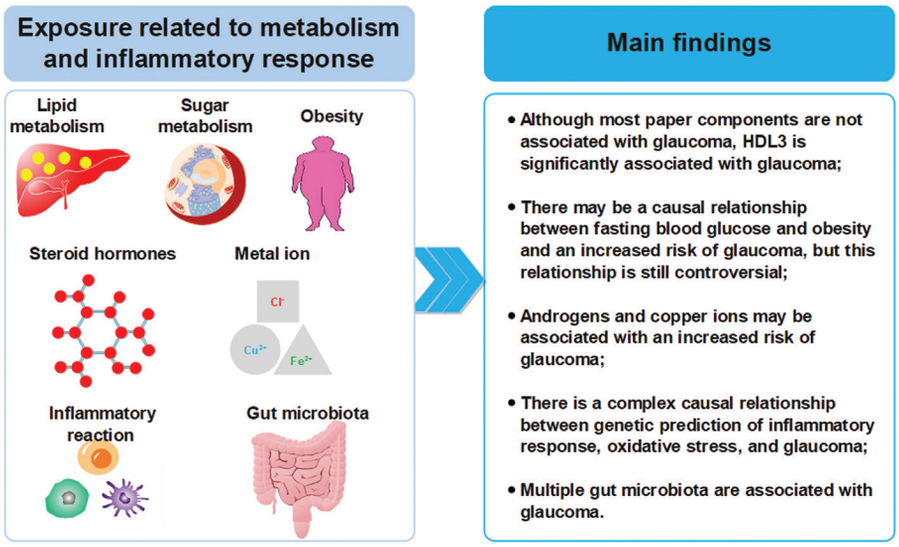

Figure 4 The causal relationship between metabolism, inflammatory response, and glaucoma HDL3: High density lipoprotein 3.

MR analysis indicates a lack of causative association between the majority of lipid components and POAG; implying supplementation with fatty acids or lipid components may not confer protective benefits against POAG. Interestingly, HDL3 exhibits a significant correlation with POAG, pointing to a potential vital role of cholesterol transport in the glaucoma pathogenesis[55].

Glucose metabolism Previous observational studies have signposted a significant association between elevated fasting glucose levels and POAG incidence. Within the framework of a Meta-analysis, it was elucidated that diabetic individuals bear a 48% elevated risk of encountering glaucoma as opposed to those without diabetes. Nonetheless, due to pronounced heterogeneity across the studies, such a causative nexus is deemed unreliable[56]. An MR study tailored to the Japanese demographic demonstrated no significant correlation between fasting glucose, C-peptide levels, and glycosylated hemoglobin with POAG[57]. Through an MR analysis leveraging datasets from Europe and East Asia, Hu *et al*[58] discerned a relationship between type 2 diabetes and POAG within the European cohort [odds ratio (OR)=1.07, *P*=0.028], a correlation absent in the East Asian pool.

Further dissecting the cause-and-effect dynamics between type 2 diabetes and 111 ocular conditions, Chen *et al*[59] uncovered a definitive positive causal relationship with glaucoma (OR=1.08, *P*=0.00481). Utilizing multi-ethnic GWAS datasets for MR analysis, Seo and Lee[60] observed a positive correlation between type 2 diabetes, fasting glucose, and glaucoma. Conversely, glycosylated hemoglobin did not share this association, attributing to the instrumental variables' heterogeneity and pleiotropy, which may tilt the findings toward bias. Tan *et al*[61] employed diverse GWAS datasets for their MR analysis, which unfolded no causative link between type 1 diabetes, type 2 diabetes, and fasting glucose levels with glaucoma. However, a correlation between fasting glucose levels and elevated IOP was identified [β: 0.80, 95% confidence interval (CI): 0.38-1.22, *P*=12e-4]. Utilizing GWAS data comprising lipid metabolism, blood pressure, and glucose metabolism as exposures, AlDarrab *et al*[56] conducted both univariate and multivariate MR analyses. They found a significant correlation between type 2 diabetes and glaucoma, with systolic blood pressure, fasting glucose, and glycosylated hemoglobin also showing some degree of association.

The MR analyses investigating the relationship between diabetes or fasting glucose levels with glaucoma have rendered varying verdicts across studies. Predominantly, in East Asian populations, metabolic dysregulation associated with glucose appears unrelated to glaucoma, while in European cohorts, the conclusions vary. This discrepancy could stem from genetic differences between ethnic groups; additionally, the variability in GWAS data sources comprising different populations or the sequencing methods employed in European contexts might contribute to these differences. Furthermore, the presence of a nonlinear relationship between blood glucose and glaucoma could lead to divergent outcomes. In summary, the causative relationship between glucose metabolism and glaucoma remains debatable, necessitating further, more in-depth investigation.

Obesity Obesity represents a profound global public health issue, closely intertwined with metabolic processes and a plethora of diseases[62]. A prospective cohort study in Korea[63] elucidated that individuals possessing a body mass index (BMI) ≥30 kg/m^2 are at an increased risk of developing POAG. In contrast, research conducted in India[64] indicated an association between lower BMI and heightened glaucoma risk. This variability could be attributed to confounding factors

within observational studies. Through the application of two-sample MR analysis, Lin *et al*[65] identified a significant positive causal relationship between BMI (OR=1.909, *P*=0.0042), waist circumference (OR=1.647, *P*=0.044), and hip circumference (OR=2.199, *P*=0.003) with POAG. Conversely, Julian *et al*[66] employed a more extensive POAG dataset for a full-phenotype MR analysis, uncovering that waist circumference (OR=0.79, *P*=1.66e-5) acts as a protective element against POAG, suggesting a potential inverse correlation between abdominal obesity and the risk of developing POAG.

The MR analysis results concerning the association between obesity and glaucoma, particularly POAG, currently exhibit a degree of contention, which can be attributed to the datasets involved in MR studies. Moreover, the potential for a nonlinear relationship between obesity and POAG might contribute to the observed inconsistencies in conclusions. In the future, more comprehensive research is necessary to fully explore the intricate relationship between obesity and POAG.

Steroid hormones Steroid hormones play a pivotal role in metabolic processes and are crucial for maintaining homeostasis, cellular proliferation, and inflammatory responses. However, due to the complexity of their types and mechanisms of action, assessing the impact of a single hormone on disease is challenging. Liu *et al*[67] deployed a two-sample MR approach to investigate the causal relationships between various steroid hormones and retinal neurodegenerative diseases. Data analysis from FinngenR5 demonstrated a positive correlation between the ratio of testosterone to 17β-estradiol and the risk of glaucoma (OR=1.11, *P*=0.03). This association was confirmed with a validation dataset from the MRC-IEU consortium, indicating a risk link between the ratio of testosterone to 17β-estradiol and glaucoma.

Metal ion metabolites The concentration of metal substances in the blood also affects health. Yang *et al*[68], through a large-scale MR analysis involving 21 metal ions as exposure factors, identified a significant causal relationship between serum copper levels and the risk of glaucoma (OR=1.107, *P*=0.005). However, no causal association was found between other metal ions and glaucoma.

In summary, MR analysis has elucidated the varied impacts of different metabolites on glaucoma, offering new perspectives and insights for treating and preventing this condition. At present, most studies focus on the etiology of POAG, which may be related to the availability of data. Subsequent researchers should pay more attention to other analyses of glaucoma to provide a more detailed explanation of the pathogenesis of glaucoma.

Immunoinflammatory Responses and Oxidative Stress Immunologic and inflammatory responses play a crucial role in the pathophysiological processes within the organism[69]. Components of blood cells are easily obtainable quantitative markers. Observational studies have pointed out that lymphocyte abnormalities, platelet parameters, *etc*., are associated with glaucoma risk[70-71]. Utilizing MR analysis, Song *et al*[72] identified that lymphocyte count, eosinophil count, platelet crit, and platelet count could be risk factors for glaucoma. After adjusting for confounding factors with multivariable MR analysis using superoxide dismutase and C-reactive protein, blood cell traits still exhibited a causal relationship with glaucoma.

Beyond blood cell traits, inflammatory factors may also be correlated with glaucoma. In a study reporting on aqueous humor testing in glaucoma patients, elevated levels of interferon-γ, interleukin (IL)-5, IL-12, IL-15, and monocyte chemoattractant protein (MCP)-1α were found in patients with glaucoma[73]. Using MR analysis on 41 circulating inflammatory factors and GWAS data from UK Biobank glaucoma patients, Teng *et al*[74] demonstrated a potential risk association between interferon-γ inducible protein and glaucoma, whereas reverse MR analysis indicated that glaucoma does not trigger changes in systemic inflammatory factors. Zhang *et al*[75] conducted MR analyses with different glaucoma datasets, revealing that MCP-1, IL1ra, IL-1β, and IL-9 act as protective factors for glaucoma, whereas IL2ra presents a risk factor.

Tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE1) and tyrosine kinase endothelial receptor (TEK) play significant roles in angiogenesis and immunoinflammatory responses[76-77]. Emerging research indicates that the signaling pathways of TIE1 and TEK can be targeted for treating elevated IOP[78]. MR analyses conducted by Rajasundaram *et al*[79] revealed that elevated levels of circulating TIE1 led to a reduction in IOP, and similarly, a reduction in circulating TEK also resulted in a decrease in IOP, but there is no direct causal relationship with POAG. This suggests that the TIE1/TEK signaling pathway plays a crucial role in POAG.

Additionally, oxidative stress may have an impact on the development of glaucoma. However, observational studies have shown contradictory conclusions. Some studies indicate an increase in oxidative stress markers in the aqueous humor and serum of glaucoma patients, suggesting that supplementation of vitamin C may reduce the risk of developing glaucoma. Yet, other Meta-analyses show no association between vitamin C supplementation and glaucoma[80-81]. Shi *et al*[82] performed bidirectional two-sample MR analysis using data relating to 11 oxidative stress markers, including vitamin C, retinol, superoxide dismutase, and catalase, as exposures, and glaucoma along with its three

subtypes from FinnGen as outcomes. The results indicated a correlation between a reduced risk of POAG and catalase (OR=0.915, *P*=0.022), a reduced risk of primary angle-closure glaucoma (PACG) with superoxide dismutase, and an increased risk for PACG with myeloperoxidase. Reverse MR analyses showed a negative correlation between total bilirubin (OR=0.961, *P*=0.039), glutathione peroxidase (OR=0.934, *P*=0.006), paraoxonase (OR=0.883, *P*=0.005), and albumin (OR=0.988, *P*=0.014) in glaucoma patients. Moreover, Hysi *et al*[83] identified significant benefits of O-methylascorbate for IOP reduction through machine learning and MR analysis, further validating the benefits of antioxidants in glaucoma management.

The complex causal links between immune-inflammatory responses, oxidative stress, and glaucoma are evident despite the efforts of MR analysis to minimize the impact of confounding factors. However, discrepancies remain between the findings of various studies, which may be attributed to differences in data types, sources of study populations, regional variations, and inconsistencies in sequencing methods. These factors underline the importance of considering the context and methodology of each study when interpreting results and emphasize the need for further research to clarify these relationships.

Gut Microbiota The gut microbiota is diverse and complex, playing a crucial role in immune responses and metabolism within the host. The concept of the gut-eye axis proposed by Vujkovic-Cvijin *et al*[84] highlights the role of the gut flora in ocular diseases, such as glaucoma. Studies have revealed that patients with POAG exhibit an increased abundance of Escherichia coli and Prevotella in their gut microbiota compared to healthy individuals; significant differences in microbiota composition have also been observed between POAG and angle-closure glaucoma[85-86]. Utilizing exposure data from 119 types of gut microbiota for MR analysis, Li and Lu[87] found that the weighted median results suggest that *Eubacterium* (nodatum group, *P*=0.041), *Lachnospiraceae* (NC2004 group) (*P*=0.026), and *Roseburia* (*P*=0.028) may be risk factors for glaucoma. Similarly, inverse variance weighted results indicated an increased risk factor associated with *Ruminococcaceae* (UCG004, *P*=0.029) for glaucoma. Bidirectional MR analysis showed that glaucoma and *Eubacterium* (nodatum group) are risk factors for each other.

Wu *et al*[88] conducted MR analysis using the gut microbiome dataset from MiBioGen, revealing that *Ruminiclostridium* 9 (OR=1.258, *P*=0.003) and *Lachnospiraceae* UCG010 (OR=1.002, *P*=0.033) are risk factors for glaucoma, consistent with the findings of Li and Lu[87]. Additionally, Wu *et al*[88] identified *Oxalobacteraceae* (OR=0.900, *P*=0.002) and *Eggerthella* (OR=0.881, *P*=0.003) as potential protective genera against glaucoma, while *Bilophila* (OR=1.202, *P*=0.001) could be a risk factor. Moreover, Zhou *et al*[89] undertook a more in-depth investigation by taking glaucoma-related traits as outcomes, finding that *Rumnicoccaceae* UCG009 and *Ruminiclostridium* 9 are associated with an increased risk of glaucoma. In contrast, *Lacchnospiraceae* is considered a protective factor for POAG. They also confirmed the mediating effect of IOP, vertical cup-disc ratio, and central corneal thickness between the gut microbiota and POAG using multivariable MR analysis.

In summary, MR analysis has significantly explored the causal association between gut microbiota and glaucoma. MR analysis has meticulously examined the role of various bacterial genera in glaucoma etiology, with some results showing consistency across different studies. However, discrepancies remain in some of the conclusions drawn from MR studies, which may be attributable to the plethora of confounding factors affecting the gut microbiota. It is hoped that future research will include larger-scale GWAS to delve deeper into this subject.

Causal Relationships Between Other Diseases and Glaucoma The correlation between diseases has always been a focus of MR analysis, and the causal relationship between other diseases and glaucoma is a top priority for researchers. This can help people to carry out targeted health management and prevention. At present, MR analysis of other diseases and glaucoma mainly involves mental disorders, autoimmune diseases, and other ophthalmic diseases such as myopia and age-related macular degeneration (Figure 5).

Causal Relationship Between Immune-Related Diseases and Glaucoma Autoimmunity is considered a risk factor for glaucoma, and autoimmune diseases have also been found to be correlated with glaucoma[90-91]. Li *et al*[92] used public data from MR-Base for MR analysis and discovered a positive correlation between ankylosing spondylitis and the risk of POAG (OR=1.45, *P*=8.80e-4) and PACG (OR=1.91, *P*=3.88e-2). Teng *et al*[93] used MR methods to analyze the causal relationship between rheumatoid arthritis and glaucoma. Their results indicated that, in European populations, rheumatoid arthritis is a risk factor for glaucoma, but this was not observed in East Asian populations.

Meng *et al*[94] utilized both univariate and multivariate MR methods to study the relationship between six common rheumatic diseases, including ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, dermatomyositis, and gout, and their correlation with POAG and PACG. Univariate MR analysis showed that ankylosing spondylitis positively correlated with the risk of POAG (OR=1.28, *P*=1.1e-4) and PACG (OR=1.55, *P*=1.4e-2). The other rheumatic diseases showed no correlation with glaucoma. After adjusting for potential confounding

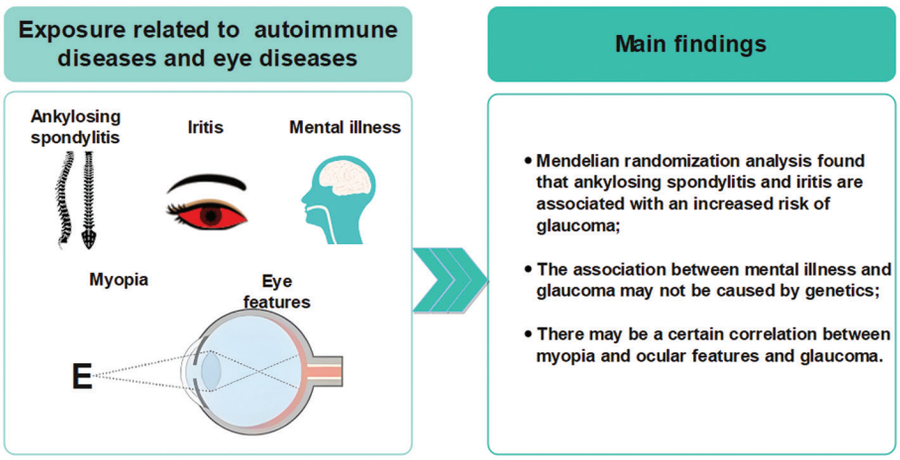

Figure 5 The causal relationship between autoimmune diseases, eye diseases, and ocular features with glaucoma.

factors such as smoking, coronary heart disease, and diabetes, the multivariate MR analysis still indicated a significant relationship between ankylosing spondylitis and both types of glaucoma. This is consistent with the findings of Li *et al*[92] and others but shows some differences from the conclusions of Teng *et al*[74].

Iritis and choroiditis have also been found to have a certain correlation with glaucoma. Seo and Lee[95] conducted MR analysis and found a significant positive correlation between iritis and glaucoma in East Asians and multiple ethnicities. However, due to the lack of significant instrumental variables for choroiditis, the MR analysis did not yield reliable conclusions.

Previous studies have also suggested that atopic dermatitis may be related to POAG, but strong evidence is lacking. Luan *et al*[96] used bidirectional MR analysis to explore the causal relationship between atopic dermatitis and POAG, finding no causal relationship between the two.

Moreover, Han *et al*[97] conducted a large-scale genome-wide association analysis, identifying 312 gene loci associated with POAG. Subsequent MR analysis suggested systemic lupus erythematosus might be a protective factor for glaucoma.

In summary, autoimmune diseases may have a risk association with glaucoma, especially patients with ankylosing spondylitis and iritis should pay more attention to traits related to glaucoma, monitor IOP as regularly as possible to reduce or avoid the vision impairment caused by glaucoma.

Myopia and Other Ocular Diseases Previous observational studies have identified a correlation between myopia and an increased risk of POAG, though the genetic basis of this correlation remains ambiguous. Research conducted by Choquet *et al*[98] elucidated a risk association between myopia or high myopia and POAG. Furthermore, cross-trait linkage disequilibrium regression analysis revealed shared genetic influences between POAG and myopia, a conclusion corroborated by subsequent MR analyses. Simcoe *et al*[99] carried out a GWAS, uncovering new loci associated with pigmentary glaucoma while demonstrating, *via* MR analysis, a risk correlation between myopia and pigmentary glaucoma. Moreover, analyses of various MR models by Chong *et al*[100] indicated a significant bidirectional causal relationship between myopia and POAG, suggesting that anti-hypertensive ocular therapy in glaucoma patients could potentially benefit the treatment of myopia.

Seo and Lee[101] conducted an MR analysis on age-related ocular diseases, revealing no causal relationship between cataracts, glaucoma, and age-related macular degeneration; however, a potential causal relationship between glaucoma and cataracts was identified, aligning with observational studies.

In summary, MR analyses have confirmed a causal linkage between myopia and POAG, providing genetic-level evidence. Such analyses offer novel strategies for risk stratification of POAG amongst patients with myopia.

Psychiatric Disorders Recent studies have indicated that individuals with psychiatric disorders have a higher likelihood of developing glaucoma compared to the general population[102-103]. Furthermore, it has been shown that both glaucoma and many psychiatric disorders are influenced by genetic factors[104-105]. Zhang *et al*[106] utilized a two-sample MR analysis to investigate the causal relationships between psychiatric disorders—including insomnia, depression, and schizophrenia—and glaucoma, along with its subtypes (PACG and POAG). The results indicated that there are no causal relationships between psychiatric disorders and glaucoma in both European and East Asian populations. Zhang *et al*[107] explored the shared genetic architecture between glaucoma and psychiatric disorders. Although their findings revealed shared genetic variants between glaucoma and various psychiatric disorders, MR analyses did not support a direct causal relationship between glaucoma and psychiatric disorders.

The outcomes from MR analyses concerning the causal relationships between psychiatric disorders and glaucoma

do not align with the majority of observational studies. This discrepancy may be related to the pleiotropy of genetic variants; it also suggests that the association between these conditions might be more significantly influenced by environmental or other modifiable factors. This implies the necessity of increasing attention towards ocular diseases and mental health, conducting more in-depth research to comprehend this relationship fully, and thereby enhancing ocular healthcare for patients with psychiatric disorders.

Several Ocular Features and Their Causal Associations with Glaucoma

Corneal thickness Studies indicate a significant genetic correlation between central corneal thickness and glaucoma. Choquet *et al*[108] performed a genome-wide analysis across different populations, unveiling 41 novel loci associated with central corneal thickness, including RAPSN rs3740685 which is notably related to glaucoma. However, subsequent MR analyses revealed no direct causal link between central corneal thickness and POAG, suggesting the association between corneal thickness and glaucoma may be attributed to pleiotropy.

Corneal biomechanics The corneal hysteresis and corneal resistance factor reflect the biomechanical properties of the cornea. Research indicated potential correlations between alterations in these indices and glaucoma[109-110]. Simcoe *et al*[111] conducted a genome-wide association analysis identifying over 200 genetic loci associated with corneal biomechanical properties, suggesting a significant genetic influence on these traits. Further, MR analyses demonstrated a causal effect of IOP on both corneal resistance factor and hysteresis, where an increase in IOP leads to decreased corneal hysteresis and increased corneal resistance factor.

Retina The gradual loss of retinal ganglion cells leading to progressive optic neuropathy marks a principal characteristic of POAG. Hamel *et al*[112] carried out MR and colocalization analyses, revealing several e/sGenes within retinal expression quantitative trait locus, including TMCO1, GAS7, and LMX1B, significantly associated with both POAG and IOP.

ADVANTAGES AND LIMITATIONS OF MENDELIAN RANDOMIZATION ANALYSIS IN GLAUCOMA ETIOLOGY

Advantages of Mendelian Randomization Analysis in Glaucoma Etiology MR is a method that utilizes genetic variability as an instrumental variable to assess the causal relationships between exposures and outcomes. Compared to traditional observational studies, it offers several advantages.

1) Accurate understanding of causal relationships: by using genetic variations associated with the risk of glaucoma as instrumental variables, MR studies can disclose if certain biomarkers (such as inflammatory factors, plasma proteins, *etc*.), lifestyle choices (diet, physical activity, *etc*.), environmental factors, or other diseases genuinely impact the risk of glaucoma, rather than merely exhibiting associations. Additionally, since the genetic variations in the hypothesis are solely related to the exposure under study, MR analysis effectively circumvents the presence of reverse causality.

2) Reduction of confounding bias: Traditional observational studies are susceptible to confounding factors. In contrast, MR studies use genetics as instrumental variables. The random genetic allocation at the point of conception, allows MR analysis to effectively minimize the impact of confounding bias.

3) Identification of new therapeutic targets: MR analysis can help researchers identify new targets for treatment or drugs by pinpointing specific biomarkers or signaling pathways involved in the development of glaucoma. For instance, a significant causal relationship between certain inflammatory proteins and an increased risk of glaucoma suggests that anti-inflammatory treatments may serve as novel therapeutic targets, indicating the potential of MR analysis in revolutionizing glaucoma treatment strategies.

4) Simplification of research processes: MR analysis does not require long-term follow-up studies and can utilize data from existing large-scale genetic and epidemiological databases, making the process more efficient. This efficiency can help researchers conserve resources and may provide momentum for investigating the genetic underpinnings of complex diseases like glaucoma, accelerating knowledge translation and reducing the gap between basic research and clinical application.

5) Clinical translation: MR analysis can provide a basis for screening and early intervention in clinical decision-making. Through MR analysis, researchers can identify potential intervention targets, such as biomarkers and signaling pathways related to glaucoma risk. At the same time, the genetic susceptibility to glaucoma can be assessed through MR analysis, which helps identify high-risk patients and guide individualized screening. Additionally, MR analysis can assist in formulating preventive measures for glaucoma. For example, in terms of lifestyle habits, we found that behaviors such as drinking coffee, alcohol, and smoking may be associated with an increased risk of glaucoma. Therefore, appropriately controlling the intake of coffee, alcohol, and cigarettes can help prevent the occurrence of glaucoma.

In summary, the MR method closely integrates genetics with epidemiology, offering a unique and powerful tool to scientifically unravel the causal relationships underlying the complex disease of glaucoma.

Limitations of Mendelian Randomization Studies in Glaucoma Etiology Despite the significant advantages of MR over observational studies, it inherently possesses certain limitations.

1) Validity of instrumental variables: The central issue in MR analysis lies in the selection and validity of instrumental variables. The correct instrumental variable must fulfill the strong relevance assumption, ensuring a clear genetic association with the exposure factor. However, in practice, the related genes often account for only 3%–20% of the variance, making it challenging to achieve a strong correlation[113]. With the expansion of databases worldwide and continuous advancements in statistical methods, this issue is believed to be resolved soon.

2) Heterogeneity and pleiotropy of instrumental variables: Exclusion of heterogeneity and pleiotropy is another core assumption in MR analysis. Researchers typically use various sensitivity analyses to test for pleiotropy, such as MR-Egger regression, Weighted Median, MR-PRESSO, *etc*. In addition, researchers often employ methods like stepwise exclusion of instrument variables potentially affected by pleiotropy, and use different statistical models to ensure the robustness and reliability of the results. However, due to the current limited understanding of biological genetic information, it is often challenging to determine whether a genetic variant is associated with other confounding factors in practice. Moreover, genes may have pleiotropic effects on diseases, which could lead to biased results. Although combining multiple sensitivity analyses can significantly reduce heterogeneity and pleiotropy, it is often difficult to completely resolve these issues. It is hoped that with deeper research into genetics, the problems of heterogeneity and pleiotropy in instrument variables will be thoroughly addressed.

3) Ethnic differences: There may be genetic differences between different ethnic groups. Due to varying environmental factors across different populations, the probability of expression for the same traits can differ, which may lead to differences in the prevalence of certain genes. For example, certain genetic variants associated with glaucoma may have significantly different effects in Asian and European populations. This necessitates stratified MR analysis across different populations to ensure the broad applicability of the research findings. Additionally, we suggest that future MR studies should place more emphasis on cross-ethnic comparative analysis. By combining large-scale GWAS data from different ethnic groups, researchers can gain a more comprehensive understanding of the genetic mechanisms of diseases like glaucoma. Furthermore, cross-ethnic MR analysis can help identify genetic factors unique to certain populations, providing more accurate genetic risk assessments for personalized medicine. It is worth noting that cultural and environmental differences between regions may influence MR research outcomes to some extent. For instance, lifestyle and dietary habits could interact with genetic backgrounds to jointly affect the onset and progression of diseases like glaucoma. Therefore, MR research should not only focus on genetic backgrounds but also take into account environmental factors within populations to further enhance the accuracy and generalizability of the findings.

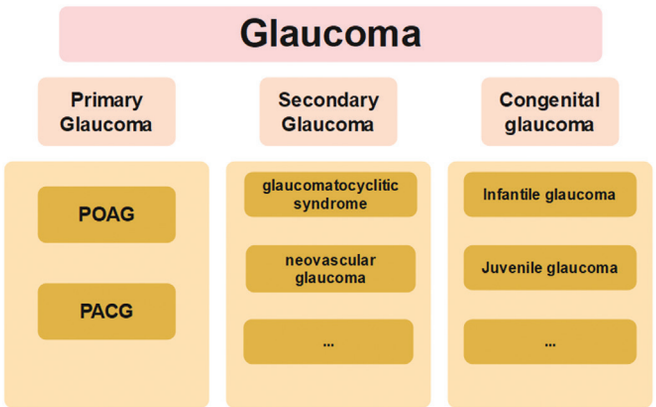

Figure 6 Glaucoma typing POAG: Primary open angle glaucoma; PACG: Primary angle-closure glaucoma.

4) Operability and interpretability: MR analysis requires researchers to possess a profound knowledge of statistics, genetics, and epidemiology. This demands not only the ability to operate complex statistical analysis software but also an understanding of the biological mechanisms of gene expression and the capability to elucidate genetic data and statistical outcomes. Additionally, although MR analysis can clarify the causal relationship between exposure and outcome, it is challenging to detail the pathophysiological mechanisms between them, which might require further foundational research for verification. Furthermore, when focusing on the same exposure and outcome, different MR studies might yield inconsistent results, potentially due to a non-linear relationship between the outcome and exposure. MR studies typically interpret linear causal connections, and once a non-linear relationship exists between exposure and outcome, the interpretative power of MR could diminish.

5) Glaucoma typing: Glaucoma is a disease characterized by complex heterogeneity, encompassing various subtypes such as primary open-angle glaucoma, primary angle-closure glaucoma, congenital glaucoma, and secondary glaucoma (Figure 6). In current MR studies related to glaucoma, a majority focus on POAG, while some do not differentiate among glaucoma subtypes; this may lead to inaccuracies or misinterpretations of research findings, for instance, a genetic variant associated with POAG might not necessarily be relevant to congenital glaucoma. Therefore, to enhance the accuracy and reliability of MR analysis in glaucoma etiology, it is pivotal to conduct stratified research according to the specific types of glaucoma. Additionally, we suggest that future researchers consider using relevant data from the Finngen

database for their studies, as this database provides a more detailed analysis of glaucoma. Future researchers should pay more attention to the stratification and categorical management of glaucoma. It is anticipated that more comprehensive GWAS detailing glaucoma subtypes will be available in the future, addressing the gaps in MR analysis for glaucoma typing research, and providing more precise genetic guidance for the individualized prevention and treatment of glaucoma.

6) Non-significant results: This study found that multiple MR analyses indicated a non-significant association between exposure factors and glaucoma. We believe this may be related to insufficient sample size. If the sample size is too small, it may fail to detect the true causal relationship between exposure and outcome. Even if a true causal relationship exists, inadequate statistical power may prevent reaching significance. Therefore, researchers should ensure an adequate sample size when designing MR studies to enhance statistical power. Future studies should consider using larger sample sizes, particularly when studying complex diseases like glaucoma, where small sample sizes may limit the reliability of the findings. At the same time, methodological limitations may also contribute to non-significant results. For instance, MR analysis typically assumes a linear relationship between exposure and outcome, but if a non-linear relationship exists, traditional MR methods may fail to capture this relationship effectively, leading to non-significant results. In such cases, we recommend considering more flexible statistical models in the study design, such as non-linear MR models, to better explore complex causal relationships. Additionally, non-significant results can still provide valuable insights for research. First, they can offer directions for future studies. Non-significant results may indicate that certain exposure factors do not have a significant impact on diseases like glaucoma, but these findings still guide future research. Researchers can use these results to adjust their hypotheses or design new studies to explore potential causal relationships more deeply. Even though the causal relationship of certain exposure factors may not be significant in existing studies, this does not mean they are entirely unrelated. Future research, by increasing sample size, selecting stronger instrumental variables, or applying new analytical methods, may reveal hidden causal relationships. It is also worth noting that non-significant results can sometimes highlight the limitations of the research methodology, prompting researchers to rethink how to improve study design. For example, more refined variable selection, better instrument variable configuration, or more complex statistical models may be needed to account for non-linear relationships.

CONCLUSION

In summary, MR analysis has explored the causal relationships between glaucoma and various traits or characteristics, including dietary factors, antihypertensive medications, addictive substances, behavioral habits, metabolic traits, immune responses and oxidative stress, gut microbiome, immune-related diseases, myopia and other eye diseases, mental disorders, and ocular features. Although certain results still require further validation, this provides new insights and potential intervention strategies for preventing, diagnosing, and treating glaucoma. Moreover, while MR offers a powerful tool for causal inference in glaucoma etiology, its application is not without limitations, necessitating appropriate statistical strategies and analysis methods to minimize their impact. With continuous advancements in research methodologies and the increasing wealth of big data, these limitations are expected to be overcome, suggesting a promising future for the MR analysis in glaucoma etiology.

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