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Research article

Genetic evidence implicating circulating lipids and lipid drug targets in pterygium

Yuchen Cai [a,b,1], Fei Fang [a,b,1], Tianyi Zhou [a,b,1], Wenjun Shi [c], Xueyao Cai [c,*], Yao Fu [a,b,**]

[a] Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Shanghai Key Laboratory of Orbital Diseases and Ocular Oncology, Shanghai, China
[c] Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

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ABSTRACT

There is limited knowledge about the impact of circulating lipids and lipid-modifying drugs on pterygium development, with conflicting results reported. Our study aimed to address these questions by applying the Mendelian randomization (MR) approach. A two-step MR model was developed. In the first step, bidirectional two-sample MR was employed to establish the causal relationship between circulating lipids and pterygium risk. In the second step, drug-target MR analysis was conducted to assess the causal effect of proprotein convertase subtilisin/kexin type 9 (PCSK9) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) inhibitors on pterygium outcomes. Genetically predicted low-density lipoprotein cholesterol (LDL-c) levels were found to be significantly associated with an increased risk of pterygium (Inverse variance weighted [IVW] odds ratio [OR] = 2.227; $P = 1.53 \times 10^{-4}$). Similarly, higher total cholesterol (TC) levels exhibited a suggestive association with greater susceptibility to pterygium (IVW OR = 1.806; $P = 1.70 \times 10^{-3}$). Through drug-target MR, a positive causal association was noted between HMGCR-mediated LDL-c levels and pterygium (IVW OR = 6.999; $P = 0.016$), suggesting that statins may be effective in reducing pterygium risk. The present findings suggest that circulating TC and LDL-c are risk factors for pterygium. Additionally, the results indicate that HMGCR inhibitors, which lower LDL-c levels, have a potential protective effect on pterygium outcomes. Further research is warranted to elucidate the underlying mechanisms involved in pterygium pathogenesis, with a particular focus on cholesterol metabolism.

1. Introduction

Pterygium, characterized by abnormal growth of the conjunctiva, typically appears as a wing-shaped migration of epithelial and fibrovascular tissue into the central cornea [1]. It is a common chronic inflammatory disease of the ocular surface, with a prevalence ranging from 0.07% to 53% in diverse populations and geographical locations [2–6]. Pterygium can cause discomfort such as redness, irritation, dryness, tearing, and foreign body sensations in the eye, and in severe cases, lead to recurrent inflammation and visual impairment, posing a significant threat to ocular health [7,8]. Potential irritants such as UV exposure, dust, and smoking are believed to trigger pterygium, which may partly explain the geographic variation in prevalence. However, the exact nature of the disease remains uncertain, especially with regard to environmental and nutritional factors [9,10]. Although surgical excision is the prevailing method for symptom alleviation, it is accompanied by a

Abbreviations: CAD, coronary artery disease; CI, confidence interval; eQTLs, expression quantitative trait loci; HDL-c, high-density lipoprotein cholesterol; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IVs, instrumental variables; IVW, inverse-variance weighted; GWAS, genome-wide association study; LD, linkage disequilibrium; LDL-c, low-density lipoprotein cholesterol; MAF, minor allele frequency; MR, Mendelian randomization; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9; RCT, randomized controlled trial; SE, standard error; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; WM, weighted median.

* Correspondence to: Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhi-Zao-Ju Road, Huangpu District, Shanghai 200011, China.

** Correspondence to: Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhi-Zao-Ju Road, Huangpu District, Shanghai 200011, China.

E-mail addresses: kevin89459@alumni.sjtu.edu.cn (X. Cai), fuyao@sjtu.edu.cn (Y. Fu).

[1] Contributed equally

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substantial recurrence rate [11]. Adjuvant therapies such as mitomycin-C, 5-fluorouracil, and beta-irradiation have been employed, but these interventions may result in complications and adverse effects [12]. From a clinical perspective, further study of the triggering factors is critical to enhance our understanding of pterygium pathogenesis and pave the way for new therapies and prevention measures for recurrence.

Circulating lipids, including high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), and triglycerides (TG), are essential components of blood plasma, serving to maintain the structure and function of cells, tissues, and organs in the human body. The analysis of circulating lipids has been of great interest in the field of medical research, as abnormal levels of these lipids may lead to a variety of pathological conditions, such as cardiovascular disease, diabetes, and obesity. Although existing studies have suggested an important role for cholesterol metabolism in ocular surface diseases, the potential impact of circulating lipids on pterygium remains largely unexplored, with inconsistent evidence available to date [13–16]. A recent research in Hebei Province, China, reported a correlation between high HDL-c levels and an increased risk of developing grade 2 or higher pterygium in males [17]. Similarly, a large-scale cross-sectional study showed that elevated levels of HDL-c and LDL-c are both significant risk factors for developing pterygium, especially in individuals aged 50 or with a normal body mass index (BMI) [18]. Conversely, two retrospective case-control studies suggested a negative association between pterygium and HDL-c [19,20]. However, it must be noted that these observational studies are inherently subject to the potential influence of confounding factors and selection bias, which ultimately hinders our understanding of the actual relationship between circulating lipids and pterygium.

Mendelian randomization (MR) is a statistical technique in epidemiology that uses instrumental variables (IVs) to investigate causal relationships between exposure and outcome [21]. Unlike randomized controlled trials (RCTs), MR overcomes the challenges of confounding and reverse causality through the employment of single nucleotide polymorphisms (SNPs) as IVs, which are randomly allocated during meiosis and are not influence by sociodemographic or behavioral factors [22,23]. In certain circumstances, MR can provide causal evidence where RCTs are impractical or unethical, and can use existing genetic data, saving time and resources compared to large-scale RCTs [24,25]. In addition, MR studies can provide valuable information for pharmaceutical development, including the prediction of therapeutic effects and the identification of adverse reactions of targeted drugs. This method uses cis-expression quantitative trait loci (cis-eQTLs) of the drug target gene as genetic proxies, which act as regulators that affect gene expression. While drug-target MR analyses have been applied in diverse disease contexts, genomic evidence of potential drug targets for pterygium remains unexplored [26–30].

The objective of the present investigation was to assess the potential implications of circulating lipids and lipid-modifying drugs on pterygium development. A bidirectional two-sample MR analysis was employed to investigate the possible causal relationship between blood lipid traits and the risk of pterygium. Additionally, drug-target MR analysis was conducted using expression quantitative trait loci (eQTLs) as instruments to explore the therapeutic potential of lipid-modifying agents for pterygium, specifically 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

2. Methods

2.1. Study design

Our 2-sample MR study was based on summary statistics from independent genome-wide association studies (GWAS). A total of four types of serum lipids were included, including HDL-c, LDL-c, TC, and TG. A forward MR analysis was performed to assess the causal associations of serum lipids with pterygium. The following reverse MR analysis was conducted to investigate the reverse causation between pterygium and different circulating lipids. Based on the established evidence of lipid effects on pterygium outcomes, two classes of U.S. Food and Drug Administration (FDA) approved LDL-c-lowering pharmaceuticals (HMGCR and PCSK9 inhibitors) were selected as exposures to evaluate their association with pterygium risk by performing the drug-target MR. The GWAS dataset of coronary artery disease (CAD) was used as a positive control to test instrument validity for PCSK9 and HMGCR. All these studies were approved by the relevant ethics committees and all participants provided informed consent. The overall design of the study is illustrated in Fig. 1.

2.2. Selection of genetic instruments

All single-nucleotide polymorphisms (SNPs) associated serum lipids were available from the MRC Integrative Epidemiology Unit (IEU) OpenGWAS database (https://gwas.mrcieu.ac.uk/datasets/). Four lipid traits were collected from independent GWAS of European ancestry, including HDL-c (dataset ieu-b-109; n = 403,943), LDL-c (dataset ieu-b-5089; n = 201,678), TC (dataset ebi-a-GCST002221; n = 94,595), and TG (dataset ieu-b-4850; n = 78,700) (Table 1). Genetic associations with pterygium were also retrieved from IEU OpenGWAS with 203,880 participants of European ethnicity (dataset finn-b-H7_PTERYGIUM, ncase = 363, ncontrol = 203,517), together with the positive control CAD dataset (dataset ieu-a-7; n = 184,305, ncase = 60,801, ncontrol = 123,504) (Table 1). Significant SNPs were selected on a p-value $< 5 \times 10^{-8}$. To assess genetic linkage among SNPs, linkage disequilibrium (LD) was applied with a window size = 1 megabase (Mb) and a threshold value of $r^2 < 0.001$.

Based on the observed positive association between LDL-c levels and pterygium risk, a drug-target MR analysis was conducted using eQTLs for targets that modify LDL-c levels, specifically PCSK9 and HMGCR, as proxies for exposure to lipid-lowering drugs. SNPs for HMGCR and PCSK9 were obtained from the eQTLGen Consortium (https://www.eqtlgen.org/), which is comprised of individuals of European ancestry (Table 2). We selected significant eQTLs with a p-value less than 5×10^{-8} for HMGCR and PCSK9 concentrations. Subsequently, we identified common eQTLs (minor allele frequency [MAF] > 0.01) associated with the expression of HMGCR or PCSK9 in blood as IVs for drug-target MR analyses. Only cis-eQTLs were included, defined as eQTLs located within 1 Mb on either side of the target gene.

2.3. MR statistical analysis

Two-sample MR analysis was conducted to estimate the causal impact of genetically determined circulating lipid traits on pterygium risk using TwoSampleMR v4.1.1 R packages. Inverse variance weighted (IVW) was chosen as the primary approach to establish causality, with weighted median (WM), MR-Egger, weighted mode, and simple mode methods used as complementary techniques. The IVW method is the most common approach in two-sample MR, providing the best unbiased estimate assuming IVs are valid and without pleiotropy [31]. The WM method is employed to integrate various genetic variants into a causal estimate and is applicable when up to 50% of IVs are invalid [32]. The MR-Egger model is used to calculate the pleiotropy effect across genetic variants and can provide a robust estimation in the case of weaker IVs [33]. The weighted mode-based method is a reliable analytical model for overall causal estimates provided that individual estimates are primarily obtained from valid IVs [34]. Estimates were considered significant based on the IVW method ($p < 0.05$) and the other MR methods displayed effects in the identical direction.

For drug-target MR, eQTLs were used as an instrument to investigate the association between pterygium risk and expression levels of two target genes, PCSK9 and HMGCR. To ensure the instrument validity of PCSK9 and HMGCR, a two-sample MR was performed using CAD as a

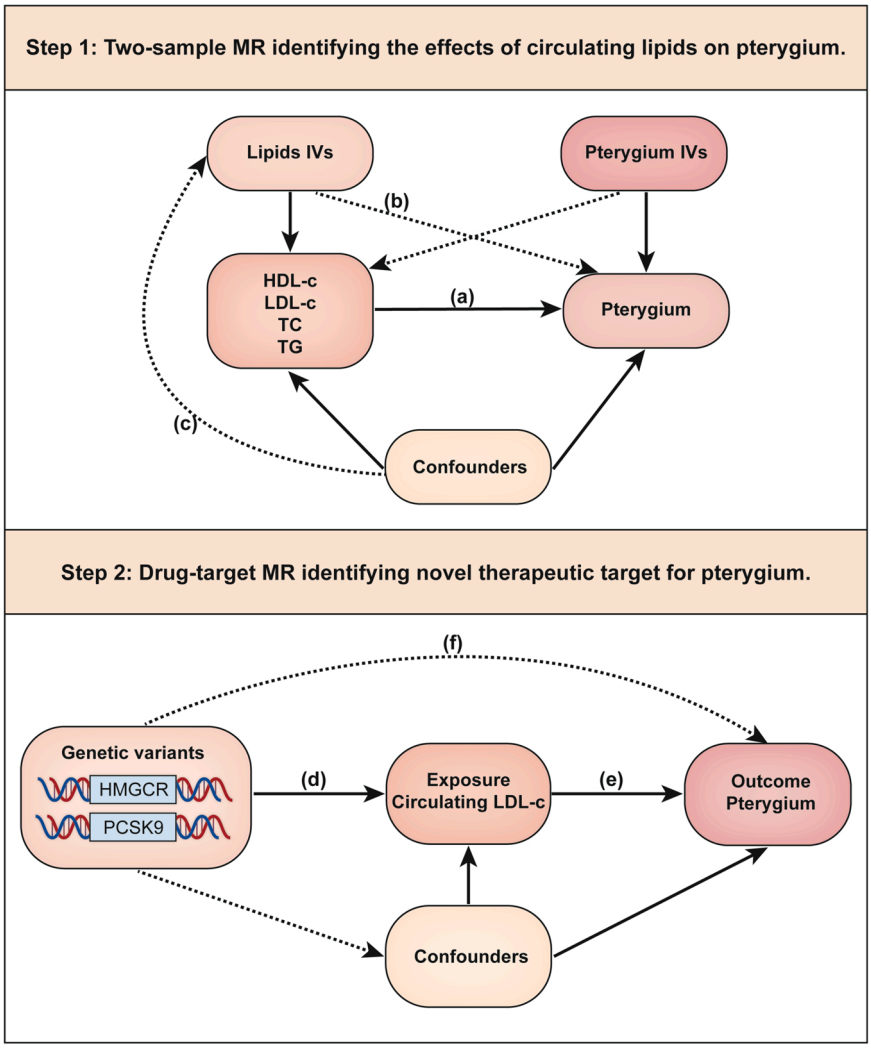

Fig. 1. Study overview and Mendelian randomization (MR) model. In step 1, we aimed to identify the causal relationship between circulating lipid traits and pterygium risk using a bidirectional two-sample MR approach. (a) Forward MR; (b) IVs for lipid traits are not related to pterygium; (c) IVs for lipid traits should not have an association with measured or unmeasured confounding. In step 2, we aimed to identify novel LDL-c-modifying targets for pterygium. (d) Effect of genetically proxied PCSK9 and HMGCR on circulating LDL-c; (e) Association of circulating LDL-c on pterygium mediated by drug targets PCSK9 and HMGCR; (f) Effect of genetically proxied PCSK9 and HMGCR on pterygium risk. HDL-c: high-density lipoprotein cholesterol; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IVs, Instrumental variables; LDL-c: low-density lipoprotein cholesterol; PCSK9: proprotein convertase subtilisin/kexin type 9; TC; total cholesterol; TG: triglycerides.

Table 1
Description of GWAS Summary Statistics for Circulating Lipids and Pterygium.

Traits	Accession	Sample Sizes	Number of SNPs	Ethnicity
HDL-c	ieu-b-109	403,943	12,321,875	European
LDL-c	ieu-b-5089	201,678	12,321,875	European
TC	ebi-a-GCST002221	94,595	2,418,562	European
TG	ieu-b-4850	78,700	7,892,037	European
Pterygium	finn-b-H7_PTERYGIUM	203,517	16,380,437	European
CAD	ieu-a-7	184,305	9,455,779	European

GWAS summary datasets were obtained from the IEU OpenGWAS (https://gwas.mrcieu.ac.uk/). CAD was used as a positive control for drug-target Mendelian randomization (MR). CAD: coronary artery disease; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides; SNP: single nucleotide polymorphism.

Table 2
Genetic Instruments of LDL-c-Lowering Drugs.

Exposure	Genetic variants associated with eQTLs	Genetic variants associated with LDL-c
HMGCR inhibitors	921 common cis-eQTLs (MAF > 0.01) in blood for HMGCR gene ($p < 5.0 \times 10^{-8}$)	7 common SNPs (MAF > 0.01) in low LD ($r^2 < 0.30$), associated with LDL-c ($p < 5.0 \times 10^{-8}$), located within a 1 Mb window from HMGCR gene
PCSK9 inhibitors	24 common cis-eQTLs (MAF > 0.01) in blood for PCSK9 gene ($p<5.0 \times 10^{-8}$)	15 common SNPs (MAF > 0.01) in low LD ($r^2 < 0.30$), associated with LDL-c ($p < 5.0 \times 10^{-8}$), located within a 1 Mb window from PCSK9 gene

eQTLs: expression quantitative trait loci; HMGCR: HMG-CoA reductase; LD: linkage disequilibrium; LDL-c: low-density lipoprotein cholesterol; MAF: minor allele frequency; PCSK9: proprotein convertase subtilisin/kexin type 9; SNP: single nucleotide polymorphism.

positive control, as CAD is the main indication for PCSK9 and HMGCR inhibitors. Subsequently, drug target instrument exposures were harmonized with the pterygium dataset, and IVW MR analysis was conducted alongside MR-Egger, WM, weighted mode, and simple mode methods. IVW $p < 0.05$ was used to assess statistical significance, and evidence strength was determined based on odds ratio (OR) and 95% confidence interval (CI).

3. Results

3.1. Causal effects of circulating lipids on pterygium

To analyze the causal associations of circulating lipids with pterygium, a total of 332, 78, 85, and 43 SNPs were selected as IVs for HDL-c, LDL-c, TC, and TG, respectively (Table 3). Our analysis revealed a significant and positive association between genetically predicted levels of LDL-c and the incidence of pterygium, as confirmed by the IVW (OR = 2.227; $P = 1.53 \times 10^{-4}$), MR Egger (OR = 3.261; $P = 1.71 \times 10^{-4}$), WM (OR = 2.381; $P = 4.17 \times 10^{-3}$), and weighted mode method (OR = 2.412; $P = 2.21 \times 10^{-3}$) (Fig. 2A and Table 3). The leave-one-out sensitivity analysis showed no outliers (Fig. 2B). As depicted in Fig. 2C, the scatter plot revealed a significant and positive correlation between genetically determined LDL-c levels and pterygium risk. The funnel plots displayed no significant heterogeneity for both IVW and MR Egger models in the analysis of LDL-c and pterygium (Fig. 2D).

Furthermore, a genetically determined higher TC level was found to be significantly associated with an increased risk of developing pterygium (Fig. 3A). Effect estimates were broadly consistent between IVW (OR = 1.806; $P = 1.70 \times 10^{-3}$) and other complementary approaches, including MR Egger (OR = 3.238; $P = 0.010$), WM (OR = 2.213; $P = 2.81 \times 10^{-3}$), and weighted mode (OR = 2.302; $P = 1.99 \times 10^{-3}$) (Table 3). The leave-one-out analysis revealed no outliers, and Egger intercept calculations showed no evidence of directional horizontal pleiotropy (Fig. 3B). The scatter plot revealed the consistent direction of the estimated MR effect for the IVW, MR Egger, and WM method (Fig. 3C), while the funnel plots visualized the distribution of the effect of a single SNP (Fig. 3D). A subsequent analysis of the potential relationship between HDL-c and TG levels and pterygium risk was undertaken, and no statistical correlation was identified (Table 3). Taken together, these findings provide support for a causal relationship between LDL-c and TC and the risk of developing pterygium.

3.2. Causal effects of pterygium on circulating lipids

We performed the reverse MR by calculating the effect of pterygium on circulating lipid levels. A total of 14, 14, 32, and 56 independent SNPs were used as outcome IVs to investigate the causal link between pterygium and four lipid parameters, HDL-c, LDL-c, TC, and TG (Table 4). Despite our analysis, genetically determined pterygium showed no association with circulating lipids using any of the methods tested (Table 4).

3.3. Genetically proxied HMGCR and PCSK9 inhibitors on the risk of pterygium

In light of the finding that LDL-c levels are positively associated with pterygium risk, we conducted a comprehensive investigation into the potential effects of HMGCR and PCSK9 inhibitors on pterygium outcomes. Our analysis of eQTLGen data revealed a total of 921 and 24 cis-eQTLs associated with the target genes, HMGCR and PCSK9, respectively. In a positive control study, significant associations were observed between the use of these drugs and CAD using the proposed eQTLs-based instruments, validating the effectiveness of our selected genetic instruments. (Tables 5 and 6).

Analysis of protein levels using eQTLs identified a positive correlation between genetically determined HMGCR levels and pterygium risk, as demonstrated by IVW (OR = 6.999; $P = 0.016$) and WM (OR = 7.660; $P = 0.034$) (Table 5, Fig. 4). No significant correlation was observed between PCSK9 expression and pterygium risk (IVW OR = 1.479; $P = 0.322$) (Table 6, Fig. 4). A schematic illustration of the drug-target MR analysis is shown in Fig. 5.

4. Discussion

To the best of our knowledge, this is the first comprehensive MR study to investigate the causal impact of genetically determined circulating lipid profiles on pterygium risk. Using independent GWAS summary statistics, this study found that genetically predicted circulating TC and LDL-c levels were positively associated with pterygium risk, providing evidence for the potential significance of dietary modifications or medications targeting LDL-c and TC in the prevention of pterygium development. To provide additional insights, drug-target MR analyses were conducted, indicating that HMGCR inhibitors may reduce pterygium risk by lowering LDL-c levels. However, PCSK9 inhibitors, another LDL-c-regulating drug, were not found to have equivalent effects.

Although the exact nature of pterygium remains largely unknown, increasing evidence suggests that pterygium is a chronic inflammatory disorder in which complex interactions exist between cell proliferation and cholesterol metabolism. A growing body of research indicates that imbalance in cholesterol metabolism plays a crucial role in the development and progression of ocular surface inflammation. Recent studies

Table 3
Causal Associations of Circulating Lipid Traits with Pterygium.

	MR method	SNP (n)	Beta	SE	OR	95% CI	*P* value
HDL-c	IVW	332	0.025	0.180	1.025	0.720, 1.459	0.891
	WM		0.591	0.303	1.808	0.997, 3.276	0.051
	MR Egger		-0.124	0.274	0.883	0.516, 1.513	0.652
	Weighted mode		0.420	0.300	1.522	0.846, 2.738	0.162
	Simple mode		0.317	0.664	1.374	0.374, 5.047	0.633
LDL-c	IVW	78	0.801	0.211	2.227	1.15, 2.33	**1.53×10^{-4}**
	WM		0.867	0.303	2.381	1.315, 4.309	**4.17×10^{-3}**
	MR Egger		1.182	0.299	3.261	1.815, 5.860	**1.71×10^{-4}**
	Weighted mode		0.881	0.278	2.412	1.399, 4.160	**2.21×10^{-3}**
	Simple mode		0.926	0.580	2.525	0.810, 7.871	0.114
TC	IVW	85	0.591	0.188	1.806	1.248, 2.613	**1.70×10^{-3}**
	WM		0.794	0.266	2.213	1.314, 3.727	**2.81×10^{-3}**
	MR Egger		0.806	0.307	2.238	1.225, 4.089	**0.010**
	Weighted mode		0.834	0.261	2.302	1.380, 3.841	**1.99×10^{-3}**
	Simple mode		0.544	0.517	1.723	0.625, 4.744	0.296
TG	IVW	43	0.148	0.212	1.160	0.765, 1.757	0.485
	WM		-0.212	0.311	0.809	0.440, 1.487	0.495
	MR Egger		0.248	0.353	1.282	0.642, 2.559	0.486
	Weighted mode		-0.076	0.307	0.927	0.508, 1.693	0.806
	Simple mode		0.230	0.667	1.258	0.340, 4.651	0.732

CI: confidence interval; HDL-c: high-density lipoprotein cholesterol; IVW: inverse-variance weighted; LDL-c: low-density lipoprotein cholesterol; MR: Mendelian randomization; TC: total cholesterol; TG: triglycerides; OR: odds ratio; SE: standard error; SNP: single nucleotide polymorphism; WM: weighted median.

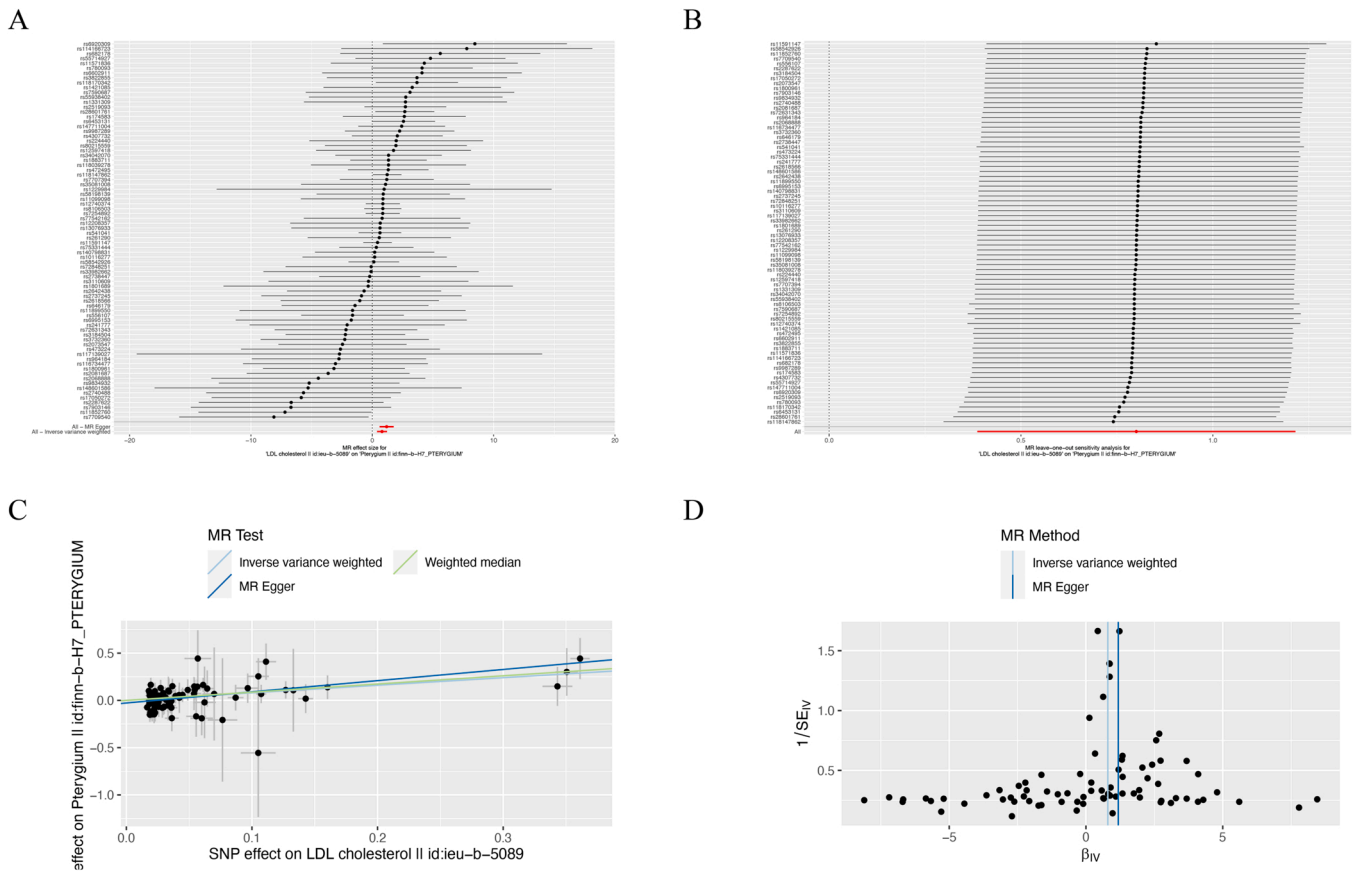

Fig. 2. Mendelian randomization (MR) for the causal effect of circulating LDL-c levels on pterygium risk. (A) Forest plot, (B) leave-one-out sensitivity analysis, (C) scatter plot, and (D) funnel plot of the genetically risk of LDL-c on pterygium. LDL-c: low-density lipoprotein cholesterol; SNP: single nucleotide polymorphism.

have found that hyperlipidemia induces inflammation of the meibomian glands (MGs). Using the ApoE knockout mouse model, which is characterized by a significant increase in plasma TC levels, the researchers found a marked increase in inflammatory cell infiltration into the surrounding microenvironment of MGs [16]. In addition, studies have consistently demonstrated that a high-fat diet and dysregulated cholesterol metabolism cause dry eye-like corneal epithelial barrier disruption and ocular surface inflammation by elevating the expression of inflammatory factors and activation of oxidative stress [35,36]. Our MR analysis confirms that high levels of circulating TC and LDL-c are significant risk factors for pterygium. Therefore, it is essential to implement proactive measures, such as dietary modifications and medication administration, to mitigate the risk of developing pterygium.

To further expand on our research findings and enhance their clinical implications, we conducted a drug-target MR to explore the causal relevance of LDL-c-modifying HMGCR and PCSK9 inhibitors on pterygium outcomes. HMGCR plays a vital role in catalyzing the conversion of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonic acid, which serves as the rate-limiting step in the cholesterol biosynthetic pathway [37]. HMGCR inhibitors, commonly known as statins, have been used for decades to control hypercholesterolemia. Statins can suppress cholesterol biosynthesis and enhance the expression of LDL receptors on the surface of hepatic cells [38]. These receptors are responsible for the capture and clearance of LDL-c from the bloodstream, an essential mechanism that helps prevent the accumulation of atherosclerotic plaques within the arterial wall and reduces the likelihood of cardiovascular disease [39]. Studies have demonstrated that statins may possess therapeutic potential in a variety of ophthalmic conditions, including dry eye, corneal ulcer scarring, uveitis, and other ocular inflammatory states [40–42]. In the present investigation, we proposed the potential efficacy of statins in the management of pterygium. These lipid-lowering agents have been shown to suppress pro-inflammatory cytokine levels, potentially reducing tissue damage associated with ocular inflammation [43–45]. Furthermore, statins have demonstrated the ability to inhibit oxidant-induced reactive oxygen species (ROS) and prevent cell apoptosis via a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and/or p38 mitogen-activated protein kinases (MAPK)-dependent mechanism [46]. These antioxidant properties may hold the potential to reduce tissue inflammation and injury commonly observed in pterygium. PCSK9 is another protein-coding gene that modulates LDL-c levels in the bloodstream by binding to LDL receptors and promoting their degradation. Although PCSK9 inhibitors have demonstrated potential in clinical trials for reducing the risk of atherosclerotic cardiovascular disease [47,48], our drug-target MR analysis suggested that HMGCR inhibitors exhibit a significant ability to reduce pterygium risk, while PCSK9 inhibitors have not yielded satisfactory results in the same context.

Our study demonstrates several notable strengths. First, we systematically investigated the correlation between circulating lipids and pterygium risk through comprehensive MR analysis. There is currently no conclusive evidence from clinical trials that lowering serum lipid levels can reduce the incidence of pterygium. This is the first large-scale genetic study to report a causal relationship between higher circulating TC and LDL-c levels and increased risk of pterygium. Second, our study offers a novel perspective on the potential efficacy of lipid-lowering therapy as a treatment for pterygium. The findings are independent of reverse causation and are less susceptible to various sources of bias than observational studies. In addition, we conducted extensive sensitivity analyses to confirm the credibility and validity of the results. Third, we chose significant eQTLs as instruments to demonstrate the association between target genes (PCSK9 and HMGCR) and pterygium risk. Our drug-target MR indicated a positive association between HMGCR

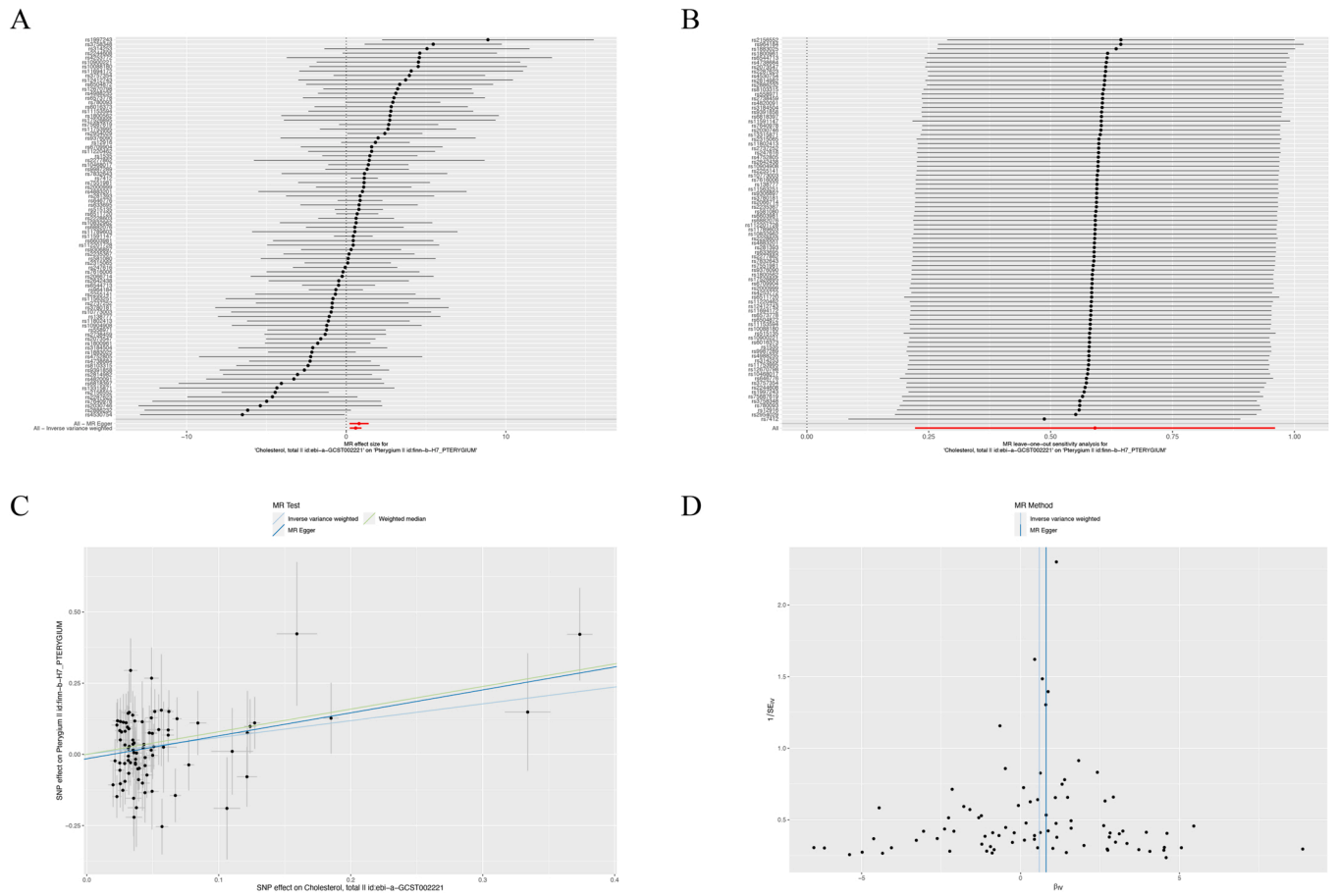

Fig. 3. Mendelian randomization (MR) for the causal effect of circulating TC levels on pterygium risk. (A) Forest plot, (B) leave-one-out sensitivity analysis, (C) scatter plot, and (D) funnel plot of the genetically risk of TC on pterygium. TC: total cholesterol; SNP: single nucleotide polymorphism.

Table 4
Causal Associations of Pterygium with Circulating Lipid Traits.

	MR method	SNP (n)	Beta	SE	OR	95% CI	*P* value
HDL-c	IVW	14	-1.69×10^{-3}	1.23×10^{-3}	0.998	0.996, 1.001	0.168
	WM		-5.32×10^{-4}	1.56×10^{-3}	0.999	0.996, 1.003	0.733
	MR Egger		-8.63×10^{-4}	1.97×10^{-3}	0.999	0.995, 1.003	0.671
	Weighted mode		-9.16×10^{-6}	1.63×10^{-3}	1.000	0.997, 1.003	0.996
	Simple mode		-4.60×10^{-4}	2.36×10^{-3}	1.000	0.995, 1.004	0.849
LDL-c	IVW	14	-2.58×10^{-3}	1.75×10^{-3}	0.997	0.994, 1.001	0.139
	WM		-2.56×10^{-3}	2.68×10^{-3}	0.997	0.992, 1.003	0.340
	MR Egger		-2.71×10^{-3}	2.73×10^{-3}	0.997	0.992, 1.003	0.342
	Weighted mode		-2.20×10^{-3}	2.76×10^{-3}	1.001	0.994, 1.008	0.440
	Simple mode		1.03×10^{-3}	3.70×10^{-3}	1.001	0.994, 1.008	0.786
TC	IVW	32	1.98×10^{-3}	2.68×10^{-3}	1.002	0.997, 1.007	0.458
	WM		4.06×10^{-3}	3.90×10^{-3}	1.004	0.996, 1.012	0.299
	MR Egger		-0.011	7.92×10^{-3}	0.989	0.974, 1.004	0.168
	Weighted mode		1.37×10^{-3}	6.89×10^{-3}	1.001	0.988, 1.015	0.843
	Simple mode		7.54×10^{-3}	7.36×10^{-3}	1.008	0.993, 1.022	0.313
TG	IVW	56	8.89×10^{-4}	1.79×10^{-3}	1.001	0.997, 1.004	0.619
	WM		2.73×10^{-3}	2.74×10^{-3}	1.003	0.997, 1.008	0.320
	MR Egger		-9.72×10^{-4}	2.86×10^{-3}	0.999	0.993, 1.005	0.736
	Weighted mode		2.87×10^{-3}	3.33×10^{-3}	1.003	0.996, 1.009	0.392
	Simple mode		2.44×10^{-4}	5.43×10^{-3}	1.000	0.990, 1.011	0.964

CI: confidence interval; HDL-c: high-density lipoprotein cholesterol; IVW: inverse-variance weighted; LDL-c: low-density lipoprotein cholesterol; MR: Mendelian randomization; TC: total cholesterol; TG: triglycerides; OR: odds ratio; SE: standard error; SNP: single nucleotide polymorphism; WM: weighted median.

expression and pterygium risk. The HMGCR inhibitor statins are widely used to treat hypercholesterolemia and various cardiovascular diseases [49]. With extensive research on the pharmacological effects of statins, our study can provide a basis for future clinical trials on statins as a therapeutic intervention for pterygium. Finally, by limiting our study population to individuals of European descent, we reduced the potential impact of population stratification bias on our results.

Nevertheless, the present study has several limitations. First, given the limited sample size, our current study may not have sufficient statistical power to draw any definitive conclusions about the relationship

Table 5
Genetically Proxied HMGCR on Pterygium.

Outcome	MR method	SNP (n)	Beta	SE	OR	95% CI	*P* value
CAD	IVW	7	0.451	0.104	1.569	1.280, 1.924	$\mathbf{1.45 \times 10^{-5}}$
	WM		0.476	0.129	1.610	1.251, 2.073	$\mathbf{2.17 \times 10^{-4}}$
	MR Egger		0.595	0.458	0.883	1.280, 1.924	0.251
	Weighted mode		0.542	0.155	1.719	1.268, 2.331	**0.013**
	Simple mode		0.490	0.199	1.632	1.104, 2.412	**0.049**
Pterygium	IVW	7	1.946	0.808	6.999	1.436, 34.114	**0.016**
	WM		2.036	0.960	7.660	1.166, 50.316	**0.034**
	MR Egger		3.280	3.627	26.582	0.022, 32500	0.407
	Weighted mode		2.077	1.119	7.978	0.890, 71.502	0.113
	Simple mode		1.287	1.492	3.622	0.195, 67.377	0.421

CAD: coronary heart disease; CI: confidence interval; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IVW: inverse-variance weighted; MR: Mendelian randomization; OR: odds ratio; SE: standard error; SNP: single nucleotide polymorphism; WM: weighted median.

Table 6
Genetically Proxied PCSK9 on Pterygium.

Outcome	MR method	SNP (n)	Beta	SE	OR	95% CI	*P* value
CAD	IVW	14	0.793	0.094	2.211	1.840, 2.657	$\mathbf{2.54 \times 10^{-17}}$
	WM		0.747	0.123	2.110	1.658, 2.685	$\mathbf{1.26 \times 10^{-9}}$
	MR Egger		0.696	0.193	2.006	1.374, 2.928	$\mathbf{3.61 \times 10^{-3}}$
	Weighted mode		0.745	0.132	2.106	1.625, 2.730	$\mathbf{8.20 \times 10^{-5}}$
	Simple mode		0.723	0.200	2.061	1.392, 3.054	$\mathbf{3.18 \times 10^{-3}}$
Pterygium	IVW	15	0.391	0.395	1.479	0.682, 3.206	0.322
	WM		0.429	0.514	1.535	0.561, 4.201	0.404
	MR Egger		0.628	0.566	1.873	0.618, 5.682	0.286
	Weighted mode		0.576	0.432	1.779	0.763, 4.149	0.204
	Simple mode		1.084	0.731	2.958	0.706, 12.392	0.160

CAD: coronary heart disease; CI: confidence interval; IVW: inverse-variance weighted; MR: Mendelian randomization; OR: odds ratio; PCSK9: proprotein convertase subtilisin/kexin type 9; SE: standard error; SNP: single nucleotide polymorphism; WM: weighted median.

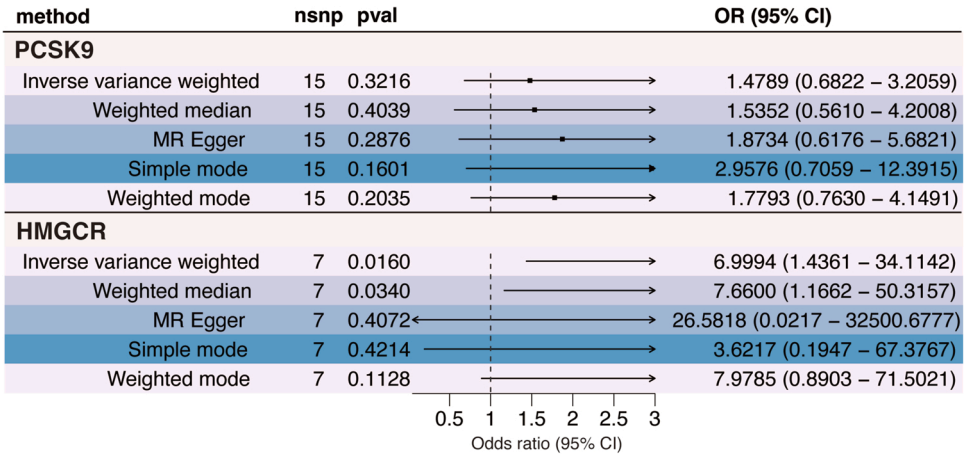

Fig. 4. Drug-target Mendelian randomization (MR) for the causal effect of PCSK9 and HMGCR on pterygium risk using expression quantitative trait loci (eQTLs) as instruments. CI: confidence interval; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; OR: odds ratio; PCSK9: proprotein convertase subtilisin/kexin type 9; SNP: single nucleotide polymorphism.

between pterygium and circulating lipid levels. Further research with a larger sample size is warranted to provide more definitive evidence of this potential relationship. Second, the genetic associations between pterygium and four lipid traits were all derived from GWAS studies conducted on individuals of European descent. Consequently, caution should be exercised when attempting to generalize our findings to other populations. For greater credibility and applicability, information on the statistics of various ethnic groups could be included. Third, due to the existence of conflicting evidence surrounding the correlation between certain lipid traits and pterygium, further clinical trials are recommended to substantiate our results. A combination of RCTs and MR studies can yield additional data to support our findings and aid decision-making in medical fields. Fourth, while statins provide many benefits, their use in pterygium should be treated with caution for potential ocular toxicity, such as extraocular muscle myositis caused by ATP depletion, which may be mitigated by concurrent coenzyme administration [50,51]. Finally, it is critical to conduct further research to unravel the molecular mechanism underlying LDL-c and pterygium, providing further support for our findings.

5. Conclusions

In conclusion, our MR analysis suggested that elevated serum LDL-c and TC levels may be associated with an increased risk of pterygium. In addition, our drug-target MR study revealed the potential protective effect of statins, which are HMGCR inhibitors, in preventing pterygium

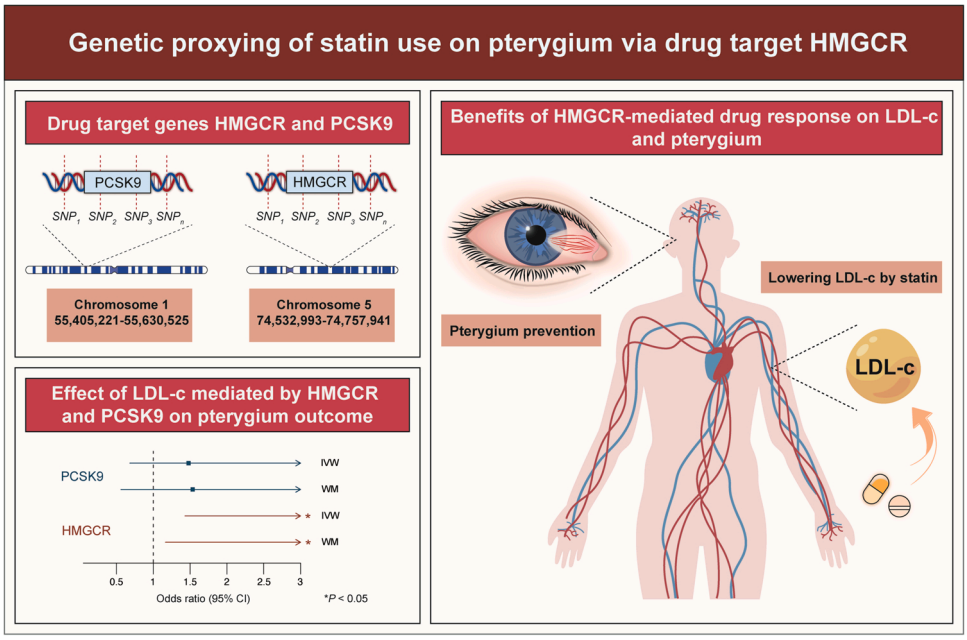

Fig. 5. Genetic proxying of statin therapy on pterygium via inhibition of the drug target gene HMGCR. SNPs were extracted from the drug target gene position in GWAS of circulating LDL-c. Drug-target Mendelian randomization (MR) was performed to investigate the causal relevance of PCSK9 and HMGCR in pterygium using expression quantitative trait loci (eQTLs) as instruments. The data shown are standardized MR effect estimates and 95% CI corresponding to pterygium risk via the drug target PCSK9 and HMGCR. GWAS: genome-wide association studies; CI: confidence interval; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL-c: low-density lipoprotein cholesterol; PCSK9: proprotein convertase subtilisin/kexin type 9; SNP: single nucleotide polymorphism.

risk. These findings highlight the importance of maintaining healthy LDL-c levels through dietary interventions and considering statin therapy as a preventive measure for this ocular condition. Further research is warranted to fully elucidate the role of cholesterol metabolism in pterygium pathogenesis.

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CRediT authorship contribution statement

Yuchen Cai: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Fei Fang:** Conceptualization, Investigation, Resources. **Tianyi Zhou:** Investigation, Writing – review & editing. **Wenjun Shi:** Writing – review & editing. **Xueyao Cai:** Supervision, Methodology, Writing – review & editing. **Yao Fu:** Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

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References

[1] Paganelli B, Sahyoun M, Gabison E. Conjunctival and limbal conjunctival autograft vs. amniotic membrane graft in primary pterygium surgery: a 30-year comprehensive review. Ophthalmol Ther 2023. https://doi.org/10.1007/s40123-023-00689-x.
[2] Rezvan F, Khabazkhoob M, Hooshmand E, Yekta A, Saatchi M, Hashemi H. Prevalence and risk factors of pterygium: a systematic review and meta-analysis. Surv Ophthalmol 2018;63:719–35. https://doi.org/10.1016/j.survophthal.2018.03.001.
[3] Ang M, Li X, Wong W, Zheng Y, Chua D, Rahman A, et al. Prevalence of and Racial Differences in Pterygium. Ophthalmology 2012;119:1509–15. https://doi.org/10.1016/j.ophtha.2012.02.009.
[4] Fekadu SA, Assem AS, Adimassu NF. Prevalence of pterygium and its associated factors among adults aged 18 years and above in Gambella town, Southwest Ethiopia, May 2019. PLOS ONE 2020;15:e0237891. https://doi.org/10.1371/journal.pone.0237891.
[5] Tano T, Ono K, Hiratsuka Y, Otani K, Sekiguchi M, Konno S, et al. Prevalence of pterygium in a population in Northern Japan: the Locomotive Syndrome and Health Outcome in Aizu Cohort Study. Acta Ophthalmol (Copenh) 2013:91. https://doi.org/10.1111/aos.12044.
[6] Oke I, Elze T, Miller JW, Lorch AC, Hunter DG, Traish AS, et al. The prevalence and recurrence risk of bare sclera pterygium surgery in the United States. Ocul Surf 2023;29:547–9. https://doi.org/10.1016/j.jtos.2023.05.006.
[7] Chu WK, Choi HL, Bhat AK, Jhanji V. Pterygium: new insights. Eye 2020;34:1047–50. https://doi.org/10.1038/s41433-020-0786-3.
[8] Clearfield E, Muthappan V, Wang X, Kuo IC. Conjunctival autograft for pterygium. Cochrane Database Syst Rev 2016;2:CD011349. https://doi.org/10.1002/14651858.CD011349.pub2.
[9] Moran DJ, Hollows FC. Pterygium and ultraviolet radiation: a positive correlation. Br J Ophthalmol 1984;68:343–6. https://doi.org/10.1136/bjo.68.5.343.
[10] Shahraki T, Arabi A, Feizi S. Pterygium: an update on pathophysiology, clinical features, and management. 251584142110201 Ther Adv Ophthalmol 2021;13. https://doi.org/10.1177/25158414211020152.
[11] Clearfield E, Hawkins BS, Kuo IC. Conjunctival autograft versus amniotic membrane transplantation for treatment of pterygium: findings from a cochrane systematic review. Am J Ophthalmol 2017;182:8–17. https://doi.org/10.1016/j.ajo.2017.07.004.
[12] Chu WK, Choi HL, Bhat AK, Jhanji V. Pterygium: new insights. Eye Lond Engl 2020;34:1047–50. https://doi.org/10.1038/s41433-020-0786-3.
[13] Ambaw YA, Timbadia DP, Raida M, Torta F, Wenk MR, Tong L. Profile of tear lipid mediator as a biomarker of inflammation for meibomian gland dysfunction and ocular surface diseases: standard operating procedures. Ocul Surf 2022;26:318–27. https://doi.org/10.1016/j.jtos.2020.09.008.
[14] Ambaw YA, Chao C, Ji S, Raida M, Torta F, Wenk MR, et al. Tear eicosanoids in healthy people and ocular surface disease. Sci Rep 2018;8:11296. https://doi.org/10.1038/s41598-018-29568-3.

[15] Lim A, Wenk MR, Tong L. Lipid-based therapy for ocular surface inflammation and disease. Trends Mol Med 2015;21:736–48. https://doi.org/10.1016/j.molmed.2015.10.001.
[16] Bu J, Wu Y, Cai X, Jiang N, Jeyalatha MV, Yu J, et al. Hyperlipidemia induces meibomian gland dysfunction. Ocul Surf 2019;17:777–86. https://doi.org/10.1016/j.jtos.2019.06.002.
[17] Pan Z, Cui J, Shan G, Chou Y, Pan L, Sun Z, et al. Prevalence and risk factors for pterygium: a cross-sectional study in Han and Manchu ethnic populations in Hebei, China. BMJ Open 2019;9:e025725. https://doi.org/10.1136/bmjopen-2018-025725.
[18] Zang S, Chen Y, Guo H, Zhang M, Zhang G, Zhang L, et al. High HDL-C and high LDL-C are risk factors of pterygium in a population-based cross-sectional study in Southern China: the Dongguan Eye Study. BMJ Open 2022;12:e058649. https://doi.org/10.1136/bmjopen-2021-058649.
[19] Kurtul BE, Kabatas EU, Ozates S. The correlation of routine hematological indices with pterygium. 2515841419848922 Ther Adv Ophthalmol 2019;11. https://doi.org/10.1177/2515841419848922.
[20] Kilic D, Guven S. Does systemic inflammation play a role in patients with pterygium. Int Ophthalmol 2020;40:2307–14. https://doi.org/10.1007/s10792-020-01414-4.
[21] Kintu C, Soremekun O, Kamiza AB, Kalungi A, Mayanja R, Kalyesubula R, et al. The causal effects of lipid traits on kidney function in Africans: bidirectional and multivariable Mendelian-randomization study. EBioMedicine 2023;90:104537. https://doi.org/10.1016/j.ebiom.2023.104537.
[22] Li X, Meng X, Timofeeva M, Tzoulaki I, Tsilidis KK, Ioannidis JP, et al. Serum uric acid levels and multiple health outcomes: umbrella review of evidence from observational studies, randomised controlled trials, and Mendelian randomisation studies. BMJ 2017;357:j2376. https://doi.org/10.1136/bmj.j2376.
[23] Gupta V, Walia GK, Sachdeva MP. "Mendelian randomization": an approach for exploring causal relations in epidemiology. Public Health 2017;145:113–9. https://doi.org/10.1016/j.puhe.2016.12.033.
[24] Gala H, Tomlinson I. The use of Mendelian randomisation to identify causal cancer risk factors: promise and limitations. J Pathol 2020;250:541–54. https://doi.org/10.1002/path.5421.
[25] Liu D, Meng X, Tian Q, Cao W, Fan X, Wu L, et al. Vitamin D and multiple health outcomes: an umbrella review of observational studies, randomized controlled trials, and mendelian randomization studies. Adv Nutr 2022;13:1044–62. https://doi.org/10.1093/advances/nmab142.
[26] Yarmolinsky J, Bull CJ, Vincent EE, Robinson J, Walther A, Smith GD, et al. Association between genetically proxied inhibition of HMG-CoA reductase and epithelial ovarian cancer. JAMA 2020;323:646. https://doi.org/10.1001/jama.2020.0150.
[27] Bell AS, Rosoff DB, Mavromatis LA, Jung J, Wagner J, Lohoff FW. Comparing the relationships of genetically proxied PCSK9 inhibition with mood disorders, cognition, and dementia between men and women: a drug-target mendelian randomization study. J Am Heart Assoc 2022;11:e026122. https://doi.org/10.1161/JAHA.122.026122.
[28] Schmidt AF, Finan C, Gordillo-Marañón M, Asselbergs FW, Freitag DF, Patel RS, et al. Genetic drug target validation using Mendelian randomisation. Nat Commun 2020;11:3255. https://doi.org/10.1038/s41467-020-16969-0.
[29] Schmidt AF, Swerdlow DI, Holmes MV, Patel RS, Fairhurst-Hunter Z, Lyall DM, et al. PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. Lancet Diabetes Endocrinol 2017;5:97–105. https://doi.org/10.1016/S2213-8587(16)30396-5.
[30] Rosoff DB, Bell AS, Jung J, Wagner J, Mavromatis LA, Lohoff FW. Mendelian randomization study of PCSK9 and HMG-CoA reductase inhibition and cognitive function. J Am Coll Cardiol 2022;80:653–62. https://doi.org/10.1016/j.jacc.2022.05.041.
[31] Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658–65. https://doi.org/10.1002/gepi.21758.
[32] Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14. https://doi.org/10.1002/gepi.21965.
[33] Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol 2017;32:377–89. https://doi.org/10.1007/s10654-017-0255-x.
[34] Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol 2017;46:1985–98. https://doi.org/10.1093/ije/dyx102.
[35] Bu J, Zhang M, Wu Y, Jiang N, Guo Y, He X, et al. High-fat diet induces inflammation of meibomian gland. Invest Ophthalmol Vis Sci 2021;62:13. https://doi.org/10.1167/iovs.62.10.13.
[36] Wu Y, Wu J, Bu J, Tang L, Yang Y, Ouyang W, et al. High-fat diet induces dry eye-like ocular surface damages in murine. Ocul Surf 2020;18:267–76. https://doi.org/10.1016/j.jtos.2020.02.009.
[37] Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science 2001;292:1160–4. https://doi.org/10.1126/science.1059344.
[38] Haines BE, Wiest O, Stauffacher CV. The increasingly complex mechanism of HMG-CoA reductase. Acc Chem Res 2013;46:2416–26. https://doi.org/10.1021/ar3003267.
[39] Stoekenbroek RM, Lambert G, Cariou B, Hovingh GK. Inhibiting PCSK9 - biology beyond LDL control. Nat Rev Endocrinol 2018;15:52–62. https://doi.org/10.1038/s41574-018-0110-5.
[40] Ooi KG-J, Khoo P, Vaclavik V, Watson SL. Statins in ophthalmology. Surv Ophthalmol 2019;64:401–32. https://doi.org/10.1016/j.survophthal.2019.01.013.
[41] Chun YH, Kim HR, Han K, Park Y-G, Song HJ, Na K-S. Total cholesterol and lipoprotein composition are associated with dry eye disease in Korean women. Lipids Health Dis 2013;12:84. https://doi.org/10.1186/1476-511X-12-84.
[42] Borkar DS, Tham VM, Shen E, Parker JV, Uchida A, Vinoya AC, et al. Association between statin use and uveitis: results from the Pacific Ocular Inflammation study. Am J Ophthalmol 2015;159:707–13. https://doi.org/10.1016/j.ajo.2015.01.009.
[43] Kim JH, Chun YS, Kim JC. Clinical and immunological responses in ocular demodecosis. J Korean Med Sci 2011;26:1231–7. https://doi.org/10.3346/jkms.2011.26.9.1231.
[44] Wei Y, Gadaria-Rathod N, Epstein S, Asbell P. Tear cytokine profile as a noninvasive biomarker of inflammation for ocular surface diseases: standard operating procedures. Invest Ophthalmol Vis Sci 2013;54:8327–36. https://doi.org/10.1167/iovs.13-12132.
[45] Kumar NR, Praveen M, Narasimhan R, Khamar P, D'Souza S, Sinha-Roy A, et al. Tear biomarkers in dry eye disease: progress in the last decade. Indian J Ophthalmol 2023;71:1190–202. https://doi.org/10.4103/IJO.IJO_2981_22.
[46] Qian J, Keyes KT, Long B, Chen G, Ye Y. Impact of HMG-CoA reductase inhibition on oxidant-induced injury in human retinal pigment epithelium cells. J Cell Biochem 2011;112:2480–9. https://doi.org/10.1002/jcb.23173.
[47] Bittner VA, Giugliano RP, Brinton EA, Guyton JR. PCSK9 inhibitors for prevention of atherosclerotic cardiovascular disease. J Clin Lipido 2018;12:835–43. https://doi.org/10.1016/j.jacl.2018.06.013.
[48] Seidah NG, Prat A, Pirillo A, Catapano AL, Norata GD. Novel strategies to target proprotein convertase subtilisin kexin 9: beyond monoclonal antibodies. Cardiovasc Res 2019;115:510–8. https://doi.org/10.1093/cvr/cvz003.
[49] Sirtori CR. The pharmacology of statins. Pharm Res 2014;88:3–11. https://doi.org/10.1016/j.phrs.2014.03.002.
[50] Bahrami B, Rajak S, Selva D. Orbital myositis secondary to statin therapy. Med J Aust 2015;203:365. https://doi.org/10.5694/mja15.00503.
[51] Deichmann R, Lavie C, Andrews S. Coenzyme q10 and statin-induced mitochondrial dysfunction. Ochsner J 2010;10:16–21.