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Allergic disease and keratoconus: A two-sample univariable and multivariable Mendelian randomization study

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ABSTRACT

Background: There is accumulating evidence that allergy is a risk factor for keratoconus. Nonetheless the association between allergic disease and keratoconus remains controversial. We performed a two-sample Mendelian randomization (MR) study to determine the putative causal association of 4 allergic diseases (allergic conjunctivitis, allergic asthma, allergic rhinitis and atopic dermatitis) with keratoconus.

Methods: Summary statistics were obtained from genome-wide association studies (GWAS) of allergic conjunctivitis (AC) (20,958 cases and 356,319 controls), allergic asthma (AA) (9631 cases and 210,122 controls), allergic rhinitis (AR) (11,009 cases and 359,149 controls), atopic dermatitis (AD) (13,473 cases and 336,589 controls), keratoconus (KC) (2116 cases and 24,626 controls) and 91 circulating inflammatory cytokines (n = 14,824). Two-sample univariable and multivariable MR analyses were performed. A two-step MR was then applied to determine whether systemic inflammatory cytokines mediated the effect of allergic disease on keratoconus.

Results: The causal odds ratio (OR) estimate of genetically determined KC was 1.66 (95% CI: 1.32–2.08; $P < 0.001$) for AC, 1.29 (95% CI: 1.10–1.51, $P = 0.0014$) for AA, 1.39 (95% CI: 1.15–1.68; $P < 0.001$) for AR and 1.30 (95% CI: 1.17–1.45, $P < 0.001$) for AD. Multivariable MR indicated a suggestive association between AC and KC after conditioning on other allergic diseases (OR 1.61; 95% CI: 1.10–2.34; P adjusted = 0.054). Two-step MR revealed that the effect was not mediated by systemic inflammatory cytokines.

Conclusions: Our findings provide evidence of a potential causal relationship between AC and KC. The effect of AC on KC may be mediated via other systemic inflammatory cytokines not included in the present study, or by alternative mechanisms. These findings may offer insight for prevention and intervention strategies to lower the risk of KC in patients with AC.

Keywords: Keratoconus, Allergic disease, Allergic conjunctivitis, Mendelian randomization

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INTRODUCTION

Keratoconus (KC) is a chronic ectatic keratopathy characterized by impaired vision due to irregular astigmatism. The prevalence and incidence rates of KC have been estimated to be between 0.2 and 4790 per 100,000 persons and 1.5 and 25 cases per 100,000 persons/year respectively, with highest rates typically occurring in those aged 20–30 years.[1] It is a potential hazard to adolescent visual health and in the late stage inevitably requires surgical intervention. This affects quality of life and places an economic burden on families and society. Although KC has been classically defined as a degenerative disease with mechanical stretches accelerating its course,[2,3] a recent growing number of genetic and clinical studies have described the inflammatory trait of KC.[4,5] Epidemiologic studies have reported an increased risk of KC with atopy and distinct allergic diseases such as allergic rhinitis, asthma, eczema and atopic dermatitis, among which allergic conjunctivitis has been studied in depth.[6–9] In addition, elevated levels of inflammatory mediators in tears along with immune responses characterized in corneal tissues by genomics and transcriptomics method in KC patients were broadly reported.[4,5,10–12] Nonetheless, correlation of KC with specific atopic conditions as well as inflammatory cytokines has been inconsistent and remains controversial.[13–17] Different allergies have complex interactions with each other and comorbid conditions are common,[18] so identification by observational studies of potential causality between AC and KC remains elusive due to confounding factors and reverse causality.

Mendelian randomization (MR) involves the application of genetic variants as instrumental variables (IVs) to test for a causal association between a risk factor and an outcome.[19] Limitations of confounding bias and reverse causations can be mitigated because alleles linked to different traits are randomly assigned at conception.

In the present study, we performed a univariable and multivariable MR to assess the comorbidity of distinct allergic diseases on KC susceptibility using genetic associations from large genome-wide association studies (GWASs). Also, a subsequent two-step MR was assigned to evaluate the effects of serum level of inflammatory cytokines in mediating the effect of specific allergic disease on KC. Establishing the causal contribution of allergic disease and systemic cytokine levels in KC is crucial for understanding the potential role of these factors in the disease. The broader implications of these findings may inform preventive and therapeutic strategies.

METHOD

Study design

We first conducted a multi-exposure two-sample univariable MR (UVMR) analysis to explore the potential causal relationship between allergic diseases (allergic conjunctivitis (AC), allergic asthma (AA), atopic dermatitis (AD) and allergic rhinitis (AR)) and keratoconus (KC). Then, we adjusted for potential interactions and coexistence phenomena between these allergic diseases through multivariable MR (MVMR) analysis. Finally, we explored the role of inflammatory cytokines in mediating the onset of keratoconus through a two-step MR analysis. Fig. 1 shows an overview of the research design. Our investigation followed the reporting recommendations outlined in the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR).[20] The assumptions of MR analysis design include: (1) The genetic variants are robustly associated with the risk factor under investigation; (2) There are no unmeasured confounders of the relationship between genetic variants and outcome; (3) The genetic variants on the outcome are only mediated by the risk factor, with no independent effects on the outcome.[21] To enhance accessibility of the technical terms used in this study, we have provided a glossary of key MR terms in Table S16, largely sourced from the Mendelian randomization dictionary (https://mr-dictionary.mrcieu.ac.uk/, accessed on August 28, 2024) and the STROBE-MR statement.[20,22]

This study utilized data from published studies and publicly available datasets, eliminating the need for further ethics approval or informed consent from participants.

Data sources of exposures, outcome and mediators

Genetic predictors for 4 allergic diseases including AC, AA, AR, and AD of European ancestry

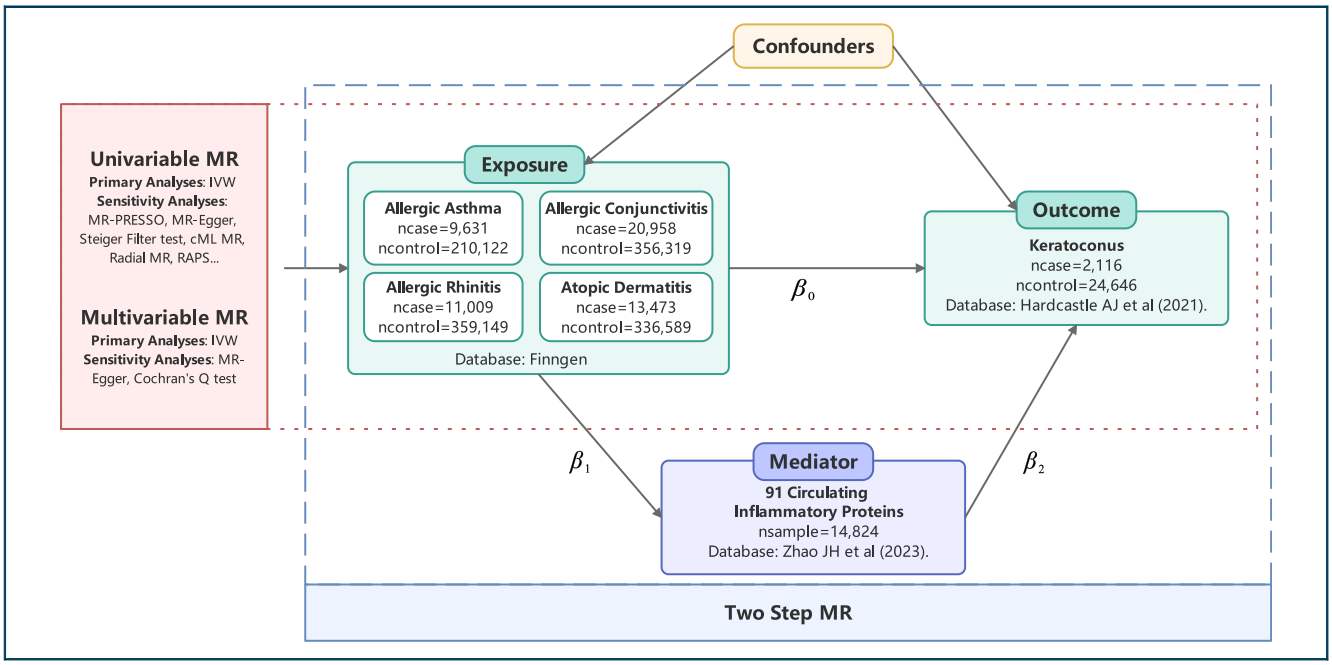


Fig. 1 Flowchart of two-sample, multivariable and two-step mendelian randomization to evaluate the effects of allergic diseases (allergic asthma, allergic conjunctivitis, allergic rhinitis and atopic dermatitis) on keratoconus and the effects of inflammatory cytokines as mediators. *MR* mendelian randomization, *MVMR* multivariable mendelian randomization. First, a multi-exposure two-sample univariable MR (UVMR) analysis was performed to investigate the causal relationship between allergic diseases and keratoconus. Next, we adjusted for potential interactions and coexistence of these allergic diseases using multivariable MR (MVMR) analysis. Finally, we examined the role of inflammatory cytokines in mediating keratoconus onset through a two-step MR analysis

were extracted from FinnGen dataset (https://www.finngen.fi/fi, accessed on August 20, 2023). These cases were defined mainly by the International Statistical Classification of Diseases and Related Health Problems (ICD) 10. The respective sample sizes were as follows: AC (20,958 cases and 356,319 controls), AA (9631 cases and 210,122 controls), AR (11,009 cases and 359,149 controls), AD (13,473 cases and 336,589 controls). The population from the FinnGen database has undergone rigorous data screening, retaining only individuals of European descent, specifically those of Caucasian ancestry.

Similarly, the outcome variable data for KC was derived from a GWAS study by Hardcastle et al[23] that involved 2116 KC cases and 24,626 healthy controls. The data of KC are all from Europe and Australia, which largely ensures that the population is of Caucasian descent. The GWAS data for 91 circulating inflammatory cytokines were sourced from a published meta-analysis by Zhao et al.[24] This study included 14,824 participants from 11 cohorts (accession numbers GCST90274758 to GCST90274848), primarily of European ancestry, with the majority originating from cohorts in Sweden, Germany, Croatia, Estonia, and the United Kingdom). Two of the 11 cohorts encompassing diverse ethnicities, namely STABILITY[25] and ARISTOTLE,[26,27] were likely dominated by Caucasian participants within their broader geographic regions. As the exposure and outcome data originate from different cohorts, there is no risk of sample overlap affecting the analysis result.

Selection of genetic instruments

In UVMR and MVMR analysis between allergic disease and KC, independent genome-wide significant ($P < 5 \times 10^{-8}$) genetic instruments were selected. The threshold for linkage disequilibrium (LD) was set to $r^2 < 0.001$ and distance>10,000 kb based on the phase 3 data of the European 1000 Genome Project reference panel.[28] In MVMR analysis, additional de linkage disequilibrium analysis was performed between IVs of the 4 allergic diseases with the same threshold. To avoid bias in weak instrument bias, we used the R^2 value of each single nucleotide polymorphism (SNP) to calculate the proportion of variance in exposure and the F-statistic to evaluate the strength of the SNP-exposure association. To

mitigate weak instrument bias, only SNPs exceeding an F-statistic threshold of 10 were included in the analysis, which ensures a sufficiently strong association between the SNP and the exposure of interest, as measured by the effect allele frequency (EAF) in the specific dataset and the estimated coefficient of correlation β between the SNP and exposure:[29]

$$R^2 = \beta^2 \times (1 - EAF) \times 2EAF$$

$$F = (N - 2) \times \frac{R^2}{(1 - R^2)}$$

In UVMR and MVMR analyses investigating the relationship between allergic disease and KC, proxy SNPs were employed when a specific SNP was absent from the outcome dataset. These proxies were identified using LDlink (https://ldlink.nci.nih.gov/) and required a LD threshold of $R^2 > 0.8$. During data harmonization, we prioritized inferring the positive strand alleles based on allele frequency information, particularly for palindromic sequences. Overlapping SNPs between different allergic diseases were excluded to avoid interference and potential pleiotropy in the UVMR analysis.

To evaluate the mediating effect of inflammatory cytokines between allergic disease and KC through two-step MR, specifically, a strict genome-wide significant threshold of 5×10^{-8} would rule out most SNPs. Therefore, the significance level of 5×10^{-6} was applied for selecting IVs for each inflammatory cytokine, following established practices.[30,31] Other SNP selection standards, such as LD, IV strength and palindrome sequence related thresholds, were the same as the previous settings.

Statistical analysis

For MR analysis, the inverse variance weighted (IVW) analysis[32] with random effects model was applied to evaluate the association of exposures with outcome as the main analysis. Although IVW is the most efficient method and provides greatest statistical power, it assumes that all genetic variants are valid IVs and with balanced pleiotropy.[33] Therefore, several robust methods and sensitivity analyses were also employed to give a consistent estimate of a causal parameter and included simple mode, weighted mode, weighted-median, MR-Egger, MR-PRESSO, Radial MR, MR-RAPS, and cML MR methods. To evaluate heterogeneity among the estimates from each SNP, we used the Cochrane's Q-statistic from IVW and Egger.[34] For pleiotropy, MR-Egger regression intercept[35] was performed to identify the presence of horizontal pleiotropy and a potentially invalid IVW estimate. The MR pleiotropy residual sum and outlier (MR-PRESSO) test were used to detect possible horizontal pleiotropic outliers, assuming >50% of the instruments were valid, and obtain the corrected estimation via outlier removal.[36] Additionally, we estimated associations using weighted-median method in which SNP-specific estimates provide valid estimates when more than 50% of the information is contributed from valid SNPs.[37] Meanwhile, the radial plot with IVW and MR-Egger methods were also shown to aid detection of outliers responsible for differences between IVW and MR-Egger estimates, and results after outlier removal also obtained.[38] Finally, we performed 2 robust methods, robust adjusted profile score (RAPS) and constrained maximum likelihood-based MR (cML MR).[39] The former demonstrated robust performance for potential pleiotropy in the data, while the latter reduced the impact of variants with heterogeneity and pleiotropy. Simple mode and weighted mode[40] analyses were also applied as the sensitivity analyses. In addition, to clarify the direction of the causal relationship between exposure and outcome, we conducted a Steiger direction test.[41] When a stronger association between IVs and outcomes than exposure was detected, Steiger directional filtering was used to exclude these IVs and reevaluate their causal estimates. A broad consistency in the results of these methods suggests a robust validity of the causal effect estimates. To assess the stability of the effect size and identify potentially influential SNPs, a leave-one-out sensitivity analysis was conducted. This analysis involved sequentially removing each SNP and re-estimating the effect size using the IVW method on the remaining SNPs.

Due to the genetic similarity between and coexistence of allergic diseases such as AC, AA, AR, and AD, we conducted an MVMR analysis.[42] In MVMR, the associations for each genetic instrument of each exposure were conditioned on one another through IVW and MR-Egger

method. Modified Cochran's Q statistics were used to measure the heterogeneity in causal effect estimates obtained using each genetic variant. The conditional F-statistic through minimization of Q-statistics was calculated to assess IVs strength, a conventional F-statistic threshold of 10 is considered to exclude weak IVs.

To determine whether systemic inflammatory cytokines mediated the associations between exposures and KC, we conducted a two-step MR analysis and calculate the magnitude of their mediating effect. First, we estimated the effect of allergic conjunctivitis (AC), which showed a statistically significant causal association with KC in MVMR analysis, on 91 inflammatory cytokines using UVMR. The effects were estimated as beta coefficients (β_1). At the second step, those inflammatory cytokines showing a significant association with the exposure (AC) were selected and their effects on KC estimated, recorded as β_2. The proportion of mediation by each inflammatory cytokine in the association of the exposure with KC was calculated as the product of β_1 and β_2 divided by the total effect of exposure on KC.

For the binary outcome of KC, the associations between the allergic diseases as exposures and KC are presented as odds ratios (ORs) with their 95% confidence intervals (CIs). These ORs represent the change in the risk of keratoconus per 1 standard deviation (SD) increase in the genetically-predicted levels of allergic diseases or inflammatory cytokines. In the first step of the two-step MR, we examined the associations between allergic conjunctivitis (a binary variable) with the levels of inflammatory cytokines (continuous variable). The β-coefficients and standard errors (SEs) from this analysis reflect the change in the SD of inflammatory cytokine levels associated with a one-unit increase in the log-odds of AC.

A Benjamini-Hochberg correction was adopted due to the number of exposures. Results with P values less than 0.05 before multiple comparison correction, although not statistically significant after correction (adjusted P value $\geq$ 0.05), were considered suggestive of potential associations warranting further investigation.

All analyses were performed using R (Version 4.3.2) with the R packages 'TwoSampleMR' (version 0.5.9), 'MendelianRandomization' (version 0.9.0), 'MR-PRESSO' (version 1.0), 'mr.raps' (version 0.4.1), 'RadialMR' (version 1.1) and 'MRcML' (version 0.0.0.9000).

RESULTS

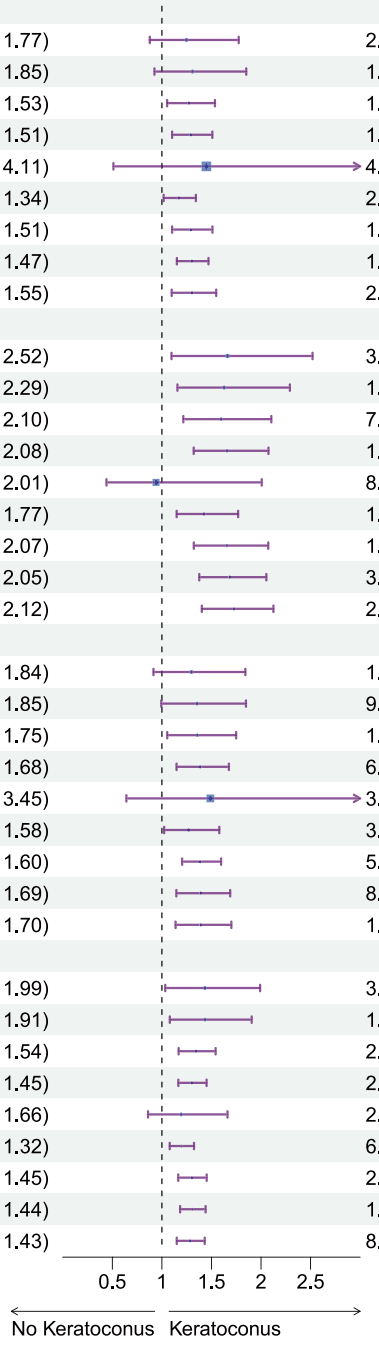
Causal effect of allergic disease on keratoconus

In UVMR study, we identified a number of independent SNPs associated at $P < 5 \times 10^{-8}$ with each AD: 13 SNPs associated with AC, 18 for AA, 8 for AR and 31 for AD. SNPs that were absent in the KC GWAS data were replaced by proxy SNPs following the standard of $r^2 > 0.8$, leaving 2 of them excluded in the subsequent analysis due to lack of proxy SNPs (Table S2). SNPs that overlapped between different allergic diseases were excluded as shown in Table S1 and Fig. S1D. In MVMR, 10 SNPs for AC, 13 SNPs for AA, 8 SNPs for AR and 30 SNPs for AD were selected as IVs following the same standard. Full details of the SNPs associated with each trait are provided in Table S1 and Table S10. And SNPs associated with 91 inflammatory cytokines are shown in Table S12. F-statistics of all selected SNPs in UVMR and MVMR analysis were >10 (Table S1, Table S11 and Table S12), indicating that no weak instruments were employed. Individuals in the exposure, mediation and outcome samples shared a European ethnic background so heterogeneity of SNPs was less likely to cause bias.

Univariable MR analysis revealed that all allergic diseases investigated were strongly associated with a higher risk of KC. MR estimates for the effects of different allergic diseases on KC risk are presented in Fig. 2A and Table S3. AC had an IVW OR of 1.66 (95% CI: 1.32–2.08; $P < 0.001$) for KC, with the corresponding value for AA being 1.29 (95% CI: 1.10–1.51, $P = 0.0014$), for AR being 1.39 (95% CI: 1.15–1.68; $P < 0.001$) and for AD being 1.30 (95%CI: 1.17–1.45, $P < 0.001$). Similarly, when tested by Radial MR, cML MR and RAPS, the causal relationship remained significant with no substantive change to these estimates. Details of cML MR and Radial MR methods are shown in Tables S4–S5 and Fig. S1B. Scatter plots of allergic disease and KC risk association for the IVs are presented in Fig. S1A. The Cochran's Q statistic indicated no evidence of heterogeneity across instrument effect except for AA (Table S7). Similarly, MR-PRESSO detected horizontal pleiotropy for AA but not for other allergic diseases

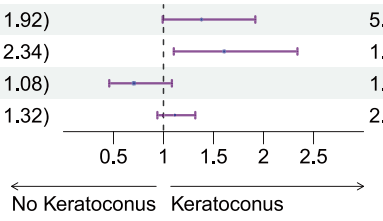
A

Exposure	Sample Size	OR(95% CI)	P value	P value(adjusted)
allergic asthma	219753			
Simple mode		1.25(0.88 − 1.77)	2.34e−01	2.34e−01
Weighted mode		1.31(0.92 − 1.85)	1.47e−01	1.47e−01
Weighted median		1.27(1.05 − 1.53)	1.26e−02	1.68e−02
IVW		1.29(1.10 − 1.51)	1.44e−03	1.44e−03
MR Egger		1.45(0.51 − 4.11)	4.96e−01	6.61e−01
Steiger filter		1.17(1.02 − 1.34)	2.69e−02	3.05e−02
RadialMR ivw		1.29(1.10 − 1.51)	1.44e−03	1.44e−03
cML MR		1.30(1.15 − 1.47)	1.88e−05	2.51e−05
RAPS		1.30(1.10 − 1.55)	2.39e−03	2.39e−03
allergic conjunctivitis	377277			
Simple mode		1.66(1.10 − 2.52)	3.38e−02	7.74e−02
Weighted mode		1.63(1.16 − 2.29)	1.61e−02	3.68e−02
Weighted median		1.60(1.22 − 2.10)	7.93e−04	1.59e−03
IVW		1.66(1.32 − 2.08)	1.18e−05	2.36e−05
MR Egger		0.94(0.44 − 2.01)	8.77e−01	8.77e−01
Steiger filter		1.43(1.15 − 1.77)	1.25e−03	2.50e−03
RadialMR ivw		1.66(1.32 − 2.07)	1.18e−05	1.57e−05
cML MR		1.68(1.38 − 2.05)	3.37e−07	6.74e−07
RAPS		1.73(1.40 − 2.12)	2.52e−07	1.01e−06
allergic rhinitis	370158			
Simple mode		1.30(0.92 − 1.84)	1.87e−01	2.34e−01
Weighted mode		1.35(0.99 − 1.85)	9.61e−02	1.28e−01
Weighted median		1.36(1.05 − 1.75)	1.80e−02	1.80e−02
IVW		1.39(1.15 − 1.68)	6.99e−04	9.32e−04
MR Egger		1.49(0.64 − 3.45)	3.90e−01	6.61e−01
Steiger filter		1.27(1.02 − 1.58)	3.05e−02	3.05e−02
RadialMR ivw		1.39(1.20 − 1.60)	5.00e−06	1.00e−05
cML MR		1.39(1.15 − 1.69)	8.65e−04	8.65e−04
RAPS		1.39(1.14 − 1.70)	1.25e−03	1.67e−03
atopic dermatitis	350062			
Simple mode		1.44(1.03 − 1.99)	3.87e−02	7.74e−02
Weighted mode		1.44(1.08 − 1.91)	1.84e−02	3.68e−02
Weighted median		1.34(1.17 − 1.54)	2.91e−05	1.16e−04
IVW		1.30(1.17 − 1.45)	2.43e−06	9.72e−06
MR Egger		1.20(0.86 − 1.66)	2.97e−01	6.61e−01
Steiger filter		1.20(1.08 − 1.32)	6.10e−04	2.44e−03
RadialMR ivw		1.30(1.16 − 1.45)	2.43e−06	9.72e−06
cML MR		1.31(1.18 − 1.44)	1.21e−07	4.84e−07
RAPS		1.28(1.15 − 1.43)	8.07e−06	1.61e−05



B

Exposure	Sample Size	OR(95% CI)	P value	P value(adjusted)
allergic asthma	219753	1.38(0.99 − 1.92)	5.58e−02	0.112
allergic conjunctivitis	377277	1.61(1.10 − 2.34)	1.34e−02	0.054
allergic rhinitis	370158	0.70(0.46 − 1.08)	1.11e−01	0.148
atopic dermatitis	350062	1.11(0.94 − 1.32)	2.17e−01	0.217



(Table S9). The MR-Egger intercept test did not show evidence of directional pleiotropy (Table S8) and Radial MR, cML MR and RAPS yielded consistent results (Tables S3–S5). Plots of leave-one-out analyses are shown in Fig. S1C and reveal no potentially influential SNPs driving the causal link between allergic disease and KC. No evidence of reverse causality across the analyses was revealed by the MR Steiger test (Table S6).

Comorbidity of distinct allergic diseases is common among patients, suggesting that allergic diseases may be related to each other. As a result, we performed an MVMR to simultaneously estimate the direct effect on KC of the 4 allergic diseases causatively related to KC in a univariable MR. When 4 allergic diseases were assessed together in MVMR, only AC (IVW OR 1.61; 95% CI: 1.10–2.34; P = 0.013) retained a robust, potentially causal relationship with KC (Fig. 2B and Table S11) and was suggestive significant with adjusted P value of 0.054. Similar results were obtained with MR-Egger analysis. The effect estimated for AC on KC was comparable to the univariable IVW estimate. The multivariable MR-Egger intercept test found no conclusive evidence for horizontal pleiotropy (P = 0.946). Similarly, Cochran's Q statistic indicated no existence of significant heterogeneity (P = 0.269), shown in Table S11.

Causal effect of allergic conjunctivitis on keratoconus through inflammatory cytokines

In the first step, the causal effects of AC on 91 inflammatory cytokines were calculated (Table S13). Results indicated that AC was correlated with higher plasma level of cystatin D (IVW OR = 1.12, 95% CI: 1.03–1.22, P = 0.008) and interleukin-1-alpha (IVW OR = 1.16, 95% CI: 1.01–1.33, P = 0.032). In the second step, the estimate of the impact of inflammatory cytokines on KC was obtained (Table S14). Several inflammatory cytokines were observed to be positively correlated with KC, including fibroblast growth factor 5 (IVW OR = 1.10, 95% CI: 1.01–1.21, P = 0.037), interleukin-17C (IVW OR = 1.33, 95% CI: 1.02–1.75, P = 0.037) and sulfotransferase 1A1 (IVW OR = 1.22, 95% CI: 1.06–1.39, P = 0.005). Others were found to be negatively correlated with KC, including eukaryotic translation initiation factor 4E-binding protein 1 (IVW OR = 0.77, 95% CI: 0.61–0.98, P = 0.03) and C-X-C motif chemokine 10 (IVW OR = 0.80, 95% CI: 0.68–0.95, P = 0.010). Nonetheless, all these associations were suggestive in view of the adjusted P value, indicating that none of the systemic inflammatory cytokines was significantly correlated with AC and KC simultaneously in this two-step MR analysis. They were therefore not potential factors that would mediate the effect of AC on KC (Tables S13–15).

DISCUSSION

This MR study suggests a causal link between allergic conjunctivitis (AC) and keratoconus (KC), independent of other allergic diseases in the multivariable Mendelian randomization (MVMR) analysis. This is the first study to apply MR to analyze the association between AC and KC. In the initial univariable Mendelian randomization (UVMR), all 4 allergic diseases were significantly associated with KC. In contrast, the causal effect of other allergic diseases on KC was absent in our MVMR study. We also conducted a two-step MR analysis to estimate the role of systemic inflammatory cytokines as potential mediators and it revealed that the causal effect of AC on KC was not mediated by any of them.

The relationship between AC and KC has long been recognized and broadly studied by researchers and ophthalmologists. Comparative studies have revealed a higher incidence of KC and abnormal patterns of corneal topography in patients with AC, and a correlation between the severity of KC and comorbidity of vernal keratoconjunctivitis has also been established.[9,43–45] A nationwide case-control study that included 2051 KC cases and 12,306 age- and sex-matched controls implicated an association of KC with allergic rash, asthma, bronchial hyperresponsiveness and inflammatory skin conditions.[6]

Fig. 2 MR estimates of the effects of allergic disease on keratoconus. (A) MR estimates of the effects of allergic disease on keratoconus based on univariable Two-sample Mendelian randomization. (B) MR estimates of the effects of allergic disease on keratoconus based on multivariable Mendelian randomization. *OR* Odds ratio, *95% CI* 95% confidence interval, *P value (adjusted)* P value adjusted by Benjamini-Hochberg correction, *IVW* inverse variance weighting, cML MR constrained maximum likelihood-based MR, *RAPS* Robust Adjusted Profile Score

Another population-based case-control study in Taiwan that involved 5055 patients with KC and 20,220 matched controls revealed that AR and asthma were positively associated with KC.[7] Additionally, the hazard ratio of severe AD for KC was estimated to be 10.01 (95% CI, 5.02–19.96) according to a Danish nationwide study. Other studies have yielded contradictory results.[8] A retrospective longitudinal cohort study of 16,053 KC patients and controls reported an insignificant association of KC with AR.[13] Another nationwide study reported a protective effect of AR for KC.[16] A nationwide longitudinal cohort analysis in Korea that included 34,375 AD patients showed no significant correlation of AD with KC, later confirmed by an MR analysis.[14,46] On the contrary, in our UVMR study, AD was positively associated with KC with convincing significance. This was probably due to a larger sample size of 2116 KC cases in the GWAS used in the present study, compared with the previous one of only 477 KC cases.[46]

Although 4 allergic diseases were significantly correlated with KC in our UVMR analysis, only AC retained a robust causal effect in the subsequent MVMR analysis. These results may imply that the correlation presented in previous observational studies of AA, AR and AD with KC may have been due to the comorbidity of AC.[6-9] Previous study determined that more than 90% of inflammatory cytokines in tear fluid were similar between AC and KC patients.[47] Some genomics and transcriptomics analyses[4,5] of corneal tissue have demonstrated the involvement of an inflammatory response in KC progression. Based on the result of our two-step MR, we hypothesize that the effect of AC on KC is not mediated by systemic inflammation, but is attributed to local inflammatory responses. Although the exact effect of AC on KC was not specified, our MR results may offer insight for clinical practice. Given that AC is common among adolescents,[48-50] close attention to and regular examination of corneal topography among these patients are suggested. For treatment, standard and effective anti-inflammatory therapy[49-51] is warranted for AC patients with the aim of relieving symptoms and lowering the risk of KC. Respiratory physicians and dermatologists should be alert to the comorbidity of AC in patients diagnosed with AA, AR and AD. Timely referral to an ophthalmologist for screening and periodic monitoring may be necessary.

Although most studies of risk factors for KC mentioned in this paper were nationwide with considerable sample size, results were conflicting. This may be partly attributed to confounding factors that are inevitable in observational studies. In addition, most studies were cross-sectional, making it difficult to assess the direction of the association. An MR study design has inherent advantages.[52] By using genetic variants as IVs, MR studies can effectively avoid confounding bias since SNPs are randomly assigned at conception. In effect, MR analysis can simulate a randomized controlled trial in an observational setting by limiting the interference of confounding variables and reverse causation, and thus generate less confounded estimates of causal relationships. Moreover, European ancestry was predominant in all our datasets so population stratification for ethnicity was not a potential bias.

To ensure that our genetic instruments were strongly associated with the risk factors of interest, we used genome-wide significant genetic variants, defined as those meeting a threshold of $P < 5 \times 10^{-8}$, as instruments. To mitigate weak instrument bias, we calculated the proportion of variance in the exposure explained by each SNP using the R^2 value, and assessed the strength of the SNP-exposure association with the F-statistic. We included only those SNPs with an F-statistic greater than 10 in our analysis, ensuring that the genetic instruments have a sufficiently strong association with the exposure of interest. Additionally, we removed SNPs in LD related to chromosome locus distribution, which helps eliminate confounding factors caused by non-Mendelian inheritance patterns. We also excluded overlapping SNPs between allergic diseases to avoid interference and potential pleiotropy in UVMR analysis. To address pleiotropy, we conducted a range of sensitivity analyses to ensure the robustness of our findings. Firstly, we employed MR-RAPS, weighted median based method and cML MR in accordance with the guidelines for sensitivity analysis by Burgess et al.[53] These methods adhere to different assumptions (i.e. Instrument Strength Independent of Direct Effect assumption (InSIDE),

majority valid, and plurality valid), and results meeting these 3 criteria are generally considered reliable. Secondly, MR-Egger regression for multiple-exposure UVMR and MVMR, along with MR-PRESSO for MVMR (Fig. 2, Tables S7, S9, S11), were also used to indicate that no significant pleiotropy or outliers were present in our analysis. Lastly, cML MR was employed to correct for violations of the 3 IV hypotheses and evaluated data perturbations, further mitigating the impact of heterogeneity and pleiotropy, and demonstrating strong robustness. Additionally, we conducted Steiger directional filtering and testing to ensure the directionality of causal associations by excluding SNPs with stronger associations with the outcomes than with exposure. The Cochrane's Q test and the leave-one-out test were used to assess heterogeneity and strong SNP interference. Through the rigorous methodologies described above, we believe our analyses demonstrate the robustness of our main results.

Some limitations of our study should be acknowledged. First, all GWAS data derived from European populations. Black persons and those of Latino ethnicity are reported to have higher odds of being diagnosed with KC while Asian persons have reduced odds.[13] Whether our findings can be extrapolated to other populations remains to be investigated. Second, KC is reported to have distinct incidence and prevalence among males compared with females and was most commonly seen in adolescents.[1] We were unable to investigate the associations of AC with KC in subpopulations due to the lack of individual-level data. Further analyses are warranted to investigate the correlation stratified by age and sex. Similarly, phenotypes of outcome GWAS data were not classified according to KC severity. Whether the risk ratio of AC differs for stable KC and progressive KC remains unknown. The problem may be solved when data stratified by KC severity and progression are available. Third, the sample size of our outcome GWAS was relatively small and so does the number of IVs used in UVMR and MVMR. This may have decreased our statistical power. Further research with GWAS data for larger sample sizes is warranted to confirm our conclusion. Lastly, while our analysis employed robust MR methods and sensitivity analyses to mitigate pleiotropy, we acknowledge the potential influence of unmeasured confounding factors, such as serum levels of immunoglobulin E and educational level. Future research could explore these factors using rigorous clinical cohort designs or advanced causal inference methods to further clarify the causal relationship between allergic diseases and keratoconus.

CONCLUSION

In conclusion, the findings from our UVMR and MVMR analyses suggest a potential causal relationship between AC and KC. The results also imply that the correlation presented in previous observational studies of AA, AR and AD with KC might be attributable to the co-occurrence of AC. These insights hold promise for the development of preventative and interventional strategies aimed at reducing KC risk in AC patients. Furthermore, the influence of AC on KC might be mediated by additional inflammatory cytokines not examined in this study, or through alternative mechanisms altogether. Future research efforts should be directed towards elucidating the underlying pathological and physiological processes of this relationship.

Abbreviations

KC: keratoconus; MR: Mendelian randomization; IVs: instrumental variables; GWASs: genome-wide association studies; UVMR: univariable Mendelian randomization; AC: allergic conjunctivitis; AA: allergic asthma; AD: atopic dermatitis; AR: allergic rhinitis; MVMR: multivariable Mendelian randomization; SNP: single nucleotide polymorphism; LD: linkage disequilibrium; EAF: effect allele frequency; IVW: inverse variance weighted; MR-PRESSO: MR pleiotropy residual sum and outlier; RAPS: robust adjusted profile score; cML MR: constrained maximum likelihood-based MR; OR: odds ratio; CI: confidence interval; SD: standard deviation; SE: standard error

Authors' consent for publication

All authors have given consent for publication.

Availability of data and materials

The datasets analyzed during the current study are available in the following databases.

1. GWAS summary statistics of allergic diseases from FinnGen database:

a) allergic conjunctivitis: https://r9.finngen.fi/pheno/H7_ALLERGICCONJUNCTIVITIS

b) allergic asthma: https://r9.finngen.fi/pheno/ALLERG_ASTHMA

c) allergic rhinitis: https://r9.finngen.fi/pheno/ALLERG_RHINITIS

d) atopic dermatitis: https://r9.finngen.fi/pheno/L12_ATOPIC

2. GWAS summary statistics of keratoconus from GWAS Catalog database: https://www.ebi.ac.uk/gwas/studies/GCST90013442

3. GWAS summary statistics of 91 circulating inflammatory cytokines from GWAS Catalog database: Full per-protein GWAS summary statistics are available for download at https://www.phpc.cam.ac.uk/ceu/proteins and the EBI GWAS Catalog (accession numbers GCST90274758 to GCST90274848).

Author contributions

Hanlu Xu and Dan Jiang conceived and designed the study. Hanlu Xu and Yajing Wen performed data analysis and wrote the manuscript. Huikang Zheng assisted in data analysis and writing. Dan Jiang and Wei Chen revised the manuscript. All authors edited and proofread the manuscript.

Ethics statement

Ethical approval was not required for this MR study as it is based on publicly available genome-wide association studies (GWAS) data without the direct engagement of participants.

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Declaration of competing interest

The authors report no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.100993.

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