

Heritability of Corneal Parameters in Nuclear Families With Keratoconus

Yawen Wang^{1,*}, Liyan Xu^{1,2,*}, Shaopei Wang³, Kaili Yang², Yuwei Gu², Qi Fan², Qing Wang¹, Meng Zhu⁴, Kai Guo⁴, Chenjiu Pang², Shengwei Ren^{1,2}, and Dongqing Zhao^{1,2}

¹ Henan University People's Hospital, Henan Provincial People's Hospital, Henan Eye Hospital, Henan Eye Institute, Zhengzhou 450003, China

² Henan Provincial People's Hospital, Henan Eye Hospital, Henan Eye Institute, People's Hospital of Zhengzhou University, Henan University People's Hospital, Zhengzhou 450003, China

³ Xinxiang Medical University, Henan Provincial People's Hospital, Henan Eye Hospital, Henan Eye Institute, Zhengzhou 450003, China

⁴ Zhengzhou University People's Hospital, Henan Provincial People's Hospital, Henan Eye Hospital & Henan Eye Institution, Zhengzhou, 450003, China

Correspondence: Shengwei Ren and Dongqing Zhao, Henan Provincial People's Hospital, Henan Eye Hospital, Henan Eye Institute, People's Hospital of Zhengzhou University, Henan University People's Hospital, Zhengzhou 450003, China. e-mail: shengweiren1984@163.com, ykszdq@163.com

Received: April 22, 2022

Accepted: June 25, 2022

Published: July 15, 2022

Keywords: keratoconus; heritability; corneal parameters; nuclear family

Citation: Wang Y, Xu L, Wang S, Yang K, Gu Y, Fan Q, Wang Q, Zhu M, Guo K, Pang C, Ren S, Zhao D. Heritability of corneal parameters in nuclear families with keratoconus. *Transl Vis Sci Technol.* 2022;11(7):13. <https://doi.org/10.1167/tvst.11.7.13>

Purpose: This study aimed to investigate the heritability of corneal parameters obtained by Pentacam in nuclear families with keratoconus (KC).

Methods: A total of 82 patients with KC and their biological parents ($n = 164$) were recruited in the current study. All subjects underwent corneal tomography with Pentacam. Family units were analyzed to calculate the heritability of corneal parameters by linear mixed effects model using the R statistical software.

Results: The pachymetry at apex, pupil, and thinnest point were all significantly heritable at 43.26%, 42.63%, and 43.09%, respectively. The heritability of flat meridian keratometry, steep meridian keratometry, and mean keratometry in the anterior surface were 10.36%, 9.05%, and 10.21%, respectively, and that of flat meridian keratometry, steep meridian keratometry, and mean keratometry in the posterior surface were 8.44%, 9.67%, and 9.06%, respectively. The posterior radius of curvature had higher heritability in comparison with anterior radius of curvature (19.16% vs. 14.37%). Moreover, among combined topometric indices, the heritability of index of vertical asymmetry was the highest (19.49%), and that of central keratoconus index was the lowest (6.64%).

Conclusions: The present study demonstrated a substantial heritability of corneal parameters in nuclear families with KC. The pachymetric indices are heritable and may be suitable as KC endophenotypes, suggesting a necessity to discover the genes associated with corneal thickness in KC.

Translational Relevance: The pachymetric indices are heritable and may be suitable as KC endophenotypes, indicating that the pachymetric indices might be a corneal characteristic to predict the occurrence of KC.

Introduction

Keratoconus (KC) is a progressive disease characterized by gradual corneal thinning and ectasia, resulting in irregular astigmatism, myopia, and mild to severe impairment in the quality of vision.^{1,2} Although it is possible to estimate the global prevalence of KC, as

Hashemi et al.³ did, obtaining 138 per 100,000 population, this actually paints an unrealistic picture of the condition, owing to the extremely wide difference in prevalence between some geographic regions and others, with a range between 0.3 per 100,000 inhabitants in the north of Russia and up to 4000 per 100,000 inhabitants in Iran.¹ The exact pathogenesis of KC remains unknown. Several studies have indicated an

association between environmental factors, such as eye rubbing, allergies, asthma, sleeping position, and diabetes with KC.⁴⁻⁶ In addition, a positive family history of 5% to 23% in KC cases,¹ and the higher concordance rate in monozygotic twins⁷ suggest that genetic factors play a role in the development of KC. Genome-wide association analysis, linkage analysis, and candidate gene analysis revealed KC susceptibility genes and associated loci,^{4,8-11} as well as changes in mitochondrial genome and epigenetics,¹²⁻¹⁴ but how these sites and changes affect the disease remains unclear.

Based on the background, we introduced heritability to explain the role of genetic factors in clinical traits of KC. In genetic epidemiology, heritability plays a central role in understanding the factors that lead to individual differences within a population. Heritability provides a clear answer of what percentage of variation in observed traits is due to genetic factors. The greater the heritability, the greater the contribution of genetic factors to the cause.¹⁵ Knowledge of relative genetic contributions to diseases or traits helps researchers to decide whether to conduct a more detailed analysis of genetic variation at the molecular level.¹⁶

Nuclear family studies, those that comprise both proband and their biological parents, are valuable in genetic epidemiology because they help to delineate the familial aggregation of a disorder as well as its potential etiological mechanisms that explain familial risk. The investigation of the heritability of corneal parameters in nuclear families with KC is helpful to determine the endophenotype and related genes of KC, further understanding the potential genetic mechanism of KC. To date, the heritability of corneal parameters in nuclear families with KC has not been reported. The purpose of this study was to investigate the heritability of corneal parameters in nuclear families with KC and to illustrate the role of genetic factors in clinical traits of KC.

Methods

This study was conducted following the Declaration of Helsinki guidelines and approved by the Institutional Review Board of Henan Eye Hospital [ethical approval number: HNEECKY-2019 (5)]. Written informed consent was obtained from each subject.

This study recruited 82 patients with KC and their 164 biological parents. The diagnosis of KC was based on asymmetric bowtie pattern with or without skewed axes revealed by corneal tomography, and the

presence of characteristics detected by slit-lamp examination, such as localized stromal thinning, conical protrusion, Vogt's striae, Fleischer's ring, or anterior stromal scar. Patients secondary to trauma, surgery, and other diseases were excluded from the study. All subjects underwent corneal tomography with Pentacam HR (Oculus, Lynnwood, WA). The Pentacam uses a monochromatic blue light-emitting diode with a wavelength of 475 nm and a Scheimpflug camera that rotates around the corneal axis. Only scans with a quality specification of "OK" were taken for analysis. Subjects with a history of other eye problems, surgery or trauma, and significant corneal scar were excluded. Subjects wearing contact lenses or rigid contact lenses were asked to stop wearing contact lenses for 2 weeks or rigid contact lenses for 4 weeks before examinations.

The data from the right eye or affected eye were included if patients had bilateral or unilateral KC. Owing to the good correlation between the left and right eyes of the parents (Supplementary Table S1), only data from the right eye were included in the analysis. Corneal parameters assessed in this study included corneal pachymetric indices (the pachymetry at apex, pupil and thinnest point), corneal curvature parameters (flat meridian keratometry [K1], steep meridian keratometry [K2], mean keratometry [Km], maximum keratometry, anterior radius of curvature [ARC], and posterior radius of curvature [PRC]), and some corneal indices indicating KC. These corneal indices indicating KC include index of surface variance (ISV), index of height decentration (IHD), Belin/Ambrosio enhanced ectasia display final D value (BADD), index of height asymmetry (IHA), keratometric power difference (KPD), keratoconus index (KI), central keratoconus index (CKI), and index of vertical asymmetry (IVA).

Descriptive characteristics of the sample were expressed as mean \pm standard deviation for continuous variables. A linear mixed effects model was used to calculate the heritability. Heritability = Genetic variance / Genetic variance + Noise variance, where genetic variance is the same as cluster variance and noise variance is the same as residual variance in a linear mixed effects model. To control for the confounding effects of variables on the parameters, multiple linear regression was used to evaluate the effect of age and gender on these parameters. Heritability was calculated for these parameters by eliminating the effect of confounding factors that showed a significant relationship with the parameters. The bootstrap method was used to calculate the confidence limits of heritability.¹⁷ All analyses were done using the R statistical software (The R Foundation, Vienna, Austria).

Results

The final analysis of 82 nuclear families, including 82 KC probands and 164 parents, were performed. In the current study, 22 probands (26.83%) were female and 60 (73.17%) were male. The mean age of the probands was 17.56 ± 4.38 years. The mean age of the 164 parents was 44.35 ± 5.53 years. The description of corneal parameters for KC patients and their parents are presented in Table 1. To control for the confounding effects of variables on the indices, we adopted multiple linear regression to evaluate the effect of age and gender on evaluated indices, and the corresponding results are shown in Table 2.

Table 3 shows the heritability of corneal parameters obtained by Pentacam. The pachymetry at apex, pupil, and thinnest point were all significantly heritable at 43.26%, 42.63%, and 43.09%, respectively. The heritability of K1, K2, and Km in the anterior surface were 10.36%, 9.05%, and 10.21%, respectively, and that of K1, K2 and Km in the posterior surface were 8.44%, 9.67%, and 9.06%, respectively. The PRC had a

higher heritability in comparison with ARC (19.16% vs. 14.37%). Moreover, the highest and lowest heritability was related to IVA (19.49%) and CKI (6.64%) among combined topometric indices.

Discussion

The present study evaluated the heritability of corneal parameters obtained by Pentacam in nuclear families with KC. In this study, corneal thickness had high heritability of more than 40%. PRC had a higher heritability in comparison with ARC. Assessment of the combined topometric indices showed that IVA had the highest and CKI had the lowest heritability.

KC was reported to be a complex non-Mendelian disease.¹⁸ Currently, there were large number of studies exploring associated genes for KC. However, no convincing mutations associated with disease has been found so far.¹⁹ The issue of the necessity of thorough phenotyping in patients is common. Dissecting the genetic structure of heterogeneous diseases such as KC can be achieved by considering the individual

Table 1. Clinical Characteristics of Participants

	Parents (<i>n</i> = 164)	Probands (<i>n</i> = 82)
Gender (male/female)	82/82	60/22
Age (years)	44.35 ± 5.53	17.56 ± 4.38
Flat meridian keratometry in 3 mm (diopter)	43.41 ± 1.30	46.87 ± 4.83
Steep meridian keratometry in 3 mm (diopter)	44.22 ± 1.43	50.86 ± 6.14
Mean keratometry (diopter)	43.81 ± 1.32	48.76 ± 5.34
Flat meridian keratometry posterior (diopter)	-6.18 ± 0.22	-6.97 ± 0.95
Steep meridian keratometry posterior (diopter)	-6.45 ± 0.26	-7.74 ± 1.10
Mean keratometry posterior (diopter)	-6.31 ± 0.23	-7.33 ± 1.00
Maximum keratometry (diopter)	44.85 ± 1.49	57.71 ± 9.73
Maximum keratometry zonal mean 3 mm (diopter)	44.34 ± 1.42	53.67 ± 7.10
Maximum keratometry zonal mean 4 mm (diopter)	44.25 ± 1.44	53.22 ± 6.86
Maximum keratometry zonal mean 5 mm (diopter)	44.13 ± 1.45	52.43 ± 6.39
Anterior radius of curvature (mm)	7.70 ± 0.23	6.60 ± 0.77
Posterior radius of curvature (mm)	6.27 ± 0.24	4.94 ± 0.71
Corneal thickness at the pupil center (μ m)	527.07 ± 32.31	478.02 ± 43.73
Corneal thickness at the apex (μ m)	527.99 ± 32.19	467.84 ± 47.3
Corneal thickness at the thinnest point (μ m)	522.66 ± 32.41	459.26 ± 46.58
Belin/Ambrosio enhanced ectasia display final D value	1.20 ± 0.15	9.28 ± 5.21
Keratometric power difference (diopter)	15.22 ± 5.86	2.32 ± 0.86
Index of surface variance	0.12 ± 0.06	84.68 ± 40.86
Index of vertical asymmetry (mm)	1.02 ± 0.03	0.78 ± 0.37
Keratoconus index	1.00 ± 0.01	1.22 ± 0.12
Central keratoconus index	4.78 ± 3.75	1.09 ± 0.07
Index of height asymmetry (μ m)	0.01 ± 0.01	26.79 ± 22.46
Index of height decentration (μ m)	1.31 ± 0.85	0.11 ± 0.06

Table 2. Multiple Linear Regression Results for Corneal Parameters

Corneal Parameters	Age		Gender	
	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value
Flat meridian keratometry in 3 mm (diopter)	-0.106 (-0.135 to -0.077)	<0.001	-0.008 (-0.807 to 0.791)	0.984
Steep meridian keratometry in 3 mm (diopter)	-0.210 (-0.247 to -0.173)	<0.001	-0.050 (-1.074 to 0.973)	0.923
Mean keratometry (diopter)	-0.155 (-0.187 to -0.122)	<0.001	-0.026 (-0.913 to 0.861)	0.954
Flat meridian keratometry posterior (diopter)	0.025 (0.019 to 0.030)	<0.001	0.003 (-0.153 to 0.159)	0.967
Steep meridian keratometry posterior (diopter)	0.041 (0.034 to 0.047)	<0.001	0.025 (-0.161 to 0.211)	0.793
Mean keratometry posterior (diopter)	0.032 (0.026 to 0.038)	<0.001	0.020 (-0.146 to 0.187)	0.811
Maximum keratometry (diopter)	-0.405 (-0.464 to -0.347)	<0.001	-0.508 (-2.122 to 1.105)	0.535
Maximum keratometry zonal mean 3 mm (diopter)	-0.292 (-0.336 to -0.249)	<0.001	-0.246 (-1.448 to 0.956)	0.687
Maximum keratometry zonal mean 4 mm (diopter)	-0.281 (-0.323 to -0.239)	<0.001	-0.213 (-1.377 to 0.951)	0.719
Maximum keratometry zonal mean 5mm (diopter)	-0.260 (-0.299 to -0.220)	<0.001	-0.144 (-1.235 to 0.947)	0.795
Anterior radius of curvature (mm)	0.035 (0.030 to 0.040)	<0.001	-0.013 (-0.150 to 0.124)	0.851
Posterior radius of curvature (mm)	0.042 (0.037 to 0.047)	<0.001	0.005 (-0.131 to 0.141)	0.944
Corneal thickness at the pupil center (µm)	1.436 (1.073 to 1.799)	<0.001	-0.076 (-10.098 to 9.945)	0.988
Corneal thickness at the apex (µm)	1.776 (1.395 to 2.158)	<0.001	2.049 (-8.492 to 12.590)	0.702
Corneal thickness at the thinnest point (µm)	1.872 (1.490 to 2.255)	<0.001	2.566 (-7.999 to 13.131)	0.633
Belin/Ambrosio enhanced ectasia display final D value	-0.244 (-0.276 to -0.212)	<0.001	-0.732 (-1.635 to 0.171)	0.111
Keratometric power difference (diopter)	-0.035 (-0.04 to -0.03)	<0.001	-0.091 (-0.235 to 0.052)	0.212
Index of surface variance	-2.170(-2.424 to -1.915)	<0.001	-6.200(-13.221 to 0.822)	0.083
Index of vertical asymmetry (mm)	-0.021(-0.023 to -0.018)	<0.001	-0.039 (-0.104 to 0.026)	0.238
Keratoconus index	-0.006 (-0.007 to -0.005)	<0.001	-0.013 (-0.035 to 0.008)	0.22
Central keratoconus index	-0.003 (-0.003 to -0.002)	<0.001	-0.010 (-0.021 to 0.002)	0.108
Index of height asymmetry (µm)	-0.677 (-0.811 to -0.544)	<0.001	1.375 (-2.315 to 5.065)	0.464
Index of height decentration (µm)	-0.003 (-0.004 to -0.003)	<0.001	-0.009 (-0.020 to 0.002)	0.113

Table 3. The Heritability of Corneal Parameters

Corneal Parameters	Heritability (95% CI)*
Flat meridian keratometry in 3 mm (diopter)	10.36 (0.28–20.61)
Steep meridian keratometry in 3 mm (diopter)	9.05(0.00–21.30)
Mean keratometry (diopter)	10.21 (0.00–21.25)
Flat meridian keratometry posterior (diopter)	8.44 (0.00–17.14)
Steep meridian keratometry posterior (diopter)	9.67 (0.00–24.04)
Mean keratometry posterior (diopter)	9.06 (0.00–21.18)
Maximum keratometry (diopter)	10.62 (0.00–23.19)
Maximum keratometry zonal mean 3 mm (diopter)	13.31 (0.57–26.30)
Maximum keratometry zonal mean 4 mm (diopter)	13.18 (0.46–26.36)
Maximum keratometry zonal mean 5 mm (diopter)	13.04 (0.09–26.22)
Anterior radius of curvature (mm)	14.37 (0.82–26.97)
Posterior radius of curvature (mm)	19.16 (4.74–33.69)
Corneal thickness at the pupil center (µm)	42.63 (25.81–56.84)
Corneal thickness at the apex (µm)	43.26 (26.73–57.59)
Corneal thickness at the thinnest point (µm)	43.09 (27.13–57.37)
Belin/Ambrosio enhanced ectasia display final D value	13.45 (0.00–26.39)
Keratometric power difference (diopter)	9.04 (0.00–19.90)
Index of surface variance	16.28 (2.89–31.15)
Index of vertical asymmetry (mm)	19.49 (8.25–32.13)
Keratoconus index	15.87 (3.88–29.18)
Central keratoconus index	6.64 (0.00–16.83)
Index of height asymmetry (µm)	9.48 (3.09–17.37)
Index of height decentration(µm)	16.28 (5.15–29.14)

* Adjusted for age.

quantitative traits or endophenotypes underlying the disease. Endophenotypes are quantitative traits that are genetically correlated with the disease and hypothesized to be less complex and closer to the underlying genetics than the disease of interest, because they reflect just one of many pathophysiological pathways that contribute to disease susceptibility.²⁰ A significant genetic correlation between disease and a quantitative trait is a mandatory prerequisite for recognition of the trait as an endophenotype for disease. In this study, heritability was used to measure the genetic correlation between diseases and quantitative traits.

Previous study highlighted the importance of corneal thickness in relation to several ocular conditions.^{21–23} Multiple studies support a genetic component for corneal thickness determination including data from heritability and genome sequencing studies.^{24–27} Corneal thinning is one of the characteristics in patients with KC.² Therefore, we evaluated the heritability of corneal thickness in nuclear families with KC to explore the genetic component for corneal thickness determination in KC. The results showed that the pachymetry at apex, pupil, and thinnest point were all significantly heritable at 43.26%, 42.63%, and 43.09%, respectively. The findings revealed that genetic factors play an important role in the changes of corneal thickness in patients with KC. However, several previous studies also evaluated the heritability of corneal thickness, and found the heritability was more than 90% in the twin studies^{28–30} and from 68% to 85% in the family studies,^{25,31,32} which was much higher than our findings. The inconsistent results might be attributed to the differences in study design and study population. Except for the heritability studies on corneal thickness, studies on genetic sequencing have also achieved some success in identifying associated genetic loci with central corneal thickness in KC. Multiple studies explored genetic variants associated with central corneal thickness in KC and found several significant variants, such as rs2371597, rs56009602, rs10453441, and rs2721051.^{22,26,27,33–35} However, the mechanisms that drive the increased KC risk at these loci remain to be elucidated. The present study found that the heritability of pachymetric indices in nuclear families with KC were more than 40%, indicating that the pachymetric indices obtained by Pentacam had a great genetic correlation with KC. Therefore, we speculated that the pachymetric indices might be considered as strong endophenotype of KC. Further identification of heritable quantitative trait loci in patients with KC using corneal thickness as endophenotype is needed in the future.

The increased steepness of anterior and posterior corneal surface is one of the characteristics of corneal

tomography in patients with KC. In our study, the heritability of K1, K2, and Km in the anterior surface were 10.36%, 9.05%, and 10.21%, respectively, which were lower than the 55% reported in the meta-analysis by Sanfilippo et al.¹⁶ The result suggests that genetic factors may play a lesser role in the change of anterior surface curvature in patients with KC. The heritability in this study is lower than Heydarian's study,²⁴ 10.36% versus 58.61% (K1), 9.05% versus 55.82% (K2), and 10.21% versus 39.27% (Km). The difference may be due to the different sample populations used in the two studies (KC nuclear families vs. normal families). Therefore, this study is more convincing in explaining the genetic factors of curvature changes in patients with KC. In this study, the heritability of PRC was found to be 19.16%, higher than that of ARC (14.37%), which was consistent with the study by Mahroo et al.²⁸ Compared with the ARC, the greater heritability of PRC suggests that genetic factors play a more significant role, which is consistent with that posterior corneal tomography is of great relevance and can be affected earlier in corneal ectatic diseases.³⁶ However, this result did not occur when comparing the heritability of curvature in anterior and posterior corneal surface (K1, K2, and Km). Therefore, it is uncertain whether genetic factors play a more important role in the variation of posterior corneal curvature than the anterior corneal curvature, and further studies are needed. This study found that the heritability of corneal curvature parameters obtained by Pentacam were not high, suggesting these parameters having the less the contribution of genetic factors may be inappropriate considered as endophenotypes of KC.

In the current study, a large number of corneal parameters that were of potential interest in KC were investigated, including topometric indices provided by Pentacam software combining anterior corneal surface, posterior corneal surface and corneal thickness. In this study, IVA (19.49%), ISV (16.28%), and IHD (16.28%) had the higher heritability, whereas the heritability of BADD, KPD, KI, CKI, and IHA ranged from 6.64% to 15.87%. The low heritability of these corneal tomographic parameters suggest that they may be less influenced by genetic factors. This finding may be due to the fact that these combined parameters are not actually inherent attributes and do not fit as clinical traits of KC in heritability analysis. Heydarian et al.²⁴ found that ISV, IVA, KI, CKI, and IHD all had high heritability, except for KPD and IHA, which was different from our study. This finding may be due to the different sample populations used in the two studies (normal families vs. KC nuclear families). Insufficient research is available on this subject, and further

studies on the heritability of these parameters are required.

This study had some potential limitations. First, heritability is spatially and temporally dependent, specific to a given population at a given time.¹⁶ Therefore, the results of this study may not be applicable to other ethnic groups of patients with KC. Second, nuclear family study designs are the less powerful of genetic designs compared with twin and adoption studies. It is difficult to study heritability in KC twins, however, owing to the low prevalence of KC and the rarity of twins with KC. The KC nuclear family was used in this study to study the heritability of corneal parameters because of the relative ease of obtaining subjects.

Conclusions

The present study evaluated the heritability of corneal parameters in nuclear families with KC and demonstrated a substantial heritability. The pachymetric indices are heritable and may be suitable as endophenotypes of KC. This study highlights the necessity of further genetic and molecular studies to discover the genes associated with KC and understand the causes of KC.

Acknowledgments

Supported by Special Program for Basic Research of Henan Eye Hospital (No. 20JCZD003), Henan Young Health Science and Technology Innovation Outstanding Program (No. YXKC2020023), Henan Provincial Building Key Program (No. SBGJ202002028, SBGJ202102051), Henan Provincial Medical Science and Technology Joint Program (No. LHGJ20200066, LHGJ20210080), Henan Provincial Scientific and Technological Research Project (No. 222102310307, 222102310599), Youth Special Program for Basic Research of Henan Eye Hospital (No. 21JCQN006, 21JCQN008).

Disclosure: **Y. Wang**, None; **L. Xu**, None; **S. Wang**, None; **K. Yang**, None; **Y. Gu**, None; **Q. Fan**, None; **Q. Wang**, None; **M. Zhu**, None; **K. Guo**, None; **C. Pang**, None; **S. Ren**, None; **D. Zhao**, None

* YW and LX Contributed equally.

References

1. Rabinowitz YS, Galvis V, Tello A, Rueda D, Garcia JD. Genetics vs chronic corneal mechanical trauma in the etiology of keratoconus. *Exp Eye Res.* 2021;202:108328.
2. Gomes JA, Tan D, Rapuano CJ, et al. Global consensus on keratoconus and ectatic diseases. *Cornea.* 2015;34:359–369.
3. Hashemi H, Heydarian S, Hooshmand E, et al. The prevalence and risk factors for keratoconus: a systematic review and meta-analysis. *Cornea.* 2020;39:263–270.
4. Lucas SEM, Burdon KP. Genetic and environmental risk factors for keratoconus. *Annu Rev Vis Sci.* 2020;6:25–46.
5. Gatinel D, Galvis V, Tello A, et al. Obstructive sleep apnea-hypopnea syndrome and keratoconus: an epiphenomenon related to sleep position? *Cornea.* 2020;39:e11–e12.
6. Mazharian A, Panthier C, Courtin R, et al. Incorrect sleeping position and eye rubbing in patients with unilateral or highly asymmetric keratoconus: a case-control study. *Graefes Arch Clin Exp Ophthalmol.* 2020;258:2431–2439.
7. Tuft SJ, Hassan H, George S, Frazer DG, Willoughby CE, Liskova P. Keratoconus in 18 pairs of twins. *Acta Ophthalmol.* 2012;90:e482–486.
8. Hardcastle AJ, Liskova P, Bykhovskaya Y, et al. A multi-ethnic genome-wide association study implicates collagen matrix integrity and cell differentiation pathways in keratoconus. *Commun Biol.* 2021;4:266.
9. Gajecka M, Radhakrishna U, Winters D, et al. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. *Invest Ophthalmol Vis Sci.* 2009;50:1531–1539.
10. Sargazi S, Moudi M, Heidari Nia M, Saravani R, Malek Raisi H. Association of KIF26B and COL4A4 gene polymorphisms with the risk of keratoconus in a sample of Iranian population. *Int Ophthalmol.* 2019;39:2621–2628.
11. da Silva DC, Gadelha BNB, Feitosa AFB, et al. Analysis of VSX1 variations in Brazilian subjects with keratoconus. *J Ophthalmic Vis Res.* 2018;13:266–273.
12. Xu L, Yang K, Fan Q, Zhao D, Pang C, Ren S. Whole mitochondrial genome analysis in Chinese patients with keratoconus. *Mol Vis.* 2021;27:270–282.
13. Vallabh NA, Romano V, Willoughby CE. Mitochondrial dysfunction and oxidative stress in corneal disease. *Mitochondrion.* 2017;36:103–113.

14. Kabza M, Karolak JA, Ryzanicz M, et al. Multiple differentially methylated regions specific to keratoconus explain known keratoconus linkage loci. *Invest Ophthalmol Vis Sci.* 2019;60:1501–1509.
15. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era—concepts and misconceptions. *Nat Rev Genet.* 2008;9:255–266.
16. Sanfilippo PG, Hewitt AW, Hammond CJ, Mackey DA. The heritability of ocular traits. *Surv Ophthalmol.* 2010;55:561–583.
17. Ukoumunne OC, Davison AC, Gulliford MC, Chinn S. Non-parametric bootstrap confidence intervals for the intraclass correlation coefficient. *Stat Med.* 2003;22:3805–3821.
18. Kriszt A, Losonczy G, Berta A, Vereb G, Takacs L. Segregation analysis suggests that keratoconus is a complex non-mendelian disease. *Acta Ophthalmol.* 2014;92:e562–568.
19. Valgaeren H, Koppen C, Van Camp G. A new perspective on the genetics of keratoconus: why have we not been more successful? *Ophthalmic Genet.* 2018;39:158–174.
20. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J of Psychiatry.* 2003;160:636–645.
21. Dimasi DP, Burdon KP, Craig JE. The genetics of central corneal thickness. *Br J Ophthalmol.* 2010;94:971–976.
22. Yang Z, Yang J, Liu D, Yu W. Mendelian randomization analysis identified genes pleiotropically associated with central corneal thickness. *BMC Genomics.* 2021;22:517.
23. Naderan M, Shoar S, Rezagholizadeh F, Zolfaghari M, Naderan M. Characteristics and associations of keratoconus patients. *Contact Lens Anterior Eye.* 2015;38:199–205.
24. Heydarian S, Hashemi H, Yekta A, et al. Heritability of corneal curvature and Pentacam Topometric indices: a population-based study. *Eye Contact Lens.* 2019;45:365–371.
25. Hashemi H, Yekta A, Heydarian S, et al. Heritability of pachymetric indices using Pentacam Scheimpflug imaging. *Br J Ophthalmol.* 2020;104:985–988.
26. Hosoda Y, Miyake M, Meguro A, et al. Keratoconus-susceptibility gene identification by corneal thickness genome-wide association study and artificial intelligence IBM Watson. *Commun Biol.* 2020;3:410.
27. Iglesias AI, Mishra A, Vitart V, et al. Cross-ancestry genome-wide association analysis of corneal thickness strengthens link between complex and Mendelian eye diseases. *Nat Commun.* 2018;9:1864.
28. Mahroo OA, Oomerjee M, Williams KM, O’Brart DP, Hammond CJ. High heritability of posterior corneal tomography, as measured by Scheimpflug imaging, in a twin study. *Invest Ophthalmol Vis Sci.* 2014;55:8359–8364.
29. Zheng Y, Ge J, Huang G, et al. Heritability of central corneal thickness in Chinese: the Guangzhou Twin Eye Study. *Invest Ophthalmol Vis Sci.* 2008;49:4303–4307.
30. Toh T, Liew SH, MacKinnon JR, et al. Central corneal thickness is highly heritable: the twin eye studies. *Invest Ophthalmol Vis Sci.* 2005;46:3718–3722.
31. Landers JA, Hewitt AW, Dimasi DP, et al. Heritability of central corneal thickness in nuclear families. *Invest Ophthalmol Vis Sci.* 2009;50:4087–4090.
32. Freeman EE, Roy-Gagnon MH, Descovich D, Masse H, Lesk MR. The heritability of glaucoma-related traits corneal hysteresis, central corneal thickness, intraocular pressure, and choroidal blood flow pulsatility. *PLoS One.* 2013;8:e55573.
33. Gao X, Nannini DR, Corrao K, et al. Genome-wide association study identifies WNT7B as a novel locus for central corneal thickness in Latinos. *Human Mol Genet.* 2016;25:5035–5045.
34. Lu Y, Vitart V, Burdon KP, et al. Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat Genet.* 2013;45:155–163.
35. Hao XD, Chen P, Chen ZL, Li SX, Wang Y. Evaluating the association between keratoconus and reported genetic loci in a Han Chinese population. *Ophthalmic Genet.* 2015;36:132–136.
36. Belin MW, Khachikian SS. An introduction to understanding elevation-based topography: how elevation data are displayed - a review. *Clin Exp Ophthalmol.* 2009;37:14–29.