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Iris colour in relation to myopia among Chinese school-aged children

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Abstract

Purpose: Understanding the association of iris colour and myopia may provide further insights into the role of the wavelength of lights in the pathophysiology of myopia. We aim to assess the association of iris colour and myopia in a school-based sample of Chinese students.
Methods: Two thousand three hundred and forty-six Year 7 students from 10 middle schools (93.5% response rate) aged 13–14 years in Mojiang, a small county located in Southwestern China, participated in the study. We obtained standardised slit lamp photographs and developed a grading system assessing iris colour (higher grade denoting a darker iris). Refractive error was measured after cycloplegia using an autorefractor by optometrists or trained technicians. An IOLMaster (www.zeiss.com) was used to measure ocular biometric parameters including axial length (AL).
Results: Of all the study participants, 693 (29.5%) were affected by myopia with the prevalence estimates being higher in girls (36.8%; 95% confidence interval [CI]: 34.0, 39.6) than in boys (22.8%; 95% CI: 20.4, 25.1) ($p < 0.001$). After adjusting for gender, height, parental history of myopia, time spent on computer, time spent watching TV, time spent outdoors, and time spent reading and writing, participants with a darker iris colour tended to have a higher prevalence of myopia, a more myopic refraction and a longer AL. Dose-response relationships were observed in all regression models (p for trend <0.05).
Conclusions: Darker iris colour was associated with more myopic refractive errors and longer ALs among Chinese school-aged children and this association was independent of other known myopia-related risk factors.

Introduction

Myopia is the most prevalent vision disorder among children and adolescents[1] and the prevalence is increasing rapidly throughout the world.[2–4] Myopia is considered to be driven by both genetic and environmental factors.[5] Despite genetic influence, multiple evidences have revealed that environmental factors play an essential role in myopia development.[6,7] Recent research regarding environmental risk factors of myopia emphasised on the role of light,[8] as time outdoors has been confirmed to be a major protective factor which could reduce the incidence of myopia in children.[9] Light intensity has been proposed to play a major role in the relationship between time outdoors and myopia development. An experimental animal study found that chicks exposed to high illuminances significantly slowed compensation for negative lenses compared with those under low illuminances.[10] However, the light intensity hypothesis has been contradicted by other investigators, who suggested that the spectral composition of lights, instead of the intensity, is the primary cause of the tendency for myopia associated with less time outdoors.[11] They proposed that the wavelength of the lights entering the eyeball is the major determinant for myopia.

Irides with different colours have different filtering rates for lights with different wavelengths passing through the iris.[12] For example, when light enters eyes with blue irides which contain very little melanin, the light with longer wavelengths penetrates the iris and is absorbed while light with shorter wavelengths is reflected back to the surface and scattered by the iris stroma. Iris colour has been proposed to be a risk factor for myopia.[13,14] To the best of our knowledge, only one study had assessed the relationship between iris colour and myopia, and non-significant findings were reported.[15] However, refractive status in this study was self-reported and may be subject to information biases, making the findings less reliable.

In this study, we aim to determine the association of iris colour and myopia in a school-based sample of Chinese students. The results may provide further insights into the role of the wavelength of lights in the pathophysiology of myopia.

Methods

Study population

The Mojiang Myopia Progression Study is a school-based cohort study aiming to longitudinally observe the onset and progression of myopia, as well as other major childhood ocular diseases in school-aged children in rural China. The baseline survey was conducted in 2016 and all the participants will be followed annually. Mojiang, a small county located in Southwestern China with a population of 0.36 million and an area of 5312 km^2, was chosen as the study site due to its relatively stable demographic structure and similar socioeconomic status to the average of rural China. In addition, residents in Mojiang had been living there for hundreds of years and there were few immigrants in this area. Thus, residents in Mojiang were considered to have similar genetic ancestries. The compulsory schooling system is well executed in Mojiang, with an enrollment rate of 99% for elementary and middle schools in 2014. Thus, school-based samples in Mojiang are highly representative of the local population.

All the Year 7 students from middle schools in Mojiang were invited to participate in this study. For the baseline survey, the student roster was obtained from each school's principal to ascertain the eligibility of the study participants, that is, students should have been living in Mojiang for at least 1 year and planned to live there for at least 4 years. A cell phone message was sent to the parents to explain the nature of the study and invite them to participate. For those who didn't agree to participate or didn't respond, telephone interviews were made available to let them better understand the nature of the study and the importance of their children's vision development. If the parents could not be reached by cell (mobile) phone message or by telephone, home visits were made. At the end of the study, a total of 2346 (93.5%) Year 7 students participated in the baseline survey.

Ethics committee approval was obtained from the Institutional Review Board of Kunming Medical University. We carried out the study according to the tenets of the Declaration of Helsinki involving human participants and the approved guidelines. Additionally, we obtained written informed consent from at least one parent or legal guardian of each participant.

Refractive error and ocular biometry measurement

Each participant's refractive status was measured before and after cycloplegia by optometrists or trained technicians using the RM-8000 Autorefractor (www.topcon.co.jp). For cycloplegia, each participant was first administered two drops of 1% cyclopentolate (www.alcon.com) after a five minute interval. Thirty minutes later, a third drop was administered if the pupillary light reflex was still present or the pupil size was <6.0 mm. The first five valid readings of autorefraction were used and averaged using vector methods to generate a single estimate of refractive error. All five readings were required to be within 0.50 dioptres (D) in both the spherical and cylinder components. Myopia was defined as spherical equivalent (S.E.) at three different levels, less than −0.50 D, −0.75 D or −1.00 D, for statistical analysis.

An IOLMaster (www.zeiss.com) was used to measure axial length (AL), anterior chamber depth (ACD) and corneal curvature (CC). Three repeated readings were obtained and averaged before cycloplegia.

Iris colour grading

Colourful iris photographs were only taken among Year 7 students as they could cooperate well with the examination. Iris colour grading protocol in this study was the same with that described in a previous study on Asians.[16,17] In brief, colour photographs of both eyes' irides were taken using the DC-3 slit lamp digital camera (www.topconmedical.com) in a dark room. Photographs were saved in JPEG format (RGB 3120 pixels * 4160 pixels) and viewed using the software Photoshop CS (www.adobe.com).

Two graders were masked to participants' demographic information and clinical diagnosis and independently graded the colour of all the iris photographs. A panel of

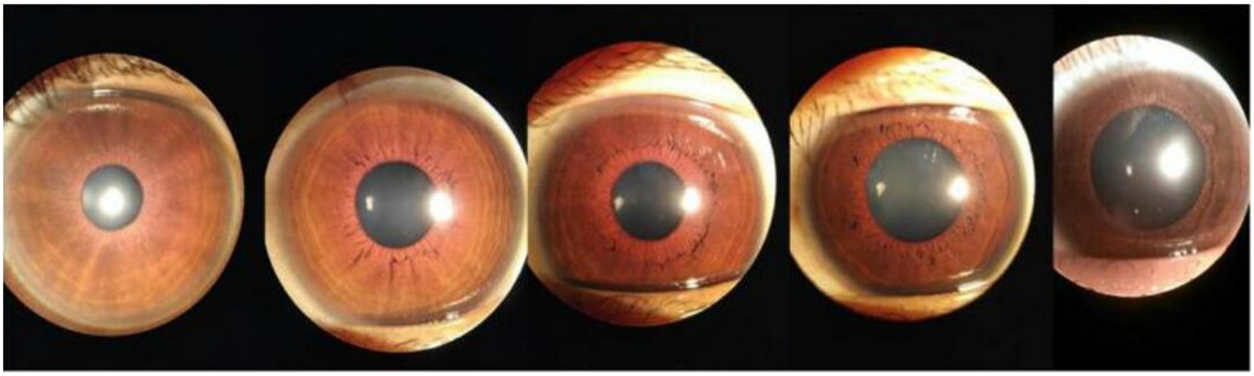

Figure 1. Reference photographs on different grades of iris colour. [Colour figure can be viewed at wileyonlinelibrary.com]

reference photographs best representing the variations observed in the study population was selected (*Figure 1*). By comparing with the reference panel, iris colour was graded from 1 to 5 based on the overall colour of the iris. Grade 1 denoted the lightest colour while Grade 5 denoted the darkest. The higher grade was assigned if a photo was considered to be between two consecutive grades. If there were inconsistencies on the observations between the two graders, the third grader made a judgment. The kappa index was 0.74 between the two graders, indicating that inter-grader agreement between the two graders was relatively good. In addition, one grader repeated the grading of 50 photographs after 2 weeks to assess the intra-grader agreement, which was 0.88 in this study.

Questionnaires

The questionnaires used in this study were similar to many previous myopia epidemiological studies on Chinese children.[18–21] The questionnaires were completed by the parents or legal guardians of the children. We collected detailed information regarding socioeconomic status, parental education, parental history of myopia, medical history, and lifestyle-related factors such as time spent on near work and on outdoor activities.

Statistical analysis

As the correlation of S.E.s ($r = 0.90$) and ALs ($r = 0.98$) between two eyes was high, and the results of analysis in both eyes were similar, only data for the right eye were presented in this paper. We compared the baseline characteristics of the participants with different iris colours, using the chi-square test, or analysis of variance, as appropriate. We performed the following pre-specified analyses to examine the association between iris colour and myopia. We calculated the odds ratio (OR) and 95% confidence interval (CI) associated with different grades of iris colour, with Grade 3 as the reference for three myopia-related outcomes: (1) the presence of myopia; (2) per D increase in S.E.s; and (3) per mm increase in ALs in multivariable regression models. For each outcome, we only adjusted for gender in the first model. In the second model, we additionally adjusted for covariates known to be associated with myopia, such as parental history of myopia, height, time spent on near work activities and time spent outdoors. We also performed subgroup analyses to test whether our results were consistent across categories of possible confounders. All statistical analyses were performed using the commercial statistical software package IBM SPSS (www.ibm.com).

Results

A total of 2346 Year 7 students from 10 middle schools who had undertaken iris photographs were included in the current analysis. The study cohort consisted of 1213 (51.7%) boys and 1133 (48.3%) girls with a mean age of 13.8 ± 0.8 years (mean ± standard deviation). Of all the study participants, 693 [29.5% (95% CI: 27.7, 31.4)] were affected by myopia (S.E. < -0.50) with the prevalence estimates being higher in girls (36.8%; 95% CI: 34.0, 39.6) than in boys (22.8%; 95% CI: 20.4, 25.1) ($p < 0.001$). *Figure 2* depicts the frequency distribution of different grades of iris colours in the study population. More than 70% of the study participants had an iris colour of Grade 3.

The trends of S.E.s and ALs associated with iris colour are shown in *Figure 3* and *Figure 4*. More myopic refractive error was found in children with darker iris colour. For example, the mean refraction was +0.40 D in children with iris colour of Grade 1 while it was −0.89 D in those with iris colour of Grade 5 ($p < 0.001$). Similarly, longer AL was found in children with darker iris colour (23.2 mm vs 23.9 mm; comparing Grade 1 with Grade 5; $p < 0.001$).

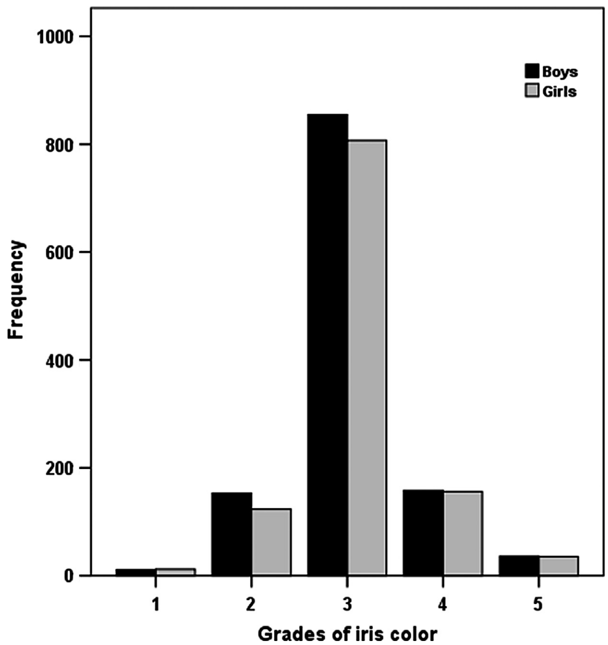

Figure 2. Frequency distribution of iris colour among Grade 7 school students in Mojiang Myopia Progression Study.

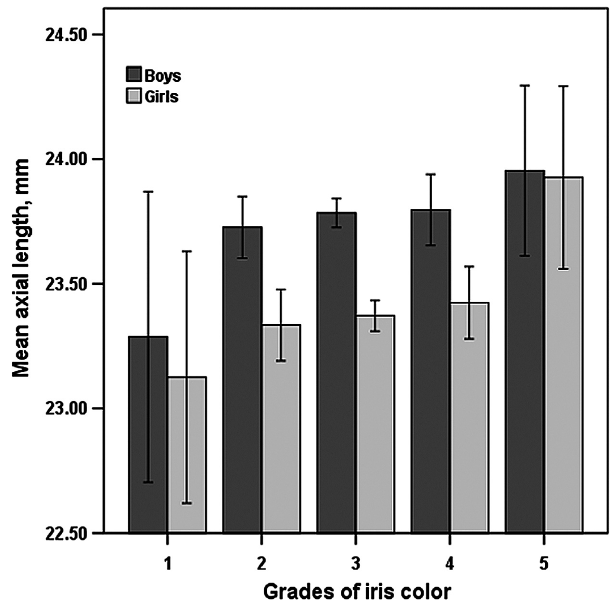

Figure 4. Mean axial length by different grades of iris colour; error bars correspond to 95% confidence intervals.

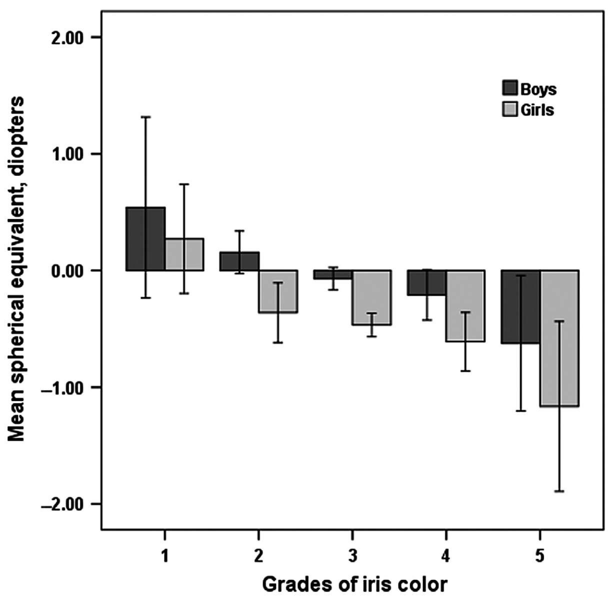

Figure 3. Mean spherical equivalent by different grades of iris colour; error bars correspond to 95% confidence intervals.

Table 1 shows the distributions of myopia-related variables among participants with different grades of iris colour. There were no significant differences in terms of gender, height, and parental history of myopia, time spent on computer use, time spent outdoors, and time spent on reading and writing, among participants with different grades of iris colour (all $p > 0.05$). Although there was a statistically significant difference in time spent watching TV per day ($p = 0.03$), the magnitude of difference was small and the trend was unclear.

Multiple logistic/linear regression analyses were performed to determine the associations of iris colour with the presence of myopia, S.E. and AL after adjusting for gender and other myopia-related risk factors. Myopia was defined using three criteria which were commonly used in epidemiological studies. In the first model, we only adjusted for gender and found that darker iris colour was associated with an increasing trend of myopia prevalence (p for trend <0.05 in all three models). After additionally adjusting for other known myopia-related covariates including height, parental history of myopia, time on computer use, time on watching TV, time outdoors, and time on reading and writing, the trend was similar and did not change significantly (*Table 2*). Besides, similar trends were observed in the analyses of S.E. and AL. In multiple linear regression models, more myopic refractive errors and longer ALs were associated with darker iris colour. For example, compared with students with iris colour of Grade 1 (the lightest), children with Grade 5 (the darkest) had a more myopic refraction of 1.14 D (95% CI: 0.46, 1.82) in S.E.s and a 0.61 mm (95% CI: 0.20, 1.02) longer in ALs after controlling for other myopia-related covariates (*Table 3*). Interaction terms including iris colour × sex and iris colour × time outdoors were not statistically significant (p for interaction >0.10).

Table 1. Distribution of myopia-related variables among children with different grades of iris colour

Variables	Grade 1 (n = 23)	Grade 2 (n = 276)	Grade 3 (n = 1662)	Grade 4 (n = 314)	Grade 5 (n = 71)	p*
Girls (%)	12 (52.2)	123 (44.6)	807 (48.6)	156 (49.7)	35 (49.3)	0.74
Height (cm)	149.9 (7.5)	152.8 (8.2)	153.0 (7.6)	152.2 (7.9)	154.2 (8.1)	0.07
Having myopic father (%)	0 (0.0)	9 (3.3)	58 (3.5)	8 (2.6)	4 (5.8)	0.60
Having myopic mother (%)	0 (0.0)	11 (4.1)	50 (3.0)	6 (1.9)	1 (1.4)	0.45
Time spent on computer per day (h)	0.4 (0.7)	0.6 (0.9)	0.7 (0.8)	0.7 (0.9)	0.9 (0.8)	0.05
Time spent on watching TV per day (h)	1.3 (0.8)	1.4 (0.9)	1.4 (0.9)	1.5 (0.9)	1.5 (0.9)	0.03
Time spent on reading and writing after school (h)	0.8 (0.6)	0.9 (0.7)	1.0 (0.7)	0.9 (0.7)	0.8 (0.6)	0.23
Time spent outdoors per day (h)	0.9 (0.3)	0.9 (0.3)	0.9 (0.3)	0.9 (0.3)	0.8 (0.3)	0.20

Data presented are means (S.D.s) or n (%), as appropriate for variable.
*p value, comparing the differences among participants with different grades of iris colour, based on chi-square test or analysis of variance (ANOVA) test, as appropriate.

Discussion

In this study, we observed an association of iris colour with refractive errors and ALs among Year 7 school-aged students in rural China. After controlling for the effect of gender and known myopia-related factors, children with darker iris colour tended to have more myopic refractive errors and longer ALs and dose-response relationships were observed. Our study provided novel information on the predictors for myopia in schoolchildren.

Our study hypothesis was based on the fact that darker iris colour is associated with longer wavelengths of light entering the eye. The colour of the iris is mainly determined by the amount and type of melanin in the iris pigment cells. There are two different types of iris pigment cells: the iris pigment epithelial cells (IPE) and iridal melanocytes (IM). The IPE contains a high amount of melanin as compared with that in the IM. However, based on our previous studies (DNH) and other researchers' work, there were no differences in the quantity of melanin in IPE from eyes with different coloured irides or from different races.[22–25] IM are less pigmented than that of IPE, but the quantity of melanin in IM varies among eyes of different iris colours.[22–24] Therefore, the variation of iris colour in normal subjects is mainly determined by the quantity of melanin in the IM.[26–28] The type of melanin also plays a role in the determination of iris colour. Two different types of melanin are present in the human iris, that is, eumelanin (brown to black in colour) and pheomelanin (yellow to red in colour).[22–24] IPE contains essentially eumelanin and the amount of eumelanin is relatively consistent in different coloured irides.[22] IM contains both eumelanin and pheomelanin and the ratio of eumelanin vs pheomelanin varies in different coloured irides, so that the variation of iris colour is also determined by the type of melanin in the IM.[23] The main difference in photoscreen effects between eumelanin and pheomelanin is that eumelanin absorbs a much higher quantity of light than pheomelanin. In the absorbance of different wavelengths of light, there are also differences between eumelanin and pheomelanin, using the absorbance at 650 nm (A650) and 500 nm (A500) as a parameter. The A650/A500 ratio in eumelanin is higher than that of pheomelanin, indicating that EM absorbs long wavelengths of light more than PM.[29] However, it is also likely that the absorbance of quantity of light may differ through irides with different colours, especially in a limited range of colour such as in Chinese subjects (from brown to dark brown colour).

It is well-known that iris colour was associated with genes, and polymorphisms in the genes, including TYR, TYRP1, OCA2-HERC2, SLC45A2, ASIP, SILV, IRF4, SLC24A4, DSCR9, LYST, and NPLOC4 have been reported to be associated with iris colour.[30,31] Until now, these genes have not been found to be related to refractive errors, so it is less likely that iris colour and myopia are connected through genetic pathways. Our study suggested that wavelengths of light entering the eyeball might have impacts on eye growth or refractive development leading to myopia among schoolchildren, but the biological mechanism underlying the association remains unclear. Longitudinal chromatic aberration, caused by the dispersion of the ocular media, makes a single object form multiple chromatic images within the eye, located at different distances from the retina. For an emmetropic human eye looking at a distant object, the focal image for each wavelength is usually formed at a different location, with short wavelengths (blue light) focussed in front of the retina, long wavelengths (red light) behind the retina (this may stimulate the development of myopia), and middle wavelengths (green or yellow light) focussed at the retina. In the human eye, the image focal planes for the whole visual spectrum vary over a range of approximately two diopters.[32] It has been assumed that

Table 2. Associations of grades of iris colour with myopia

	Myopia < −0.5 D				Myopia < −0.75 D				Myopia < −1.0 D			
	Gender-adjusted		Multivariate-adjusted*		Gender-adjusted		Multivariate-adjusted*		Gender-adjusted		Multivariate-adjusted*	
Grades of iris colour	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
1	0.49 (0.16, 1.44)	0.19	0.52 (0.17, 1.56)	0.24	0.43 (0.13, 1.45)	0.17	0.46 (0.13, 1.57)	0.21	0.32 (0.07, 1.37)	0.13	0.35 (0.08, 1.50)	0.16
2	0.88 (0.66, 1.18)	0.39	0.89 (0.66, 1.19)	0.42	0.87 (0.64, 1.18)	0.38	0.86 (0.63, 1.18)	0.36	0.87 (0.63, 1.20)	0.39	0.85 (0.61, 1.19)	0.34
3	Reference		Reference		Reference		Reference		Reference		Reference	
4	1.10 (0.84, 1.43)	0.50	1.11 (0.84, 1.44)	0.49	1.18 (0.90, 1.54)	0.24	1.17 (0.89, 1.55)	0.26	1.11 (0.83, 1.47)	0.49	1.11 (0.83, 1.48)	0.49
5	1.48 (0.90, 2.43)	0.13	1.35 (0.81, 2.26)	0.25	1.71 (1.04, 2.82)	0.04	1.70 (1.00, 2.85)	0.05	1.91 (1.15, 3.17)	0.01	1.72 (1.01, 2.91)	0.04
p for trend	0.03		0.06		0.005		0.01		0.005		0.01	

*Multivariate models adjusted for gender, parental myopia, time spent on watching TV, time spent on reading and writing, time spent on computers and time spent outdoors.
D, Dioptre; OR, odds ratios; CI, confidence interval.

Table 3. Associations of grades of iris colour with spherical equivalent and axial length

	Spherical equivalent (D)				Axial length (mm)			
	Gender-adjusted		Multivariate-adjusted*		Gender-adjusted		Multivariate-adjusted*	
Grades of iris colour	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
1	0.68 (0.09, 1.27)	0.03	0.61 (0.02, 1.20)	0.04	−0.37 (−0.73, −0.01)	0.04	−0.33 (−0.69, 0.03)	0.08
2	0.17 (−0.01, 0.36)	0.07	0.18 (−0.10, 0.37)	0.06	−0.05 (−0.16, 0.06)	0.40	−0.05 (−0.17, 0.06)	0.38
3	Reference		Reference		Reference		Reference	
4	−0.14 (−0.31, 0.03)	0.11	−0.15 (−0.32, 0.03)	0.11	0.03 (−0.07, 0.14)	0.55	0.02 (−0.09, 0.12)	0.78
5	−0.63 (−0.96, −0.28)	<0.001	−0.55 (−0.90, −0.20)	0.002	0.36 (0.15, 0.57)	0.001	0.30 (0.09, 0.51)	0.006
p for trend	<0.001		<0.001		0.001		0.008	

*Multivariate models adjusted for gender, parental myopia, time spent on watching TV, time spent on reading and writing, time spent on computers and time spent outdoors.
D, Dioptre; β, regression coefficient; CI, confidence interval.

light with various wavelengths may have different effects on the ocular refraction status. However, experimental animal studies examining the chromatic effects on the emmetropisation processes obtained conflicting results. To examine the chromatic hypothesis, one study was performed to assess the relationship between colour vision and incident myopia in Chinese students. Colour vision is mediated by three types of cones and two chromatic opponent mechanisms. Absence of any cone type causes colour vision deficiency. The most common colour vision deficiency is red-green colour blindness which has two subtypes: deutan and protan. The deutan subtype is characterised by missing or defective M-cones, with a shift in peak sensitivity towards that of L-cones and more sensitive to longer wavelengths. Eyes with higher luminance sensitivity in the L-cone would be more strongly stimulated by images behind the retina, and respond with greater accommodation and an axial elongation to maximise L-cone contrast. The protan subtype is characterised by missing or defective L-cones and is assumed to have a tendency to be hyperopia. Students with colour vision deficiency had a lower incidence of myopia than the students with normal colour vision.[32] The similarity of the refractive data for the two different colour vision deficiency groups seems to challenge the chromatic hypothesis and suggests that these results cannot be explained by a simple model. Thus, further clarification in this area is needed.

An alternative hypothesis regarding the observations in this study needs to be considered, namely that the degree of myopia might be associated with incomplete cycloplegia in children with darker iris colour. Melanin is a polyanion and has an affinity for substances with cationic properties (e.g., metals and amines), such as cycloplegic agents (muscarinic antagonists).[33] Therefore, achieving complete cycloplegia in children with darker irides may be more difficult than in those with light coloured irides, as cycloplegic agents could be conjugated to melanin.[34] However, this hypothesis is less likely to be possible. In this study, we found that both S.E. and AL were associated with iris colour (*Table 3*). The measurements of S.E. could be affected by the effectiveness of the cycloplegia, but the measurements of AL with the IOLMaster should not be. Therefore, the relationship between iris colour and AL would support the initial hypothesis that light filtered by the iris pigments enters the eye and changes the balance of wavelengths that impinge on the retina, leading to the change of the rates of eye growth.

In this study, we noted that height was close to statistical significance in association with iris colour ($p = 0.07$, *Table 1*). However, including height as a covariate in multivariate analysis did not significantly alter the association between iris colour and refractive error, indicating that height may not be a confounder. Similarly, the association between iris colour and refractive error was not affected if age was included as a covariate in multivariate analysis.

Although our study is the first epidemiological study assessing the potential association between iris colour and myopia in school-aged children, it has a few limitations. First, the grading protocol for iris colour was subjective in nature and therefore was subject to measurement bias. Nevertheless, we have assessed the intra-observer agreement of the two grades and it was relatively good, with a kappa index of 0.74. In addition, the graders were masked to the subjects' clinical characteristics while performing the grading. We acknowledged that a more objective method for quantifying iris colour may help to achieve more precise and reliable measurements. Furthermore, our analysis was based on cross-sectional data and causal relationship cannot be determined. However, it is unlikely that the change of refractive status predates the formation of iris colour. It is also unlikely that the diagnosis of refractive errors may affect iris colour grading. Thus, despite the cross-sectional design, the temporal sequence between the exposure and outcome variables is clear in this study. In the future, we will follow up with these children and look into the potential relationship between iris colour and incidence or progression of myopia. Last but not least, our study participants were all Chinese, and had the same variations in iris colour (light brown to dark brown). Whether these findings could be directly extrapolated to other ethnic groups who have a larger variation in iris colour remains unclear.

In conclusion, our study suggested that iris colour as a risk factor might contribute to the development of myopia, indicating that wavelengths of light entering the eyes may influence eye growth and refractive development among school-aged children.

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Disclosure

The authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

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