BMC Ophthalmology

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Correlation of contrast sensitivity at low spatial frequencies with myopic shift in Chinese children

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Abstract

Purpose To investigate the correlation of contrast sensitivity function (CSF) with myopic shift in Chinese children.

Methods This prospective case-series study included 62 eyes (31 children) who visited the Eye and ENT Hospital of Fudan University in January 2022 and were followed up for 6 months. Routine ophthalmic examinations and quantitative CSF (qCSF) tests without refractive correction were performed. Differences in CSF parameters, including the area under the log CSF (AULCSF), CSF acuity, and contrast sensitivity (CS) at 1.0–18.0 cpd, were compared between two groups stratified according to the myopic shift based on mydriatic spherical equivalent (<-0.50 D or ≥-0.50 D) during follow-up.

Results The myopia progressed by 0.13 ± 0.24 and 1.18 ± 0.75 D in the stabilized (28 eyes) and advanced (34 eyes) groups, respectively. Compared with the advanced group, the stabilized group showed significantly lower baseline qCSF test results for CSF acuity and CS at 1.0 and 1.5 cpd. The qCSF readings for CSF acuity and CS at 1.0, 1.5, and 3.0 cpd increased significantly during the 6-month follow-up in the stabilized group, while these values showed non-significant decreases in the advanced group. CS at 3.0 cpd was significantly correlated with myopic shift. Compared with the advanced group, participants in the stabilized group with higher myopia showed relatively significantly lower CS (baseline CSF acuity and CS at 1.0, 1.5, and 3.0 cpd).

Conclusions Children with relatively slower myopic shift showed lower contrast sensitivity at low spatial frequencies, which might be an effective factor in myopia control.

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Key messages

What was Known:

- Contrast sensitivity assesses visual function more comprehensively than visual acuity testing.
- The quantitative contrast sensitivity (qCSF) test is a faster test with good accuracy and reliability compared with conventional contrast sensitivity test.

What this paper adds:

- Children with relatively slower myopic shift showed lower contrast sensitivity at low spatial frequencies.

How this study might affect research, practice or policy:

- Lower contrast sensitivity at low spatial frequencies may play a protective role in myopic shift. QCSF screening and contrast sensitivity interventions for children with relatively high CS at low spatial frequencies may contribute to accurate myopia prevention and control.

Keywords Myopia control, Quantitative contrast sensitivity function, Spatial frequency, Children

Introduction

Myopia is an increasingly serious public health problem that causes significant visual impairment in individuals and populations. The global myopia population is estimated to reach 4758 million by 2050, including 938 million patients with high myopia [1]. Southeast Asia and China have the highest prevalence rate of myopia [2]. In addition to the inconvenience in performing daily activities caused by corrective spectacles, myopia-related complications such as retinal detachment, choroidal neovascularization, and macular hemorrhage may result in an irreversible loss of vision [3, 4]. The risk of lifetime maculopathy is reduced by 40% for every 1 diopter (D) decrease in myopia [5]. Delaying myopia occurrence and controlling myopia progression are key for reducing the global burden [6]. Increasing outdoor activities, optical management choices, and low-concentration atropine are proven methods to control myopia progression and axial length growth [7].

Contrast sensitivity (CS) refers to the contrast required to separate objects of a specific size from their background. Contrast sensitivity function (CSF) is usually used to express the relationship between contrast threshold and spatial frequency, and reflects the ability to distinguish objects in daily life. CS is an important feature of visual function and is affected by the optical conditions in the retina or visual pathway and neural processing [8]. While age is the major factor associated with CSF in adults with spectacles-aided refraction correction [9, 10], it has no or weak effects on CSF in children [11]. We previously demonstrated that refraction sphere and spherical equivalent are the main factors in children, and observed a ladder-like downward trend in quantitative CSF (qCSF) test results with increasing degree of myopia in children without refractive correction [12].

The contrast hypothesis suggests that the accommodative eye has less vergence-related blur during indoor activities and that the higher contrast sensitivity signal produced may be related to myopia progression [13]. Diffusion optics technology (DOT) lenses designed by Sight Glass Vision based on this hypothesis effectively controlled myopia progression during a one-year follow-up [14]. The present prospective study further explored the correlation between contrast sensitivity and myopic shift in children for accurate myopia prevention and control in the context of contrast sensitivity.

Methods

This prospective case-series study included children who visited the Eye and ENT Hospital of Fudan University in January 2022. The study complied with the principles of the Declaration of Helsinki and was reviewed by the Ethics Committee of the Eye and ENT Hospital of Fudan University. Informed consent was obtained from all the participants and their legal guardians.

The inclusion criteria were children in primary and junior high school (6–14 years of age); no use of soft contact lenses for >2 weeks or rigid gas permeable contact lenses for >4 weeks. The exclusion criteria were a history of ocular trauma or ophthalmic surgery; strabismus, amblyopia, keratopathy, cataracts, and other ophthalmic diseases; systemic diseases such as connective tissue disease; and severe psychological or mental illnesses.

This study enrolled a total of 31 participants (62 eyes). Their demographic data are shown in Table 1. Figure 1[A] shows their age and equivalent sphere (SE) distributions.

Examinations

The mean corneal curvature, axial length (AL), anterior chamber depth (ACD), lens thickness (CT), central corneal thickness (CCT), and white to white (WTW) were measured using a Humphrey IOL Master700 instrument (Carl Zeiss Meditec, Germany).

Compound tropicamide eye drops were used for ciliary paralysis (three times and 10 every minute for each) and phoropter was performed 30 min after the last administration. The manifest refraction and corrected visual acuity were obtained using optometry (RT-5100, Nidek Technologies, Japan).

Table 1 Patient demographics: baseline and 6-month follow-up, analyzed by generalized Linear Model

Characteristic	Baseline	6-mon follow-up	*p*
	Mean ± SD	Mean ± SD	
Age(years)	9.68 ± 2.01		
Gender(male/female)	18/13		
Axial length(mm)	**24.34 ± 1.05**	**24.65 ± 1.09**	**< 0.001**
RS(D)	-1.44 ± 2.06	-2.13 ± 2.15	**< 0.001**
RC(D)	-0.51 ± 0.6	-0.56 ± 0.57	0.517
SE(D)	**-1.69 ± 2.21**	**-2.42 ± 2.2**	**< 0.001**
CDVA (LogMAR)	0 ± 0.02	0 ± 0.02	0.783
K-mean (D)	43.17 ± 1.45	43.26 ± 1.47	0.830
AULCSF	0.35 ± 0.36	0.35 ± 0.35	0.741
CSF Acuity	7.08 ± 6.39	7.32 ± 6.53	0.675
CS (1.0 cpd)	0.75 ± 0.42	0.78 ± 0.38	0.503
CS (1.5 cpd)	0.7 ± 0.43	0.71 ± 0.4	0.664
CS (3.0 cpd)	0.48 ± 0.48	0.47 ± 0.46	0.786
CS (6.0 cpd)	0.28 ± 0.4	0.24 ± 0.39	0.317
CS (12.0 cpd)	0.08 ± 0.2	0.09 ± 0.19	0.468
CS (18.0 cpd)	0.02 ± 0.06	0.02 ± 0.06	0.749

RS: Refraction Sphere, RC: Refraction Cylinder, SE: Spherical equivalent, CDVA: Corrected Distance Visual Acuity

Values with statistical significance ($p < 0.05$) are shown in bold

qCSF tests

The qCSF test tested under daily refractive correction method of the enrolled children was more representative. The number of children wearing glasses was comparable to that without refractive correction in each group. Consequently, the qCSF tests were performed with ciliary paralysis and without refraction correction. The digits were displayed on an NEC P403 monitor (Gension & Waltai Digital Video System Co., Ltd. China) with a size of 116.84 × 77.89 cm, a resolution of 1920 × 1080 pixels, a maximum brightness of 700 cd/m^2, a standard brightness of 550 cd/m^2, and a contrast of 4000:1. The test stimuli were 10 digits with spatial frequencies ranging from 1.4 to 36.2 cpd. The participants viewed the stimuli horizontally from 3 m away from the display using one eye with the contralateral eye covered. The other eye was then separately tested similarly. The participants were asked to read out the three stimulus numbers on the screen, which the examiner immediately recorded on a notepad as: "no answer", "wrong answer", or "correct answer". The program automatically progressed to the next stimuli until the end of the test. The test results included area under the line of contrast sensitivity function (AULCSF), CSF acuity (cutoff spatial frequency), and contrast sensitivity values (log units) of spatial frequencies of 1.0, 1.5, 3.0, 6.0, 12.0, and 18.0 cpd. The specific testing methods and principles were performed as described previously [10, 12].

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). The data are expressed as means ± standard deviation and the Shapiro–Wilk test was used to evaluate data normality. The participants were categorized according to the degree of myopic shift (>-0.50 D [32 eyes], The advanced group; or <-0.50 D [28 eyes], the stabilized group). Spearman correlation analysis was performed to assess the correlation between parameters. A generalized linear model was used for multi-level correlation and significance analysis to account for the correlation between fellow eyes and other correlated parameters. $P < 0.05$ indicated statistical significance.

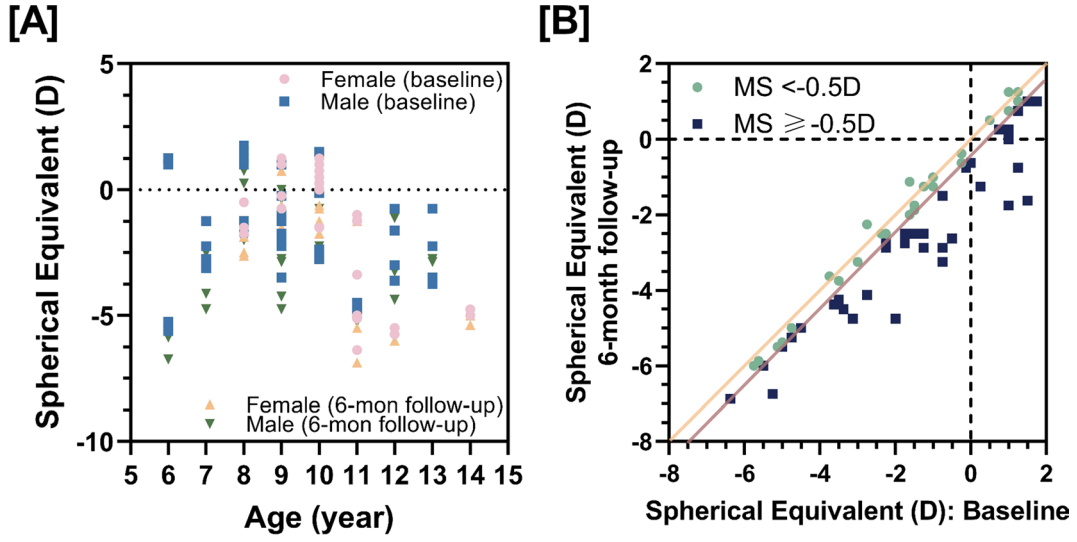

Fig. 1 Patient distributions and myopic shift during follow-up [**A**] Distributions of patient age and spherical equivalents [**B**] Myopic shift (MS) in spherical equivalents: 6-month follow-up vs. baseline

Table 2 Clinical and biological parameters stratified with myopic shift (< 0.5D or ≥ 0.5D): baseline and 6-month follow-up, analyzed by generalized Linear Model

Characteristic	Myopic shift <-0.5D (*N* = 27)		*p*	Myopic shift ≥-0.5D (*N* = 35)		*p*
	Baseline	6-month follow-up		Baseline	6-month follow-up	
RS(D)	-1.65 ± 1.96	-1.75 ± 2.1	0.156	**-1.28 ± 2.14**	**-2.43 ± 2.16**	**< 0.001**
RC(D)	**-0.53 ± 0.59**	-0.58 ± 0.44	0.621	**-0.49 ± 0.61***	-0.55 ± 0.65	0.684
SE(D)	**-1.91 ± 2.11**	**-2.04 ± 2.18**	**0.010**	**-1.53 ± 2.31**	**-2.7 ± 2.2**	**< 0.001**
CDVA (LogMAR)	**0 ± 0**	0.01 ± 0.02	0.188	**0.01 ± 0.02***	0 ± 0.01	0.301
AL (mm)	**24.48 ± 0.98**	**24.74 ± 0.93**	**< 0.001**	**24.23 ± 1.1**	**24.59 ± 1.21**	**0.001**
K-mean (D)	43.26 ± 1.33	42.92 ± 1.62	0.223	43.1 ± 1.55	43.52 ± 1.31	0.657
ACD (mm)	3.92 ± 0.15	3.89 ± 0.21	0.466	3.85 ± 0.16	3.83 ± 0.18	0.906
LT (mm)	3.37 ± 0.09	3.36 ± 0.11	0.799	3.37 ± 0.12	3.33 ± 0.13	1.000
CCT (μm)	543.13 ± 28.54	552.67 ± 40.23	0.731	558.38 ± 22.85	555.46 ± 28.5	0.573
WTW (mm)	12.71 ± 0.46	12.35 ± 0.34	0.178	12.39 ± 0.38	12.21 ± 0.32	0.280

RS: Refraction Sphere, RC: Refraction Cylinder, SE: Spherical equivalent, CDVA: Corrected Distance Visual Acuity, AL: Axial Length, ACD, Anterior Chamber Depth, LT: Lens Thickness, CCT: Central Corneal Thickness, WTW, White to White

*, Myopic shift <-0.5D vs. that ≥-0.5D of the baseline, $p < 0.05$. (Generalized Estimating Equation analysis)

Values with statistical significance ($p < 0.05$) are shown in bold

Table 3 QCSF (quick contrast sensitivity function) results stratified with myopic shift (< 0.5D or ≥ 0.5D): baseline and 6-month follow-up, analyzed by generalized Linear Model

Characteristic	Baseline		*p*	6-month follow-up		*p*
	MS<-0.5D (*N* = 27)	MS≥-0.5D (*N* = 35)		MS<-0.5D (*N* = 27)	MS≥-0.5D (*N* = 35)	
AULCSF	0.31 ± 0.37	0.39 ± 0.35	0.096	0.37 ± 0.33	0.32 ± 0.37	0.470
CSF Acuity	**6.53 ± 6.9**	**7.51 ± 6.04**	**0.036**	**7.97 ± 6.78***	6.83 ± 6.39	0.220
CS (1.0 cpd)	**0.71 ± 0.43**	**0.79 ± 0.42**	**0.027**	**0.87 ± 0.34***	**0.72 ± 0.4**	**0.003**
CS (1.5 cpd)	**0.64 ± 0.43**	**0.74 ± 0.44**	**0.009**	**0.79 ± 0.35***	**0.66 ± 0.44**	**0.036**
CS (3.0 cpd)	**0.40 ± 0.47**	**0.55 ± 0.48**	**0.018**	**0.52 ± 0.40***	0.43 ± 0.49	0.205
CS (6.0 cpd)	0.23 ± 0.42	0.31 ± 0.38	0.567	0.24 ± 0.38	0.24 ± 0.41	0.835
CS (12.0 cpd)	0.09 ± 0.22	0.07 ± 0.18	0.627	0.10 ± 0.22	0.08 ± 0.17	0.968
CS (18.0 cpd)	0.03 ± 0.07	0.02 ± 0.06	0.746	0.03 ± 0.08	0.01 ± 0.05	0.273

AULCSF: Area Under Log CSF, CS: Contrast Sensitivity, MS: Myopic shift

*, MS <-0.5D, baseline vs. 6-month follow-up, $p < 0.05$

Values with statistical significance ($p < 0.05$) are shown in bold

Results

All participants successfully completed the 6-month follow-up with < 5% missing data.

Patient characteristics

Table 1 shows a -0.72 ± 0.78 D myopic shift and 0.31 ± 0.48 mm axial length growth, with no significant difference in corneal curvature and contrast sensitivity during the 6-month follow-up. Figure 1 shows the distribution of SE with age (Fig. 1[A]) and the changes in SE before and after the follow-up (Fig. 1[A][B]).

Group analysis

Table 2 shows myopic shift and axial length growth in the two groups during the 6-month follow-up. The myopic shift in the stabilized group was − 0.13 ± 0.24 D, significantly less than that in the advanced group (-1.18 ± 0.75 D, $p < 0.001$). Corneal curvature, anterior chamber depth, or other anterior biometric parameters did not differ significantly between the groups during the follow-up.

Comparison of baseline contrast sensitivity readings, the stabilized group showed significantly lower readings for CSF acuity and low to moderate spatial frequencies (1.0, 1.5, and 3.0 cpd) in the stabilized group compared with those in the advanced group (Table 3). During the 6-month follow-up, the contrast sensitivity in the advanced group did not change significantly (-0.69 ± 4.89 log units of CSF acuity, -0.07 ± 0.33 at 1.0 cpd, -0.08 ± 0.32 at1.5 cpd, and − 0.11 ± 0.33 at 3.0 cpd); however, the corresponding readings increased significantly in the stabilized group (1.44 ± 3.39 log units of CSF acuity, $p = 0.040$; 0.16 ± 0.34 at 1.0 cpd, $p = 0.026$; 0.15 ± 0.27 at 1.5c pd, $p = 0.010$; and 0.12 ± 0.24 at 3.0 cpd, $p = 0.018$) (Table 3 and Fig. 2).

Correlation analysis

The two groups demonstrated a significant linear correlation between baseline SE and AL with baseline CSF acuity, and CS at 1.0 cpd, 1.5 cpd, and 3.0 cpd (all $p < 0.001$). Participants showed relatively significantly lower CS for

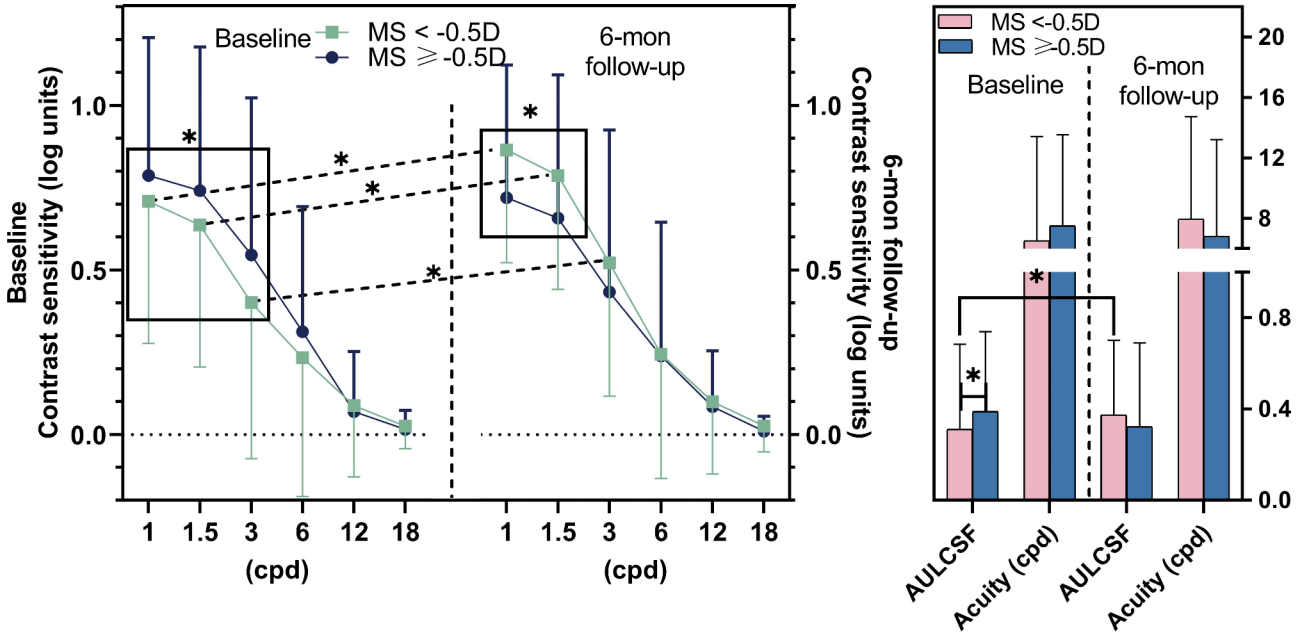

Fig. 2 Average contrast sensitivity function (qCSF) test values of the baseline and 6-month follow-up in groups stratified with myopic shift (MS, <-0.5D or ≥-0.5D). Left: Contrast sensitivity (log units) at different spatial frequencies (cpd). Right: average area under the log CSF (AULCSF) and CSF acuity (cpd). *, $p < 0.05$

the corresponding parameters (bigger positive slope of the regression line) in the stabilized group compared with the advanced group (Fig. 3). The intersection points of regression lines were (-1.51, 0.91) for CSF Acuity-SE (Fig. 3[A]), (-0.67, 0.91) for CS at 1.0 cpd-SE (Fig. 3[B]), (1.02, 1.13) for CS at 1.5 cpd-SE (Fig. 3[C]), and (2.41, 1.20) for CS at 3.0 cpd-SE (Fig. 3[D]).

Discussion

This prospective study first observed qCSF results under different degrees of myopic shift. Our previous findings suggested that RS and SE are the main factors affecting qCSF in children without refractive correction [12]. The findings of the present study confirmed these results and additionally revealed that children with stable refraction had lower CSF acuity and contrast sensitivity at 1.0–3.0 cpd during the 6-month follow-up. These results suggest that a lower contrast sensitivity may be correlated to a relatively slower myopic shift. Thus, contrast intervention may be practicable in children with controlled myopia. Meanwhile, the change rate of RS or SE with contrast sensitivity differed between the two groups. The different intersection positions of fitting lines of the two groups in Fig. 3 may indicate the starting point of appropriate intervention on contrast. At the right of the intersection point, the hyperopic children with myopic shift > 0.50 D in the future had lower contrast sensitivity, which may be correlated to the accelerated emmetropization in hyperopia state under the close-range work background.

The follow-up period in the present study coincided with the coronavirus 2019 (COVID-19) home quarantine in Shanghai in 2022. The long-term indoor environment and the difficulty of prescribing low-concentration atropine magnified the proportion of contrast sensitivity factors in myopic shift in the enrolled participants, which led to more prominent contrast sensitivity results in this study. Previous studies reported stronger contrast adaptation in participants with myopia compared with those with emmetropia, with the largest difference (0.106 log units) observed for 4.4 cpd [15]. Although the myopic shift was significantly greater in the advanced group in the present study, the decrease in contrast sensitivity was not significant, contrary to previous reports [12]. Meanwhile, the stabilized group showed that the contrast sensitivity at low spatial frequency was significantly higher than that of the baseline readings. These might be explained by the long-time close-range work which changed the emmetropization process in participants with contrast adaptation [13]. It remains further researches whether the contrast-adaptation-induced relatively high contrast sensitivity would lead to an acceleration in the future myopic shift. The decrease in retinal activity caused by contrast adaptation led to changes in neurotransmitter and neuromodulator release, changes in the physiological function of the sclera, and eventually increased axial length and myopia progression [16, 17]. Therefore, the relatively higher contrast sensitivity of the two groups during the 6-month follow-up may be related to the contrast sensitivity adaptation caused by

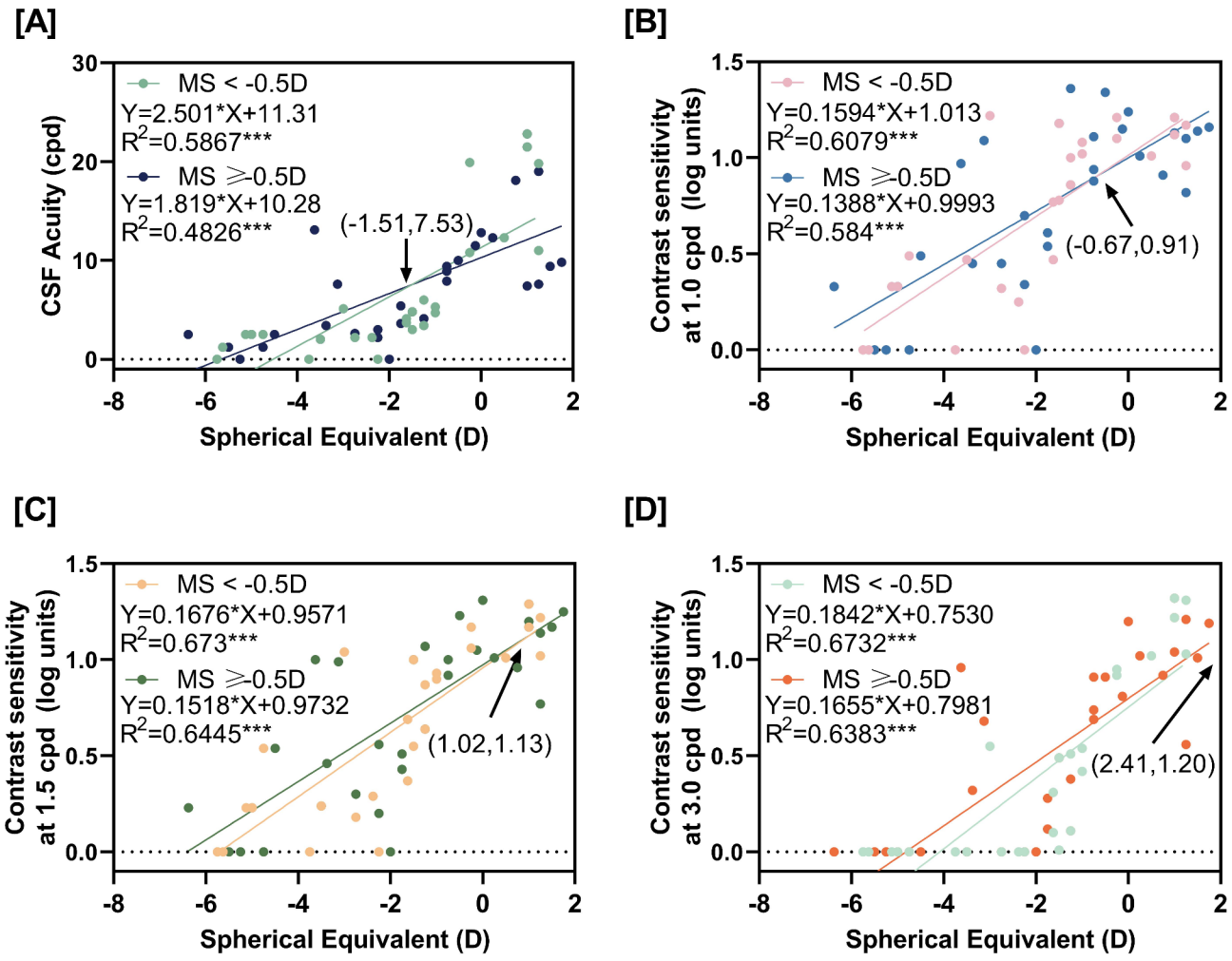

Fig. 3 Distribution and the regression line of baseline spherical equivalent and contrast sensitivity function (CSF) acuity [**A**], and contrast sensitivity at 1.0 cpd [**B**], 1.5 cpd [**C**], and 3.0 cpd [**D**] in groups stratified with myopic shift (MS, <-0.5D or ≥-0.5D). ***, $p < 0.001$ Coordination with arrow mark: intersection points of the regression lines of the stabilized (MS <-0.5D) and advanced (MS ≥-0.5D) groups

long-term indoor activities. Thus, children are encouraged to intermittently stop close-range work to reduce contrast adaptation.

From the perspective of visual pathways, the visual system is divided into ON and OFF pathways. The circular area of retinal ganglion cells is divided into the central ON structure and the surrounding OFF structure [18]. The ON and OFF pathways are correlated to axial growth and emmetropization [19]. In the natural environment, an evenly illuminated environment will not stimulate ganglion cells, and the ON pathway is in balance with the OFF pathway, which avoids unnecessary transmission of visual information to the visual cortex [20]. The ON pathway helps to identify the target in the dark, while blocking the ON pathway significantly reduces the contrast sensitivity, blocking the OFF pathway has the opposite effect [21]. In animal experiments, blocking the ON pathway through gene knockout, optics, or drugs (D, L-2-amino-4-phosphonobutyric acid [APB] and L-α-aminoadipic acid [LAA]) leads to hyperopia or lower myopia, while blocking the OFF pathway with D-α-aminoadipicacid (DAA) [22] or gene knockout [23] leads to lower hyperopia or myopia in mice. In addition, choroidal thickness may also be associated with axial growth, emmetropia, and myopia [24–26]. The stimulation of the ON pathway is correlated with local increases in dopamine levels, which lead to choroidal thickening and the slowdown of myopia progression [27]. Contrary to the choroidal thinning by 16 μm caused by 1-h reading stimulation of the OFF pathway with black text on a white background, a choroidal thickening of 10 μm was observed by stimulating the ON pathway with white text on a black background; moreover, the change in reading contrast sensitivity may affect myopia progression through the visual pathway [28]. The present study directly tested contrast sensitivity without refraction correction. The comparison of baseline parameters showed that children with lower qCSF results experienced less

myopic progression. The lower contrast may stimulate the ON pathway, leading to local dopamine release and slowing myopia progression. Therefore, qCSF measurement may provide information for early clinical interventions. Through further research, the qCSF test might be applied to screen for patients who can benefit from CS interventions and allow more accurate myopia prevention and control.

At the genetic level, amyloid precursor-like protein 2 (*APLP2*) participates in refraction development by regulating the function of amacrine cells [29, 30]. *APLP2* may regulate the retinal contrast processing through the level of synaptic transmission, thus regulating the sensitivity to retinal image degeneration [31–33]. *APLP2*-knockout mice showed lower contrast sensitivity at low spatial frequency and lower susceptibility to myopia [34]. Long-wavelength (L) and short-wavelength (M) cones mediate high visual acuity and play a key role in emmetropization by guiding the coupling of the AL and SE [35]. The opsin 1 (cone pigments), long-wave-sensitive (*OPN1LW*) and opsin 1 (cone pigments), medium-wave-sensitive (*OPN1MW*) haplotypes, especially LVAVA, that encode photoreceptors in their cells are directly related to cellular defects in cones and result in high myopia [36–38]. The missplicing of exon 3 of LVAVA leads to a sharp decrease in functional opsin in affected cones. Expression of the LVAVA haplotype and undamaged opsin in different submosaic cones increases the contrast signal between adjacent full and empty optic density cones and may stimulate axial elongation [36]. Therefore, environmental factors that produce abnormal contrast between adjacent cones may be a signal of axial growth. Based on this hypothesis, a DOT-based spectacle lens was developed, which was shown in a multicenter randomized controlled study to decrease the percentage of SER progress by 59–74% in one year [14]. The findings of the present prospective study further confirmed that low contrast sensitivity signals, especially at low spatial frequencies, may be related to the slowing of myopic shift. Moreover, the stabilized group showed increased contrast sensitivity, although the decrease in contrast sensitivity was correlated with myopic shift in the advanced group [12]. The reasons for these observations may include increased contrast in long-term indoor activities, activation of the ON pathway, and visual development related to contrast sensitivity. Although our findings cannot establish causality, the lower contrast sensitivity was correlated with relatively slower myopic shift in the context of a significant increase in indoor activities during the home quarantine. Therefore, interventions involving contrast sensitivity, especially at low spatial frequencies, may help in myopia prevention and control.

This study has several limitations. First, the sample size was relatively small, and the enrolled subjects were mainly myopic; therefore, additional studies with larger sample sizes are needed to further compare the effect of contrast sensitivity on myopic shift under various factors. Second, the follow-up time was relatively short. Longer-term follow-up comparisons may be to determine the correlation between contrast sensitivity and myopic shift at different ages, thus further contributing to accurate prevention and control. Third, Previous hypotheses have focused on the peripheral retinal region, which accounts for a significant portion of the retinal area. While, this study assessed the contrast sensitivity of the fovea. The findings concentrate on the performance of contrast sensitivity at low spatial frequencies, potentially guiding the assessment of peripheral contrast sensitivity in future investigations.

In conclusion, lower contrast sensitivity at low spatial frequencies may play a protective role in myopic shift. QCSF screening and contrast sensitivity interventions for children with relatively high CS at low spatial frequencies may contribute to accurate myopia prevention and control.

Acknowledgements
We would like to thank Editage (www.editage.cn) for the English language editing.

Author contributions
Study concept and design (YY, YX, FL, JZ, XZ); data collection (YY, FL, YX, JZ); data analysis and interpretation (YY, FL, YX, JZ); drafting of the manuscript (YY, FL, YX, JZ); critical revision of the manuscript (YY, ML, JZ, XZ); supervision (XZ and JZ). All authors have read and approved the final manuscript.

Funding
This study was supported by National Key Research and Development Program of China (2024YFC2510805); Key Project of the Regional Innovation and Development Joint Fund of the National Natural Science Foundation of China (U24A20708); National Natural Science Foundation of China (Grant No. 82271119, 82471116); Shanghai Rising-Star Program (23QA1401000); Healthy Young Talents Project of Shanghai Municipal Health Commission (2022YQ015); Shanghai Oriental Talents-Technology Platform Program (QNKJ2024055).

Data availability
The datasets generated and/or analyzed during the current study are not publicly available due to funding requirements but are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate
This study was approved by the Ethics Committee of Fudan University Eye and ENT Hospital Review Board (Shanghai, China) (2020107, Date:07/01/2021) and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants and their legal guardians.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 20 April 2024 / Accepted: 8 January 2025
Published online: 27 February 2025

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