TEMPORAL PROPERTIES OF REPEATED HYPEROPIC DEFOCUS IN THE GUINEA PIG EYE

Poster #2

Amelia J. Leotta and Sally A. McFadden

School of Psychology, The University of Newcastle, NSW 2308

INTRODUCTION

In all species studied including humans, when a growing eye wears a spectacle lens it will adjust its growth rate and change its refractive error to compensate for any imposed defocus.

Spectacle lens compensation (SLC) has been shown in many species, including chicks[1], tree shrews[2], marmosets[3], guinea pigs[4] and monkeys[5].

While the temporal properties of such spectacle lens compensation have been investigated in the chick[6,7], there have only been preliminary investigations in the mammalian eye. The data from the chick suggests that the signal that leads to ocular elongation arising from hyperopic blur decays within 30 minutes[7].

Our aim was to investigate the temporal properties of hyperopic defocus in the guinea pig.

METHOD

42 guinea pigs wore -4D lenses on their right eye for repeated 15 minute periods with varying dark intervals in between these episodes of visual experience (Fig. 1).

Ocular Measurements: At 18 days of age, refractive error (using streak retinoscopy), corneal curvature (using infrared videoretinoscopy, Fig. 2) and ocular structures (using high frequency ultrasound, Fig. 3) were measured under isoflurane anaesthesia.

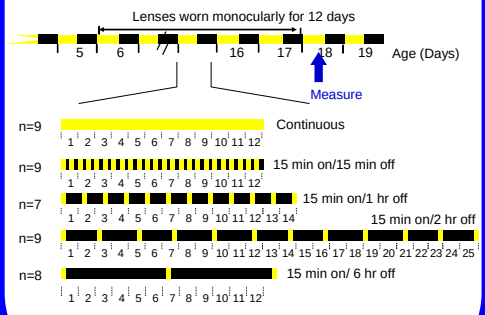


Figure 1. Light cycles for guinea pigs wearing -4D lenses

OCULAR MEASUREMENTS

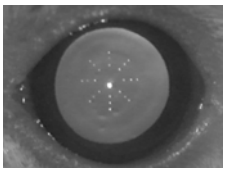


Figure 2. Example image from infrared keratometry.

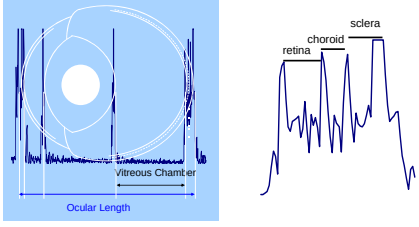
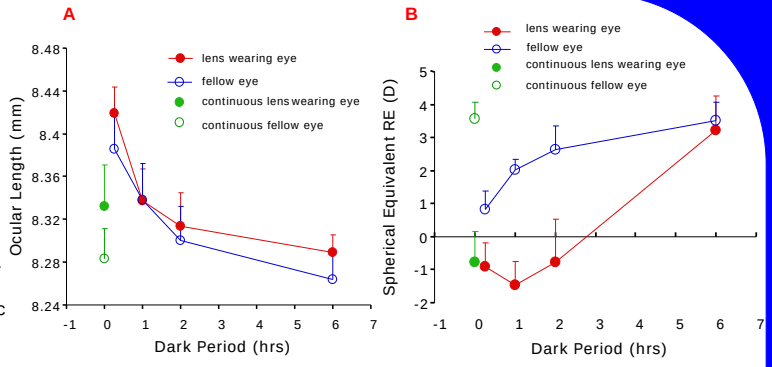


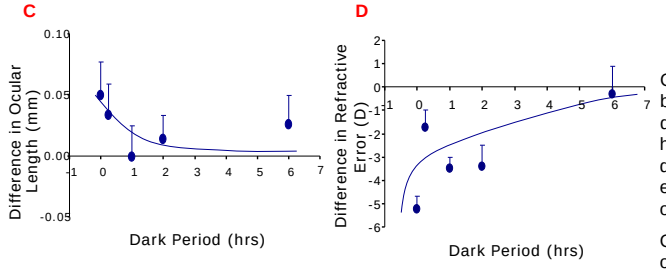
Figure 3. A-scan ultrasound of guinea pig eye

RESULTS

FREQUENCY EFFECT

As the frequency of the light periods increased, both the lens-wearing and the non-lens-fellow eye became more elongated (A) and more myopic (B).





DECAY OF HYPEROPIC DEFOCUS

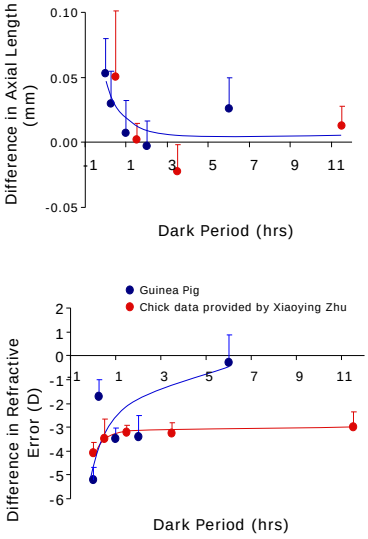
Guinea pigs who wore -4D lenses continually became most myopic (M = 4.3D). As the dark period increased in length, the hyperopic defocus signal decayed. The decay rate was more rapid for the ocular elongation (C) than for the refractive status of the eye (D).

Greater myopia and more robust SLC was observed with more frequent visual episodes of hyperopic defocus.

DECAY OF HYPEROPIC DEFOCUS IN THE CHICK AND THE GUINEA PIG

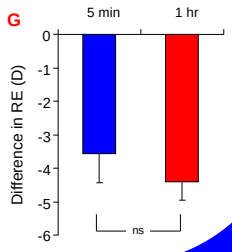
Axial elongation decays to 50% when the dark interval between episodes is approximately 30 minutes in both mammals and birds (E), suggesting that the signals that control axial elongation are phylogenetically conserved.

However, the consequences of axial elongation on refractive status varies between species (F). The myopia that results from hyperopic defocus decays much more rapidly in the mammal (50% decay at one hour) than the chick, where it appears to last several days.



RISE TIME OF HYPEROPIC DEFOCUS

Preliminary RE data shows that guinea pigs experiencing one hour dark episodes need only five minutes of light to compensate for the hyperopic defocus from -4D lenses, this being as effective as one hour of light (G). This is comparable to the short time required for chicks[7].



CONCLUSION

- Short multiple visual exposures, regardless of blur, act as a myogenic stimulus. The more frequent the exposures, the stronger the resulting myopia.
- Only five minutes of repeated episodes of hyperopic defocus are sufficient to cause spectacle lens compensation.
- In mammals, the ocular elongation caused by a minus lens decays in 30 minutes (like the chick), but the resulting myopia is more resilient and does not decay until at least an hour has passed between episodes.
- This implies that frequent exposures to blur with short periods in between will lead to myopia in humans

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CONTACT DETAILS

Amelia Jane Leotta
Vision Sciences Laboratory
School of Behavioral Sciences
Ph: 49 217310
Amelia.Leotta@newcastle.edu.au

Poster #3

The Association of the *Lumican* Gene Haplotype with High Myopia Susceptibility in Taiwanese Patients

Zoe Tzu-Yi Chen [1,2,3], I-Jong Wang [3], Yung-Feng Shih [3] and Luke Long-Kung Lin [3]

Department of Ophthalmology, Taipei City Hospital Zhongxing Branch, Taipei, Taiwan R.O.C.

College of Medicine, National Taiwan University, Taipei, Taiwan R.O.C.

Department of Ophthalmology, National Taiwan University Hospital, Taipei, Taiwan R.O.C

PURPOSE

Scleral thinning and eyeball elongation in myopic eyes are related to the remodeling of scleral extracellular matrices(1;2). Small Leucine-Rich Proteoglycans (SLRPs) in human sclera play an important role in regulating the assembly and interaction of collagen fibrils, which influence scleral mechanical properties and axial elongation(3;4). Chromosome 12q21-23 (MYP 3), linked with certain familial high myopia, includes three SLRP genes, which are *decorin*, *lumican*, and *dermatan sulfate proteoglycan 3* (*DSPG-3*)(5). Herein, we investigate genetic polymorphisms at these three *SLRP* genes in the *MYP3* loci of extremely high myopes to elucidate the role of *decorin*, *lumican* and *DSPG3* in high myopia..

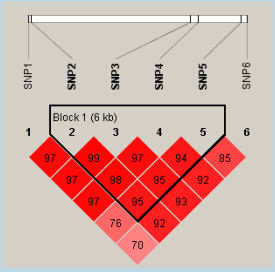
METHODS

This was a hospital-based case-control study including 120 unrelated high myopia cases with refractive errors of less than -10.0D in both eyes, and 137 emmetropia controls with refractive errors of -1.5D to 0.5D in either eye. All the subjects were of Han Chinese origin. We extracted genomic DNA from the participants' whole blood samples, which were then genotyped using PCR direct sequencing. SNP information was obtained from GenBank and Japanese SNP database(JSNP). SNPs with minor allele frequencies (MAF) > 0.05 in our study population were selected. Sequence alignment was done using BioEdit software (version 5.0.6). Case and control genotypes were assessed for Hardy-Weinberg equilibrium (HWE) using $\chi 2$ tests with a corrected significant threshold of P = 0.003 (Bonferroni method). Allele frequencies were compared between cases and controls using $\chi 2$ tests. The Haploview program was applied to estimate pairwise LD between markers and to partition haplotype blocks(6). We used a sliding-window gene-centered approach to further determine the most predictive haplotype block. Finally, the multifactor dimensionality reduction (MDR) method(7;8) was used to detect the best locus-locus and gene-gene interaction models.

RESULTS

Controls and cases were adequately matched on sex; controls had a higher mean age (p = 4.5x10-6) compared with cases. A total of 16 SNPs were selected from the *decorin* (4), *lumican* (8), and *DSPG3* genes (4). No statistically significant deviation from HWE (all p-values larger than 0.0033) was noted in the control group. The most significant finding was in the 5' UTR of lumican (rs3759223, p = 2.83x10-4, odds ratio = 8.18(2.4~29.8). To better capture the contribution of the *lumican* locus to high myopia risk, we examined six SNPs of the *lumican* gene to identify possible haplotypes. Figure 1 shows the plot of the pairwise LD (D') values for the six SNPs and LD structure of the *lumican* gene. The LD plot indicates one block (SNPs 2-5; size = 6kb) with high LD encompassing the upstream intron and putative promoter area. Table 1 summarizes the associations between frequencies of the haplotypes and risk of high myopia. Sliding-windows haplotype analyses with global score test showed statistically significant differences in the haplotype profile between cases and controls for SNP2-SNP3-SNP4-SNP5, SNP3-SNP4-SNP5, and SNP4-SNP5 blocks.

Figure 1.
Graphical representation of the SNP locations and LD structure of LUM gene. The arrow illustrates the transcription direction of the gene. 3' ← 5'



SNP1: rs2300588 SNP4: rs3741834
SNP2: rs7135740 SNP5: rs3759223
SNP3: rs3741835 SNP6: rs3759222

Figure 2
The MDR models and interaction dendrogram for gene-gene interaction on high myopia risk

A.Summary of MDR interaction models

No. of factors	Best candidate models	Testing accuracy (%)	P value	CV consistency
1	rs3759223	61.5	0.0107	10/10
2	rs2300588 rs3741834	64.0	0.0010	7/10
3	rs2300588 rs3741834 rs1920748	62.0	0.0547	4/10
4	rs2070985 rs2300588 rs7135740 rs3759223	66.5	0.0010	5/10

B.Interaction dendrogram.
The color indicates the strength of the dependence: green is weak, and red is strong. The hierarchical cluster analysis placed LUM rs2300588(SNP1) and rs3740834(SNP4) on the same branch.

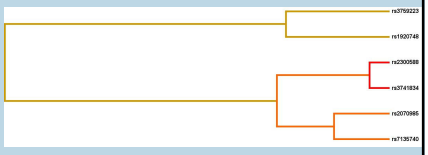


Table 1.

Estimated Haplotype Frequencies of the LUM Gene and Associations between Haplotypes in Each Block and High Myopia Risk

		Global Score test P value	Haplotype specific test P value	Haplotype frequency Controls : Cases	Odds ratio (95% C.I.)	Adjusted Odds ratio (95% C.I.)
SNP2-SNP3-SNP4-SNP5		1.2591×10^{-7}				
SNP2-SNP3-SNP4		0.053695				
SNP3-SNP4-SNP5		$1.6659e\times10^{-7}$				
SNP2-SNP3		0.070622				
SNP3-SNP4		0.068853				
SNP4-SNP5	C-T	1.0725×10^{-6}	0.77855	0.64 : 0.63	1.0000 (reference)	1.0000 (reference)
	T-T		0.57271	0.0073 : 0.0136	1.95 (0.31-12.15)	1.20 (0.175-8.276)
	C-C		1.0823×10^{-6}	0.0037 : 0.1095	33.89 (4.46-257.04)	19.32 (2.55-146.54)
	T-C		0.015029	0.35 : 0.25	0.55 (0.36-0.85)	0.69 (0.46-1.04)

Whereas only haplotypes constituted by SNP4-SNP5 were all observed among both case and control groups, the analysis of SNP4-SNP5 haplotype block was of greatest significance: haplotype C-C (OR=19.32, 95% CI=2.55-146.54) was significantly associated with high myopia risk, while haplotype T-C (OR=0.69, 95% CI=0.46-1.04) was inversely associated with high myopia risk. Total 16 SNPs in *decorin*, *lumican*, and *DSPG3* genes were included in the MDR analysis. Figure 2A summarizes the best interaction models obtained from the MDR analysis. Consistent with the individual SNP analyses, in the one-locus model, *lumucan* gene rs3759223: T>C was the best attribute for predicting high myopia risk (testing accuracy = 61.5%; CVC = 10; p=0.01). The best interaction model was a two-locus model composed of *lumican* gene rs2300588 and rs3741834 (testing accuracy = 64.0%; CVC = 7; p=0.001). The figure 2B interaction dendrogram determines a strong interaction effect of these two loci in the *lumican* gene on modulating risk of high myopia.

CONCLUSIONS

Our results indicate variants in the regulatory region of lumican in Chinese Hans may be associated with high myopia susceptibility and worth further investigation.

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Poster #5

Parental myopia - genetic or cultural transmission? An extended twin parent study

Mingguang He, Jian Zhang, Xiaohu Ding, Yingfeng Zheng, Jian Ge

Zhongshan Ophthalmic Center, Guangzhou, China.

INTRODUCTION

➢Individual difference in myopia tend to cluster in families.
➢Growing evidence suggests parental history of myopia is an important predictor for myopia.
➢ The effects of parental myopia could be due to genetic relatedness, environmental similarities, cultural transmission from one generation to the next, or a combination of these mechanisms.
➢ Extended twin study provides an unique opportunity to detect the presence of cultural transmission.

PURPOSE

To determine whether cultural transmission is involving in population variation of myopia, and to estimate the extent to which cultural transmission influence myopia phenotypic variance.

METHODS

1. Study population:
- In 2005 we initiated a population-based twin registry in Guangzhou city in southern China. To date, over 9700 pairs of twin born from 1987 to 2000 were enrolled in the database.
- The study subjects were recruited from this twin registry. All twins aged 7 to 15 years on July 1st 2006 living in two districts close to the Zhongshan Ophthalmic Center, where the examination was set up, were invited for the examination together with their parents.

2. Examinations:
Cycloplegic refraction was measured by three times using an autorefractor (KR8800, Topcon Corp, Tokyo, Japan) on the twins. The refraction was further refined by subjective refraction using standard protocol. Non-cyclopegic refraction was administrated on parents. Spherical equivalent (SE), representing degree of refractive error, was calculated as spherical power plus half of cylinder power.

3. Zygostic ascertainment:
Zygosity of all same-sex twin pairs was assessed by 16 multiplex STR (Powerplex 16 system, Promega, Madison, US). This is to classify the twin pairs as monozygotic (MZ) and dizygotic (DZ).

4. Statistical analysis
The right eye was arbitrarily selected to represent the myopia phenotypic characteristics of the specific individual. We computed correlations among monozygotic (MZ), dizygotic (DZ) twins and parents, and carried out model-fitting analyses using a model assuming phenotypic assortment and cultural transmission. Parents pass their genes to their children, with the factor loading 1/2. In the children, part of the genetic variance is explained by transmission from the parents. The remaining residual additive genetic variance represents the variance that results from recombination. Dominance effects are not assumed to be transmitted from parents to offspring.

Table 1. Model-Fitting for extended twins design

Model	Description
1	Full model assuming phenotypic assortment and cultural transmission
2	Same as Model 1, but drop dominant genetic effect
3	Same as Model 1, but drop dominant genetic effect and assortment mating
4	Same as Model 1,but drop dominant genetic effect, cultural transmission, G-E correlation

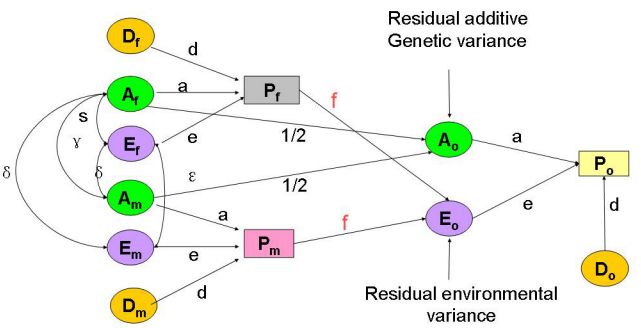


Figure 1 (above) Path model for the spouse and parent-offspring correlations under the assumption of cultural transmission with γ representing genotypic correlation between parents, ε environmental correlation between parents, δ correlation between environmental of one parents with genotype of other parent, f cultural transmission, s genotype environmental correlation, f father, m mother, o offspring, A additive genetic value, E environmental value, D dominance variation. For clarity reasons, we put twins in **Figure 2** below.

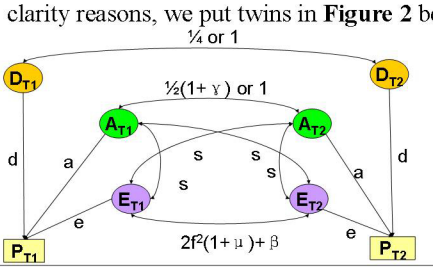


Figure 2 (left) Path model with twins. β representing residual environmental covariance not explained by cultural transmission. T1 first born twin, T2 second born twin.

RESULTS

561 twin families were available for analysis, which included 357 MZ, 204 DZ pairs, 427 fathers, and 516 mothers. MZ twin correlations were significantly greater than DZ twin correlations, suggesting substantial genetic influences for myopia. Parents-offspring correlations were moderate, indicating that there are paternal or maternal effects on myopia. The most parsimonious model is the one with additive genetic effects, environmental effects, and cultural transmission (f=0.48, 95% confidence interval 0.41, 0.53). Additive genetic effects accounted for 79.6% (95%CI: 76.5%, 82.5%) of population variance.

Table 2 Monozygotic and dizygotic twins and parent-offspring correlations for spherical equivalent (95%CI)

Relationship	First born twin	Second born twin
Father	0.116 (0.021, 0.209)	0.174 (0.081, 0.265)
Mother	0.140 (0.054, 0.223)	0.221 (0.138, 0.302)
MZ	0.889 (0.865, 0.909)	
DZ	0.394 (0.271, 0.504)	

CONCLUSION

A model assuming cultural transmission fitted the data better than the models assuming otherwise. The main influence on myopia variation is genetic, with evidence of cultural transmission between generations.

Correspondence: Mingguang He MD PhD, Zhongshan Ophthalmic Center, Guangzhou, China. Email: mingguang_he@yahoo.com

The authors have no competing interests to declare.

Poster #6

Is there shared genetic determinant between height and axial length in children: the Guangzhou Twin Eye Study

Mingguang He, Ke Feng, Jian Zhang, Xiaohu Ding, Jian Ge

Zhongshan Ophthalmic Center, Guangzhou, China.

INTRODUCTION

- It is difficult to understand the risk factors and natural history of myopia without recourse to ocular biometry.
- Taller people have longer axial length (AL) and probably higher rates of myopia than shorter persons.
- The age cessation of axial elongation of the eye is similar to the age of cessation of increase in height.
- Twin study provides an optimal method to detect shared additive genetic effects between height and ocular biometry.

PURPOSE

To determine whether common sets of genes are involving in the myopia-related traits and height, and to estimate the extent to which common genetic and/of environmental factors influence the relationships.

METHODS

1. Study population:

- In 2005 we initiated a population-based twin registry in Guangzhou city in southern China. To date, over 9700 pairs of twin born from 1987 to 2000 were enrolled in the database.
- The study subjects were recruited from this twin registry. All twins aged 7 to 15 years on July 1st 2006 living in two districts close to the Zhongshan Ophthalmic Center, where the examination was set up, were invited for the examination.

2. Examinations:

AL was measured using a Zeiss IOLMaster. Height was measured according to standard protocol..

3. Zygostic ascertainment:

Zygosity of all same-sex twin pairs was assessed by 16 multiplex STR (Powerplex 16 system, Promega, Madison, US). This is to classify the twin pairs as monozygotic (MZ) and dizygotic (DZ).

4. Statistical analysis

The right eye was arbitrarily selected to represent the AL phenotypic characteristics of the specific individual. We computed twin correlations and cross-trait cross-twin correlations for monozygotic (MZ) and dizygotic (DZ) twins and carried out model-fitting analyses using a multivariate Cholesky model. The first Cholesky factors (i.e., A1, D1, & E1) exert influences on both AL and height, although they predominantly impact AL. The second Cholesky factors (i.e., A2, D2, & E2) have effects unique on height. The A, D, and E covariance matrices were computed by the product of their respective Cholesky factor loading matrix and its transpose. Akaike information criterion (AIC = X2 – 2df) and the likelihood-ratio chi-square test (LRT) were used to select the best-fitting model.

Table 1. Cholesky Model-Fitting

Model	Description
1	Full ACE cholesky
2	Full ADE cholesky
3	Same as Model 2, but drop shared environmental var-covariances among AL, Height
4	Same as Model 3,but drop additive genetic variance unique to AL
5	Same as Model 3, but drop additive genetic variance unique to Height
6	Same as Model 3, but drop additive genetic covariances among AL,Height
7	Same as Model 3, but drop nonshared environmental covariances among AL, Height

RESULTS

565 twins aged 7-15 years were available for analysis, which included 359 MZ and 206 DZ pairs. The phenotypic correlation between height and AL was 0.47. MZ twin correlations were significantly greater than DZ twin correlations, suggesting substantial genetic influences for both height and AL. MZ cross-twin cross-trait correlations were only slightly greater than the corresponding DZ correlations, indicating that limited shared genetic factors in the covariations. When we standardized the Cholesky additive genetic factor loadings, of 88% of heritability for AL, 3% (95%CI: 0.01-0.06) were shared with the height phenotype. The effects of common random environmental factors were minimal (0.4%) and were not statistically significant.

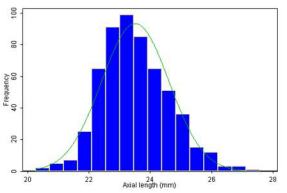


Figure 1 Distribution of axial length in the first born twin

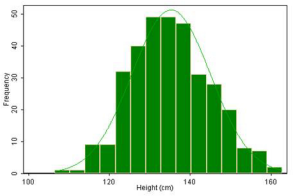


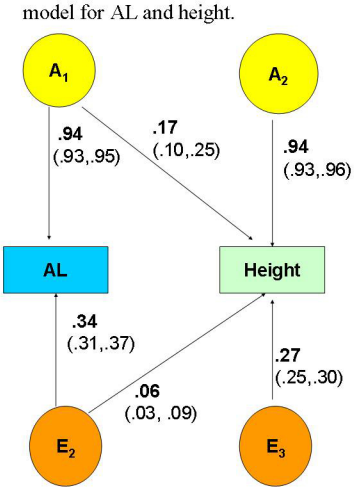
Figure 2 Distribution of height in the first born twin aged 7-11 years

Table 2 Phenotypic correlations, and cross-twin cross-trait correlations for AL and Height for MZ and DZ twins.

Phenotypic correlations (N = 1134)		
	AL	Height
AL	1.0	
Height	0.47	1.0
Twin correlations and Cross-twin cross-trait correlations		
	AL1	Height1
	MZ twins (N=359 pairs)	
AL2	0.91	0.45
Height2	0.45	0.98
	DZ twins (N=206 pairs)	
	AL1	Height1
AL2	0.47	0.40
Height2	0.44	0.86

AL = axial length. "1" and "2" refer to measures made on the twin and co-twin. The elements on the diagonals represent twin correlations. The off-diagonal elements are cross-twin cross trait correlations

Table 3 The standardized parameter estimates in the best-fitting Cholesky model for AL and height.



A=additive genetic factors, E=random environmental factors. AL=axial length

CONCLUSION

Genetic covariance for height and myopia is limited although it is statistically significant, suggesting the phenotypic association is likely due to environmental effects.

Correspondence: Mingguang He MD PhD, Zhongshan Ophthalmic Center, Guangzhou, China. Email: mingguang_he@yahoo.com

The authors have no competing interests to declare.