Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Investigation of the association between all-*trans*-retinol dehydrogenase (*RDH8*) polymorphisms and high myopia in Chinese*

Yan-shu YU[§1], Lin-ling WANG[§1], Ye SHEN[1], Maurice K. H. YAP[2], Shea-ping YIP[3], Wei HAN[†‡1,2,3]

([1]*Department of Ophthalmology, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*)

([2]*School of Optometry, The Hong Kong Polytechnic University, Hong Kong SAR, China*)

([3]*Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China*)

[†]E-mail: hanweidr@hotmail.com

Received Dec. 29, 2009; Revision accepted Mar. 28, 2010; Crosschecked Oct. 14, 2010

Abstract: Retinoic acid level in the retina/choroid is altered in induced myopia models. All-*trans*-retinol dehydrogenase (RDH8) is an important enzyme of retinoic acid metabolism. This study aimed to investigate the association of the *RDH8* gene with high myopia. Three single nucleotide polymorphisms (SNPs) [RDH851 (rs2233789), RDH8E5a (rs1644731), and RDH855b (rs3760753)] were selected, based on the linkage disequilibrium pattern of *RDH8* from a previous study, and genotyped for 160 Han Chinese nuclear families with highly myopic (−10 diopters or worse) offspring as well as in an independent group with 166 highly myopic cases (−10 diopters or worse) and 211 controls. Family-based association analysis was performed using the family-based association test (FBAT) package, and genotype relative risk (GRR) was calculated using the GenAssoc program. Population-based association analysis was performed using Chi-square test. These SNPs were in linkage equilibrium with each other. SNPs RDH851 (rs2233789) and RDH8E5a (rs1644731) both did not show association with high myopia. SNP RDH855b (rs3760753) demonstrated significant association (P=0.0269) with a GRR of 0.543 (95% confidence interval=0.304–0.968, P=0.038). The association became statistically insignificant, however, after multiple comparison correction. Haplotype analysis did not show a significant association either. Population-based association analysis also showed no significant association (P>0.05). Our family- and population-based data both suggest that the *RDH8* gene is unlikely to be associated with high myopia in Chinese.

Key words: Myopia, All-*trans*-retinol dehydrogenase (RDH8), Single nucleotide polymorphisms, Association study, Linkage disequilibrium, Genotype relative risk

doi:10.1631/jzus.B1000001 **Document code:** A **CLC number:** R778

1 Introduction

Myopia is very common worldwide, particularly in Asian populations (Saw, 2003; Xu *et al*., 2005), and has become a serious public health concern. Family aggregation for myopia and a higher prevalence of myopia in Asian than in Caucasian and African populations suggest a role for genes in the etiology of myopia (Lyhne *et al*., 2001; Saw, 2003; Young, 2009). In most cases, myopia is a complex trait in which multiple genes, environmental factors, and their interactions are involved (Saw *et al*., 2000; Lyhne *et al*., 2001). Identification of the susceptibility genes will lead to a better understanding of the mechanisms underlying myopia, and hence help to find effective ways to prevent the onset, or control the progression, of myopia.

Image defocus or blur on the retina can signal the eye to develop myopia in animals, whereas animals raised in a dark environment do not develop significant myopia (Hung *et al*., 1995; Norton and Siegwart,

‡ Corresponding author

§ The two authors contributed equally to this work

* Project supported by the National Natural Science Foundation of China (No. 30600693), the Qianjiang Talent Project of Zhejiang Province (No. 2010R10068), and the Hong Kong Polytechnic University (No. J-BB7P), China

1995; Yoshino *et al*., 1997). Therefore, myopia might be triggered at the level of photoreceptors or visual metabolism. The level of retinoic acid in retinal and choroid layers has also been shown to change significantly in animal myopia models (Seko *et al*., 1998; Bitzer *et al*., 2000; Mertz and Wallman, 2000; McFadden *et al*., 2004). Thus, it is speculated that retinoid acid might provide signals to modulate eye growth and play a role in the onset and development of myopia (Morgan, 2003). Enzymes involved in the metabolism of retinoid acid might contribute to the onset or severity of myopia. Retinoic acid is the oxidation product of retinol. All-*trans*-retinol dehydrogenase (RDH8), also known as photoreceptor RDH, catalyzes the reduction of all-*trans*-retinal to all-*trans*-retinol, the first reaction step of the rhodopsin regeneration pathway, and also the rate-limiting step in the visual cycle (Saari *et al*., 1998; Rattner *et al*., 2000). This indicates that the activity of RDH8 may influence the level of retinol and subsequently the synthesis of retinoic acid. In view of the important role of RDH8 in the visual biological function, we hypothesized that the gene encoding RDH8 might be a potential candidate responsible for the susceptibility to high myopia.

The human *RDH8* gene, located on 19p13, has six exons spanning about 9 kb (Rattner *et al*., 2000). In previous work, we identified single nucleotide polymorphisms (SNPs) within and around the *RDH8* gene, established the linkage disequilibrium (LD) pattern of common SNPs, and determined the tag SNPs for use in association studies involving the *RDH8* gene in a Han Chinese population (Han *et al*., 2004). In the present study, we investigated the association between the *RDH8* gene and high myopia in a group of Han Chinese nuclear families with highly myopic offspring. Recently, replication of association analysis in an independent population has been considered to be critically important to achieve greater confidence in the association found. Thus, we also tested the family-based association analysis data in an independent group of case-control Han Chinese subjects.

2 Patients and methods

2.1 Subjects

Nuclear families were recruited in a method reported previously (Han *et al*., 2006). Case-control subjects were also recruited from the Department of Ophthalmology, the First Affiliated Hospital in Hangzhou, China, with written informed consent. Briefly, all subjects were Han Chinese from southern China. Each nuclear family consisted of two parents and one or more affected myopic offspring. For all affected offspring and cases, the entry criterion for high myopia was a spherical equivalence (SE) of −10.0 diopters (D), or worse, for both eyes, where SE (spherical power plus half cylindrical power) was calculated from the refraction measured. For all emmetropia controls, the entry criterion was an SE ≥−0.75 D and ≤+1.0 D for both eyes.

2.2 SNP genotyping

DNA was extracted from blood samples with commercial kits (Han *et al*., 2006). Our previous study established the LD patterns of SNPs within and around *RDH8* and identified three tag SNPs for use in association studies (Han *et al*., 2004). These three tag SNPs were RDH855b (−1715G>A; rs3760753), RDH851 (−472C>T; rs2233789), and RDH8E5a (7826T>C; rs1644731). The genotypes were determined using denaturing high-performance liquid chromatography (HPLC) as described before (Han *et al*., 2004). ElDorado (http://www.genomatix.de/en/produkte/genomatix-software-suite.html#1) was used to search the promoter region for potential transcription factor binding sites of the *RDH8* gene.

2.3 Statistical analysis

Two LD measures (standardized Lewontin's LD parameter $D'=D/D_{\max}$; $r^2=D^2/[P_AP_B(1-P_A)(1-P_B)]$, where the P_A and P_B are the frequencies of alleles A and B at two different loci, respectively) were calculated for the parents of the recruited families (Han *et al*., 2006). The family-based association test (FBAT) software package Version 1.5.5 (http://www.biostat.harvard.edu/~fbat/fbat.htm) was used for the association test (Laird *et al*., 2000). The association of both single marker SNPs and multiple marker haplotypes was examined. The linkage phase was resolved using an expectation-maximization algorithm.

A matched case-control dataset was generated with each affected offspring matched to three possible pseudocontrols created from the untransmitted parental allele (Cordell *et al*., 2004). As a measure of the effect size of the marker genotype on the disease risk, the genotype relative risk (GRR) and its 95% confidence interval (CI) were calculated using conditional

logistic regression from this case-pseudocontrol dataset. Conditional logistic regression was performed with the GenAssoc package (http://www-gene.cimr.cam.ac.uk/clayton/software/).

Chi-square test was used to test the association between the cases and controls with Haploview software 3.3.2 (http://www.broadinstitute.org/haploview).

3 Results

In total, 160 nuclear families were recruited. Of these families, 61 (38.1%) had one myopic (−0.75 D or less) parent and 31 (19.4%) two myopic parents. The average age of the myopic offspring at entry was 20.93 years and their average onset age of myopia was 7.02 years. Their mean refractive error in SE was −12.02 D. For case-control subjects, altogether 166 high myopia subjects were recruited with mean SE of (−11.03±2.86) D and 211 normal control subjects with mean SE of (−0.25±0.75) D. Detailed clinical data are listed in Table 1.

Search with ElDorado showed that RDH851 (rs2233789) was in the promoter region of the *RDH8* gene. RDH855b was not involved in the promoter or regulatory binding sites of *RDH8*.

Among 320 unrelated parents of the recruited families, pairwise LD measures (absolute value of D' plus r^2 in brackets) were 0.04 (0.00) for RDH855b-RDH851, 0.22 (0.01) for RDH855b-RDH8E5a, and 0.30 (0.07) for RDH851-RDH8E5a. This indicates that all three SNPs were in linkage equilibrium.

Both SNPs RDH851 and RDH8E5a were found not to be associated with high myopia under all three genetic models tested (Table 2). It is interesting to note the reciprocal relationship of Z scores between the dominant and the recessive models for bi-allelic markers. For RDH855b, the minor allele (A) was found to show reduced transmission to the myopic offspring under both additive (Z=−2.213, P=0.0269) and dominant (Z=−2.098, P=0.0359) models (Table 2). Thus, the A allele seems to be protective for high myopia. The global statistic, however, was significant only under the additive model (P=0.0269), and not the dominant model (P=0.0863). Analysis of the case-pseudocontrol dataset for RDH855b with GenAssoc gave a GRR of 0.543 (95% CI=0.304–0.968; P=0.038) for combined genotypes of G/A and A/A with reference to G/G. When multiple comparisons (three markers and/or three genetic models) were taken into account, the significance level for the additive model did not confirm an association. Haplotype analysis of all three markers together did not reveal any significant association with high myopia (Table 3).

Table 1 Clinical data of high myopia subjects recruited

Parameter	Value*
Age at entry (year)	20.93±12.35
Sex (male/female)	173/163 (P>0.05)
Onset age of myopia (year)	7.02±3.02
MSE (D)	
Affected siblings	−11.31±3.57
Highly myopic cases	−11.03±2.86
AXL (mm)	27.56±2.02 (r=−0.75, P<0.001)
CP (D)	43.11±1.36 (r=−0.37, P=0.015)
ACD (mm)	3.65±0.29 (r=−0.30, P<0.001)
LT (mm)	3.66±0.27 (r=−0.15, P=0.07)

n=336. * Values are expressed as mean±standard deviation (SD) or number. The values in brackets are the partial correlation test between mean spherical equivalent (MSE) and ocular indices of axial length (AXL), corneal power (CP), anterior chamber depth (ACD), and lens thickness (LT). AXL has the most significant correlation to MSE

Table 2 Summary of genetics data in parents and tests of association by FBAT under different genetic models in 160 nuclear families for three *RDH8* SNPs

SNP	Allele[a]	AF[b]	FBAT-additive model				FBAT-dominant model				FBAT-recessive model			
			n[c]	Z score	P value	Global statistics[d]	n	Z score	P value	Global statistics	n	Z score	P value	Global statistics
RDH855b (rs3760753)	1 (G)	0.887	58	2.213	0.0269	$\chi^2(1)$=4.898, P=0.0269	11	0.962	0.3359	$\chi^2(2)$=4.900, P=0.0863	58	2.098	0.0359	$\chi^2(2)$=0.490, P=0.0863
	2 (A)	0.113	58	−2.213	0.0269		58	−2.098	0.0359		11	−0.962	0.3359	
RDH851 (rs2233789)	1 (C)	0.539	103	−0.266	0.7901	$\chi^2(1)$=0.071, P=0.7901	78	0.063	0.9495	$\chi^2(2)$=0.267, P=0.8751	65	−0.499	0.6180	$\chi^2(2)$=0.267, P=0.8751
	2 (T)	0.461	103	0.266	0.7901		65	0.499	0.6180		78	−0.063	0.9495	
RDH8E5a (rs1644731)	1 (C)	0.555	108	−0.430	0.6670	$\chi^2(1)$=0.185, P=0.6670	79	−0.506	0.6129	$\chi^2(2)$=0.260, P=0.8782	73	−0.134	0.8932	$\chi^2(2)$=0.260, P=0.8782
	2 (T)	0.445	108	0.430	0.6670		73	0.134	0.8932		79	0.506	0.6129	

[a] Alleles 1 and 2 are the major and minor alleles, respectively; [b] The allele frequency (AF) data refer to those of the parents (n=320); [c] "n" refers to the number of informative families in which there is at least one heterozygous parent; [d] The degree of freedom for χ^2 test is shown within brackets as χ^2 (x) where x=1 or 2. Note the reciprocal relationship between the dominant and the recessive models for bi-allelic markers

Table 3 Summary of genetics data in parents and tests of association by FBAT under different genetic models in 160 nuclear families for *RDH8* haplotypes

RDH8 haplotype[a]			HF[b]	FBAT-additive model			FBAT-dominant model			FBAT-recessive model		
RDH855b	RDH851	RDH8E5a		*n*[c]	*Z* score	*P* value	*n*	*Z* score	*P* value	*n*	*Z* score	*P* value
1 (G)	2 (T)	2 (T)	0.259	78	0.399	0.6900	69	0.095	0.9245	21	0.688	0.4913
1 (G)	1 (C)	2 (T)	0.235	68	0.623	0.5330	60	1.331	0.1832	16	−0.970	0.3320
1 (G)	2 (T)	1 (C)	0.218	64	0.493	0.6221	59	0.537	0.5911	17	0.161	0.8718
1 (G)	1 (C)	1 (C)	0.161	61	0.392	0.6952	60	0.868	0.3852	8	−1.095	0.2733
Global statistics[d]				χ^2 (6)=5.071, *P*=0.5347			χ^2 (6)=5.989, *P*=0.4244			χ^2 (4)=2.448, *P*=0.6539		

[a] Alleles 1 and 2 are the major and minor alleles, respectively; [b] The haplotype frequency (HF) data refer to those of the parents (*n*=320); [c] "*n*" refers to the number of informative families in which there is at least one heterozygous parent; [d] The degree of freedom for χ^2 test is shown within brackets as χ^2 (*x*) where *x*=4 or 6. Results are shown only for haplotypes with frequencies >10%

Table 4 Case-control association analysis for the *RDH8* gene by Chi-square test

SNP	Allele[a]	Case		Control		Association analysis	
		Allele frequency	HWE *P*[b]	Allele frequency	HWE *P*	χ^2 (1)[c]	*P*
RDH855b (rs3760753)	1 (G)	0.834	0.6010	0.851	0.9767	0.377	0.5390
	2 (A)	0.166		0.149			
RDH851 (rs2233789)	1 (C)	0.741	0.8381	0.702	0.3211	0.399	0.5276
	2 (T)	0.259		0.298			
RDH8E5a (rs1644731)	1 (C)	0.590	0.1727	0.633	0.6016	0.103	0.7486
	2 (T)	0.410		0.367			

[a] Alleles 1 and 2 are the major and minor alleles, respectively; [b] HWE refers to Hardy-Weinberg equilibrium; [c] The degree of freedom for χ^2 test is shown within brackets as χ^2 (1)

For case-control analysis, all three tagging SNPs in the *RDH8* gene showed no significant association (*P*>0.05) (Table 4).

4 Discussion

On the basis of our previous work of LD pattern in the *RDH8* gene (Han *et al*., 2004), we selected three SNPs as tag markers to test the association of the *RDH8* gene with high myopia. In line with the LD pattern in a random population of Han Chinese, the three tag SNPs selected were also found to be in linkage equilibrium in the parental population of the recruited Han Chinese nuclear families with severely myopic offspring.

Searches with ElDorado indicate that RDH851 is involved in the promoter region of the *RDH8* gene and might thus influence the level of gene transcription. RDH8E5a located in exon 5 is a non-synonymous polymorphism (Met202Thr) (Han *et al*., 2004) which may result in a variation in the protein function. Neither RDH851 nor RDH8E5a, however, showed any evidence of association with high myopia (Table 2). RDH855b showed mild significant association with high myopia under an additive model, but this association became insignificant after adjustment for multiple comparisons for multiple markers and/or multiple genetic models. It is well-known that analysis using haplotypes of multiple linked markers is more informative than that using single markers (Zhang *et al*., 2003). In the present study, analysis based on haplotypes involving all three SNPs also did not show any evidence of association with high myopia (Table 3). The same was true for analysis of subhaplotypes involving any two of these three SNPs (data not shown). In this study, we also used population-based association analysis to replicate the results in the family-based study in an independent sample group. Our independent case-control data also did not show any significant associations (Table 4). Thus, our replication data suggest that the *RDH8* locus does not play a major role in the susceptibility to high myopia in the Han Chinese population.

Genetic association study is currently the method of choice for mapping genes involved in complex diseases like high myopia (Risch, 2000; Tang *et al*., 2008). Family-based association studies can avoid

spurious association due to population stratification and heterogeneity in population-based case-control association studies (Ewens and Spielman, 1995; Risch, 2000). Therefore, we employed the family-based association study design, despite the difficulties with nuclear family recruitment. The population-based association study is more powerful than the family-based approaches, assuming no confounding from the population stratification. Replication in an independent population is also critically important for drawing a more convincing result from the association study. Therefore, we also involved the population-based case-control data to replicate and confirm our family-based analysis results in the present study.

High myopia is usually defined as a refractive error of −6.00 D or worse (Curtin, 1985). The present study adopted a refractive error of −10 D or worse with early onset age (earlier than 12 years old) as the entry criterion for all myopic offspring so as to provide a higher likelihood of strong genetic background (Iribarren *et al*., 2005). More importantly, myopia is a complex disease/trait involving several ocular components, for which the underlying genetic mechanisms may be quite different. The equivocal inheritance modes observed in different studies also highlight the complexity of genetic profiles for myopia (Farbrother *et al*., 2004). Therefore, the clinical assessment of high myopia is crucial for minimizing the disease heterogeneity which may confound the association study. High myopia is mainly due to the elongation of axial length, which is the primary ocular component found to be under genetic control (Goss *et al*., 1997). The strong correlation between axial length and refractive error in diopters demonstrates that the major ocular component for our myopic subjects is axial length, not other ocular refractive components such as corneal power (Han *et al*., 2006). All of these are important determinants for providing a strong and homogeneous genetic background for the myopic offspring in our study (Han *et al*., 2006).

5 Conclusions

Taken together, we investigated the association of polymorphic markers and their haplotypes of the *RDH8* gene with high myopia with both the family-based and population-based association studies. Our results in the two independent groups suggest that the *RDH8* gene is unlikely to be associated with high myopia.

References

Bitzer, M., Feldkaemper, M., Schaeffel, F., 2000. Visually induced changes in components of the retinoic acid system in fundal layers of the chick. *Exp. Eye Res.*, **70**(1): 97-106. [doi:10.1006/exer.1999.0762]

Cordell, H.J., Barratt, B.J., Clayton, D.G., 2004. Case/pseudocontrol analysis in genetic association studies: a unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet. Epidemiol.*, **26**(3):167-185. [doi:10.1002/gepi.10307]

Curtin, B.J., 1985. The Myopias: Basic Science and Clinical Management. Harper & Row, Philadelphia, p.102-105.

Ewens, W.J., Spielman, R.S., 1995. The transmission/disequilibrium test: history, subdivision, and admixture. *Am. J. Hum. Genet.*, **57**(2):455-464.

Farbrother, J.E., Kirov, G., Owen, M.J., Guggenheim, J.A., 2004. Family aggregation of high myopia: estimation of the sibling recurrence risk ratio. *Invest. Ophthalmol. Vis. Sci.*, **45**(9):2873-2878. [doi:10.1167/iovs.03-1155]

Goss, D.A., van Veen, H.G., Rainey, B.B., Feng, B., 1997. Ocular components measured by keratometry, phakometry, and ultrasonography in emmetropic and myopic optometry students. *Optom. Vis. Sci.*, **74**(7):489-495. [doi:10.1097/00006324-199707000-00015]

Han, W., Yip, S.P., Wang, J., Yap, M.K.H., 2004. Using denaturing HPLC for SNP discovery and genotyping, and establishing the linkage disequilibrium pattern for the all-*trans*-retinol dehydrogenase (*RDH8*) gene. *J. Hum. Genet.*, **49**(1):16-23. [doi:10.1007/s10038-003-0100-9]

Han, W., Yap, M.K., Wang, J., Yip, S.P., 2006. Family-based association analysis of hepatocyte growth factor (*HGF*) gene polymorphisms in high myopia. *Invest. Ophthalmol. Vis. Sci.*, **47**(6):2291-2299. [doi:10.1167/iovs.05-1344]

Hung, L.F., Crawford, M.L., Smith, E.L., 1995. Spectacle lenses alter eye growth and the refractive status of young monkeys. *Nat. Med.*, **1**(8):761-765. [doi:10.1038/nm0895-761]

Iribarren, R., Balsa, A., Armesto, A., Chiaradia, P., Despontin, L., Fornaciari, A., Pfortner, T., 2005. Family history of myopia is not related to the final amount of refractive error in low and moderate myopia. *Clin. Exp. Ophthalmol.*, **33**(3):274-278. [doi:10.1111/j.1442-9071.2005.01009.x]

Laird, N.M., Horvath, S., Xu, X., 2000. Implementing a unified approach to family-based tests of association. *Genet. Epidemiol.*, **19**(S1):S36-S42. [doi:10.1002/1098-2272(2000)19:1+<::AID-GEPI6>3.0.CO;2-M]

Lyhne, N., Sjølie, A.K., Kyvik, K.O., Green, A., 2001. The importance of genes and environment for ocular refraction and its determiners: a population based study among

20–45 year-old twins. *Br. J. Ophthalmol.*, **85**(12):1470-1476. [doi:10.1136/bjo.85.12.1470]

McFadden, S.A., Howlett, M.H., Mertz, J.R., 2004. Retinoic acid signals the direction of ocular elongation in the guinea pig eye. *Vision Res.*, **44**(7):643-653. [doi:10.1016/j.visres.2003.11.002]

Mertz, J.R., Wallman, J., 2000. Choroidal retinoic acid synthesis: a possible mediator between refractive error and compensatory eye growth. *Exp. Eye Res.*, **70**(4):519-527. [doi:10.1006/exer.1999.0813]

Morgan, I.G., 2003. The biological basis of myopic refractive error. *Clin. Exp. Optom.*, **86**(5):276-288. [doi:10.1111/j.1444-0938.2003.tb03123.x]

Norton, T.T., Siegwart, J.T.Jr., 1995. Animal models of emmetropisation: matching axial length to the focal plane. *J. Am. Optom. Assoc.*, **66**(7):405-414.

Rattner, A., Smallwood, P.M., Nathans, J., 2000. Identification and characterization of all-*trans*-retinol dehydrogenase from photoreceptor outer segments, the visual cycle enzyme that reduces all-*trans*-retinal to all-*trans*-retinol. *J. Biol. Chem.*, **275**(15):11034-11043. [doi:10.1074/jbc.275.15.11034]

Risch, N., 2000. Searching for genetic determinants in the new millennium. *Nature*, **405**(6788):847-856. [doi:10.1038/35015718]

Saari, J.C., Garwin, G.G., van Hooser, J.P., Palczewski, K., 1998. Reduction of all-*trans*-retinal limits regeneration of visual pigment in mice. *Vision Res.*, **38**(10):1325-1333. [doi:10.1016/S0042-6989(97)00198-3]

Saw, S.M., 2003. A synopsis of the prevalence rates and environmental risk factors for myopia. *Clin. Exp. Optom.*, **86**(5):289-294. [doi:10.1111/j.1444-0938.2003.tb03124.x]

Saw, S.M., Chua, W.H., Wu, H.M., Yap, E., Chia, K.S., Stone, R.A., 2000. Myopia: gene-environment interaction. *Ann. Acad. Med. Singapore*, **29**(3):290-297.

Seko, Y., Shimizu, M., Tokoro, T., 1998. Retinoic acid increases in the retina of the chick with form deprivation myopia. *Ophthalmic Res.*, **30**(6):361-367. [doi:10.1159/000055496]

Tang, W.C., Yap, M.K., Yip, S.P., 2008. A review of current approaches to identifying human genes involved in myopia. *Clin. Exp. Optom.*, **91**(1):4-22. [doi:10.1111/j.1444-0938.2007.00181.x]

Xu, L., Li, J., Cui, T., Hu, A., Fan, G., Zhang, R., Yang, H., Sun, B., Jonas, J.B., 2005. Refractive error in urban and rural adult Chinese in Beijing. *Ophthalmology*, **112**(10):1676-1683. [doi:10.1016/j.ophtha.2005.05.015]

Yoshino, Y., Funata, M., Akazawa, Y., Tokoro, T., 1997. Visual deprivation myopia with translucent and black goggles. *Ophthalmologica*, **211**(1):4-7. [doi:10.1159/000310857]

Young, T.L., 2009. Molecular genetics of human myopia: an update. *Optom. Vis. Sci.*, **86**(1):E8-E22. [doi:10.1097/OPX.0b013e3181940655]

Zhang, S., Sha, Q., Chen, H.S., Dong, J., Jiang, R., 2003. Transmission/disequilibrium test based on haplotype sharing for tightly linked markers. *Am. J. Hum. Genet.*, **73**(3):566-579. [doi:10.1086/378205]

2009 JCR of Thomson Reuters for *JZUS-B*