Muscarinic acetylcholine receptor 1 gene polymorphisms associated with high myopia

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Purpose: Numerous studies, including those using animal models of myopia development and human clinical trials, have shown that the non-selective muscarinic antagonist atropine is effective in preventing the axial elongation that leads to myopia development. Among all of the muscarinic acetylcholine receptors (mAChRs), mAChR 1 (M1) was the most effective in preventing myopic eye change. Our specific aim in this study was to examine the association between high myopia and polymorphisms within the muscarinic acetylcholine receptors 1 gene (CHRM1).

Methods: The participants comprised of a high myopia group (n=194; age range, 17–24 years) having a myopic spherical equivalent greater than 6.5 diopters (D) and a control group (n=109; age range, 17–25 years) having a myopic spherical equivalent less than 0.5 D. Genotyping was performed using an assay-on-demand allelic discrimination assay. Polymerase chain reaction (PCR) was performed using 96 well plates on a thermal cycler. The polymorphisms detected were S1 (CHRM1 rs11823728), S2 (CHRM1 rs544978), S3 (CHRM1 rs2186410), and S4 (CHRM1 rs542269).

Results: There was a significant difference in the distribution of S2 and S4 between the high myopia and control groups (p=2.4×10^-6 and 2.38×10^-8, respectively). The odds ratios of AA genotype of S2 and GG genotype of S4 were both 0.08 (95% confidence interval [CI]: 0.02–0.39 and 0.02–0.36, respectively). Logistic regression test revealed S1, S2, and S4 CHRM1 as all being significant in the development of high myopia. Moreover, the distributions of haplotype 4 (H4; C/A/A/A) differed significantly between the two groups (p=3.4×10^-5, odds ratio: 0.1, 95% CI: 0.03–0.34).

Conclusions: Our results suggest that the S2 and S4 polymorphisms of CHRM1 are associated with susceptibility for developing high myopia. S1, S2, and S4 CHRM1 had a co-operative association with high myopia.
subtype is the most dominant type to regulate scleral growth [16].

Many studies have suggested that myopia is a complex disease with multiple causes including the interaction of multiple genes with environmental stimuli [17]. Therefore, to understand myopia, it is necessary to take both genes and environment into account [17]. There is evidence supporting the premise that myopia and refractive errors are in large part genetically determined. This comes from twin studies and from studies of refractive errors in parents and their children [18,19]. A well conducted study has shown that refractive errors were much more strongly correlated in monozygotic twins than in dizygotic twins [19]. In the present study, we investigated the association of genetic polymorphisms in M1 and high myopia. The gene encoding the M1 receptor, muscarinic acetylcholine receptors 1 gene (CHRM1), is localized to 11q13. Four genetic polymorphisms were selected using HapMap, genotypes were analyzed within Haploview and tag SNPs were selected using the Tagger function. The polymorphisms were rs11823728 in the 3′UTR and rs544978, rs2186410, and rs542269 in the intron.

METHODS

Patients: Between February to November 2004, we measured the refractive error in 3,000 volunteers. All of the participants were medical students, unrelated, and Taiwan-born Han Chinese. The enrolled participants in this study were aged 16–25 years (mean age of 18±3.2 years), and the male to female ratio was 1.8:1.0. All participants had a visual acuity with distance correction of 0.2 logMAR (20/32) or better. Refractive error was measured in diopters (D) and determined by the mean spherical equivalent (SE) of the two eyes of each individual after administering one drop of cycloplegic drug (1% mydriacyl; Alcon, Berlin, Germany). Individuals with myopia greater than or equal to 6.5 D (both eyes) were included in the high myopia group, and those with myopia less than 0.5 D and hyperopia less than 1.0 D (both eyes) were included in the control group. Patients with astigmatism with myopia such as Strickler or Marfan syndromes. Clinical examinations included visual acuity, refraction error, slit-lamp examination, ocular movements, intraocular pressure, and fundoscopy. Patients with organic eye disease, a history of cataract, glaucoma, retinal disorders, or laser treatment were excluded. As with all data collection procedures, auto-refraction (Auto-refractor/auto-keratometer [ARK 700A; Nikon, Tokyo, Japan]) was conducted on both eyes by experienced optometrists who were trained and certified on the study protocols. Refractive data, sphere (s), negative cylinder (c), and axis measurements were analyzed by calculating SEM refractive error.

Genotype determinations: To select the most representative SNPs to capture the majority genetic variation, SNP genotype information was downloaded in December, 2007 from the HapMap (haplotype map of the human genome project) HCB (Han Chinese in Beijing, China) or JPN (Japanese) population from an extended 30Kb region of Chromosome 11. HapMap genotypes were analyzed within Haploview and Tag SNPs were selected using the Tagger function. Four tag SNPs were selected for each gene with r²≥0.80 to capture 80% of genotype information in the region. The average tag SNP was with r²=0.901. Genotyping was achieved using an assay-on-demand allelic discrimination assay and a detection system according to the manufacturer’s instructions (Applied Biosystems Co, Foster City, CA). The polymerase chain reaction (PCR) reaction contained 10 ng of genomic DNA, 10 μl TaqMan master mix, and 0.125 μl of 40X assay mix. PCR was performed using 96 well plates on a thermal cycler (ABI 9700; Applied Biosystems). Reaction conditions were 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Four polymorphisms met the criteria and were selected in this study. They were S1 (CHRM1 rs11823728), S2 (CHRM1 rs544978), S3 (CHRM1 rs2186410), and S4 (CHRM1, rs542269). Haplotypes were inferred from unphased genotype data using the Bayesian statistical method available in the software program, Phase 2.1. All five SNPs were analyzed with the Phase 2.1 software [20].

Statistical analysis: Genotypes were obtained by direct counting with subsequent calculation of allele frequencies. Data were analyzed using the χ² test or Fisher’s exact test, and p values were calculated using the Minitab program (Minitab Inc., San Jose, CA). A p value less than 0.05 was considered statistically significant. The odds ratios (OR) and the corresponding 95% confidence intervals (CI) were calculated for associations concerning allele and genotype. Bonferroni corrections for multiple comparisons were performed [21]. All of the detected SNPs were assessed for Hardy–Weinberg equilibrium (HWE) using the χ² test [22]. Stepwise logistic regression was used to determine if any one accounted for the effects of others. This was performed using the STATA package (version 8.2; Stata Corp., College Station, TX).

RESULTS

Volunteers enrolled in this study were on the basis of the following data: age, 16–25 years (mean, 18±3.3 years); male-to-female ratio, 1.48:1.0; mean axial length (AXL), 24.8 mm;
and mean spherical equivalent (SE), ~4.5D. There were no significant difference between the control and cases groups with respect to age, gender, cornea diopter (CD), anterior chamber depth (ACD), and LT (lens thickness; Table 1). The study group comprised of 194 patients with high myopia, and the control group consisted of 109 individuals with normal eyes. Four polymorphisms selected for this study are as described in Table 2: S1 (CHRM1 rs11823728), S2 (CHRM1 rs544978), S3 (CHRM1 rs2186410), and S4 (CHRM1 rs542269). The genotype distributions of the CHRM1 polymorphism for both the high myopia group and controls are shown in Table 2. There was significant difference in genotype and allele distribution of the S2 (CHRM1 rs544978) polymorphism between high myopia patients and normal controls \((p=2.40\times10^{-6}\) and \(0.004\), respectively) after Bonferroni correction (Table 2 and Table 3). For S2 (CHRM1 rs544978), the genotype frequencies of G/G, A/G, and A/A were 74.74%, 23.71%, and 1.55%, respectively, in the high myopia group and 98.62% and 1.38%, respectively, in the control group. There were no significant differences in the genotype and allele distributions of the S1 (CHRM1 rs11823728) polymorphism between the high myopia and control groups \((p=2.96\) and 2.176, respectively; Table 2 and Table 3). There were also no significant differences in the genotype distributions or allele frequencies of the S3 polymorphism (CHRM1 rs2186410) between the two groups. The \(p\) values of S3 (CHRM1 rs2186410) genotype and allele were \(p=0.12\) and 3.98, respectively (Table 2 and Table 3).

Moreover, we selected four haplotypes by phase software v2.1.1 (Table 4). The haplotypes distributions of the H1t (G/G/A/A), H2t (G/G/G/A), H3t (G/A/A/G), and H4t (C/A/A/A) were also compared between the two groups. The haplotype H4t (C/A/A/A) was significantly different between the two groups \((p=3.4\times10^{-3}, \text{OR: } 0.1, 95\% \text{ CI: } 0.03–0.34\). The haplotypes frequencies of H1t, H2t, and H3t did not differ significantly between the two groups \((p=0.258, 0.6, \text{and } 1.56, \text{respectively}; \text{Table } 4)\).

A logistic regression was used to determine the strength of association of the four SNPs (Table 5) with high myopia. Significant associations were found by the backward stepwise procedure for all SNPs except S3 \((p=0.9219)\). S2 had the highest degree of association in the model \((p=0.000)\) followed by S4 \((p=0.002)\) and then S1 \((p=0.031)\) (Table 5). In the test of HWE, there were departures from HWE for S1 in both the control and high myopia groups \((p=0.048 \text{ and } 0.023, \text{respectively})\) and for S2 in the control group \((p=0.012)\). The S3 and S4 polymorphisms were both in HWE.

### Table 1. Characteristics of the Study Subjects and the Partial Correlation between Spherical Equivalent and Other Ocular Components.

| Characteristics | Control (SD) n=109 | Cases (SD) n=194 | All subjects (SD) n=303 |
|-----------------|-------------------|-----------------|------------------------|
| Age, mean(SD), years | 18 (2.8) | 18 (3.5) | 18 (3.3) |
| Female, n (%) | 44 (40.4%) | 78 (40.2%) | 122 (40.3%) |
| SE, mean (SD), D | 43.7 (0.8) | 44.2 (1.8) | 43.9 (1.2) |
| CD, mean (SD), mm | 3.57 (0.24) | 3.89 (0.32) | 3.62 (0.4) |
| ACD, mean (SD), mm | 3.8 (0.64) | 4.0 (0.59) | 3.9 (0.6) |
| LT, mean (SD), mm | -8.82 (2.4) | -4.5 (2.1) | -8.82 (2.4) |
| AXE, mean, mm | 23.8 (0.78) | 26.8 (1.8) | 24.8 (1.2) |
| Female, n (%) | 44 (40.4%) | 78 (40.2%) | 122 (40.3%) |
| Age, mean(SD), years | 18 (2.8) | 18 (3.5) | 18 (3.3) |
| SE: spherical equivalent (spherical equivalent equal to the sphere plus 1/2 the cylinder) spherical equivalent, AXL: axial length, CD: cornea diopter, ACD: anterior chamber depth, LT: lens thickness.

**DISCUSSION**

Genetic studies have uncovered some polymorphisms, and separate loci are correlated with high myopia including those on chromosomes 18p and 12q [23,24] and on the genes of myocilin [25], TGF (transforming growth factor) [26], PAX6 (paired box gene 6) [27], and COL1A1 (collagen, type I, alpha 1) [26]. This indicates that there is a genetic predisposition to developing high myopia. However, no one gene can be solely...
responsible for the development of myopia, especially considering the wide variability in the prevalence of myopia across different ethnic groups [27-31]. The mechanisms by which myopia develops are further obscured by the uncertainty regarding the role of environmental factors. There is a higher prevalence of myopia among individuals with higher education levels than among members of the general population. Therefore, medical students were chosen as participants in the present study. Because both the high myopia and control groups constituted medical students, we believed that the bias associated with environmental influences would be minimal.

### Table 2. Association between genotype distributions of *CHRM1* polymorphisms and individuals with high myopia.

| Polymorphisms      | Individuals with myopia > 6.0D (%) | Individuals with myopia < 0.5D (%) | OR  | 95% CI* | p value/Cp-value       |
|--------------------|------------------------------------|------------------------------------|-----|---------|------------------------|
| rs11823728 (S1)    |                                    |                                    |     |         |                        |
| G/G                | 187 (96.3)                         | 106 (97.2)                         | 1   | -       | 0.74/2.96              |
| A/G                | 6 (3.1)                            | 3 (2.8)                            | 1.13| 0.28–4.63|                        |
| A/A                | 1 (0.5)                            | 0 (0)                              | -   | -       |                        |
| rs544978 (S2)      |                                    |                                    |     |         |                        |
| G/G                | 145 (74.7)                         | 76 (69.7)                          | 1   | -       | 5.99×10⁻⁷/2.40×10⁻⁶   |
| A/G                | 46 (23.7)                          | 14 (12.8)                          | 1.72| 0.89–3.33|                        |
| A/A                | 3 (1.5)                            | 19 (17.4)                          | 0.08| 0.02–0.29|                        |
| rs2186410 (S3)     |                                    |                                    |     |         |                        |
| A/A                | 150 (77.3)                         | 89 (81.7)                          | 1   | -       | 0.03/0.12              |
| A/G                | 24 (12.3)                          | 4 (3.7)                            | 3.56| 1.2–10.59|                        |
| G/G                | 20 (10.3)                          | 16 (14.7)                          | 0.74| 0.37–1.51|                        |
| rs542269 (S4)      |                                    |                                    |     |         |                        |
| A/A                | 149 (76.8)                         | 91 (83.5)                          | 1   | -       | 5.96×10⁻⁹/2.38×10⁻⁸   |
| A/G                | 43 (22.2)                          | 3 (2.8)                            | 8.75| 2.64–29.04|                       |
| G/G                | 2 (1.0)                            | 15 (13.8)                          | 0.08| 0.02–0.36|                        |

Genotype frequencies were compared between individuals with myopia greater than 6.00 D and less than 0.5 D. The asterisk indicates CI=confidence interval. The χ² test or Fisher's exact test was performed to obtain the p value. It was considered statistically significant if p was less than 0.05. Cp-value, p-value corrected by Bonferroni correction.

### Table 3. Association between allelic frequencies of *CHRM1* polymorphisms and individuals with high myopia.

| Alleles | Individuals with myopia > 6.0D (%) | Individuals with myopia < 0.5D (%) | OR  | 95% CI* | p value/Cp-value       |
|---------|------------------------------------|------------------------------------|-----|---------|------------------------|
| rs11823728 |                                   |                                    |     |         |                        |
| G       | 380 (97.9)                         | 215 (98.6)                         | 1   | -       | 0.544/2.176           |
| A       | 8 (2.0)                            | 3 (1.4)                            | 1.51| 0.4–5.75|                        |
| rs544978 |                                   |                                    |     |         |                        |
| G       | 336 (86.6)                         | 166 (76.1)                         | 1   | -       | 0.001/0.004           |
| A       | 52 (13.4)                          | 52 (23.9)                          | 0.49| 0.32–0.76|                       |
| rs2186410 |                                  |                                    |     |         |                        |
| A       | 324 (83.5)                         | 182 (83.5)                         | 1   | -       | 0.995/3.98            |
| G       | 64 (16.5)                          | 36 (16.5)                          | 1   | 0.64–1.56|                       |
| rs542269 |                                   |                                    |     |         |                        |
| A       | 341 (87.9)                         | 185 (84.9)                         | 1   | -       | 0.291/1.164           |
| G       | 47 (12.1)                          | 33 (15.1)                          | 0.77| 0.48–1.25|                       |

The frequencies of alleles were compared between individuals with myopia greater than 6.00 D and less than 0.5 D. The asterisk indicates CI=confidence interval. The χ² test or Fisher's exact test was performed to obtain the p value. It was considered statistically significant if p was less than 0.05. Cp-value, p-value corrected by Bonferroni correction.
Clinical trials in school-aged patients have shown the effectiveness of daily atropine administration, which reduces the progression of myopia by approximately 60% at least in the first year of treatment [27,28]. The effect of atropine was suspected by depression of neuronal activity and leads to an increase in the general release of retinal neurotransmitters [22]. Furthermore, there is evidence that pirenzepine and himbacine inhibit myopia in a dose-dependent fashion, suggesting that these drugs mediate their effects through a receptoral mechanism [13,14]. Different muscarinic antagonists have been investigated for their individual effectiveness in reducing myopia. The efficacy of atropine, pirenzepine (M1 selective antagonist), himbacine (M4/M2 selective antagonist), and the nonselective muscarinic antagonist, oxyphenonium, were all effective in the inhibition of myopic eye growth [8,9,13,14], but individual mechanisms were still unknown. Since the results of an in vitro study suggest that the M1 mAChR subtype is most likely involved in regulating scleral growth [16], we selected it as the target gene in this study.

With stepwise logistic regression, we found an association between three of the mAChR polymorphisms (S1: CHRM1 rs11823728, S2: CHRM1 rs544978, and S4: CHRM1 rs542269) and high myopia. No association was found for S3 (rs2186410). The mAChR polymorphism most strongly associated with high myopia was S2 (CHRM1 rs544978; p=0.000) followed by S4 (CHRM1 rs542269; p=0.002) and then S1 (CHRM1 rs11823728; p=0.031). This was compatible with the genotype study. The frequencies of the AA genotype of S2 and the GG genotype of S4 were significantly less in the control group than in the study group, that is, they had protective effects of subjects suffering from high myopia (OR for both: 0.08; 95% CI: 0.02–0.29 and 0.02–0.36, respectively). Allele analysis revealed that the “A” allele of S2 had a significant protective effect against the development of high myopia (p=0.004; OR: 0.49; 95% CI: 0.32–0.76). However, the distribution of the “G” allele of S4 was not significantly different between the high myopia and control groups. These data indicate that the “GG” homologous genotype of S4 protects against the development of high myopia, and this function did not establish when only one “G” allele existed. Moreover, we observed that the frequencies of the haplotype Ht4 (G/A/A/A) differed significantly between the two groups (p=3.4×10^{-5}). The Ht4 haplotype occurred more often in the control group than in the high myopia group (7.3% and 0.34%, respectively). Allele analysis revealed that the “A” allele of S4 was not significantly different between the high myopia and control groups. These data indicate that the “GG” homologous genotype of S4 protects against the development of high myopia, and this function did not establish when only one “G” allele existed. Moreover, we observed that the frequencies of the haplotype Ht4 (G/A/A/A) differed significantly between the two groups (p=3.4×10^{-5}). The Ht4 haplotype occurred more often in the control group than in the high myopia group (7.3% and 0.34%, respectively). The Ht4 haplotype might play a role in preventing the development of high myopia (OR: 0.1, 95% CI: 0.03–0.34). In conclusion, S1, S2, and S4 co-contributed to the genetic background of high myopia.
Our study design has strengths and limitations. We screened a large number of study subjects, and our study population was relatively homogenous in terms of ethnicity, geographic location, and age. The limitation was that the target polymorphisms detected in this study were in the 3’UTR and in the intron. These polymorphisms might contribute to the physiologic function insignificantly. 3’UTR may alter the RNA stability and/or its translational efficiency, and the intron may alter mRNA splicing. All these implications should be confirmed by experimental data. We minimized errors in genotyping and validated our findings by repeating the genotyping analysis several times and obtaining consistent results. Therefore, occurrence of genotyping errors in this study was kept to a minimum. In the HWE test, however, two of the SNPs considered in our study were not in HWE. The deviations from HWE might be a sign of mutation and may increase possible association with myopia. This disequilibrium became other evidence of the association of the polymorphisms in CHRM1 with high myopia. Moreover, we tried to trace the ancestry background of controls and patients. Eighty-five percent are of Minnan descendants, 5% of them are Hakka descendants, and the remaining 10% are a mixed population of Minnan, Hakka, and Canton descendants. According to the paper published by Pan et al. [32], they mentioned that the SNP profiles in the major histocompatibility complex (MHC) region (6p21.3) showed no significant difference among Minnan descendants, Hakka descendants and mixed population of Minnan and Hakka descendants, which indicate the homogeneity of the population. Thus population stratification should not produce in this study [32].

In the future, further studies are needed to confirm a role of CHRM1 in high myopia. Indeed, we intend to replicate the present study in a separate population group such as a non-student sample. We also plan to analyze the genotypes of the parents of the highly myopic participants of the current study to minimize bias. Further, we intend to analyze data across the spectrum of refractive error as obtained from all 3,000 participants screened to further understand the relationship between CHRM1 and the severity of myopia. Complex diseases such as diabetes, cancer, asthma, and arthritis are probably caused by subtle changes in multiple genes combined with environmental and lifestyle factors. Myopia is considered a complex and multigenic disease involving several overlapping signaling pathways, each mediated by a group of distinct genetic profiles. Therefore, studying genetic polymorphisms that are related to the mechanisms of myopia can help to further clarify the relationship between genetics and myopia. The discovery of associations between myopia and various genetic markers has helped increase the knowledge needed for the prevention and treatment of myopia. As with CHRM1, CHRM4 encodes a receptor important in the progression of myopia and also needs to be investigated.

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REFERENCES
1. Au Eong KG, Tay TH, Lim MK. Race, culture and Myopia in 110,236 young Singaporean males. Singapore Med J 1993; 34:29-32. [PMID: 8266124]
2. Mavracanas TA, Mandalos A, Peios D, Goliás V, Megalóu K, Gregoriadou A, Delidou K, Katsougiamopoulos B. Prevalence of myopia in a sample of Greek students. Acta Ophthalmol Scand 2000; 78:656-9. [PMID: 11167226]
3. Rose K, Smith W, Morgan I, Mitchell P. The increasing prevalence of myopia: implications for Australia. Clin Experiment Ophthalmol 2001; 29:116-20. [PMID: 11446448]
4. Seet B, Wong TY, Tan DT, Saw SM, Balakrishnan V, Lee LK, Lim AS. Myopia in Singapore: taking a public health approach. Br J Ophthalmol 2001; 85:521-6. [PMID: 11316705]
5. McKanna JA, Casagrande VA. Atropine affects lid suture myopia development. Doc Ophthalmol Proc Ser 1981; 28:187-92.
6. Tigges M, Iuvone PM, Fernandes A, Sugrue MF, Mallorga PJ, Laties AM, Stone RA. Effects of muscarinic cholinergic receptor antagonists on postnatal eye growth of rhesus monkeys. Optom Vis Sci 1999; 76:397-407. [PMID: 10416935]
7. McBrien NA, Moghaddam HO, Reeder AP. Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism. Invest Ophthalmol Vis Sci 1993; 34:205-15. [PMID: 8425826]
8. Stone RA, Lin T, Laties AM. Muscarinic antagonist effects on experimental chick myopia. Exp Eye Res 1991; 52:755-8. [PMID: 1855549]
9. Luft WA, Ming Y, Stell WK. Variable effects of previously untested muscarinic receptor antagonists on experimental myopia. Invest Ophthalmol Vis Sci 2003; 44:1330-8. [PMID: 12601066]
10. Nathanson NM. Molecular properties of the muscarinic acetylcholine receptor. Annu Rev Neurosci 1987; 10:195-236. [PMID: 2436543]
11. Caulfield MP, Birdsell NJ. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 1998; 50:279-90. [PMID: 9647869]
12. Peralta EG, Ashkenazi A, Winslow JW, Smith DH, Ramachandran J, Capon DJ. Distinct primary structures, ligand-binding properties and tissue- specific expression of four human muscarinic acetylcholine receptors. EMBO J 1987; 6:293-9. [PMID: 3440953]
13. Leech EM, Cottriall CL, McBrien NA. Pirenzepine prevents form deprivation myopia in a dose dependent manner. Ophthalmic Physiol Opt 1995; 15:351-6. [PMID: 8524553]
14. Cottriall CL, Truong HT, McBrien NA. Inhibition of myopia development in chicks using himbacine: a role for M4 receptors? Neuroreport 2001; 12:2453-6. [PMID: 11496128]
15. Cottriall CL, McBrien NA. The M1 muscarinic antagonist pirenzepine reduces myopia and eye enlargement in the tree
16. Lind GJ, Chew SJ, Marzani D, Wallman J. Muscarinic Acetylcholine Receptor Antagonists Inhibit Chick Scleral Chondrocytes. Invest Ophthalmol Vis Sci 1998; 39:2217-31. [PMID: 9804129]

17. Feldkamper M, Schaeffel F. Interactions of genes and environment in myopia. Dev Ophthalmol 2003; 37:34-49. [PMID: 12876828]

18. Lyhne N, Sjolie AK, Kyvik KO, Green A. 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 2001; 85:1470-6. [PMID: 11734523]

19. Hammond CJ, Snieder H, Gilbert CE, Spector TD. Genes and environment in refractive error: the twin eye study. Invest Ophthalmol Vis Sci 2001; 42:1232-6. [PMID: 11328732]

20. Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 2005; 76:449-62. [PMID: 15700229]

21. Pertile KK, Schäche M, Islam FM, Chen CY, Dirani M, Mitchell P, Baird PN. Assessment of TGIF as a candidate gene for myopia. Invest Ophthalmol Vis Sci 2008; 49:49-54. [PMID: 18172074]

22. Falconer DS, Mackay TFC. Introduction to quantitative genetics. 4th ed. Essex (UK): Longman; 1996.

23. Young TL, Ronan SM, Alvear AB, Wildenberg SC, Getting WS, Atwood LD, Wilkin DJ, King RA. A second locus for familial high myopia maps to chromosome 12q. Am J Hum Genet 1998; 63:1419-24. [PMID: 9792869]

24. Farbrother JE, Kirov G, Owen MJ, Pong-Wong R, Haley CS, Guggenheim JA. Linkage Analysis of the Genetic Loci for High Myopia on 18p, 12q, and 17q in 51 U.K. Families. Invest Ophthalmol Vis Sci 2004; 45:2879-85. [PMID: 15326098]

25. Tang WC, Yip SP, Lo KK, Ng PW, Choi PS, Lee SY, Yap MK. Linkage and association of myocilin (MYOC) polymorphisms with high myopia in a Chinese population. Mol Vis 2007; 13:534-44. [PMID: 17438518]

26. Inamori Y, Ota M, Inoko H, Okada E, Nishizaki R, Shiota T, Mok J, Oka A, Ohno S, Mizuki N. The COL1A1 gene and high myopia susceptibility in Japanese. Hum Genet 2007; 122:151-7. [PMID: 17557158]

27. Bedrossian RH. The effect of atropine on myopia. Ophthalmology 1979; 86:713-9. [PMID: 545205]

28. Lee JJ, Fang PC, Yang IH, Chen CH, Lin PW, Lin SA, Kuo HK, Wu PC. Prevention of myopia progression with 0.05% atropine solution. J Ocul Pharmacol Ther 2006; 22:41-6. [PMID: 16503774]

29. Luft WA, Ming Y, Stell WK. Variable effects of previously untested muscarinic receptor antagonists on experimental myopia. Invest Ophthalmol Vis Sci 2003; 44:1330-8. [PMID: 12601066]

30. Schwahn HN, Kaynak H, Schaeffel F. Effects of atropine on refractive development, dopamine release, and slow retinal potentials in the chick. Vis Neurosci 2000; 17:165-76. [PMID: 10824671]

31. Teikari J, O'Donnell J, Kaprio J, Koskenvuo M. Genetic and environmental effects on oculometric traits. Optom Vis Sci 1989; 66:594-9. [PMID: 2797713]

32. Pan WH, Fann CS, Wu JY, Hung YT, Ho MS, Tai TH, Chen YJ, Liao CJ, Yang ML, Cheng AT, Chen YT. Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. Hum Hered 2006; 61:27-30. [PMID: 16534213]