Education influences the association between genetic variants and refractive error: a meta-analysis of five Singapore studies

Qiao Fan1, Robert Wojciechowski5,6, M. Kamran Ikram1,2,7,8,9,10, Ching-Yu Cheng1,2,7, Peng Chen1, Xin Zhou1, Chen-Wei Pan1,7, Chiea-Chuen Khor1,9,11, E-Shyong Tai3, Tin Aung1,2,7, Tien-Yin Wong1,2,7, Yik-Ying Teo1,4,11 and Seang-Mei Saw1,2,7,10,*

1Saw Swee Hock School of Public Health, 2Department of Ophthalmology, 3Department of Medicine and 4Department of Statistics and Applied Probability, National University of Singapore, Singapore, Singapore 5Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA 6Department of Ophthalmology, Johns Hopkins School of Medicine, Baltimore, MD, USA 7Singapore Eye Research Institute, Singapore National Eye Centre, Singapore, Singapore 8Memory Aging and Cognition Centre, National University Health System, Singapore, Singapore 9Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands 10Duke-National University of Singapore Graduate Medical School, Singapore, Singapore 11Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore

Received May 20, 2013; Revised and Accepted September 3, 2013

Refractive error is a complex ocular trait governed by both genetic and environmental factors and possibly their interplay. Thus far, data on the interaction between genetic variants and environmental risk factors for refractive errors are largely lacking. By using findings from recent genome-wide association studies, we investigated whether the main environmental factor, education, modifies the effect of 40 single nucleotide polymorphisms on refractive error among 8461 adults from five studies including ethnic Chinese, Malay and Indian residents of Singapore. Three genetic loci SHISA6-DNAH9, GJD2 and ZMAT4-SFRP1 exhibited a strong association with myopic refractive error in individuals with higher secondary or university education (SHISA6-DNAH9: rs2969180 A allele, β = 0.33 D, P = 3.6 × 10^-6; GJD2: rs524952 A allele, β = 0.31 D, P = 1.68 × 10^-5; ZMAT4-SFRP1: rs2137277 A allele, β = 0.47 D, P = 1.68 × 10^-4), whereas the association at these loci was non-significant or of borderline significance in those with lower secondary education or below (P for interaction: 3.82 × 10^-4 to 4.78 × 10^-4). The evidence for interaction was strengthened when combining the genetic effects of these three loci (P for interaction = 4.40 × 10^-8), and significant interactions with education were also observed for axial length and myopia. Our study shows that low level of education may attenuate the effect of risk alleles on myopia. These findings further underline the role of gene–environment interactions in the pathophysiology of myopia.

INTRODUCTION

Myopia is the most common cause of visual impairment worldwide (1, 2). The global prevalence of myopia has been rising steadily over the past few decades, especially amongst urban East and Southeast Asian populations (3, 4). In Singapore, for example, the prevalence of myopia in young adults increased from an estimated 26% in the late 1970s to up to 80% by the middle 1990s (5, 6). This rapid rise of myopia prevalence in Asia may be attributed to changes in environmental factors, such as the increasing intensity of education, and possibly to gene and environment (G × E) interaction (7).

Recent genome-wide association studies (GWAS) have greatly advanced our understanding of the genetic architecture...
of myopia (8–14), with two large GWAS reporting >30, partially overlapping, genetic loci associated with refractive phenotypes (9, 10). Many of these recently identified genes are linked to known visually triggered signaling pathways in the development of human myopia, such as neuronal signaling in the retina and scleral extracellular matrix remodeling (i.e. GJD2, RASGRF1, BMP2 and BMP3), while others point to novel pathways involved in neuronal development and ion transport (i.e. KCNJ2, KCNMA1 and CD55).

The most solid evidence for environmental influences in adults studies on myopia is education, with a large number of studies reporting a strong correlation between the occurrence of myopia and a higher level of education (4, 5, 15–18). In Asian populations, persons aged 40 years or older with university education are nearly four times more likely to have myopia compared with those with primary and no formal education (18). Education level has largely been considered a surrogate of lifetime cumulative near work activities such as reading and writing, although other lifestyle factors including outdoor play patterns may be involved in myopia development (19–21). So far, most studies have investigated environmental and genetic factors for myopia separately. Whether there are interactions between genetic markers and environmental exposures on the risk of myopia remains to be elucidated.

In the present study, we examined whether education modifies the association between recently discovered single nucleotide polymorphisms (SNPs) and refractive error using data from five population-based cohorts in Singapore.

RESULTS

Table 1 shows the baseline characteristics of subjects included from five Singapore cohorts. After applying stringent quality-control procedures and retaining those with valid phenotype data, the present analyses were based on a total of 8461 participants aged 25 years and older, including Chinese from Singapore Chinese Eye Study (SCES) (n = 1710), SP2 (n = 1665) and Study on Strabismus, Amblyopia and Refractive Error in Singapore Children (STARS) (n = 741), as well as Malays from Singapore Malay Eye Study (SiMES) (n = 2257) and Indians from Singapore Indian Eye Study (SINDI) (n = 2088). Across all studies, individuals are more myopic in the higher education stratum (mean spherical equivalent (SE) = −2.3 D; SD = 3.0 D), compared with the lower education stratum (mean SE = −0.7 D; SD = 2.5 D). Chinese subjects, especially the young adults from STARS (mean SE = −2.8 D; SD = 2.85 D), tended to be more myopic compared with Malay (mean SE = −0.03 D; SD = 1.81 D) and Indian participants (mean SE = 0.04 D; SD = 2.07 D).

To test the hypothesis that education level could influence the genetic effect of specific variants on SE, we evaluated 40 candidate SNPs recently identified from three GWAS (see Supplementary Material, Table S1), where the current study sample was partially included in the two of three: GWAS on SE (9) and ocular axial length (22) by the Interactional Consortium for Refractive Error and Myopia (CREAM). In the current meta-analysis, 19 out of the 40 SNPs were associated with SE within one of the education strata. The risk alleles of these 19 SNPs were positively associated with a refraction shift towards myopia, consistent with the direction of the main association effect on SE in previous GWAS (Table 2; Supplementary Material, Table S1). Seven SNPs showed evidence of interaction at a P<0.05, representing five loci: SHISA6-DNAH9, GJD2, ZMAT4-SFRP1, RBFOX1 and PRSS56. These SNPs that showed an interaction had larger main effects on myopic refractive error than those that did not display an interaction (average β = −0.12 versus β = 0.07, respectively; Supplementary Material, Fig. S1 and Table S1).

The associations at SNPs rs2969180 in SHISA6-DNAH9, rs524952 in GJD2 and rs2137277 in ZMAT4-SFRP on SE were highly significant in the higher education stratum (rs2969180 A allele, β = −0.33 ± 0.07 D, P = 3.60 × 10⁻⁶; rs524952 A allele, β = −0.31 ± 0.07 D, P = 1.68 × 10⁻⁵; rs2137277 A allele, β = −0.47 ± 0.12 D, P = 1.46 × 10⁻⁴, Table 2), while the signals were greatly attenuated in the lower education stratum (all P ≥ 0.041). The strongest predictor in the main model was level of education, as higher educational level was associated with a significant shift towards myopia (β = −1.27, P = 6.00 × 10⁻⁶ for all models; Supplementary Material, Table S2). SNPs at three loci also showed significant main effects on SE (SNP main effect: rs2969180 A allele, β = −0.11 ± 0.03 D, P = 8.47 × 10⁻⁴; rs524952 A allele, β = −0.12 ± 0.03 D, P = 1.53 × 10⁻⁴; rs2137277 A allele, β = −0.13 ± 0.06 D, P = 1.94 × 10⁻³). Among these genetic variants, SNP rs2969180 in SHISA6-DNAH9 exhibited the most significant interaction with education (βINT for interaction = −0.28 ± 0.08 D, PINT for interaction: 4.78 × 10⁻⁴). SNPs rs524952 in GJD2 and rs2137277 in ZMAT4-SFRP showed nominal significance (βINT = −0.23 ± 0.08 D, PINT = 0.0038; βINT = −0.42 ± 0.14 D, PINT = 0.0021; respectively).

Table 1. Characteristics of participants in five Singapore cohorts

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Male (%)</th>
<th>Age (years)</th>
<th>SE (D)</th>
<th>Lower education group</th>
<th>Male (%)</th>
<th>Age (years)</th>
<th>SE (D)</th>
<th>Higher education group</th>
<th>Male (%)</th>
<th>Age (years)</th>
<th>SE (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCES</td>
<td>1710</td>
<td>51.6</td>
<td>57.5 (9.0)</td>
<td>−0.72 (2.69)</td>
<td>1346</td>
<td>49.2</td>
<td>58.4 (9.1)</td>
<td>−0.3 (2.2)</td>
<td>364</td>
<td>60.4</td>
<td>54.4 (7.7)</td>
<td>−2.4 (3.1)</td>
</tr>
<tr>
<td>SP2</td>
<td>1665</td>
<td>40.4</td>
<td>47.6 (10.9)</td>
<td>−1.62 (2.87)</td>
<td>957</td>
<td>34.7</td>
<td>51.2 (10.0)</td>
<td>−0.7 (2.4)</td>
<td>708</td>
<td>48.0</td>
<td>42.8 (9.9)</td>
<td>−2.9 (3.0)</td>
</tr>
<tr>
<td>STARS</td>
<td>741</td>
<td>52.4</td>
<td>38.5 (5.2)</td>
<td>−2.80 (2.85)</td>
<td>328</td>
<td>50.9</td>
<td>39.5 (5.8)</td>
<td>−1.8 (2.4)</td>
<td>413</td>
<td>53.5</td>
<td>37.6 (4.5)</td>
<td>−3.6 (2.9)</td>
</tr>
<tr>
<td>SiMES</td>
<td>2257</td>
<td>49.1</td>
<td>58.0 (10.8)</td>
<td>−0.03 (1.81)</td>
<td>2105</td>
<td>47.8</td>
<td>58.6 (10.8)</td>
<td>0.02 (1.8)</td>
<td>152</td>
<td>67.1</td>
<td>49.1 (6.6)</td>
<td>−0.81 (2.3)</td>
</tr>
<tr>
<td>SINDI</td>
<td>2088</td>
<td>51.5</td>
<td>55.8 (8.8)</td>
<td>−0.04 (2.07)</td>
<td>1618</td>
<td>47.1</td>
<td>56.6 (8.9)</td>
<td>0.23 (1.9)</td>
<td>470</td>
<td>66.6</td>
<td>53.2 (8.2)</td>
<td>−0.63 (2.4)</td>
</tr>
<tr>
<td>Total</td>
<td>8461</td>
<td>53.6</td>
<td>53.6 (11.3)</td>
<td>−0.71 (2.54)</td>
<td>6354</td>
<td>46.1</td>
<td>56.0 (10.7)</td>
<td>−0.18 (2.1)</td>
<td>2107</td>
<td>56.7</td>
<td>46.5 (10.3)</td>
<td>−2.3 (3.0)</td>
</tr>
</tbody>
</table>

SE, spherical equivalent; SCES, Singapore Chinese Eye Study; SP2, Singapore Prospective Study Program; STARS, Study on Strabismus, Amblyopia and Refractive Error in Singapore Children; SiMES, Singapore Malay Eye Study; and SINDI, Singapore Indian Eye Study.

aData presented are mean (s.e.).
Table 2. SNPs with suggestive association with SE in either education group (P < 0.05) and SNP × education interactions in relation to SE from a meta-analysis of five Singapore studies

<table>
<thead>
<tr>
<th>Ref</th>
<th>Gene</th>
<th>SNP</th>
<th>Chr</th>
<th>BP</th>
<th>Allele</th>
<th>Lower education group (n = 6354)</th>
<th>Higher education group (n = 2107)</th>
<th>SNP × education (n = 8461)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B  s.e.  P</td>
<td>β  s.e.  P</td>
<td>β&lt;sub&gt;INT&lt;/sub&gt;  s.e.  P&lt;sub&gt;INT&lt;/sub&gt;  P&lt;sub&gt;HET&lt;/sub&gt;</td>
</tr>
<tr>
<td>(9)</td>
<td>CD55</td>
<td>rs1652333</td>
<td>1</td>
<td>20740460</td>
<td>G/A</td>
<td>−0.12 0.04 0.001</td>
<td>−0.04 0.07 0.549</td>
<td>0.08 0.08 0.333</td>
</tr>
<tr>
<td>(11)</td>
<td>ZC3H11B</td>
<td>rs994767</td>
<td>1</td>
<td>21775432</td>
<td>G/A</td>
<td>−0.10 0.04 0.017</td>
<td>−0.08 0.08 0.336</td>
<td>0.02 0.09 0.804</td>
</tr>
<tr>
<td>(9)</td>
<td>PRSS56</td>
<td>rs1656404</td>
<td>2</td>
<td>233379941</td>
<td>A/G</td>
<td>−0.12 0.07 0.094</td>
<td>−0.32 0.16 0.050</td>
<td>−0.19 0.18 0.276</td>
</tr>
<tr>
<td>(10)</td>
<td>CHRNG</td>
<td>rs1500904</td>
<td>2</td>
<td>233835396</td>
<td>G/A</td>
<td>−0.07 0.05 0.188</td>
<td>−0.30 0.11 0.0056</td>
<td>−0.23 0.12 0.049</td>
</tr>
<tr>
<td>(9)</td>
<td>LOC100506035</td>
<td>rs9307551</td>
<td>4</td>
<td>80530671</td>
<td>A/C</td>
<td>−0.08 0.04 0.23</td>
<td>−0.14 0.07 0.050</td>
<td>−0.05 0.08 0.501</td>
</tr>
<tr>
<td>(9)</td>
<td>KCNQ5</td>
<td>rs7744813</td>
<td>6</td>
<td>73643289</td>
<td>A/C</td>
<td>−0.11 0.04 0.013</td>
<td>−0.12 0.09 0.163</td>
<td>−0.01 0.10 0.883</td>
</tr>
<tr>
<td>(9)</td>
<td>ZMAT4-SFRP1</td>
<td>rs7829127</td>
<td>8</td>
<td>40726394</td>
<td>A/G</td>
<td>−0.07 0.06 0.251</td>
<td>−0.34 0.12 0.0029</td>
<td>−0.28 0.13 0.033</td>
</tr>
<tr>
<td>(10)</td>
<td>TOX</td>
<td>rs2137277</td>
<td>8</td>
<td>40734662</td>
<td>A/G</td>
<td>−0.04 0.06 0.487</td>
<td>−0.47 0.12 1.46 × 10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>−0.42 0.14 0.0021</td>
</tr>
<tr>
<td>(9)</td>
<td>BICC1</td>
<td>rs7084402</td>
<td>10</td>
<td>60265404</td>
<td>G/T</td>
<td>−0.12 0.04 0.002</td>
<td>−0.07 0.08 0.370</td>
<td>0.05 0.09 0.556</td>
</tr>
<tr>
<td>(10)</td>
<td>LRRC4C</td>
<td>rs1381566</td>
<td>11</td>
<td>41014607</td>
<td>G/T</td>
<td>−0.04 0.05 0.428</td>
<td>−0.19 0.09 0.048</td>
<td>−0.14 0.10 0.162</td>
</tr>
<tr>
<td>(9)</td>
<td>RDH5</td>
<td>rs3138144</td>
<td>12</td>
<td>56114769</td>
<td>G/C</td>
<td>−0.19 0.06 0.001</td>
<td>−0.01 0.11 0.925</td>
<td>0.18 0.13 0.140</td>
</tr>
<tr>
<td>(9)</td>
<td>PTPRR</td>
<td>rs1229663</td>
<td>12</td>
<td>71249996</td>
<td>A/G</td>
<td>−0.08 0.04 0.036</td>
<td>−0.12 0.08 0.116</td>
<td>−0.04 0.09 0.663</td>
</tr>
<tr>
<td>(9, 10)</td>
<td>GJD2</td>
<td>rs524952</td>
<td>15</td>
<td>35005886</td>
<td>A/T</td>
<td>−0.08 0.04 0.041</td>
<td>−0.31 0.07 1.68 × 10&lt;sup&gt;−5&lt;/sup&gt;</td>
<td>−0.23 0.08 0.0038</td>
</tr>
<tr>
<td>(9, 10)</td>
<td>RBFQ1X</td>
<td>rs1764524</td>
<td>16</td>
<td>7465983</td>
<td>C/G</td>
<td>−0.07 0.06 0.255</td>
<td>−0.37 0.12 0.0015</td>
<td>−0.29 0.13 0.026</td>
</tr>
<tr>
<td>(9)</td>
<td>SHISA6-DNAH9</td>
<td>rs2908972</td>
<td>17</td>
<td>11407259</td>
<td>A/T</td>
<td>−0.06 0.04 0.087</td>
<td>−0.30 0.07 3.45 × 10&lt;sup&gt;−5&lt;/sup&gt;</td>
<td>−0.24 0.08 0.0035</td>
</tr>
<tr>
<td>(10)</td>
<td>SHISA6-DNAH9</td>
<td>rs2969180</td>
<td>17</td>
<td>11407901</td>
<td>A/G</td>
<td>−0.05 0.04 0.165</td>
<td>−0.33 0.07 3.60 × 10&lt;sup&gt;−6&lt;/sup&gt;</td>
<td>−0.28 0.08 4.78 × 10&lt;sup&gt;−4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P, P-value of SNP effect on SE in each education stratum; P<sub>INT</sub>, P-value for interaction between SNP and education on spherical equivalent; β, beta regression coefficient of the SNP effect in each education stratum; β<sub>INT</sub>, interaction beta coefficient, representing the differences in spherical equivalent per risk allele at each SNP comparing individuals in the higher education group to the lower education group; s.e., standard error; P<sub>HET</sub>, P-value for heterogeneity P of SNP effect between five study cohorts. Allele is listed as risk allele/other allele reported from prior GWAS. Genome NCBI build 37; Three genetic loci (in bold) with P<sub>INT</sub> < 0.01.*
The magnitude of the effect on myopic refraction was at least 2.8-fold greater per risk allele in the higher education group compared with that in the lower education group. For these three loci, there was no evidence of heterogeneity in the interaction effects across studies ($Q$ test: $P$-value for heterogeneity $\geq 0.377$, $I^2 \leq 5\%$; Fig. 1).

Next, we assessed whether education modifies the combined genetic effects of carrying increasing number of risk alleles at these three loci on refractive error. The strong interaction with education on SE in the same direction was observed for combined genetic effects ($P_{\text{INT}} = 4.40 \times 10^{-18}$, Fig. 2A; Supplementary Material, Table S3). Each additional copy of risk alleles of either $SHISA6$-$DNAH9$, $GJD2$ or $ZMAT4$-$SFRP1$ was associated with a $-0.35$ D shift towards myopia in the higher education group ($P = 2.01 \times 10^{-13}$), while the corresponding figure was a $-0.06$ D myopic shift in the lower education group ($P = 0.014$). Accounting for the interaction term of the combined genetic effects with education led to an average of a 1.5-fold increase in the explained variance in SE (Chinese: 0.7% versus 0.46%; Malays: 0.1% versus 0.06% and Indians: 1.0% versus 0.6%) compared with a model with the main effects alone. Consistent with the analysis of SE, a similar effect was observed on axial length, which is one of the most important ocular biometric determinant of refraction ($P_{\text{INT}} = 6.00 \times 10^{-5}$; Fig. 2B). Furthermore, we found that subjects with increasing number of risk alleles at these three loci were more likely to have myopia within the higher education stratum compared with the lower education stratum ($P_{\text{INT}} = 0.012$ and 0.0013 for mild or moderate myopia, respectively; Fig. 2C and D). For individuals carrying one additional copy of risk allele at three loci, the odds ratios (ORs) for mild myopia were 1.24 (95% CI: 1.12–1.38, $P = 6.23 \times 10^{-5}$), for moderate myopia 1.35 (95% CI: 1.20–1.52, $P = 1.05 \times 10^{-6}$) and 1.40 for high myopia (95% CI: 1.19–1.65, $P = 4.96 \times 10^{-5}$; Supplementary Material, Table S3) in the higher education group, whereas significantly reduced ORs for mild, moderate or high myopia were observed (OR = 1.06, 95% CI: 1.00–1.12, $P = 0.034$; OR = 1.06, 95% CI: 0.98–1.15, $P = 0.15$; OR = 1.14, 95% CI: 0.99–1.32, $P = 0.79$, respectively) in the lower education group.

**DISCUSSION**

In our study, the most promising interactions between SNPs and education on refractive error were detected at three genetic loci ($SHISA6$-$DNAH9$, $GJD2$, $ZMAT4$-$SFRP1$). Similar interaction patterns were also observed for axial length and myopia. Among these three genetic loci, $GJD2$ was identified as the first robust myopia-susceptibility gene. It was first discovered in a GWAS of European participants at the genetic marker rs634990 (23), followed by replication in several studies.

![Figure 1](https://example.com/figure1.png)

*Figure 1.* Forest plot of the effects of the interaction between genetic variants at $SHISA6$-$DNAH9$, $GJD2$ and $ZMAT4$-$SFRP1$ and education on SE in a meta-analysis of five Singapore studies. The interaction beta represents the difference in diopters of SE per risk allele of rs2969180, rs524952 or rs2137227 comparing individuals within the higher education group to the lower education group. Between-study heterogeneity was quantified by the $I^2$ value, and low heterogeneity was defined as an $I^2$ value of 0–25%. SCES, Singapore Chinese Eye Study; SP2, Singapore Prospective Study Program; STARS, Study on Strabismus, Amblyopia and Refractive Error in Singapore Children; SiMES, Singapore Malay Eye Study; and SINDI, Singapore Indian Eye Study.
Both the CREAM and 23andMe GWAS recently independently pinpointed SNP rs524952 (9, 10), in strong LD with previously identified SNP rs634990 as being involved in the variation of refractive error. The \textit{GJD2} gene encodes the connexin protein and \textit{GJD2} knockout mice show defects in retinal photoreceptors, implying an important role in the retinal signal transmission (23). \textit{SHISA6-DNAH9} and \textit{ZMAT4-SFRP1} are relatively novel loci reported in two recent large GWAS (9, 10). \textit{SHISA6} (shisa, homolog 6) has no known link to vision, but the top SNP rs2969180 also lies 94 kb upstream of \textit{DNAH9}, the nearest gene to \textit{SHISA6}. The \textit{DNAH9} gene encodes an axonemal dynein heavy chain 9, a component to intraflagellar transport (IFT) responsible for cilia motility of the photoreceptor (27). IFT in the connecting cilium is critical for the survival of photoreceptors cells, and consequently defects of IFT result in retinal degeneration, such as retinitis pigmentosa (28, 29). We further examined interactions between education and genetic variants in the gene \textit{DNAH9} and found a cluster of SNPs with significant signals (SNP rs4792159, \( P \) for interaction = \( 6.37 \times 10^{-5} \); Supplementary Material, Fig. S2). The \textit{ZMAT4} gene belongs to the zinc finger family, consistent with recent reports of an involvement of several zinc finger proteins in myopia development (11, 30); a neighboring gene \textit{SFRP1} (secreted frizzled-related protein 1) is implicated in the growth of photoreceptors cells (31) and the Wnt signaling (32). These genes in or near these loci are linked to signal transduction processes in the retina and hence are likely to be involved in the development of refractive errors.

Education level is thought to reflect the accumulated effect of near work tasks during the first three decades of life (17, 33). Near work, such as reading and writing, has long been considered an important environmental risk factor for the development of myopia (20, 34). Other putative environmental risk factors (e.g. time spent outdoors) might mitigate this process as well (35), whereas the relationship between education level and outdoor activity warrants investigation. One study in Singapore found that intensive near work activity, measured by the increased number of books read per week, had a greater effect on the risk of having myopia in children of myopic parents compared with those with no myopic parents (36). When viewing near objects, eye generates extra optical power to focus the target on the retina to maintain a clear visual image. Across animal models, negative-lens-induced defocus as in near work tasks is known to promote the excessive eye elongation, resulting from the loss of protoglycans and sclera thinning through the turnover of extracellular matrix materials (37). Five genetic loci that showed interactions with education (\( P \) for interaction < 0.05; Table 2) can be placed within the biological context of the visually evoked signal transduction that begins in the retina and mediates sclera remodeling (9, 10). Three genes, \textit{DNAH9}, \textit{SFRP1} and \textit{GJD2}, play a pivotal role in the retina by maintaining photoreceptor function (27), determining photoreceptor cell degeneration (38) or enabling molecular transduction between these neuronal cells (23). An altered morphology of the photoreceptors, such as photoreceptor elongation (39) and reduced photoreceptor cells density associated with retinal stretching and retinal imaging, has been reported in myopic eyes (40). A recent study also showed that a mutation in opsin gene is responsible for both photosensitive cone dysfunction and vision defect in families with Bornholm Eye Disease and myopia (41). These findings show that genes modulating visual
imaging and neurotransmission in the process of retinal cell development play an important role in the ocular growth.

The myopia risk-increasing alleles of rs524952 in GJD2, rs2969180 in SHISA6-DNAH9 and rs2137227 in ZMAT6-SFRP1 are very common, with 60% of our subjects carrying at least three risk alleles at these loci. However, a strong association between these risk alleles and myopia was observed only in individuals with a high level of education (25% of the total sample). This conveys an important message that the influence of genes on the susceptibility to myopia could be highly dependent on which environmental factors are present. Moreover, the risk alleles of the majority of susceptibility loci (13 of 19 SNPs; Table 2) in our study had less or no influence on myopic shift in the lower education category compared with the higher education category. Thus, for certain risk allele carriers, the hereditary predisposition to myopia could be latent or suppressed, if less exposed to the myopiogenic environment associated with education level. Conversely, it is possible that increased exposure to a protective environment, such as outdoor activity, may differentially affect individuals who are more genetically predisposed to develop myopia compared with those at lower risk.

The key determinants of statistical power in the G × E interaction studies are the sample size, the magnitude of the interaction, the allele frequency, the strength of the association for the main effect and the significance threshold. The phenotypic variation accounted for by the interaction term at the individual locus is modest in our data, i.e. average $R^2$ of 0.16% for the most significant SNP rs2969180. Using the same minor allele frequency and magnitude of the interaction term and the main effect of SNP rs2969180 reported in this study, we calculated a post hoc statistical power of 79% to detect the reported interaction effect size at an alpha-level of $1.25 \times 10^{-3}$ under additive inheritance model (QUANTO (42)). To detect half of the original interaction effect size of this locus, a study of ~30 000 participants is required to have desired statistical power of 80% at the same level of the statistical rigor. Notably, the top SNPs that showed an interaction have the effect sizes larger than those that did not display an interaction (Supplementary Material, Fig. S1). Our study might not be sufficiently powered to examine G × E interactions for those SNPs with smaller effect sizes. A large-scale study will be beneficial in mapping the details of the interplay between genetics and environmental factors in this scenario.

Finally, several limitations of our study warrant some attention. First, our selection of SNPs relied on a subset of previously identified SNPs achieving genome-wide significance and several SNPs were further excluded after quality-control procedures. Thus, a number of potentially interesting loci may have been missed due to such stringent criteria for SNP inclusion. Second, the transferability of the GWAS index SNPs is influenced by many factors across different populations. Could there be other genetic loci interacting with education that failed to be detected in this study, possibly due to the linkage disequilibrium variation and allelic heterogeneity between our samples and previous GWAS cohorts. Although our study contains the largest Asian sample collection available, a much larger sample size will be required to examine the source of such interpopulation heterogeneity (43). In addition, we dichotomized education levels into two categories, higher education (high school, polytechnic or university) versus lower education (lower secondary education or below); this is somewhat arbitrary and may not adequately reflect the true underlying risk factors for myopia. The duration of education (i.e. years of formal education) would likely be a better proxy variable, but was not available in the current data.

In summary, we found that education level influenced the association between recently discovered three genetic loci (SHISA6-DNAH9, GJD2 and ZMAT6-SFRP1) and refractive error. The genetic effects of these loci on myopic refraction were significantly larger within subjects who had a higher level of education compared with a lower education level. Our findings highlight the important role of G × E interactions in the development of myopia.

**MATERIALS AND METHODS**

**Study population**

Study participants of Chinese, Malay and Indian origin were drawn from five Singapore cohorts: (i) the SCES, a population-based, cross-sectional survey on eye diseases in Chinese aged 40–80 years (n = 2226) with random sampling drawn from the residents in the Southwestern part of Singapore (18, 44); (ii) the SiMES, a population-based, random sampling survey of eye diseases among elderly Malays residing in the Southwestern part of Singapore (n = 3280) (45, 46); (iii) the SINDI, a population-based survey of eye diseases in a sample of elderly Indians in the Southwestern part of Singapore (n = 3400) (44); (iv) the Singapore Prospective Study Program (SP2), surveying a sample of individuals aged 25 years or older from the entire Singapore population (n = 2867) (47) and (v) the STARS, a population-based survey of Chinese families with children residing in the Southwestern and Western region of Singapore (n = 870; Chinese parents aged 22–58) (48). Individuals <25 years old or with anisometropia >5 D were excluded, as well as those who had undergone cataract surgery, laser refractive procedures or other intra-ocular procedures that could alter refraction.

All studies adhered to the tenets of the Declaration of Helsinki. Ethic approvals were obtained from the Institutional Review Boards of the Singapore Eye Research Institute, Singapore General Hospital, National University of Singapore and National Healthcare Group, Singapore. All participants provided written, informed consent before recruitment into the studies.

**Measurements of refractive error, axial length and covariates**

All studies used a similar protocol for ocular phenotype measurements. Non-cycloplegic refraction was measured by autorefractor (Canon RK-5, Tokyo, Japan), and ocular axial length was measured using optical laser interferometry (IOLMaster V3.01, Carl Zeiss; Meditec AG Jena, Germany) (44, 45). SE was calculated as the sphere power plus half of the cylinder power for each eye. The mean values of the right and left eyes were used as a quantitative outcome. When data from only one eye were available, the SE of this eye was used. Moderate myopia and mild myopia were defined as SE $\leq -3.0$ and SE $\leq -1.0$ D, respectively. Non-myopia controls were defined as SE $> -0.5$ D.

Age, gender, height and level of education were obtained from all participants who underwent an ophthalmologic examination.
For education, subjects chose the most appropriate level of education he/she had completed from the following options: (i) no formal education, (ii) completed primary education, (iii) completed lower secondary (iv) completed high school or polytechnic or (v) completed university. Level of education was categorized into a dichotomous variable: the lower education group including those who had completed lower secondary education or less (i)–(iii) and the higher education group comprising of high schoolers (iv) or university graduates (v).

Genotyping and imputation
We included 40 index SNPs known to be associated with refractive phenotypes (P-value < 5.0 × 10^6), identified from a recent GWAS meta-analysis of refractive error from the international CREAM (9), a large GWAS of age of onset of myopia (10), and from a GWAS meta-analysis of axial length (22). These 40 SNPs represent 31 unique genetic loci, where 11 loci were associated with refractive error, 8 loci were associated with age of myopia onset, 10 loci were associated with both phenotypes above and 1 locus was associated with axial length.

For SCES, SiMES, SINDI and STARS, the DNA samples were genotyped using the Illumina Human610 Quad Beadchips (Illumina Inc., San Diego, USA). SP2 samples were genotyped using Illumina Human 1Mduov3 and 610 Quad Beadchips. The detailed quality-control procedures of genotyping are provided elsewhere (11, 49). In brief, each study applied stringent quality-control filters to remove SNPs with: (i) per-SNP call rate <95%; (ii) minor allele frequency (MAF) <1% and (iii) Hardy–Weinberg equilibrium (HWE) P-value < 10^-6, and individuals with the following conditions: (i) per-sample call rate <95%, (ii) excessive heterozygosity, (iii) cryptic relatedness, (iv) gender discrepancies and (v) deviation in population structure. Population structure in each study was ascertained using principal component analysis (PCA) with the EIGENSTRAT program (50) (Supplementary Material, Figs. S3–S7). Due to the presence of population structure, we adjusted for the top five principal components in the association analyses for SiMES and SINDI datasets. From the PCA plots, we did not observe any evidence that eigenvalues (PC scores) along the top five eigenvectors were more likely to be clustered in any education stratum in each study. No obvious correlation between the top five eigenvectors and SE was noted in our data (data not shown). Imputation was performed using the genotyped data passed the quality-control filtering, together with the 1000 genomes phase 1 cosmopolitan panel haplotypes (March 2012 release). The Markov Chain Haploview software was used in the imputation procedure (Minimac software, http://genome.sph.umich.edu/wiki/Minimac, last accessed date on January, 2013). Two SNPs (rs994767, rs1960445) not in 1000 genomes genotypes were imputed using HapMap release 22 CHB + JAP population data using IMPUTE version 2(9). These 40 SNPs in our analysis had MAFs >0.01, good imputation quality (imputation quality score >0.44; average: 0.90) if imputed, and were in HWE (P-value > 0.10) (Supplementary Material, Table S1).

Statistical analysis
For each study, a linear regression model at each genotyped or imputed SNP was conducted to determine the association with the mean SE and the interaction with educational attainment. We assumed an additive genetic model where the number of risk allele carried is an ordinal variable (0, 1, 2). For the imputed SNP, we used risk allele dosage, a continuous variable ranging from 0 to 2. Primary analysis included age, age squared, sex and education as covariates as well as SNP and a SNP × education interaction term. Education was treated as a dichotomous variable (0 = lower education; 1 = higher education) and mean-centered age as a continuous variable. Age squared was also adjusted to address the quadratic pattern of SE with age. The beta estimates of SNP effects and corresponding standard errors (s.e.) by the level of education were derived from the primary model. Main effects of SNPs and education levels were estimated from the same primary model of linear regression, except no interaction terms included. The top five principal components of genetic ancestry were included to account for the potential effects of population substructure in SiMES and SINDI (49). The linear regression analyses of the interaction models and parameters estimates were conducted with R version 2.15.2 software (http://www.r-project.org/, last accessed date on September, 2013).

A fixed-effects meta-analysis on the regression coefficients of SNP × education interaction terms as well as SNP effects was conducted using conventional inverse-variance weighting (51). The meta-analysis was performed using METAL software (www.sph.umich.edu/csg/abecasis/metal, last accessed date on May, 2013). A Cochran’s Q test or I^2 index was used to assess heterogeneity of the beta coefficients across studies. In the meta-analysis, we tested a total of 40 index SNPs that had genome-wide significant evidence of association with refractive errors and related traits in prior GWAS. We used a conservative P-value threshold of 1.25 × 10^-8 as a stringent correction for the multiple testing of 40 SNPs at 31 unique loci to achieve a type 1 error rate of 0.05.

To evaluate the combined genetic effect of the multiple genetic loci on refractive phenotypes, we summed the number (or the dosage) of risk alleles of the SNPs carried by each individual. Relations with quantitative traits of SE and axial length were evaluated by a linear regression model, whereas relations with myopia were evaluated by a logistic regression model. Height was also adjusted for the outcome of axial length.

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

ACKNOWLEDGEMENTS
We acknowledge the Genome Institute of Singapore for the genotyping for all study populations. We thank all the participants who volunteered to take part in the studies, and the research teams from the Singapore Eye Research Institute and the National University of Singapore who collected the DNA samples and phenotyped the subjects.

Conflict of Interest statement. None declared.

FUNDING
This study was supported by the National Medical Research Council, Singapore (NMRC 0796/2003, NMRC 1176/2008,
REFERENCES


