Association of the Lumican Gene Functional 3′-UTR Polymorphism with High Myopia

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PURPOSE. The lumican gene (LUM) encodes a major extracellular component of the fibrous mammalian sclera. Alteration in the expression levels of extracellular matrix components may influence scleral shape, which in turn could affect visual acuity. Single-nucleotide polymorphisms (SNPs) in the LUM gene were determined in an investigation of whether LUM gene polymorphisms correlate with high myopia.

METHODS. Sequences spanning all three exons, intron–exon boundaries, and promoter regions were determined in 50 normal individuals. Five SNPs were identified, one of which was found to be a newly identified polymorphism. Genomic DNA was prepared from a peripheral blood obtained from 201 patients with high myopia and 86 control subjects. Genotypes of the SNPs −1554 T/C (rs35759223), −628 A/C (rs17018875), −59 CC/A (rs3852846), c.601 T/C (rs17853500), and the novel SNP c.1567 C>T were determined by polymerase chain reaction.

RESULTS. Of the five SNPs, one showed a significant difference between patients and control subjects (c.1567 C > T, P = 0.0016). Haplotype analysis revealed a significantly higher presence of polymorphisms in patients with myopia (P < 0.0001). Moreover, the c.1567 T polymorphism was determined to have lower reporter gene activity than that of c.1567 C.

CONCLUSIONS. These observations suggest that LUM gene polymorphisms contribute to the development of high myopia. (Invest Ophthalmol Vis Sci. 2010;51:96–102) DOI:10.1167/iovs.09-3612

Myopia is prevalent worldwide and has become a serious illness, particularly in Asian populations such as those in Taiwan, where prevalence may exceed 65%.1 Thus, myopia poses a public health concern.2,3 Simple myopia can be corrected with spectacles or contact lenses, whereas “high” (pathologic) myopia often predisposes subjects to an increased developmental risk for potentially blinding conditions such as retinal detachment, macular degeneration, and glaucoma.4 High myopia is typically defined as a refractive error with a spherical equivalent (SE) worse than −6 D. The prevalence of pathologic myopia has been estimated to be 1% to 3% in population-based studies.5 Moreover, the leading causes of registered blindness and partial sight are associated with high myopia. In addition to visual impairment, treatment and management of individuals affected with this disorder can have a substantial economic impact on society. Therefore, it is important to identify the etiology of high myopia. Early identification of individuals, especially children, predisposed to high myopia would enable implementation of adequate preventive measures, such as limiting the duration of unnecessary near work and engaging in outdoor activities, to facilitate the practice of good eye care habits6 that may help to delay the onset of myopia.

Myopia is a complex disease affected by both environmental and genetic factors.7–10 Determination of the genetic factors that predispose a person to myopia is challenging because myopia is a multigenetic condition involving several overlapping signaling pathways, each of which is associated with a group of distinct genetic profiles. Currently, genetic association studies are regarded as the most powerful approach to mapping of the genes underlying such complex traits.11

The sclera is the white, tough outer covering of the eye. It is a connective tissue that provides the structural framework for defining the shape and axial length of the eye. The development of high myopia causes anterior–posterior enlargement of the eye, scleral thinning, and frequent detachment of the retina, which can result from stress associated with excessive axial elongation.12,13 Scleral remodeling involves decreased production of the extracellular matrix because of diminished production of collagen and proteoglycans and increased collagen degradation. The major extracellular matrix components of the fibrous mammalian sclera comprise collagen type-I and -III and small leucine-rich proteoglycans (SLRPs), which include decorin, biglycan, lumican, and fibromodulin.14,15 Alteration in the expression levels of any of these extracellular matrix components presumably influences scleral shape, which in turn could affect visual acuity.16,17

Recently, polymorphism in the LUM gene was found to be associated with high myopia.18 Moreover, a recent mouse knockout study provided evidence of LUM as a candidate gene for high myopia.19 Majava et al.20 identified a Leu199Pro change in LUM that could have a damaging effect on its protein function. However, a c.893-105G>A polymorphism in the LUM gene may have protective effects against myopia, as is
Table 1. Summary of Studies Investigating the Relationship between LUM and High Myopia

<table>
<thead>
<tr>
<th>Study</th>
<th>Nationality of Subjects</th>
<th>Subjects (n)</th>
<th>Affected Status</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chakravarti et al.19</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>The axial length was increased by 10% in LUM*/−<em>FMOD</em>/−* mice compared with that in wild-type mice. Altered expression levels of LUM or FMOD may contribute to myopia. No polymorphism and/or mutations were found in the LUM gene. Any association between the LUM gene and myopia was excluded.</td>
</tr>
<tr>
<td>Paluru et al.21</td>
<td>American</td>
<td>Myopia: 10</td>
<td>≤ −6.0 D</td>
<td></td>
</tr>
<tr>
<td>Wang et al.18</td>
<td>Taiwanese</td>
<td>Control: 5</td>
<td>Myopia: 120</td>
<td>Rs3759223, located in the promoter region of the LUM gene, may contribute to myopia (P = 0.000283).</td>
</tr>
<tr>
<td>Marja et al.20</td>
<td>English and Finnish</td>
<td>Myopia: 125</td>
<td>≤ −6.0 D</td>
<td>Sequence variations and/or mutations in the LUM, FMOD, PRELP, and OPTC genes may have contributed to the pathogenesis of myopia.</td>
</tr>
<tr>
<td>Wang et al.22</td>
<td>Chinese</td>
<td>Control: 308</td>
<td>Myopia: 288</td>
<td>Rs 2229356 in TGFβ, rs3759223 in LUM, rs1982075 in TGFβ1, and rs3755520 in HGF were not associated with high myopia.</td>
</tr>
</tbody>
</table>

DNA Sequencing to Determine LUM SNPs

The LUM gene was sequenced to determine SNPs among 50 Taiwanese subjects. In this study, we sequenced the promoter region, 5′-UTR, 5′-UTR, and three exons of the LUM gene. Five different genomic DNAs were pooled to reduce the number of sequencing reactions performed and to exclude those SNPs with low heterozygosity. After the PCR fragments were purified (Qiagen II; Qiagen, Doncaster, VIC, Australia), they were directly sequenced for identification by determination chemistry (BigDye Dideoxy Terminator Cycle Sequencing Kit; Applied Biosystems, Inc. [ABI] Foster City, CA) on a DNA sequencer (Prism 3100; ABI).

Genotype Determinations

Four SNPs were determined by restriction enzyme (RE) digestion: −1554 T/C, −628 A/T, c.601 T/C, and c.1567 G/T. Genomic DNA was prepared from peripheral blood by using a DNA extraction kit (Extractor WB; Wako, Osaka, Japan). PCRs for LUM gene polymorphisms were performed in a 50-µL reaction mixture containing 50 ng of genomic DNA, 2 to 6 picomoles of each primer, 1× Taq polymerase buffer (1.5 mM MgCl2), and 0.25 U of Taq DNA polymerase (AmpliTaq, ABI). The primers, PCR conditions, and RE cutting sites used to determine LUM gene polymorphisms are listed in Table 2.

The c.−59 CC*/−* polymorphism was identified by using the DNA sequencer (model 3100 Prism; ABI). The DNA fragment containing the c.−59 CC*/−* polymorphism was amplified with a fluorescent FAM-labeled forward primer (Table 2). DNA fragments were separated and analyzed (Prism GenoMapper 3.0 software; ABI).

Haplotype Analysis

Haplotypes were inferred from unphased genotype data using the Bayesian statistical method available in the software program Phase 2.1.25,26 All five SNPs were analyzed with the Phase 2.1 software. Insertion and deletion SNPs (−628 A/− and −59 CC/*) were given numerical designations (insertion, 1; deletion, −1) and subsequently analyzed with Phase 2.1.

evidenced in conflicting reports by Paluru et al.21 and Wang et al.22 in which they excluded LUM as the candidate gene for high myopia. Table 1 summarizes these genetic studies in investigating the relationship between the LUM gene and high myopia. To establish whether LUM gene polymorphisms are correlated with high myopia in a Taiwanese Chinese population, sequences spanning all three exons, intron–exon boundaries, and promoter regions were determined in 50 normal individuals. Five single-nucleotide polymorphisms (SNPs) were found in the LUM gene, one of which was a new polymorphism. These polymorphisms were examined in patients with high myopia (myopia < −6.0 D) and in emmetropic volunteers by using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique to determine whether the distribution of LUM gene polymorphisms differs between control subjects and patients with high myopia.

METHODS

Participants

Refractive error was measured in 3000 volunteers, all of whom were unrelated Taiwanese Chinese selected from different parts of Taiwan. The volunteers were between 16 and 25 years of age whose visual acuity with distance correction was 0.2 logMAR (20/32) or better. Refractive error was measured in diopeters and determined by the mean SE in both eyes in each individual after administration of 1 drop of a cycloplegic drug (1% Mydriacyl; Alcon, Berlin, Germany). Individuals with myopia ≤ −6.0 D (both eyes) were included in this study, with the control group comprising individuals with a refractive error of ≤0.5 D. Our study was approved by the ethics committee of China Medical University Hospital, Taichung, Taiwan, and informed consent was obtained from all participants. The study was performed according to the tenets of the Declaration of Helsinki for research involving human subjects.

Comprehensive ophthalmic examinations were performed, and blood samples were collected from all patients. None of the participants had a history of ocular disease or ocular insult, such as retinopathy of prematurity or neonatal ocular problems. Further, no participant had a diagnosis of a genetic disease and/or connective tissue disorder associated with myopia, such as Stickler or Marfan syndrome. Clinical examination included visual acuity, refractive error, slit lamp examination, ocular movements, intraocular pressure, and funduscopy. Patients with organic eye disease; a history or evidence of intraocular surgery; and/or a history of cataract, glaucoma, retinal disorders, or laser treatment were excluded. A total of 201 patients with high myopia and 86 control subjects were enrolled from February to November 2004, with a male-to-female ratio of 1.8:1. Autorefraction (autorefractor/autokeratometer, ARK 700A; Topcon, Tokyo, Japan) was performed on both eyes of each patient by experienced optometrists who were trained and certified in the study protocols. Refractive data, sphere(s), negative cylinder, and axis measurements were analyzed by calculating the SE refractive error.
TABLE 2. Primers and PCR Conditions Used to Determine LUM Gene Polymorphisms

<table>
<thead>
<tr>
<th>Set</th>
<th>Primers Used and PCR Conditions</th>
<th>PCR Product</th>
<th>RE Cutting Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUM promoter −1554 T/C rs3759223</td>
<td>F5′-ATGTTATGAAAATTTAAAAAGGAAGA-3′&lt;br&gt;R5′-ATGTTATGAAAATTTAAAAAGGAAGA-3′&lt;br&gt;95°C × 5 min, 95°C × 30 s, and 60°C × 30 s</td>
<td>275 + 230 bp</td>
<td>Psfl</td>
</tr>
<tr>
<td>LUM promoter −628 A/rs17018757</td>
<td>F5′-GATGCTCCTCCCCAAGTTAGG-3′&lt;br&gt;R5′-CAGGAAGAGGAAATGACAGAGA-3′&lt;br&gt;95°C × 5 min, 95°C × 30 s and 60°C × 30 s</td>
<td>118 + 198 bp</td>
<td>HpyCH4V</td>
</tr>
<tr>
<td>LUM promoter −59 CC/rs3832846</td>
<td>F5′-ACACACCAAGATCCCACAAATGAC-3′&lt;br&gt;FAM labeled&lt;br&gt;R5′-AAAGCAGTAGTAGCTGACTGGAACAGA-3′&lt;br&gt;95°C × 5 min, 95°C × 30 s and 60°C × 30 s</td>
<td>173 bp</td>
<td>MspI</td>
</tr>
<tr>
<td>c.601 T&gt;C rs17853500</td>
<td>F5′-CCACCTCCTCCACCTCTGGA-3′&lt;br&gt;R5′-GCGGCAGCTTGGACAGGAT-3′&lt;br&gt;95°C × 5 min, 95°C × 30 s and 60°C × 30 s</td>
<td>447 + 108 bp</td>
<td>MspI</td>
</tr>
<tr>
<td>c.1567 C&gt;T</td>
<td>F5′-GCATGGAAATCAGCCAAGTT-3′&lt;br&gt;R5′-AACACATGATGCTGATGCGATTTGGC-3′&lt;br&gt;95°C × 5 min, 95°C × 30 s and 57°C × 30 s</td>
<td>52 + 131 + 122 + 41 bp</td>
<td>AluI</td>
</tr>
</tbody>
</table>

cDNA and promoter numbering are according to GenBank accession no. BC007038 and AF239660, respectively (http://www.ncbi.nlm.nih.gov/GenBank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). The transcribed sequence of the first exon is numbered +1. RE, restriction enzyme.

Linkage Disequilibrium Analysis of SNPs
The genotype data for the SNPs were input into JLIN software (ver. 1.60; http://www.genepi.org.au/jlin, provided in the public domain by the Laboratory for Genetic Epidemiology, Western Australian Institute for Medical Research). Lewontin's standardized linkage disequilibrium (LD) parameter (D') and r² were calculated by JLIN, and pair-wise LD maps were constructed.

Reporter Assay
The 3'UTR of the LUM gene was subcloned into the pGL4.73 vector (Promega, Madison, WI) to replace the SV40 late poly(A) signal between RE cutting sites (hLUM-SpeF: 5′-AACACTCTTTATGCATGCTTGAGAACATAA-3′ and hLUM-BamHI-R: 5′-AAGAAGATCCTGAGCGCAAGGATGACTTTTTGA-3′) produced by the PCR; the resulting plasmid was designated pGL4.73-T or pGL4.73-C. All constructs were verified by DNA sequencing to confirm that the only difference between the two copies of the LUM gene's 3'UTR was c.1567 C or T. The pGL4.70 vector (Promega) was used as a negative control. CHO-k1 cells were plated in six-well plates (10⁶ cells per well) and then transecteded with pGL4.73-T, pGL4.73-G, and 0.5 μg pTAL-SEAP per well. The cells were incubated at 37°C in 5% CO₂ for 24 hours. Cell culture supernatants and cell lysates were collected to determine secreted alkaline phosphatase activity and luciferase activities. Luciferase activity was normalized to the alkaline phosphatase activity. The results are expressed as the mean (SEM) of three independent experiments performed in triplicate.

Statistical Analysis
The genotype frequency and allelic frequency distributions of the polymorphisms in individuals with high myopia and controls were analyzed by the χ² method (SPSS ver. 10.0; SPSS, Inc., Chicago, IL). Correction for multiple comparisons was performed by the Bonferroni method. P < 0.01 was considered statistically significant. Odds ratios (ORs) were calculated from genotype and allelic frequencies with a 95% confidence interval (CI). LD was measured using the expectation maximization (EM) algorithm in the JLIN program.

RESULTS

Allele and Genotype Frequency of LUM Polymorphisms
We sequenced the promoter region, intron-exon boundaries, and the coding regions of the LUM gene of 50 normal individuals. Five SNPs were identified, one of which, c.1567 C>T, was determined to be a novel polymorphism in the LUM gene (Fig. 1). The genotype frequencies of the SNPs among the patients with myopia and normal individuals were identified, and the corresponding primers, REs, and FAM-labeled primers are listed in Table 2.

The genotype distributions and allele frequencies of the five polymorphisms are shown in Tables 3 and 4, respectively. Comparison of the genotypes between individuals with high myopia and the control group revealed no significant difference for four of five polymorphisms, including −1554 T/C, −628 A/rs17018757, −59 CC/rs3832846, and 601 T/C (Table 3); however, for one polymorphism in the 5'UTR of the LUM gene, a significant difference was found between the high myopia and control groups. Genotype distribution of the novel polymorphism (c.1567 C/T) between the high myopia and control groups showed a significant difference (P = 0.0016; heterozygous mutant T/C: OR, 3.39; 95% CI, 1.56–7.36; homozygous mutant T/T: OR, 3.61; 95% CI, 1.68–7.73).

The differences in allele frequencies of these polymorphisms between individuals with high myopia and the control group were analyzed by the χ² method (SPSS ver. 10.0; SPSS, Inc., Chicago, IL). Correction for multiple comparisons was performed by the Bonferroni method. P < 0.01 was considered statistically significant. Odds ratios (ORs) were calculated from genotype and allelic frequencies with a 95% confidence interval (CI). LD was measured using the expectation maximization (EM) algorithm in the JLIN program.

FIGURE 1. Direct sequencing data of c.1567 C/T. Arrow: a heterozygote for the polymorphism.
Table 3. Association between Genotype Distributions of LUM Gene Polymorphisms and Individuals with High Myopia*

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>High Myopia, Refractive Error ≤ −6.0 D (%)</th>
<th>Controls, Refractive Error ≥ 0.5 D (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.−1554 T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>104 (51.7)</td>
<td>57 (45)</td>
<td>1</td>
<td>—</td>
<td>0.213</td>
</tr>
<tr>
<td>T/C</td>
<td>83 (41.3)</td>
<td>45 (52.3)</td>
<td>0.66</td>
<td>0.39–1.11</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>14 (7)</td>
<td>4 (4.7)</td>
<td>1.25</td>
<td>0.39–4.02</td>
<td></td>
</tr>
<tr>
<td>c.−628 A/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>105 (52.2)</td>
<td>38 (44.2)</td>
<td>1</td>
<td>—</td>
<td>0.294</td>
</tr>
<tr>
<td>A/−</td>
<td>83 (41.3)</td>
<td>44 (51.2)</td>
<td>0.68</td>
<td>0.41–1.15</td>
<td></td>
</tr>
<tr>
<td>−/−</td>
<td>13 (6.5)</td>
<td>4 (4.6)</td>
<td>1.18</td>
<td>0.36–3.83</td>
<td></td>
</tr>
<tr>
<td>c.−59 CC/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/CC</td>
<td>99 (49.2)</td>
<td>39 (45.3)</td>
<td>0.90</td>
<td>0.35–2.44</td>
<td></td>
</tr>
<tr>
<td>t.601 T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>109 (54.2)</td>
<td>40 (46.5)</td>
<td>1</td>
<td>—</td>
<td>0.025†</td>
</tr>
<tr>
<td>T/C</td>
<td>78 (38.8)</td>
<td>45 (52.3)</td>
<td>0.12</td>
<td>0.02–0.97</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>14 (7)</td>
<td>1 (1.2)</td>
<td>0.19</td>
<td>0.02–1.53</td>
<td></td>
</tr>
<tr>
<td>c.1567 C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>16 (8)</td>
<td>20 (23.3)</td>
<td>1</td>
<td>—</td>
<td>0.0016</td>
</tr>
<tr>
<td>T/C</td>
<td>84 (42.3)</td>
<td>31 (36.1)</td>
<td>3.39</td>
<td>1.56–7.36</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>101 (50.2)</td>
<td>35 (40.6)</td>
<td>3.61</td>
<td>1.68–7.73</td>
<td></td>
</tr>
</tbody>
</table>

* Genotype frequencies were compared between individuals with myopia ≤ −6.00 D and ≥ 0.5 D by χ² tests, unless otherwise indicated. P < 0.01 was considered statistically significant.

† P < 0.01 was considered statistically significant; Fisher’s exact test.

Table 4. Association between Allelic Frequencies of LUM Gene Polymorphisms and Individuals with High Myopia*

<table>
<thead>
<tr>
<th>Alleles</th>
<th>High Myopia, Refractive Error ≤ −6.0 D (%)</th>
<th>Controls, Refractive Error ≥ 0.5 D (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.−1554 T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>291 (72.4)</td>
<td>119 (69.2)</td>
<td>1</td>
<td>—</td>
<td>0.437</td>
</tr>
<tr>
<td>T</td>
<td>111 (27.6)</td>
<td>53 (30.8)</td>
<td>0.86</td>
<td>0.58–1.27</td>
<td></td>
</tr>
<tr>
<td>c.−628 A/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>293 (72.9)</td>
<td>120 (69.8)</td>
<td>1</td>
<td>—</td>
<td>0.446</td>
</tr>
<tr>
<td></td>
<td>109 (27.1)</td>
<td>52 (30.2)</td>
<td>0.86</td>
<td>0.58–1.27</td>
<td></td>
</tr>
<tr>
<td>c.−59 CC/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>283 (70.4)</td>
<td>119 (69.2)</td>
<td>1.06</td>
<td>0.72–1.56</td>
<td></td>
</tr>
<tr>
<td>t.601 T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>296 (73.6)</td>
<td>125 (72.7)</td>
<td>1</td>
<td>—</td>
<td>0.812</td>
</tr>
<tr>
<td>C</td>
<td>106 (26.4)</td>
<td>47 (27.3)</td>
<td>0.95</td>
<td>0.64–1.42</td>
<td></td>
</tr>
<tr>
<td>c.1567 C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>116 (28.9)</td>
<td>71 (41.3)</td>
<td>1</td>
<td>—</td>
<td>0.0036</td>
</tr>
<tr>
<td>T</td>
<td>286 (71.1)</td>
<td>101 (58.7)</td>
<td>1.73</td>
<td>1.19–2.52</td>
<td></td>
</tr>
</tbody>
</table>

* Genotype frequencies were compared between individuals with myopia ≤ −6.00 D and ≥ 0.5 D by χ² tests. P < 0.01 was considered statistically significant.

Table 5. Association between LUM Gene Haplotypes and Myopia

<table>
<thead>
<tr>
<th>Haplotype †</th>
<th>−1554</th>
<th>−628</th>
<th>−59</th>
<th>601</th>
<th>1567</th>
<th>High Myopia, Refractive Error ≤ −6.0 D (%)</th>
<th>Controls, Refractive Error ≥ 0.5 D (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht1</td>
<td>C</td>
<td>A</td>
<td>CC</td>
<td>T</td>
<td>T</td>
<td>251 (57.5)</td>
<td>64 (37.2)</td>
<td>1.81 × 10⁻⁷</td>
<td>2.28 (1.58–3.29)</td>
</tr>
<tr>
<td>Ht2</td>
<td>C</td>
<td>A</td>
<td>CC</td>
<td>T</td>
<td>C</td>
<td>7 (1.74)</td>
<td>19 (11)</td>
<td>0.14 (0.06–0.35)</td>
<td></td>
</tr>
<tr>
<td>Ht3</td>
<td>C</td>
<td>A</td>
<td>−</td>
<td>T</td>
<td>T</td>
<td>53 (13.2)</td>
<td>30 (17.4)</td>
<td>0.72 (0.44–1.17)</td>
<td></td>
</tr>
<tr>
<td>Ht4</td>
<td>T</td>
<td>−</td>
<td>CC</td>
<td>C</td>
<td>C</td>
<td>38 (9.5)</td>
<td>20 (11.6)</td>
<td>0.79 (0.45–1.41)</td>
<td></td>
</tr>
<tr>
<td>Ht5</td>
<td>T</td>
<td>−</td>
<td>−</td>
<td>C</td>
<td>C</td>
<td>66 (16.4)</td>
<td>20 (11.6)</td>
<td>1.49 (0.87–2.55)</td>
<td></td>
</tr>
</tbody>
</table>

† Order of polymorphisms comprising LUM gene haplotypes: −1554 T/C, −628 A/−, −59 CC/−, 601 T/C, and 1567 C/T. The haplotypes were identified by the Bayesian statistical method available in the software program Phase 2.1.

† P < 0.01 was considered statistically significant; Pearson χ² test (5 × 2 table).
group were similar to the results of genotype frequencies (Table 4). The three promoter polymorphisms (−1554 T/C, −628 A/C, and −59 CC/−) showed no distinctions in allele frequency; however, the allele frequency of the c.1567 C/T polymorphism was significantly different between the two groups (P = 0.0036; OR, 1.73; 95% CI, 1.19−2.52), although the allele frequency of the c.601 T/C polymorphism was not (P = 0.812). Taken together, these results show a significant difference between the high myopia and control groups with regard to genotype or allele distribution for the c.1567 C/T polymorphism. Furthermore, the frequency of the T allele was significantly increased in patients with high myopia.

**Distributions of LUM Haplotypes**

Haplotype frequencies were estimated among the five identified polymorphisms. Ht1 to -5 (Pearson χ² test; P = 1.81 × 10⁻⁵; Table 5). The frequency of the most common haplotype (Ht1-CACCTT) in the control group was 37.2% compared with 57.5% in high myopia group. The haplotype Ht1 (OR, 2.28; 95% CI, 1.58−3.29) appeared to be a significant at-risk haplotype, whereas Ht2 (OR, 0.14; 95% CI, 0.06−0.35) appeared to be a protective one (Table 5). The five SNPs were input into the JLIN software and analyzed for LD, with the control and high myopia groups examined separately (Fig. 2). The LD map showed distinct differences between the two groups, and an apparent variation in the c.1567 C/T polymorphism was detected, indicating that this novel SNP may play some role in high myopia.

**Functional Analysis of the c.1567C/T Polymorphism**

To further evaluate whether the c.1567 C/T polymorphism would influence RNA stability and/or its translational efficiency and subsequent reporter gene activity, we performed reporter gene analysis. The 3'-UTR of the LUM gene was subcloned into the pGL4.73 vector to replace the SV40 late poly(A) signal sequences. The resulting plasmid was designated pGL4.73-T or pGL4.73-C. The c.1567 C polymorphism (pGL4.73-C) showed higher luciferase activity than that of c.1567 T (pGL4.73-T; Fig. 3). These results suggest that this LUM genetic variant is associated with high myopia.

**DISCUSSION**

In the present study, we found a novel SNP in the LUM gene and showed a significant association between LUM polymorphism and high-grade myopia in terms of genetic and functional aspects. Nonsyndromic high myopia is a common and complex disorder in Asian populations and results from alterations in multiple genetic factors. Several positional candidate genes were screened and found to be located at specific loci; these genes included TGIF, EMLN-2, MLCB, and CLUL1, and they map within the high-grade myopia-2 locus (MYP2) candidate interval and on the dermatan sulfate proteoglycan 3.
high myopia.\textsuperscript{31} High myopia is also caused by
in the biochemical structure of the sclera have been reported
linked to dissociation of the collagen fiber bundle, and changes
have suggested that scleral thinning in the highly myopic eye is
biomechanical properties of the sclera. Results in other studies
al.\textsuperscript{22} also excluded the possibility of the association between
individuals in these two pedigrees were screened. Wang et
subjects with myopia of \(H11002\) were unable to replicate the result of Wang et al.,\textsuperscript{18} who
found \(H11002\) to be strongly associated with high myopia. Thus, population differences between the two studies may
determine whether the gene is associated with high myopia.
We identified haplotypes that showed significant association
with the development of high myopia and suggest that genetic
variations in the \(LUM\) gene may affect collagen formation of the
scleral matrix and play some role in the progression of
myopia.

In conclusion, the present study shows that the \(c.1567\) C/T
polymorphism may be associated with high myopia. In addi-
ction, our haplotype study revealed that the \(LUM\) gene may be
a genetic risk factor for myopia in the Taiwanese population.

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References

1. Lin LL, Shih YF, Hsiao CK, Chen CJ. Prevalence of myopia in Tai-
wanese schoolchildren: 1983 to 2000. \textit{Ann Acad Med Singa-
orese. 2004;33:27–33.
refractive errors in adult Chinese in Singapore. \textit{Invest Ophthalmol
3. Zhao J, Pan X, Sui R, Munoz SR, Sperduto RD, Ellwein LB. Refrac-
tive error study in children: results from Shunyi District, China.
dominant high myopia maps to the long arm of chromosome 17.
5. Vongphanh J, Mitchell P, Wang JJ. Prevalence and progression of
myopic retinopathy in an older population. \textit{Ophtalmology. 2002;
6. Jacobsen N, Jensen H, Goldschmidt E. Does the level of physical
activity in university students influence development and progres-
sion of myopia?—a 2-year prospective cohort study. \textit{Invest Oph-
7. Lyhne N, Sjolik AK, Kvyik KO, Green A. The importance of genes
and environment for ocular refraction and its determiners: a pop-
ulation based study among 20–45 year old twins. \textit{Br J Ophthal-
8. Mutti DO, Zadnik K, Adams AJ. Myopia: the nature versus nurture
10. Saw SM, Chua WH, Wu HM, Yap E, Chia KS, Stone RA. Myopia:
11. Risch N, Merikangas K. The future of genetic studies of complex
12. Siegwart JT Jr, Norton TT. Selective regulation of MMP and TIMP
mRNA levels in tree shrew sciera during minus lens compensation
13. Tkatchenko AV, Walsh PA, Tkatchenko TV, Guinsteinch S, Raviola
E. Form deprivation modulates retinal neurogenesis in primate
4686.
14. Dunlevy JR, Rada JA. Interaction of lumican with aggrecan in the
3856.
15. Rada JA, Achen VB, Penugonda S, Schmidt RW, Mount BA. Proteo-
glycan composition in the human sciera during growth and aging.
16. McBrien NA, Lawlor P, Gentile A. Scleral remodeling during the
development of and recovery from axial myopia in the tree shrew.


