# 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 peripheral blood obtained from 201 patients with high myopia and 86 control subjects. Genotypes of the SNPs −1554 T/C (rs3759223), −628 A/−(rs17018757), −59 CC/−(rs3832846), 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

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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 habits[6] 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

| Study | Nationality of Subjects | Subjects (*n*) | Affected Status | Conclusions |
|---|---|---|---|---|
| Chakravarti et al.[19] | — | — | — | The axial length was increased by 10% in $LUM^{-/-}FMOD^{-/-}$ mice compared with that in wild-type mice. Altered expression levels of *LUM* or *FMOD* may contribute to myopia. |
| Paluru et al.[21] | American | Myopia: 10<br>Control: 5 | ≤ −6.0 D | No polymorphism and/or mutations were found in the *LUM* gene. Any association between the *LUM* gene and myopia was excluded. |
| Wang et al.[18] | Taiwanese | Myopia: 120<br>Control: 137 | < −10.0 D | Rs3759223, located in the promoter region of the *LUM* gene, may contribute to myopia ($P = 0.000283$). |
| Marja et al.[20] | English and Finnish | Myopia: 125<br>Control: 308 | ≤ −6.0 D both eyes | Sequence variations and/or mutations in the *LUM, FMOD, PRELP,* and *OPTC* genes may have contributed to the pathogenesis of myopia. |
| Wang et al.[22] | Chinese | Myopia: 288<br>Control: 208 | ≤ −6.0 D | Rs 2229336 in *TGIF,* rs3759223 in *LUM,* rs1982073 in *TGFB1,* and rs3735520 in *HGF* were not associated with high myopia. |

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 diopters 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 patients. 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.

### 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, 3′-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 (Qiaex II; Qiagen, Doncaster, VIC, Australia), they were directly sequenced for identification by dye-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/−, c.601 T/C, and c.1567 C/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 $MgCl_2$), and 0.25 U of *Taq* DNA polymerase (Ampli*Taq*; 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 GeneMapper 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.[23,24] 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.

**TABLE 2.** Primers and PCR Conditions Used to Determine *LUM* Gene Polymorphisms

| Set | Primers Used and PCR Conditions | PCR Product | RE Cutting Site |
|---|---|---|---|
| *LUM* promoter −1554 T/C rs3759223 | F5′-ATGTATGAAATTTAAAGGAAGAA-3′<br>R5′-ATGCTATGTATTAATTTTGAGTGT-3′<br>95°C × 5 min, 95°C × 30 s, and 60°C × 30 s | 275 + 230 bp | *Psi*I |
| *LUM* promoter −628 A/−rs17018757 | F5′-GAATGCTCTCCCCAAGTAAGG-3′<br>R5′-CAGGAAAACGCAAATGAACAGA-3′<br>95°C × 5 min, 95°C × 30 s and 60°C × 30 s | 118 + 198 bp | *Hpy*CH4V |
| *LUM* promoter −59 CC/−rs3832846 | F5′-ACACCACAAGATCCCCACAATGAC-3′<br>FAM labeled<br>R5′-AAAGCAGATGCACTATGGACAAGA-3′ | 173 bp | |
| c.601 T>C rs17853500 | F5′-CCACCTCCCAATCTCTGGA-3′<br>R5′-GCCGCAGCTTGGACAGGAT-3′<br>95°C × 5 min, 95°C × 30 s and 60°C × 30 s | 447 + 108 bp | *Msp*I |
| c.1567 C>T | F5′-GCATGGAAATCAGCCAAGTT-3′<br>R5′-AACACAGTGATGCCATTTGC-3′<br>95°C × 5 min, 95°C × 30s and 57°C × 30 s | 52 + 131 + 122 + 41 bp | *Alu*I |

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).[25] Lewontin's standardized linkage disequilibrium (LD) parameter ($D'$) and $r^2$ 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-*Spe*I-F: 5′-AAAACTAGTTATCTGTATCCTGGAACAATA-3′ and hLUM-*Bam*HI-R: 5′-AAAGGATCCTGCAGGCCAGAGATATCTTTTGA-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 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 ($10^6$ cells per well) and then transfected with pGL4.73-T, pGL4.73-G, and 0.5 μg pTAL-SEAP per well. The cells were incubated at 37°C in 5% $CO_2$ for 24 hours. Cell culture supernatants and cell lysates were collected to determine secreted alkaline phosphatase 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 $\chi^2$ 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/−, −59 CC/−, 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

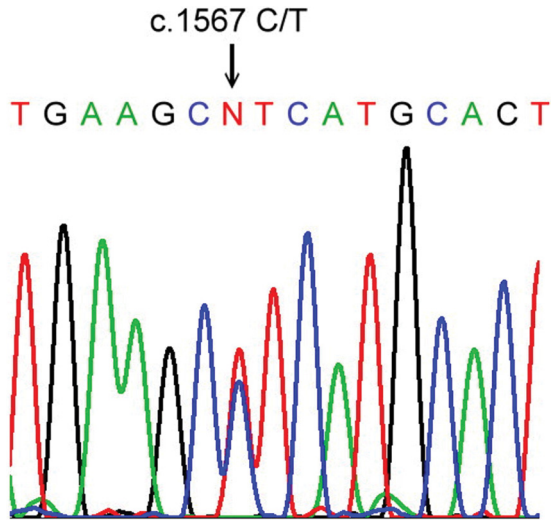


**FIGURE 1.** Direct sequencing data of c.1567 C/T. *Arrow*: a heterozygote for the polymorphism.

**TABLE 3.** Association between Genotype Distributions of *LUM* Gene Polymorphisms and Individuals with High Myopia*

| Polymorphisms | High Myopia, Refractive Error ≤ −6.0 D (%) | Controls, Refractive Error ±0.5 D (%) | OR | 95% CI | *P* |
|---|---|---|---|---|---|
| c.−1554 T/C | | | | | |
| C/C | 104 (51.7) | 37 (43) | 1 | — | 0.213 |
| T/C | 83 (41.3) | 45 (52.3) | 0.66 | 0.39–1.11 | |
| T/T | 14 (7) | 4 (4.7) | 1.25 | 0.39–4.02 | |
| c.−628 A/− | | | | | |
| A/A | 105 (52.2) | 38 (44.2) | 1 | — | 0.294 |
| A/− | 83 (41.3) | 44 (51.2) | 0.68 | 0.41–1.15 | |
| −/− | 13 (6.5) | 4 (4.6) | 1.18 | 0.36–3.83 | |
| c.−59 CC/− | | | | | |
| −/− | 17 (8.5) | 6 (7) | 1 | — | 0.686 |
| CC/− | 85 (42.3) | 41 (47.7) | 0.73 | 0.27–1.99 | |
| CC/CC | 99 (49.2) | 39 (45.3) | 0.90 | 0.33–2.44 | |
| c.601 T/C | | | | | |
| T/T | 109 (54.2) | 40 (46.5) | 1 | — | 0.025† |
| T/C | 78 (38.8) | 45 (52.3) | 0.12 | 0.02–0.97 | |
| C/C | 14 (7) | 1 (1.2) | 0.19 | 0.02–1.53 | |
| c.1567 C/T | | | | | |
| C/C | 16 (8) | 20 (23.3) | 1 | — | 0.0016 |
| T/C | 84 (41.8) | 31 (36.1) | 3.39 | 1.56–7.36 | |
| T/T | 101 (50.2) | 35 (40.6) | 3.61 | 1.68–7.73 | |

* Genotype frequencies were compared between individuals with myopia ≤ −6.00 D and ±0.5 D by $\chi^2$ 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*

| Alleles | High Myopia, Refractive Error ≤ −6.0 D (%) | Controls, Refractive Error ±0.5 D (%) | OR | 95% CI | *P* |
|---|---|---|---|---|---|
| c.−1554 T/C | | | | | |
| C | 291 (72.4) | 119 (69.2) | 1 | | 0.437 |
| T | 111 (27.6) | 53 (30.8) | 0.86 | 0.58–1.27 | |
| c.−628 A/− | | | | | |
| A | 293 (72.9) | 120 (69.8) | 1 | | 0.446 |
| — | 109 (27.1) | 52 (30.2) | 0.86 | 0.58–1.27 | |
| c.−59 CC/− | | | | | |
| — | 119 (29.6) | 53 (30.8) | 1 | | 0.772 |
| CC | 283 (70.4) | 119 (69.2) | 1.06 | 0.72–1.56 | |
| c.601 T/C | | | | | |
| T | 296 (73.6) | 125 (72.7) | 1 | | 0.812 |
| C | 106 (26.4) | 47 (27.3) | 0.95 | 0.64–1.42 | |
| c.1567 C/T | | | | | |
| C | 116 (28.9) | 71 (41.3) | 1 | | 0.0036 |
| T | 286 (71.1) | 101 (58.7) | 1.73 | 1.19–2.52 | |

* Genotype frequencies were compared between individuals with myopia ≤ −6.00 D and ±0.5 D by $\chi^2$ tests.

$P < 0.01$ was considered statistically significant.

**TABLE 5.** Association between *LUM* Gene Haplotypes and Myopia

| Haplotype* | −1554 | −628 | −59 | 601 | 1567 | High Myopia, Refractive Error ≤ −6.0 D (%) | Controls, Refractive Error ±0.5 D (%) | *P*† | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| Ht1 | C | A | CC | T | T | 231 (57.5) | 64 (37.2) | $1.81 \times 10^{-7}$ | 2.28 (1.58–3.29) |
| Ht2 | C | A | CC | T | C | 7 (1.74) | 19 (11) | | 0.14 (0.06–0.35) |
| Ht3 | C | A | — | T | T | 53 (13.2) | 30 (17.4) | | 0.72 (0.44–1.17) |
| Ht4 | T | — | CC | C | C | 38 (9.5) | 20 (11.6) | | 0.79 (0.45–1.41) |
| Ht5 | T | — | — | C | C | 66 (16.4) | 20 (11.6) | | 1.49 (0.87–2.55) |

* 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 $\chi^2$ test (5 × 2 table).

group were similar to the results of genotype frequencies (Table 4). The three promoter polymorphisms (−1554 T/C, −628A/−, 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

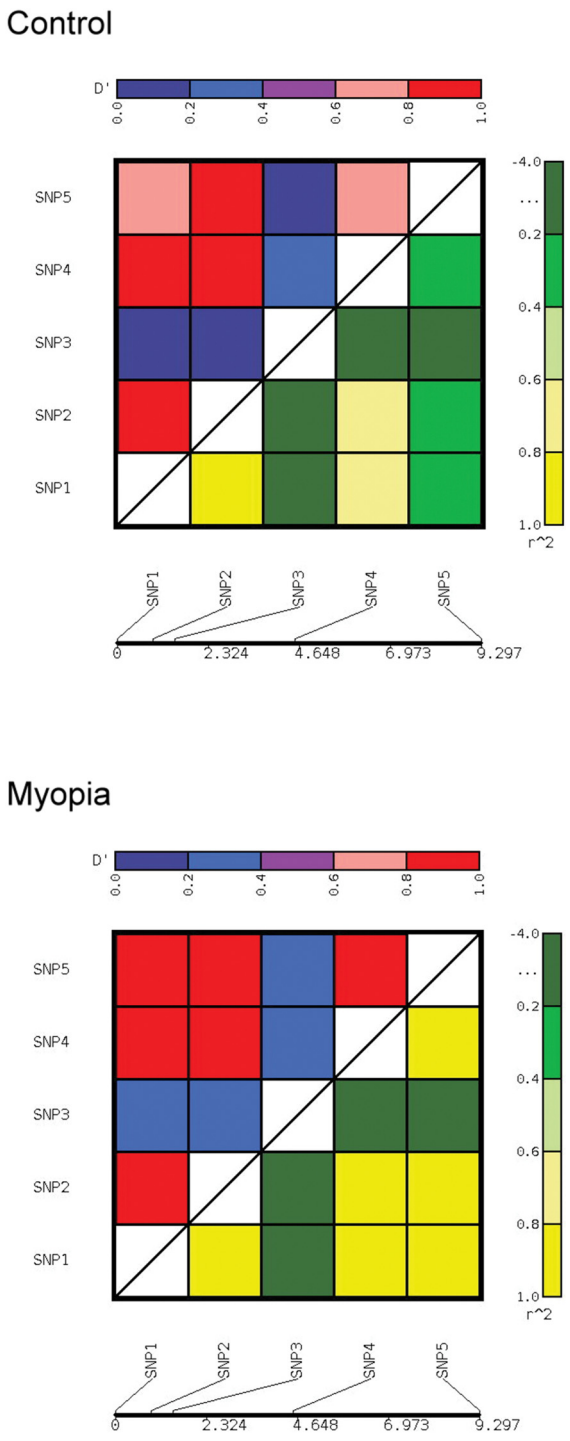


**FIGURE 2.** Pair-wise LD measures of $D'$ and $r^2$ for SNPs of the *LUM* locus. Scales beneath the charts show the sites of each SNP around the *LUM* gene region. SNP1: −1554 T/C; SNP2: −628 A/−; SNP3: −59 CC/−; SNP4: c.601 T/C; SNP5: c.1567 C/T.

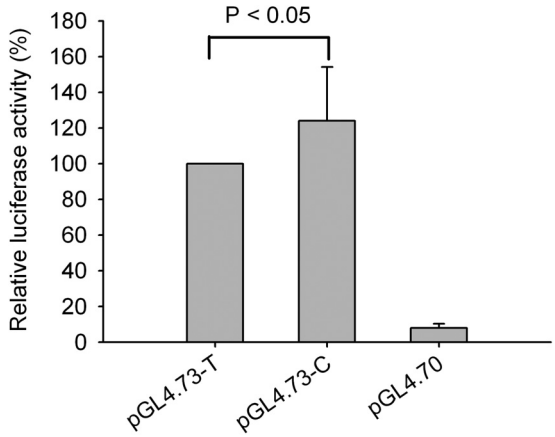


**FIGURE 3.** Effects of the c.1567 C/T polymorphism on luciferase induction. Results are expressed as the mean relative activity ± SEM.

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 $\chi^2$ test; $P = 1.81 \times 10^{-7}$; 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*, *EMLIN-2*, *MLCB*, and *CLUL1*, and they map within the high-grade myopia-2 locus (*MYP2*) candidate interval[26] and on the dermatan sulfate proteoglycan 3

(*DSPG-3*), decorin, and *LUM* genes located on *MYP3*.[27] However, there is disagreement about the role of some of these candidate genes. For example, *TGIF* was proposed as a possible gene for *MYP2*-associated high myopia because of its location and possible involvement in scleral growth[28]; however, this finding was questioned by Scavello et al.[29] Moreover, there is a similar debate on the role of *LUM* in the pathology of high myopia.

LUM is a member of the leucine-rich repeat glycoprotein family and was initially described as a corneal proteoglycan responsible for the control of collagen fibrillogenesis and interaction.[17,30] This role implicates LUM in determining the biomechanical properties of the sclera. Results in other studies have suggested that scleral thinning in the highly myopic eye is linked to dissociation of the collagen fiber bundle, and changes in the biochemical structure of the sclera have been reported in patients with high myopia.[31] High myopia is also caused by excessive axial elongation associated with altered proteoglycan synthesis. The *LUM* gene is located at 12q21-q23 (*MYP3*), which is a locus associated with high-grade myopia. Gene variation at the region encoding lumican, a major extracellular matrix component, may be associated with increased susceptibility to high myopia.

Gene-knockout studies in mice have shown that the *LUM* and fibromodulin genes may be candidate genes for high myopia, because of increased axial length in double-null mice.[19] However, Paluru et al.[21] suggested that the knockout study findings represent a false-positive result because of the "hitchhiker gene effect": Adjacent altered genes influenced the phenotype rather than the implicated candidate genes. They investigated the *MYP3* family, and 10 affected individuals in these two pedigrees were screened. Wang et al.[22] also excluded the possibility of the association between high myopia and the *LUM* gene (rs3759223). However, results of case–control studies indicated that the SNP of the *LUM* gene may be a risk factor for the pathogenesis of high myopia in Han Chinese, English, and Finnish populations.[20]

Our results show that *LUM* genetic polymorphism is associated with high myopia. After identifying five SNPs in a normal population, including a novel polymorphism, we screened 201 patients with high myopia and 86 control subjects. A genetic association study revealed that the frequency of the T allele in c.1567 was increased in patients with high myopia compared with that in the control group. The increased frequency of the T allele may further influence RNA stability or alter its translational efficiency to modulate the expression level of lumican. In haplotype studies, five SNPs in the *LUM* gene showed significant differences between the high myopia and control groups ($P = 1.81 \times 10^{-7}$). In addition, the Ht1 haplotype was present in a higher proportion of patients with high myopia than in the control group (Table 5). It is interesting to note that we were unable to replicate the result of Wang et al.,[18] who found rs3759223 to be strongly associated with high myopia in Taiwanese subjects.[18] However, the discrepancy may be due to the different selection criteria for myopia and control subjects in our study. Wang et al. selected subjects with myopia of −10.00 D in both eyes, whereas we selected subjects with myopia of −6.00 D or worse. With regard to the control group, we used a more stringent criterion (±0.5 D in both eyes) than they did (−1.5 to 0.5 D in either eye). Thus, population differences between the two studies may have contributed to the difference in the result.

Our study differs from other studies in that most studies do not investigate all polymorphisms in the *LUM* gene and frequently exclude this gene as a candidate for high myopia because of small sample size or the isolated study of one SNP. In our study, we found that investigation of the *LUM* gene haplotype was a more effective approach when attempting to 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 addition, our haplotype study revealed that the *LUM* gene may be a genetic risk factor for myopia in the Taiwanese population.

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