Association Analysis of Polymorphisms in Lumican Gene and Systemic Lupus Erythematosus in a Taiwan Chinese Han Population

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ABSTRACT. Objective. Lumican (LUM) is predominantly localized in areas of pathological fibrosis. To determine whether polymorphisms in LUM gene are associated with development of systemic lupus erythematosus (SLE), we analyzed 2 single-nucleotide polymorphisms (SNP) of LUM in a Taiwan Chinese Han population.

Methods. Participants included 168 patients with SLE and 192 age-matched controls in whom examinations had excluded SLE. Genotyping of –628 A/– (rs17018757) and c.1567 T/C polymorphisms in LUM were carried out in each patient and control using the polymerase chain reaction-restriction fragment-length polymorphism method, and validated by Taqman SNP genotyping assay. Data were correlated with the development of SLE and various clinical symptoms by chi-square analysis.

Results. Frequencies of C/C genotype and the C allele at c.1567 T/C were significantly higher in patients than controls. Polymorphism at c.1567 C/T was found to be associated with arthritis and photosensitivity in patients with SLE, which are both connective tissue-related symptoms.

Conclusion. The c.1567 T/C polymorphism of LUM is related to the development and clinical symptoms of SLE. (First Release Sept 1 2011; J Rheumatol 2011;38:2376–81; doi:10.3899/jrheum.101310)

Key Indexing Terms: LUMICAN SINGLE-NUCLEOTIDE POLYMORPHISMS SYSTEMIC LUPUS ERYTHEMATOSUS SMALL LEUCINE-RICH PROTEOGLYCAN

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease causing accumulative damage on multiple organs. Clinical manifestations of SLE include rash, arthritis, nephritis, anemia, thrombocytopenia, serositis, vasculitis, and neuropsychiatric defects. Clinical phenotypes of SLE are highly variable in patients, affecting multiple organs such as skin, joints, lungs, kidneys, hematopoietic organs, and nervous system. It is still unclear why the same disease can affect individuals with highly variable phenotypes and severities. Studies have indicated that genetic, environmental, and hormonal factors are implicated in the heterogeneity of SLE. Besides SLE, some other connective tissue diseases such as systemic sclerosis and rheumatoid arthritis (RA) share common pathogenic features including malfunction of the immune system, inflammation, and fibrosis.

Lumican (LUM) is a member of the structurally related small leucine-rich proteoglycan (SLRP) family, which regulates extracellular matrix (ECM) remodeling through coordinately modulating fibrillogenesis and collagen turnover. In various connective tissues, fibrotic ECM accumulation, accompanied by alterations of SLRP expressions...
sion profiles, is often linked to tissue destruction and pathogenic fibrosis17,18,19,20.

Histochemically, LUM is found as the primary keratan sulfate proteoglycan in the corneal stroma, and also in the ECM of skin, muscle, and cartilage21,22,23. Studies have indicated that LUM is prominently expressed in areas of pathological fibrosis24,25,26, and participates in cell signaling by regulating the activity of transforming growth factor-β (TGF-β)25,27. As an immune modulator, decreased level of TGF-β was coupled to the onset and severity of SLE28. In addition, TGF-β activation following cellular inflammatory response also causes an increase of ECM components, which in turn leads to fibrosis in various inflammatory diseases such as RA and myocarditis29,30,31. Based on these findings, we propose that LUM may play a role in the development of SLE, which exhibits symptoms of both inflammation and fibrosis.

The human LUM gene spreads over 7.5 kb of genomic DNA and is located on chromosome 12q2222. This gene consists of 3 exons separated by introns of 2.2 kb and 3.5 kb length. The shorter 5’-intron resides 21 bp upstream of the translation initiation codon, and the 3’-intron 152 bp upstream of the translation termination codon33. By sequencing polymerase chain reaction (PCR)-amplified genomic DNA, Lin, et al found that 2 SNP in the LUM gene, –628 A/– and c.1567 C/T, were associated with the development of high myopia in a Taiwan Chinese Han population34. They further demonstrated that the c.1567 C/T SNP may influence the expression of LUM34. Since myopia is also a connective tissue disease, we decided to evaluate whether these polymorphisms are associated with SLE in a Taiwan Chinese Han population with or without this disease.

**MATERIALS AND METHODS**

**Patients and sample collection.** A total of 168 patients with SLE and 192 healthy age-matched controls were recruited from the China Medical University Hospital in Taiwan. The clinical features of the selected patients met the criteria for SLE of the American Rheumatism Association35. Controls were selected from individuals undergoing regular health check-ups at the same hospital and certified as healthy based on those examinations. Blood samples were collected by venipuncture for blood cell isolation and genomic DNA preparation. This study was approved by the Institutional Review Board of the China Medical University Hospital prior to patient enrollment. All participants provided informed consent.

**Genotyping.** Genomic DNA was prepared from peripheral blood leukocytes according to standard protocols (DNA extractor WB kit; Wako Pure Chemical Industries, Osaka, Japan). PCR procedures for LUM gene polymorphisms were performed in a 50 µl reaction mixture containing 50 ng genomic DNA, 2 – 6 pmol of each primer, 1× Taq polymerase buffer (1.5 mM MgCl2), 0.25 mM dNTP, and 0.25 U of Taq DNA polymerase (AmpliTaq; Applied Biosystems, Carlsbad, CA, USA). PCR products were amplified with an initial denaturation at 95˚C for 5 min, followed by a 25-cycle program (95˚C for 30 s, 60˚C or 57˚C for 30 s, and 72˚C for 1 min). The primers, PCR conditions, and restriction enzyme cutting sites used to determine polymorphisms in LUM gene are listed in Table 1. Restriction enzyme HpyCH4V and AluI were used for the PCR products of –628 and c.1567, respectively. PCR products were digested by the restriction enzymes at 37˚C for 4 h and overnight. Gel electrophoresis of the digested DNA products confirmed that the cutting was completed within 4 h, and the prolonged digestion time did not alter the results. Sequence detections were performed in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). DNA fragments were separated and analyzed using Prism GeneMapper 3.0 (Applied Biosystems). Genotyping results from the PCR-restriction fragment-length polymorphism (RFLP) method were further validated using the Taqman SNP genotyping assay system (Applied Biosystems). Probes for the –628 and c.1567 polymorphism of LUM were custom-designed using the sequences of their flanking regions. PCR amplification conditions consisted of initial denaturation at 95˚C for 5 min, followed by 40 cycles of 95˚C for 15 s, and 60˚C for 60 s. Genetic variations were detected by reading the fluorescence signals of PCR products. A positive signal indicates a perfect match between the probe and the tested DNA, thus identifying the allele types.

**Statistical analysis.** The genotype and allelic frequency distributions of the LUM polymorphisms in patients with SLE and controls were analyzed by the chi-square method using SPSS (version 10.0; SPSS Inc., Chicago, IL, USA). P < 0.05 was considered statistically significant. Odds ratios were calculated for both genotype and allelic frequencies with a 95% CI. Genotype data were checked for deviation from Hardy-Weinberg equilibrium using the PLINK program36.

**RESULTS**

**Allelic and genotype frequencies of LUM polymorphisms.** Using the PCR-RFLP method, we determined the presence of –628 A/– (rs17018757) and c.1567 C/T polymorphisms of LUM gene. A representative gel image of the enzyme digestion product of the PCR products for both SNP is shown in Figure 1, panel A. The PCR products with deletion at –628 were digested by the enzyme HpyCH4V and generated 2 fragments. The PCR products with T allele at c.1567 were digested by ApuI and generated 4 fragments (Table 1). We further validated the genotyping results from PCR-RFLP by Taqman SNP genotyping assays. The fluorescence signal plots are shown in Figure 1, panels B and C, for –628 and c.1567, respectively.

The genotyping data revealed significant differences in both the allelic and genotype distributions of the c.1567 SNP between patients with SLE and healthy controls. The genotype and allele frequencies of the 2 polymorphisms are summarized in Table 2. Both the C/C and C/T genotypes were observed more frequently in patients with SLE compared to controls. A significant difference in the distribution of the c.1567 C/T genotype was found between patients with SLE and controls (p = 0.011; OR 2.53, 95% CI 1.24–5.18; Table 2). In addition, allelic frequencies of the c.1567 C/T polymorphism also varied significantly between patients with SLE and controls (p = 0.0029; OR 1.60, 95% CI 1.17–2.20; Table 2). This result indicated that the C allele at c.1567 may be a predisposing risk factor for development of SLE. However, the p values from chi-square tests indicated no significant differences between patients and controls in either allelic or genotype frequencies of the –628 A/– polymorphism (Table 2). P values for Hardy-Weinberg equilibrium for both –628 and c.1567 SNP were all > 0.05 in patients and controls (Table 2), indicating that the genotype distributions respect the Hardy-Weinberg equilibrium.
The association between 11 clinical features and allele distributions in patients with SLE was analyzed, and the results are summarized in Table 3. Comparing SLE patients with and without the symptoms, the c.1567 polymorphism of LUM is associated with the development of arthritis and photosensitivity (p = 0.0064 and 0.030, respectively; Table 3). Since LUM is involved in the regulation of ECM structure in various connective tissues including cartilage and skin, the c.1567 polymorphism may be capable of modifying the expression of the LUM gene. Therefore, it is not surprising that the connective tissue-related symptoms such as arthritis and photosensitivity exhibited high odds ratios in patients with the C allele at c.1567 of LUM.

**DISCUSSION**

Our discovery that the LUM gene represents a novel genetic risk factor for development of SLE provides new perspectives in the study of molecular mechanisms underlying the pathogenesis and progression of SLE. SLE is a multiorgan disease whose pathogenesis is attributed to genetic and environmental factors and immune system abnormalities. Recent advances in genome-wide association studies have identified more than 30 genes associated with SLE, most involved in the immune response-related pathways.
Knowledge of the role of LUM in immune regulation remains limited, and related studies are mostly focused on its regulation in collagen fibril organization, corneal transparency, cell migration, and tissue repair. Nevertheless, one study in 2007 demonstrated a regulatory role of LUM in the innate immune response via the Toll-like receptor (TLR) pathway. Recently, TLR have been shown to be involved in the pathogenesis of SLE through activation of immune cells and upregulation of many disease-related cytokines. The possible roles of LUM in immune regulation need to be further defined.

Correct amounts of SLRP, including LUM, are crucial for the normal function of various ECM-rich tissues such as skin, tendon, muscle, and cornea of the eye. Schaefer, et al. discovered distinct expression patterns of LUM, decorin, biglycan, and fibromodulin, the 4 members of the SLRP family, in adult human kidney cortex. Their study provided a basis for elucidating specific roles of the individual SLRP members in the pathogenesis of fibrotic kidney diseases.

In common with other connective tissues, cartilage also contains a variety of SLRP, which are a minor component of tissue considering their weight, but on a molar basis may rival the abundance of aggrecan. Along with LUM, other major SLRP all help to maintain the integrity of tissue and modulate its metabolism. LUM is a keratan sulfate-bearing type of SLRP that can bind fibrillar collagens and function in the assembly of collagen networks in connective tissues.

In our study, the functional SNP in LUM gene that have been previously linked to myopia were also found to be significantly associated with SLE, suggesting that SLE may share similar pathogenic pathways with other connective tissue diseases. The possible roles of LUM in immune regulation need to be further defined.

### Table 2. Genotype and allele frequencies of LUM polymorphisms in Taiwan Chinese Han patients with SLE and controls.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.–628 (rs17018757)</td>
<td>–/–</td>
<td>11 (6.6)</td>
<td>10 (5.2)</td>
<td>0.237</td>
<td>1.50 (0.61–3.70)</td>
</tr>
<tr>
<td></td>
<td>A/–</td>
<td>73 (44.0)</td>
<td>70 (36.5)</td>
<td>1 (reference)</td>
<td>1.42 (0.92–2.25)</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>82 (49.4)</td>
<td>112 (58.3)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>95 (28.6)</td>
<td>90 (23.4)</td>
<td>0.11</td>
<td>1.31 (0.94–1.83)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>237 (71.4)</td>
<td>294 (76.6)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>C.1567</td>
<td>C/C</td>
<td>24 (14.3)</td>
<td>15 (8.0)</td>
<td>0.86</td>
<td>2.53 (1.24–5.18)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>84 (50.0)</td>
<td>78 (41.5)</td>
<td>1.71 (1.09–2.67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>60 (35.7)</td>
<td>95 (50.5)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>132 (39.3)</td>
<td>108 (28.7)</td>
<td>0.0029*</td>
<td>1.60 (1.17–2.20)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>204 (60.7)</td>
<td>268 (71.3)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
</tbody>
</table>

† Genotype and allele frequencies were compared between patients with SLE and controls. p values are calculated by chi-square test without correction for multiple tests. * Indicates statistical significance. HWE: Hardy-Weinberg equilibrium.

### Table 3. Association study of c.1567 C/T of LUM in patients with SLE stratified by 11 clinical symptoms.

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinuclear antibody</td>
<td>82 (96.8)</td>
<td>3 (3.7)</td>
<td>0.93</td>
<td>0.92 (0.16–5.17)</td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>74 (77.9)</td>
<td>21 (22.1)</td>
<td>0.95</td>
<td>0.97 (0.46–2.05)</td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>46 (48.4)</td>
<td>49 (51.6)</td>
<td>0.45</td>
<td>1.27 (0.69–2.35)</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>10 (10.5)</td>
<td>85 (89.5)</td>
<td>0.15</td>
<td>1.96 (0.77–5.03)</td>
</tr>
<tr>
<td>Renal</td>
<td>38 (40.0)</td>
<td>57 (60.0)</td>
<td>0.65</td>
<td>1.15 (0.62–2.15)</td>
</tr>
<tr>
<td>Serositis</td>
<td>20 (21.1)</td>
<td>75 (78.9)</td>
<td>0.54</td>
<td>1.26 (0.60–2.63)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>44 (46.3)</td>
<td>51 (53.7)</td>
<td>0.0064*</td>
<td>2.38 (1.27–4.46)</td>
</tr>
<tr>
<td>Mucosal ulcer</td>
<td>34 (35.4)</td>
<td>62 (64.6)</td>
<td>0.68</td>
<td>0.88 (0.46–1.66)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>43 (46.7)</td>
<td>49 (53.3)</td>
<td>0.030*</td>
<td>2.05 (1.06–3.94)</td>
</tr>
<tr>
<td>Discoid lupus</td>
<td>13 (13.5)</td>
<td>83 (86.5)</td>
<td>0.19</td>
<td>1.75 (0.75–4.08)</td>
</tr>
<tr>
<td>Malar rash</td>
<td>48 (50.5)</td>
<td>47 (49.5)</td>
<td>0.068</td>
<td>1.78 (0.95–3.32)</td>
</tr>
</tbody>
</table>

† Comparisons between the positive and negative groups by chi-square test of the allele distributions. p values are calculated without correction for multiple tests. * Indicates statistical significance. Positive: Patients positive for a certain phenotype. Negative: Patients negative for a certain phenotype.
in such processes as we found, that the c.1567 C/T polymorphism of LUM is associated with the development of SLE disease, as well as clinical symptoms manifested in connective tissues such as arthritis and photosensitivity. Moreover, the relationship with those clinical symptoms is also consistent with the fact that LUM is crucial for maintenance of normal functioning of skin tissue. Therefore, genetic variations of LUM may act as a common pathogenic linkage between SLE and other connective tissue diseases. The pathologic effects of the genetic variations on ECM architecture may represent an indicator of LUM’s activity within a variety of tissues affected by SLE, and aid in elucidating its cellular and molecular functions.

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REFERENCES


