Mannose-Binding Lectin Gene and Promoter Polymorphism and Susceptibility to Renal Dysfunction in Systemic Lupus Erythematosus

1Mohammad Asgharzadeh, 2Hossein Samadi Kazil, 3Mohammad Ebrahim Ebrahimzadeh and 3Aboulfazl Bohloul
1Biotechnology Research Center, Tabriz University of Medical Science, Tabriz, Iran
2Department of Microbiology, School of Medicine, Tabrizz Modares University, Tehran, Iran
3Department of Urology and Nephrology, Emam Khomeini Hospital, Tabriz University of Medical Science, Tabriz, Iran

Abstract: Systemic Lupus Erythematosus (SLE) is a prototypical autoimmune disease characterized by the production of the auto antibodies, the aim of present study is to determine the distribution of the alleles of Mannose-binding Lectin (MBL) gene codon 52, 54 and 57 and promoter variants H/L, X/Y, P and Q in SLE patients while compares then with normal control and seek correlation between these variants and disease that cause renal dysfunction. Twelve SLE patients with renal failure samples were compared with thirty normal controls from Azerbaizani population of Iran. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Allelic and genotypic frequency of the polymorphism at position-550,+4 and at codon 52, 54 and 57 did not show statistical differences between SLE patients and controls. But frequency of Lx haplotype of promoter was observed in patients with SLE and Renal failure (p = 0.0518). Present findings showed that presence of Lx haplotype that cause low concentration of MBL in serum can be a risk factor for severity of systemic Lupus Erythematosus and susceptibility to renal dysfunctions.

Key words: Gene polymorphism, mannose-binding lectin, promoter, renal dysfunction, SLE

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a prototypical autoimmune disease characterized by the production of autoantibodies and the deposition of immune complexes in affected end organs (Sullivan et al., 2003; Kelsoe, 2003). There is an implied loss of both B-cell and T-cell tolerance.

Both genetic and environmental factors appear to contribute to the development of Systemic Lupus Erythematosus (Deapen et al., 1993; Kammer and Mishra, 2000). Genetic factors such as hereditary complement deficiencies contribute to disease susceptibility and inherited homozygous deficiencies of C1, C2 and C4 have been reported to be associated with a high incidence of SLE (Lachmann et al., 1991; Walport, 1993). There is a well documented association of the HMC class II and III phenotype C4Q0 with SLE both in different ethnic groups of patients with SLE (Kumar et al., 1991; Tsao, 2004; Hirose et al., 2006).

Mannose-Binding Lectin (MBL) is a member of the collectin family of proteins found in serum (Presansis et al., 2003). It binds to mannose and N-acetylglucosamine and activates the complement system independently of antibodies via two associated serine protease, MBL-associated serine protease 1 and 2 (Jack et al., 2001; Mass et al., 1998; Thiel et al., 1997). C1q and MBL, as well as lung surfactant protein A share the same phagocytic receptor, which is present on a variety of cells, including phagocytes platelets and endothelial cells (Nepomuceno et al., 1997; Nepomuceno and Tenner, 1998).

Human MBL is derived from a single gene on chromosome 10 (Sastry et al., 1989), the normal structural MBL allele is named A, while the common designation for the 3 variant structural alleles B (Mutation in codon 54, Gly to ASP), C (mutation in codon 57, Gly to Glu) and D (mutation in codon 52, Arg to Cys) are O (Hegele et al., 1999; Neth et al., 2001).

In general, individuals with a normal genotype (A/A) have MBL concentration in serum that are 6-8 times higher than those in individuals heterozygous for one of the variant alleles (A/O: A/B, A/C or A/D), while individuals with a defective genotype (2

Corresponding Author: Mohammad Asgharzadeh, Biotechnology Research Center, Tabriz University of Medical Sciences, Gohgasht Ave, Tabriz, Iran. Tel: +98 411 3357126. Fax: +98 411 3364666
variant alleles B/B, C/C, D/D, B/C, B/D or C/D) have almost undetectable MBL serum levels (Garred et al., 1999; Graudal et al., 2000; Dornelles et al., 2006).

Moreover, MBL expression is influenced by polymorphic sites in the upstream part of the MBL gene (Crosdale et al., 2001; Ip et al., 1998) nucleotides substitutions at position -550, -221 and +4 give rise to H/L, Y/X and P/Q respectively and cause to different haplotypes, while LX haplotype is associated with low MBL plasma levels (Santos et al., 2001; Soborg et al., 2003; Asgharzadeh et al., 2007). We investigated in a group of Iranian patients, whether the risk of renal dysfunction in SLE can be associated with the presence of MBL variant alleles.

**MATERIALS AND METHODS**

Blood samples were obtained from twelve SLE patients with sever Renal failure from March 2004 to July 2005 and thirty normal control from Azerbajan population of Iran. DNA was isolated from either granulocytes or mononuclear cells by the modified proteinase K, Sodium Dodecyl Sulfate (SDS), N-acetyl-N, N-trimethyl ammonium bromide (CTAB). (Asgharzadeh et al., 2007)

PCR was performed in 20 to 100 μL volumes that contained 50 to 500 ng of genomic DNA, 0.5 μm of specific primers (Table 1) in the presence of 1.5 mM MgCl2, 100 μM of each dNtp, 50 mM KCl, 20 mM tris-HCl, pH 8.4 and 1 to 2.5 unit recombinant DNA polymerase (fermentas). DNA was amplified by general PCR and sequence-specific primed polymerase chain reaction (SSP-PCR). All PCRs were initiated by a 4-min denaturating step at 94°C and completed by a 7-min extension step at 72°C. The temperature cycles for different types of PCRs were as follows: 32 cycles of 40 sec at 94°C, annealing temperature for 40 sec and 72°C for 55 sec.

Annealing temperatures which were used as bellow follow: 60, 63, 65, 66, 67, 68, 66, 67, 66, 65, 67, 65, 66, 67, 66, 65, 67 for codon 57 (wild type), 57 (mutant), 54 (wild type), 54 (mutant), 52 (wild type), 52 (mutant), allele H, L, P, Q, haplotypes H/H, L/H, X/Y amplification, respectively (Madsen et al., 1995; Crosdale et al., 2000, Sullivan et al., 1996).

In addition to SSP-PCR, B and C alleles were detected by Ban I and Mbo I restriction enzyme digestions of the 328 bp product amplified by the allele P and Q primers, respectively (Table 1), followed by a 2.5 % agarose gel electrophoresis. Ban I cleaves the A allele into two fragments allele (245 and 83 bp) and leaves the B allele undigested, while Mbo I specifically cleaves the C allele into two fragments (266 and 62 bp) (Asgharzadeh et al., 2007).

Statistical analyses were performed by χ² (Chi-square test). p-values below 0.05 were considered statistically significant.

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**Table 1: Oligonucleotides used for genotyping Mannose-binding lectin by Polymerase Chain Reaction (PCR)**

<table>
<thead>
<tr>
<th>Origin of genes</th>
<th>Sequence of primers</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 57 (wild type)</td>
<td>GAG GCT TAC ACC TAG</td>
<td>GAT ACC</td>
<td>TAT GGC GGT AG</td>
</tr>
<tr>
<td>Codon 57 (mutant)</td>
<td>CTA CTC CTA TTA</td>
<td>GCT GGT</td>
<td>CCA CCA GTT</td>
</tr>
<tr>
<td>Codon 54 (wild type)</td>
<td>GAG GCT TAC ACC TAG</td>
<td>GAC GGT</td>
<td>GGT GGT AG</td>
</tr>
<tr>
<td>Codon 54 (mutant)</td>
<td>CTA CTC CTA TTA</td>
<td>GCT GGT</td>
<td>CCA CCA GTT</td>
</tr>
<tr>
<td>Codon 52 (wild type)</td>
<td>GAG GCT TAG ACC TAC</td>
<td>GAC GGT</td>
<td>GGT GGT AG</td>
</tr>
<tr>
<td>Codon 52 (mutant)</td>
<td>CTA CTC CTA TTA</td>
<td>GCT GGT</td>
<td>CCA CCA GTT</td>
</tr>
<tr>
<td>MBL allele H</td>
<td>GCT TAC CCA GGC AGG</td>
<td>GCT TAC CCA GGC AGG</td>
<td></td>
</tr>
<tr>
<td>MBL allele L</td>
<td>GCT TAC CCA GGC AGG</td>
<td>GCT TAC CCA GGC AGG</td>
<td></td>
</tr>
<tr>
<td>MBL allele P</td>
<td>GTA GGA AGG AGG</td>
<td>GTA GGA AGG AGG</td>
<td></td>
</tr>
<tr>
<td>MBL allele Q</td>
<td>GTA GGA AGG AGG</td>
<td>GTA GGA AGG AGG</td>
<td></td>
</tr>
<tr>
<td>Haplotypes H/H</td>
<td>GCT TAC CCA GGC AGG</td>
<td>GCT TAC CCA GGC AGG</td>
<td></td>
</tr>
<tr>
<td>Haplotypes L/L</td>
<td>GCT TAC CCA GGC AGG</td>
<td>GCT TAC CCA GGC AGG</td>
<td></td>
</tr>
<tr>
<td>Haplotypes L/H</td>
<td>GTA GGA AGG AGG</td>
<td>GTA GGA AGG AGG</td>
<td></td>
</tr>
<tr>
<td>Haplotypes H/H</td>
<td>GCT TAC CCA GGC AGG</td>
<td>GCT TAC CCA GGC AGG</td>
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</table>
RESULTS

From twelve cases, ten were women and two were men, the association between MBL deficiency and renal failures can not be explain by confounding factors such as differences in age at disease onset or disease duration (Garred et al., 1999).

This overall frequencies did not differ significantly between SLE patients with renal failure and normal controls but tendency to loss of wild type allele A was higher between SLE patients (p = 0.025) (Table 2). Frequency of promoter variants and position +4 showed in Table 3 and there was not any statistical difference between SLE patients and Normal controls.

In Table 4 we showed frequency of promoter haplotypes between SLE patients with renal failure and controls and in HY and LY haplotypes there was not statistical difference, but in LX haplotypes that cause to lower concentration of MBL, frequency in SLE patients was higher than normal controls (p = 0.0518) but it had not a full reliable statistical value.

<table>
<thead>
<tr>
<th>Allele</th>
<th>SLE patients (n=12)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
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<tr>
<td>Codon 54 mutation</td>
<td>33.3</td>
<td>33.3</td>
<td>0.1356</td>
</tr>
<tr>
<td>(Allele B)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Codon 57 mutation</td>
<td>8.3</td>
<td>3.3</td>
<td>0.4918</td>
</tr>
<tr>
<td>(Allele C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codon 52 mutation</td>
<td>8.3</td>
<td>6.7</td>
<td>0.08497</td>
</tr>
<tr>
<td>(Allele D)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>50</td>
<td>76.7</td>
<td>0.0092</td>
</tr>
<tr>
<td>(Allele A)</td>
<td></td>
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* Mannose-binding lectin Variants, frequency in patients and controls | Each patient has two alleles in its genotype

<table>
<thead>
<tr>
<th>Variant</th>
<th>SLE patients (n=12)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
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<tr>
<td>H</td>
<td>25</td>
<td>36.67</td>
<td>0.4687</td>
</tr>
<tr>
<td>L</td>
<td>75</td>
<td>63.33</td>
<td>0.4918</td>
</tr>
<tr>
<td>P</td>
<td>81.33</td>
<td>73.33</td>
<td>0.04918</td>
</tr>
<tr>
<td>Q</td>
<td>16.67</td>
<td>26.67</td>
<td>0.4918</td>
</tr>
</tbody>
</table>

* Mannose-binding lectin Variants, frequency in patients and controls | Each Patient has two variants in its genotype

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>SLE patients (n=12)</th>
<th>Controls (n=30)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Hy</td>
<td>25</td>
<td>36.67</td>
<td>0.7627</td>
</tr>
<tr>
<td>Ly</td>
<td>25</td>
<td>43.33</td>
<td>0.2690</td>
</tr>
<tr>
<td>LX</td>
<td>50</td>
<td>20.00</td>
<td>0.0518</td>
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</table>

<table>
<thead>
<tr>
<th>Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 54 mutation</td>
</tr>
<tr>
<td>(Allele B)</td>
</tr>
<tr>
<td>Codon 57 mutation</td>
</tr>
<tr>
<td>(Allele C)</td>
</tr>
<tr>
<td>Codon 52 mutation</td>
</tr>
<tr>
<td>(Allele D)</td>
</tr>
<tr>
<td>Wild type</td>
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<tr>
<td>(Allele A)</td>
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</table>

<table>
<thead>
<tr>
<th>Frequency (%)*</th>
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<tbody>
<tr>
<td>Codon 41 mutation</td>
</tr>
<tr>
<td>(Allele B)</td>
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<tr>
<td>Codon 47 mutation</td>
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<tr>
<td>(Allele C)</td>
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<tr>
<td>Codon 42 mutation</td>
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<td>(Allele D)</td>
</tr>
<tr>
<td>Wild type</td>
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<tr>
<td>(Allele A)</td>
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</table>

<table>
<thead>
<tr>
<th>Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hy</td>
</tr>
<tr>
<td>Ly</td>
</tr>
<tr>
<td>LX</td>
</tr>
</tbody>
</table>

Table 2: Genotype frequency of mannose-binding lectin structural alleles in SLE patients and Healthy controls

Table 3: Frequency of promoter variants and position +4 in SLE patients and Healthy controls

Table 4: Promoter haplotypes frequency in SLE patients and healthy controls

DISCUSSION

Several previous studies investigated the possibility of interactions among SLE candidate genes. Most of them were focused on the relative contributions of HLA and complement loci on chromosome 6 (Reveille et al., 1998; Christiansen et al., 1991), present approach relied on data suggesting that multiple genes involved in systemic lupus erythematosus (Sullivan et al., 2003; Winehester et al., 1982; Soborg et al., 2003), low serum MBL levels have been reported to be associated with SLE in recent studies (Ip et al., 1998; Senaldi et al., 1995), As MBL is an acute phase protein, such results may be difficult to interpret and genetic studies may be more informative. In present study we focused in SLE patients that lost their kidney and find Relation of MBL polymorphism and renal failure in SLE patients.

Present study focused in presence of different alleles and promoter variants and as results showed there was not any difference in MBL gene allele between our patients and normal controls (Table 2) also in our study we didn’t find any differences in promoter variants and position +4 between SLE patients and normal controls (Table 3) but in promoter haplotypes frequency of LX haplotype in SLE patients with renal dysfunction was higher than normal controls. This haplotype associate with a low concentration of MBL in serum (Santos et al., 2001) and it was evident that presence of LX haplotype with MBL deficiency accounted for much of the renal failure tendency seen in the SLE patients which conclude to renal transplantation.

In previous studies such as Garred et al. (1999) studied relation of MBL polymorphism and susceptibility to infections in Systemic lupus erythematosus and found hemozoxycity for MBL variant alleles was strongly associated with infections in SLE patients, or Sendali et al. (1995) demonstrated the association of codon 54 mutation (allele B) and SLE, or Ip et al. (1998) have been showed that the low producing promoter polymorphisms of the MBL gene were associated with SLE. These observations are in keeping with present observation and low serum MBL associating with developing of SLE in these patients. Patients with SLE and renal failure are rare, therefore, with larger group of patients there will be more statistical significance.

Structural mutations of the MBL gene resulting to low serum MBL levels and in our study tendency of mutations in patients was higher in comparing with normal control but there was not statistical difference.

In conclusion present study showed that the low producing promoter polymorphism (LX) can associate
with severe renal failure in SLE patients and it can conclude
that a low serum MBL level is a risk factor for developing
of SLE to renal failure.

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