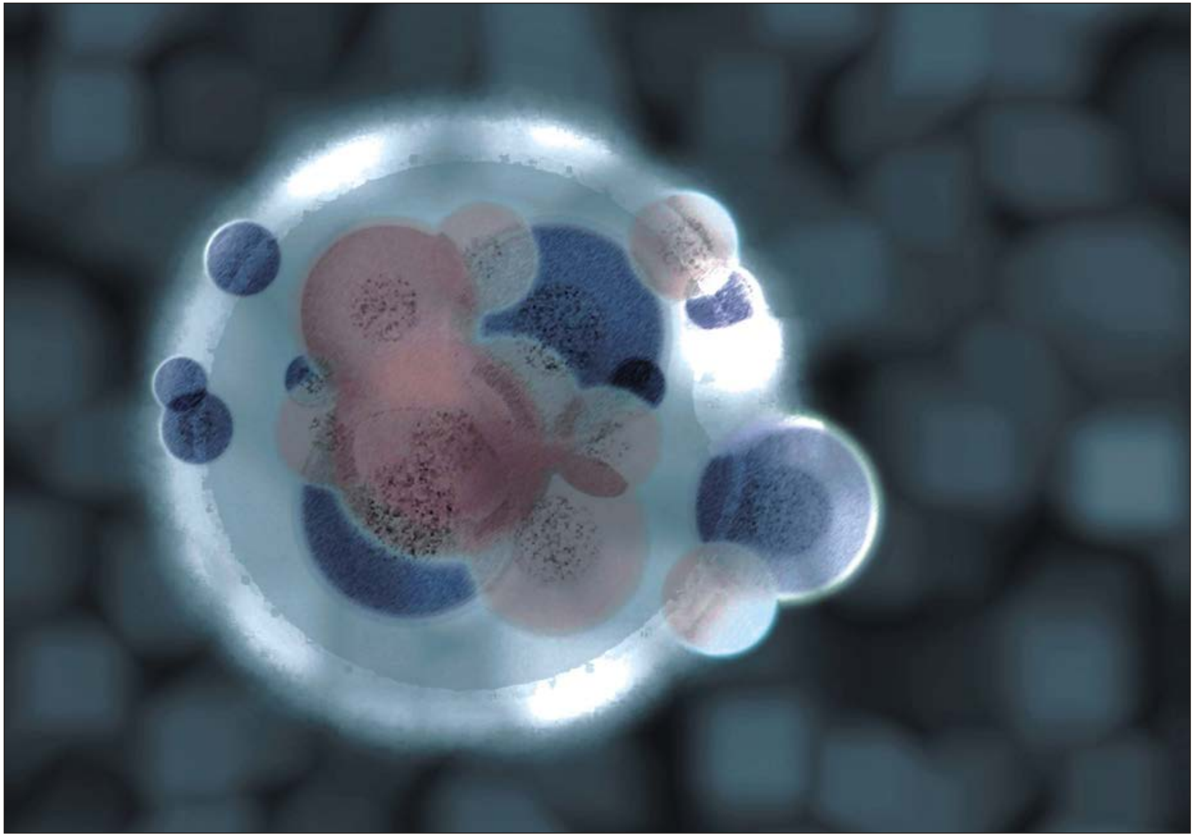

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Mannose-Binding Lectin Gene and Promoter Polymorphism and Susceptibility to Renal Dysfunction in Systemic Lupus Erythematosus

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Abstract: Systemic Lupus Erythemattosus (SLE) is a prototypical auto immuno 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 Azarbaijan 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 de 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 effected 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 MHC 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 (Presanis *et al*., 2003). It binds to mannose and N-acetylglucosamine and activates the complement system independently of antibodies via two associated serin protease, MBL-associated serin 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; Nepomoceno 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 that 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

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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/I, 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 Azarbaijan 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 denaturizing 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, 63, 62, 66, 63, 66, 67, 64, 67, 67, 65, 65 and 66 for codon 57 (wild type), 57 (mutant), codon 54 (wild type), 54 (mutant), codon 52 (wild type), 52 (mutant), allele H, L, P, Q, haplotypes Hy, Ly, Lx and Hx 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 χ^2 (Chi-square test). p-values below 0.05 were considered statistically significant.

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

Origin of genes	Sequence of primers								
Codon 57 (wild type)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	TAC	CTG	GTT	CCC	CCT	TTT	CTC	
Codon 57 (mutant)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	TAC	CTG	GTT	CCC	CCT	TTT	CTT	
Codon 54 (wild type)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	CCC	CTT	TTC	TCC	CTT	GGT	GC	
Codon 54 (mutant)	Forward	GAG	GCT	TAG	ACC	TAT	GGG	GCT	AG
	Reverse	CCC	CTT	TTC	TCC	CTT	GGT	GT	
Codon 52 (wild type)	Forward	CTT	CCC	AGG	CAA	AGA	TGG	GC	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
Codon 52 (mutant)	Forward	CTT	CCC	AGG	CAA	AGA	TGG	GT	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele H	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTG	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele L	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele P	Forward	GTA	GGA	CAG	AGG	GCA	TGC	TC	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
MBL allele Q	Forward	GTA	GGA	CAG	AGG	GCA	TGC	TT	
	Reverse	CAG	GCA	GTT	TCC	TCT	GGA	AGG	
Haplotypes Hy	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTG	
	Reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CC
Haplotypes Ly	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	Reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CC
Haplotypes Lx	Forward	GCT	TAC	CCA	GGC	AAG	CCT	GTC	
	Reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CG
Haplotypes Hx	Forward	GCT	TAC	CCA	GGC	AAG	CTT	GTG	
	Reverse	GGA	AGA	CTA	TAA	ACA	TGC	TTT	CG

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 = 0.095) (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 2: Genotype frequency of mannose-binding lectin structural alleles in SLE patients and Healthy controls

	Frequency (%)*		
Alleles	SLE patients (n =12)	Controls (n =30)	p-value
Codon 5 4mutation (Allele B)	33.3	13.3	0.1359
Codon 57 mutation (Allele C)	8.3	3.3	0.4918
Codon 52 mutation (Allele D)	8.3	6.7	08497
Wild type (Allele A)	50	76.7	0.0912

* Mannose-binding lectin *Variants frequency in patients and controls* †Each patient has two alleles on its genotype

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

	Frequency (%)*		
Variant	SLE patients (n =12) †	Controls (n =30)†	p-value
H	25	36.67	0.4687
L	75	63.33	0.4687
P	83.33	73.33	0.4918
Q	16.67	26.67	0.4918

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

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

	Frequency (%)*		
Haplotypes	SLE patients (n =12)	Controls (n =30)	p-value
Hy	25	36.67	0.7627
Ly	25	43.33	0.2690
LX	50	20.00	0.0518

* Mannose-binding lectin *Variants frequency in patients and 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 hemozygosity 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 sever 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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