Mannose-Binding Lectin is a Disease-Modifying Factor in North American Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To investigate whether development of systemic lupus erythematosus (SLE), its clinical manifestations, and autoantibody production are associated with polymorphisms of the mannose-binding lectin (MBL) gene in North American patients with SLE.

Methods. MBL gene polymorphisms in codons 52 (designated variant D, with the wild-type designated A), 54 (variant B), and 57 (variant C) were determined by polymerase chain reaction-sequence specific priming in 130 patients with SLE and 142 healthy controls. Autoantibodies against double-stranded DNA (dsDNA), Smith antigen, phospholipids, Ro/SSA, La/SSB, and RNP were tested at certified clinical pathology laboratories.

Results. A statistically significant increased likelihood of anti-Smith antibody production was observed in SLE patients with the heterozygous A/B genotype [odds ratio (OR) 5.1; 95% confidence interval (CI) 1.6–16.6; the A/A genotype as the reference group] or A/C genotype (OR 8.2; 95% CI 2.0–33.9). SLE patients with the homozygous or compound heterozygous variant genotype (O/O; O, a common designation for variant alleles) had an increased likelihood of mounting autoantibody responses against dsDNA, Ro/SSA, and La/SSB, and were more likely to have a history of renal disease (OR 4.8; 95% CI 0.9–25.2). However, differences in the frequencies of MBL variant alleles and genotypes observed between patients with SLE and controls did not reach statistical significance.

Conclusion. A significantly increased prevalence of anti-Smith antibody was associated with the heterozygous genotypes A/B and A/C. Although MBL structural gene polymorphism was not a risk factor for SLE development in this study population, homozygosity of MBL variant alleles may be a weak disease-modifying factor, particularly for renal involvement, in North American patients with SLE. (First Release June 15 2007; J Rheumatol 2007;34:1506–13)

Key Indexing Terms:
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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production and immune complexes/complement-mediated inflammatory tissue damage. While the etiopathogenesis of SLE remains elusive, a combination of genetic and environmental factors is believed to provoke development of the disease. There is compelling evidence that complete deficiency of any of the early complement components (C1q, C2, and C4) is the strongest genetic risk factor for SLE development[1-3], with a hierarchical association with disease prevalence and severity. It has been reported that 90%, 75%, and 30% of individuals deficient in C1q, C4, and C2, respectively, develop SLE[1-3]. However, homozygous complement deficiencies are extremely rare, occurring in about 1% of patients[4], suggesting the involvement of other genetic factors in the majority of patients with SLE.

Recently, the mannose-binding lectin (MBL) gene has emerged as a candidate lupus gene due to the role of MBL in innate immunity and the possible association between MBL deficiency and disease (e.g., infections and autoimmune disorders)[5,6]. MBL, which is structurally and functionally analogous to C1q, is encoded by a single 4-exon gene located on chromosome 10. MBL is a multi-chain molecule of up to 6 subunits. Each subunit consists of 3 identical 32-kDa polypep-

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tide chains that contain a cysteine–rich region, a collagenous region, a "neck" region, and a carbohydrate-binding domain encoded by exon 1, exon 2, exon 3, and exon 4, respectively[7,8]. Mutations in the coding and promoter regions dramatically affect serum levels of MBL[8-10]. Three point mutations at nucleotides 223 (C to T), 230 (G to A), and 239 (G to A) of exon 1, resulting in changes of amino acid residues 52 (Arg to Cys), 54 (Gly to Asp), and 57 (Gly to Glu), give rise to 3 variant alleles D, B, and C, respectively[11-13]. Of the 3 structural variant alleles, the B and C alleles lead to profound reduction of serum MBL levels, whereas the D allele has minimal impact. Two other variants commonly found in the promoter region, H/L (due to nucleotide substitutions at position –550) and Y/X (nucleotide substitution at position –221), also influence serum MBL levels[14]. In particular, the X allele (a G to C substitution) is associated with low serum MBL concentrations[10].

As part of the complement system, MBL is responsible for initiating the lectin pathway. In light of its structural and functional similarities to C1q and the strong association of C1q deficiency with SLE, MBL variant alleles that lead to low serum levels and/or functional deficits of MBL are postulated to contribute to the susceptibility of SLE. However, reports of the effects of MBL polymorphisms on SLE development vary considerably. MBL deficiency due to variant alleles has been identified as a risk factor for developing SLE in African American, Chinese, and Japanese populations[15-18], and the risk was greater if combined with defects in Fcγ receptors[19] or C4B null alleles[20]. In several studies using patients of different ethnic origins, MBL variant alleles have been identified either as a minor risk factor[21,22] or not a risk factor[23] for developing SLE. The influence of MBL gene variants on autoantibody production[24,25] and organ involvement[15,22] in patients with SLE also remains controversial. Such diverse results may originate from differences in sample size, homogeneity of samples, selection of controls, or ethnic background of patient populations in different studies[26].

In view of the inconsistent reports concerning the role of MBL gene polymorphism in SLE and the lack of rigorous studies of MBL variants in patients with SLE in the United States, we investigated the association of MBL polymorphisms with SLE and its clinical manifestations and autoantibody production in a large cohort of well characterized patients with SLE and healthy controls (predominantly Caucasian women) followed at a major medical center in the US.

MATERIALS AND METHODS

Patients with SLE and controls. Patients with SLE were recruited for this study during routine visits to the University of Pittsburgh Lupus Translational Research and Patient Care Center. All patients met the 1982[27] or 1997[28] American College of Rheumatology revised criteria for the classification of definite SLE. As part of their routine care, all patients underwent a history-taking and examination by one physician. Blood was drawn at their routine visits. Disease activity was assessed at the time of the visit using the Systemic Lupus Activity Measure (SLAM)[29] and the Safety of Estrogens in Lupus Erythematosus: National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI)[30]. Controls were recruited through advertisements posted on the University of Pittsburgh campus. A total of 130 patients with SLE and 142 controls were enrolled. To confirm their health status, all participants completed a brief questionnaire regarding existing medical conditions. Ethnicity was self-reported by the subjects. Informed consent was obtained from the patients and controls. The University of Pittsburgh Institutional Review Board approved our study.

Detection of MBL structural variant alleles. Genomic DNA was extracted from peripheral blood cells using reagents provided in the REDExtract-N-Amp Blood PCR Kit (Sigma, St. Louis, MO, USA) following the manufacturer's instruction. MBL variant alleles were detected using the polymerase chain reaction-sequence specific priming (PCR-SSP) technique[31] (see Table 1 for primer sequences). The B and C alleles were detected by PCR with confronting primers using modified conditions of Hamajima, *et al*[32]. The AnonD and D alleles were detected in separate PCR because of the difficulty of confronting primer design. Oligonucleotide primers encoding sequences within exon 4 of the MBL gene were used to amplify an internal control fragment. PCR were performed in a volume of 20 μl containing 1.5 mM of $MgCl_2$, 50 μM of dNTP, 0.025 U/μl of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.28 μM of internal control primers, 1.0 μM of AnonB/AnonC forward primers and B/C reverse primers, 0.8 μM of AnonB/AnonC reverse primers and B/C forward primers, and 0.4 μM of AnonD and D primers. All PCR were initiated by 3 min of denaturation at 94°C and completed by 10 min of extension at 72°C. PCR cycles were as follows: 30 cycles of 30 s at 94°C, 30 s at 56°C, and 45 s at 72°C. PCR amplicons were resolved on 2% agarose gels containing ethidium bromide and visualized by UV transillumination.

Statistical analysis. The allelic and genotypic frequencies of the MBL genes for patients with SLE and controls were compared, and Fisher's exact test was used to assess the statistical significance of differences (GraphPad, QuickCalcs, online calculator). The significance of the associations between MBL genotypes and autoantibody production or clinical features in patients with SLE was also tested using Fisher's exact test. Four dummy variables were constructed for genotypes A/B, A/C, A/D, and O/O (wild-type A/A was the referent group), and the association between the MBL genotypes and autoantibody production or clinical phenotypes was estimated by odds ratios (OR) with 95% confidence interval (CI) using logistic regression analysis by SPSS statistical software (Version 11.0 for Windows). All tests were 2-tailed. P values < 0.05 were considered statistically significant.

RESULTS

A multiethnic cohort of 130 patients with SLE (female/male = 128/2; Caucasian/African American/other races = 109/18/3; mean age = 44.35 ± 12.25 yrs) and 142 controls (F/M = 132/10; Caucasian/African American/other races = 130/7/5; mean age = 43.91 ± 11.55 yrs) were examined for single nucleotide polymorphisms at codon 52(D), codon 54(B), and codon 57(C) of exon 1 of the mbl2 gene by PCR using sequence-specific primers. As summarized in Table 2, allele frequencies and genotype distribution were comparable in patients with SLE and controls. The distribution of MBL genotypes in both controls and patients with SLE showed good probability of fitness with Hardy-Weinberg equilibrium (data not shown).

When the SLE patient cohort and control group were stratified by ethnicity, comparable allele and genotype distributions in patients with SLE versus controls were observed in both Caucasian and African American groups, with an exception for the D allele (Table 2C). The frequencies of the D

Table 1. Sequences of PCR-SSP primers for the *mbl2* gene.

Allele	Sequence		Product Size
AnonB	Forward	5' AGT CGA CCC AGA TTG TAG GAC AGA G 3"	277 bp
	Reverse	5' CCT TTT CTC CCT TGG TGC 3'	
B	Forward	5' GCA AAG ATG GGC GTG ATG A 3'	224 bp
	Reverse	5' GGG CTG GCA AGA CAA CTA TTA 3'	
AnonC	Forward	5' ACT CGA CCC AGA TTG TAG GAC AGA G 3'	287 bp
	Reverse	5' CCT GGT TCC CCC TTT TCT C 3'	
C	Forward	5' CAA AGA TGG GCG TGA TGG CAC CAA GGA 3'	222 bp
	Reverse	5' GGG CTG GCA AGA CAA CTA TTA 3'	
AnonD	Forward	5' AGT CGA CCC AGA TTG TAG GAC AGA G 3'	270 bp
	Reverse	5' TCC CTT GGT GCC ATC ACG 3'	
D	Forward	5' AGT CGA CCC AGA TTG TAG GAC AGA G 3'	271 bp
	Reverse	5' CTC CCT TGG TGC CAT CAC A 3'	
Exon 4	Forward	5' GAG TTT CAC CCA CTT TTT CAC A 3'	421 BP
	Reverse	5' GCC TGA GTG ATA TGA CCC TTC 3'	

allele and A/D genotype were higher in healthy African Americans (14.3% and 28.6%, respectively) than in African American patients with SLE (0% and 0%, respectively). The MBL allele frequencies and genotype distribution in study participants of other races were not separately analyzed due to their small numbers (3 patients with SLE, all A/A genotype; 5 healthy individuals, 3 A/A genotype and 2 A/B genotype).

Overall, there appeared to be an increase in the prevalence of the A/C genotype among this multiethnic cohort of patients with SLE (12/130 = 9.2% vs 5/142 = 3.5%; Table 2A), particularly in the African American patients (7/18 = 38.9% vs 1/7 = 14.3%; Table 2C). Such subtle, although statistically insignificant, increases in the prevalence of heterozygous MBL genotypes in patients with SLE prompted us to explore further the potential effects of the MBL variant alleles/deficiency genotypes on serological and clinical manifestations of SLE.

To examine the relationship between MBL gene polymorphisms and autoantibody production, the prevalence of several autoantibodies in SLE patients with different MBL genotypes was analyzed. The results showed that SLE patients with the O/O genotype were more likely to produce anti-dsDNA (100% in O/O vs 65.3% in A/A and 63.8% in A/O) and other autoantibodies (anti-Ro/SSA, anti-La/SSB, and anti-RNP — 50.0% in O/O vs 34.7% in A/A and 36.2% in A/O), but less likely to produce antiphospholipid antibodies (anticardiolipin and/or lupus anticoagulant — 25.0% in O/O vs 50.7% in A/A and 44.7% in A/O) and anti-Smith antibody (0% in O/O vs 8.0% in A/A and 29.8% in A/O; $p < 0.001$; Table 3A). In comparison, patients with the A/O genotype were mostly likely to develop anti-Smith antibodies. The statistically significant increase in the prevalence of anti-Smith antibody in SLE patients with the A/O genotype was further investigated. The data revealed that patients with the A/B and A/C genotype were more likely to develop anti-Smith antibody (30.8% and 41.7%, respectively) than those with the A/D genotype (12.5%) and the O/O genotype (0.0%; Table 3B). Logistic regression analysis further showed that SLE patients with the heterozygous variant genotype A/B or A/C, as compared to those with the A/A genotype, were several times more likely to have anti-Smith antibodies (OR 5.1; 95% CI 1.6–16.6 for A/B and OR 8.2; 95% CI 2.0–33.9 for A/C; Table 4).

The correlation of clinical features, including renal disease, cardiovascular disease, neurologic disease, hematologic disease, malar rash, discoid rash, photosensitivity, oral ulcers, and serositis, with MBL polymorphisms was also evaluated. The prevalence of different clinical features varied across SLE patients with different MBL genotypes. The only variable that reached statistical significance was renal disease ($p = 0.035$; Table 5). Logistic regression modeling indicated that patients with the O/O genotype were almost 5 times more likely to have renal involvement (OR 4.8; 95% CI 0.9–25.2) than patients with the A/A genotype (Table 6).

DISCUSSION

We examined MBL structural polymorphisms at codon 52 (allele D), codon 54 (allele B), and codon 57 (allele C) in a multiethnic cohort of 130 patients with SLE (84% Caucasians, 14% African Americans, and 2% other races) who were recruited at a major US medical center. Although differences in allelic and genotypic frequencies observed between patients with SLE and controls did not reach statistical significance, the D allele and A/D genotype appeared to occur less frequently in African American patients with SLE than in healthy African Americans (Table 2C). Because of the size of the African American cohort (18 patients with SLE and 7 controls), it is premature to conclude that the D allele and A/D genotype are protective against the development of SLE in African American patients. Notably, the prevalence of the A/C genotype was higher in patients with SLE (especially African American patients) than healthy controls (Tables 2A and 2C). Production of anti-Smith antibody, which is specific for lupus, was most prevalent among SLE patients with the A/C geno-

Table 2. Allelic and genotypic frequencies of MBL variants in patients with SLE and healthy controls.

2A. Analysis of the entire multiethnic cohort.

	SLE, n = 130 (%)	Healthy Control, n = 142 (%)	p*
Allele			
A	197 (75.8)	218 (76.8)	0.840
B	33 (12.7)	33 (11.6)	0.793
C	16 (6.1)	11 (3.9)	0.241
D	14 (5.4)	22 (7.7)	0.303
Genotype			
A/A	75 (57.7)	87 (61.3)	0.621
A/B	26 (20.0)	26 (18.3)	0.759
A/C	12 (9.2)	5 (3.5)	0.077
A/D	9 (6.9)	13 (9.2)	0.657
O/O†	8 (6.2)	11 (7.7)	0.642
A/O	47 (36.1)	44 (31.0)	0.372

* SLE vs controls, Fisher's exact test. † O/O distribution in SLE: B/B (1), B/C (2), B/D (3), C/C (1), D/D (1). O/O distribution in controls: B/C (2), B/D (5), C/C (1), C/D (2), D/D (1).

2B. Analysis of the Caucasian cohort.

	SLE, n = 109 (%)	Healthy Control, n = 130 (%)	p*
Allele			
A	168 (77.1)	199 (76.5)	0.914
B	31 (14.2)	31 (11.9)	0.496
C	5 (2.3)	10 (3.8)	0.433
D	14 (6.4)	20 (7.7)	0.721
Genotype			
A/A	64 (58.7)	80 (61.5)	0.692
A/B	26 (23.9)	24 (18.5)	0.340
A/C	5 (4.6)	4 (3.1)	0.736
A/D	9 (8.2)	11 (8.5)	1.000
O/O†	5 (4.6)	11 (8.5)	0.302
A/O	42 (36.7)	39 (30.0)	0.334

* SLE vs controls, Fisher's exact test. † O/O distribution in SLE: B/B (1), B/D (3), D/D (1). O/O distribution in controls: B/C (2), B/D (5), C/C (1), C/D (2), D/D (1).

2C. Analysis of the African American cohort.

	SLE, n = 18 (%)	Healthy Control, n = 7 (%)	p*
Allele			
A	23 (63.9)	11 (78.6)	0.501
B	2 (5.5)	0 (0.0)	1.000
C	11 (30.6)	1 (7.1)	0.140
D	0 (0.0)	2 (14.3)	0.074
Genotype			
A/A	8 (44.4)	4 (57.1)	0.673
A/B	0 (0.0)	0 (0.0)	1.000
A/C	7 (38.9)	1 (14.3)	0.362
A/D	0 (0.0)	2 (28.6)	0.070
O/O†	3 (16.7)	0 (0.0)	0.534
A/O	7 (38.9)	3 (42.8)	1.000

* SLE vs controls, Fisher's exact test. † O/O distribution in SLE: B/C (2), C/C (1). O/O distribution in controls: 0.

type (Tables 3B and 4). Moreover, SLE patients with the O/O genotype were prone to have anti-dsDNA autoantibodies and renal involvement (Tables 3 and 5). Together, these results suggest that heterozygosity or homozygosity of MBL variant allele may be a minor risk factor and/or a disease modifying factor for SLE in this cohort of patients with SLE.

MBL variant alleles are found frequently in the general population[9,10]. Single nucleotide substitutions occurring in both the promoter and structure-coding regions of the mbl2 gene may result in decreased synthesis of MBL or production of dysfunctional MBL variants incapable of oligomerization, thereby causing quantitative and qualitative deficiencies in MBL[10,33-35]. MBL deficiency has been implicated in susceptibility to infections and autoimmune diseases[5,11,22,36-38]. MBL has been shown to bind directly to apoptotic cells and necrotic cells and initiate their removal by macrophages[39,40]. It can therefore be postulated that insufficient levels of MBL may predispose patients to the accumulation of apoptotic cells. These cells may serve as a major source of autoantigens, and hence induce autoantibody production in patients with SLE[41-43]. Autoantibodies generated against such autoantigens target primarily nuclear components such as dsDNA, nuclear ribonucleoprotein (nRNP), Ro/SSA, La/SSB, and Smith antigen. Among these autoantibodies, anti-Smith antibody, which is against the core polypeptides of the spliceosome complex, is highly specific for SLE and is present in 15%–30% of patients with SLE. In our study, heterozygous A/B and A/C genotypes of MBL were found to be associated with an increased probability of generation of anti-Smith antibody (OR 5.1, p = 0.007 for A/B genotype; OR 8.2, p = 0.004 for A/C genotype). Interestingly, however, SLE patients with the O/O genotype were less likely to produce anti-Smith antibody, but more likely to produce anti-dsDNA and Ro/SSA, La/SSB, and RNP antibodies (Tables 3 and 4). These results, together, not only support the hypothetical link between MBL deficiency, apoptosis, and SLE, but also imply the complex interplay between genetic factors and autoantibody phenotypes in different individuals. In this context, it is postulated that the extent of MBL deficiency (more profound in patients with the O/O genotype than in those with the A/O genotypes) may influence differentially the clearance of different subsets of apoptotic debris and hence the generation of different autoantibodies in individual patients with SLE.

Quantitative or functional deficiency of MBL may also lead to insufficient activation of the complement system and inadequate clearance of immune complexes, which in turn result in immune complex deposition in tissues and cause organ damage. The factors that influence the involvement of different target organs in individual patients with SLE are currently unknown. More than 90% of people with C1q deficiency develop SLE, but only one-third of them develop glomerulonephritis[1]. The existence has been suggested of nephropathy susceptibility genes independent of SLE susceptibility genes[44]. Garred, *et al* have reported that renal involvement

Table 3. Presence of autoantibodies in patients with SLE stratified by MBL genotypes.

3A.

Autoantibodies	Genotype A/A, n = 75 (%)	A/O, n = 47 (%)	O/O, n = 8 (%)	p*
Anti-dsDNA	49 (65.3)	30 (63.8)	8 (100.0)	0.120
Anti-Smith**	6 (8.0)	14 (29.8)	0 (0.0)	< 0.001
Antiphospholipid†	38 (50.7)	21 (44.7)	2 (25.0)	0.357
Other antibodies††	26 (34.7)	17 (36.2)	4 (50.0)	0.692

3B.

Autoantibodies	Genotype A/A, n = 75 (%)	A/B, n = 26 (%)	A/C, n = 12 (%)	A/D, n = 9 (%)	O/O, n = 8 (%)	p*
Anti-dsDNA	49 (65.3)	17 (65.4)	8 (66.7)	5 (55.6)	8 (100.0)	0.332
Anti-Smith**	6 (8.0)	8 (30.8)	5 (41.7)	1 (12.5)	0 (0.0)	0.003
Antiphospholipid†	38 (50.7)	11 (42.3)	7 (58.3)	3 (33.3)	2 (25.0)	0.480
Other antibodies††	26 (34.7)	8 (30.8)	6 (50.0)	3 (33.3)	4 (50.0)	0.719

* Fisher's test. ** 129 SLE patients were tested for anti-Smith antibody. † Antiphospholipid includes anticardiolipin and lupus anticoagulant. †† Other autoantibodies include any one or combinations of SSA/Ro, SSB/La, or RNP antibody.

Table 4. Logistic regression analysis of prevalence of anti-Smith antibody in patients with SLE.

Genotypes	p*	Odds Ratio	95% CI
A/B	0.007	5.1	1.6–16.6
A/C	0.004	8.2	2.0–33.9
A/D	0.666	1.6	0.2–15.7
O/O	0.999	0.0	0.0–infinity

* Heterozygous and homozygous variant genotypes vs wild-type genotype A/A.

was more frequent in European Caucasian (Danish) SLE patients with the O/O genotypes[22]. Similarly, among patients with SLE in our multiethnic, yet Caucasian-dominant, cohort (84% American Caucasians, 14% African Americans, and 2% other races), those with the O/O genotype were more likely to report renal events (Tables 5 and 6). However, in a recent study conducted with a multiethnic cohort dominant in non-Caucasian patients (31% American Caucasians, 40% African Americans, and 29% Hispanics), it was reported that renal involvement was more likely to occur in patients with the wild-type allele (A/A) than patients with MBL variant alleles (A/O and O/O)[45]. Collectively, these seemingly inconsistent data suggest that MBL deficiency may be a weak risk factor for renal disorders in SLE patients of certain ethnicities. Nevertheless, it should be cautioned that the numbers of patients with the O/O genotype were small in the study by Garred, *et al* (n = 7; total patients studied = 91) and our study (n = 8; total patients studied = 130), which may pose considerable limitations in statistical analyses. Therefore, further

Table 5. Clinical features of SLE patients with different MBL genotypes.

Features	Genotype A/A, n = 75 (%)	A/B, n = 26 (%)	A/C, n = 12 (%)	A/D, n = 9 (%)	O/O, n = 8 (%)	p*
Malar rash	37 (49.3)	11 (42.3)	6 (50.0)	4 (44.4)	6 (75.0)	0.608
Discoid rash	10 (13.3)	2 (7.7)	4 (33.3)	1 (11.1)	3 (37.5)	0.111
Photosensitivity	38 (50.7)	15 (57.7)	4 (33.3)	5 (55.6)	6 (75.0)	0.433
Oral ulcers	44 (58.7)	10 (38.5)	7 (58.3)	5 (55.6)	4 (50.0)	0.504
Serositis†	54 (73.0)	22 (84.6)	10 (83.3)	7 (77.8)	8 (100.0)	0.326
Renal event	29 (38.7)	14 (53.8)	3 (27.3)	1 (11.1)	6 (75.0)	0.035
Cardiovascular event	14 (18.7)	6 (23.1)	5 (41.7)	1 (11.1)	3 (37.5)	0.298
Neurologic event	13 (17.3)	3 (11.5)	2 (16.7)	1 (11.1)	2 (25.0)	0.891
Hematologic event	42 (56.0)	15 (57.7)	8 (66.7)	7 (77.8)	8 (100.0)	0.122

* Fisher's test. † 129 SLE patients were tested for serositis.

Table 6. Odds ratio and 95% CI from the binary logistic regression analysis of renal events in 130 patients with SLE.

Genotypes	p*	Odds Ratio	95% CI
A/B	0.180	1.9	0.8–4.6
A/C	0.469	0.6	0.1–2.4
A/D	0.137	0.2	0.0–1.7
O/O	0.067	4.8	0.9–25.2

* Heterozygous and homozygous variant genotypes vs wild-type genotype A/A.

studies using larger patient populations are needed before definitive conclusions can be reached about the association of MBL polymorphisms with specific clinical features in ethnic patients with SLE.

Recently, a prospective study using 91 Danish patients with SLE has shown that vascular, particularly arterial thrombotic, events occurred more frequently in patients with the O/O genotype, suggesting that MBL deficiency is a risk factor for thrombotic cardiovascular disease in SLE[46]. However, disagreeing results have been reported in 2 other studies using either a German cohort[47] or a multiethnic (American Caucasian/African American/Hispanic) cohort of patients with SLE[48]. In this regard, the small number of vascular events accrued in our patient cohort prohibited the performance of meaningful statistical analyses. Further investigation of the role of MBL polymorphisms in thrombotic vascular events in SLE is warranted.

The association of MBL polymorphism/deficiency with SLE in various ethnic groups has been extensively investigated. An early study conducted in African American patients with SLE showed a significant increase in the frequencies of B and C alleles[15]. Significant increases in the frequency of the B allele in Spanish patients with SLE[20] and B/B genotype in Japanese patients with SLE[17,18] have also been reported. These results suggest that MBL deficiency, due in particular to the B variant allele, is associated with development of SLE. In contrast, Horiuchi, *et al* reported no significant increase in the frequency of the B allele in Japanese patients with SLE, concluding that MBL polymorphism is not a risk factor for SLE[23].

In other studies with various ethnic groups, modest increases in the frequency of MBL structural variant alleles in patients with SLE compared to healthy controls have been reported. For instance, increased frequencies of B and C alleles were found in South African patients[49], increased B allele frequency in Chinese[50], British Caucasian[21] and Greek Caucasian patients[51], and increased frequency of the O/O genotype in Danish Caucasian patients[22]. Most of the observed increases in MBL variant alleles in patients with SLE, however, have not proved to be statistically significant and, in some cases, were inconsistent among different studies. In an attempt to reconcile the inconsistent observations, Lee and colleagues recently conducted a metaanalysis of 15 published studies of associations between MBL gene polymorphism and SLE[26]. Overall, these authors found a significantly increased OR for association of the B allele with SLE (OR 1.41; 95% CI 1.22–1.61; $p < 0.001$) in African, Asian, and Caucasian patients. Taken together, these studies suggest that structural mutations of MBL have a significant, albeit minor, influence on the susceptibility to SLE.

If deficiency of C1q, a complement component structurally and functionally similar to MBL, is strongly associated with susceptibility to SLE, why does MBL deficiency have no significant association with SLE? C1q has been shown to play important roles in complement activation, T cell-dependent antibody production, and clearance of apoptotic cells[52,53]. Such activities may be severely compromised in the absence of C1q, leading to autoimmune reactivity and development of SLE. In comparison, MBL may play a lesser, redundant role in immune regulation. Thus, the presence of C1q may mask the effect of MBL deficiency. The extreme rarity of homozygous C1q deficiency (~20–30 cases worldwide) also underscores the detrimental effects of C1q deficiency in life and in disease development in general, in contrast to the relatively benign outcome of MBL deficiency. Deficiency/mutation of a single gene product such as MBL may not correlate with SLE because of genetic heterogeneity and epistatic interactions among different genes in different ethnic populations, unless the penetrance of a particular gene is high. These genetic variations may explain why the reported associations of MBL gene polymorphism with SLE in different ethnic groups are conflicting. MBL gene polymorphism may be important in defining specific clinical features favored by concomitant inherited or acquired risk factors.

Based on analyses of organ involvement and antibody production profiles, our study showed that MBL variant alleles/deficiency genotypes may be a weak risk factor for clinical subsets of patients with SLE. However, as in other ethnic groups previously reported, MBL variant alleles/deficiency genotypes per se do not seem to play a major role in the development of SLE in North American patients. It should be cautioned, however, that this study population consists predominantly of American Caucasians; thus, whether similar associations of MBL deficiency genotypes with renal involvement exist in African American patients with SLE warrants further investigation. Interestingly, a recent study evaluating a multiethnic (Hispanic, African American, and Caucasian) cohort of patients with SLE showed that MBL deficiency genotypes were associated with cerebrovascular events in Caucasian patients but not in Hispanic and African American patients[48], reinforcing the possibility that the relationship between MBL variant alleles/deficiency genotypes and renal disease may exist only in SLE patients of certain ethnic groups.

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