Concise Report

Association of mannose-binding lectin gene polymorphisms with antiphospholipid syndrome, cardiovascular disease and chronic damage in patients with systemic lupus erythematosus


Objective. To investigate the association of mannose-binding lectin (MBL)-deficient genotypes with cardiovascular disease in a large series of patients with systemic lupus erythematosus (SLE).

Methods. A total of 114 patients diagnosed with SLE were included in the study. MBL polymorphisms were investigated by sequencing-based DNA typing of the promoter and exon 1 of the MBL2 gene. The genotypes 0/0, 0/XA and XA/XA were considered as MBL-low genotypes.

Results. A higher prevalence of cardiovascular disease was observed in patients carrying MBL-low genotypes compared with those carrying MBL-high genotypes [30 vs 9%, \( P = 0.012 \), odds ratio (OR) 4.54, 95% confidence interval (CI) 1.20–16.46]. Patients with MBL-low genotypes also presented higher mean values for total cholesterol (228.6 vs 202.3 mg/dl, \( P = 0.017 \)) and low-density lipoprotein (LDL) cholesterol (139.9 vs 121.9 mg/dl, \( P = 0.045 \)), a higher frequency of chronic renal failure (30 vs 4%, \( P = 0.001 \)), vasculitis (30 vs 11%, \( P = 0.043 \)), heart valve lesions (71 vs 32%, \( P = 0.026 \)), cardiac valve dysfunction (57 vs 7%, \( P = 0.0004 \)) and associated APS (39 vs 12%, \( P = 0.005 \)), a higher mean Systemic Lupus International Collaborating Clinics score (2.09 vs 1.26, \( P = 0.029 \)) and a lower prevalence of low C4 levels (43 vs 71%, \( P = 0.015 \)). Multivariate analysis of genetic, clinical and immunological variables showed that only antiphospholipid syndrome (APS) was independently associated with cardiovascular events (\( P = 0.001 \)).

Conclusion. Although the prevalence of cardiovascular disease in our SLE patients carrying MBL-deficient genotypes was 3.3 times higher than in patients with non-deficient genotypes, only APS was independently associated with cardiovascular events. This suggests that the higher frequency of thrombotic events in SLE patients carrying MBL-deficient genotypes might be related to coexisting APS.

KEY WORDS: Systemic lupus erythematosus, Mannose-binding lectin, Genetic polymorphism, Cardiovascular disease, Antiphospholipid syndrome.

Introduction

Systemic lupus erythematosus (SLE) is considered the most clinically and serologically diverse autoimmune disease because it may affect any organ and display a broad spectrum of clinical manifestations [1]. Cardiovascular events have emerged as major causes of morbidity and mortality in SLE patients [2–4]. There is recent evidence that the pathogenesis of cardiovascular disease may involve components of the innate immune system, which includes specific pattern-recognition receptors such as mannose-binding lectin (MBL) [5–7]. MBL is a liver-derived serum protein that binds to sugars on the surface of pathogenic micro-organisms and triggers complement fixation. Low serum levels of MBL are found in association with single-nucleotide polymorphisms (SNPs) in the promoter region and the structural gene-coding region of the MBL2 gene [8, 9].

The role of the MBL pathway in complement activation and in the clearance of apoptotic cells suggests that genetic variability in MBL may be involved in the pathogenesis of SLE [10]. A previous report showed an association between the deficient homozygous 0/0 MBL-genotype and the development of arterial thrombosis in patients with SLE [11]. The aim of this study was to investigate the association of MBL-deficient genotypes with cardiovascular disease in a large series of Spanish patients with SLE.
MBL polymorphisms and SLE

Methods

Patient selection

A total of 114 patients, evaluated in the Department of Autoimmune Diseases between 2001 and 2003, were included in the study after providing written, informed consent. The inclusion criterion was the fulfilment of four or more of the revised American College of Rheumatology (ACR) classification criteria [12]. Blood samples were obtained from SLE patients and from 104 healthy voluntary blood donors from the Hospital Clinic (Barcelona, Spain). The study was approved by the Ethics Committee of our hospital.

Clinical and immunological variables

Cardiovascular features. Cardiovascular risk factors were defined according to previous reports [13]. Cardiovascular disease, including arterial and/or thrombotic events, was retrospectively evaluated at the study inclusion. The following venous thrombotic events were included: (i) deep vein thrombosis, confirmed by Doppler studies and/or phlebography; (ii) pulmonary embolism, diagnosed by ventilation/perfusion pulmonary scintigraphy and (iii) cerebral venous thrombosis, confirmed by computed tomography and/or magnetic resonance imaging scans. The following arterial thrombotic events were included: (i) cerebrovascular accident confirmed by computed tomography and/or magnetic resonance imaging scans; (ii) myocardial infarction confirmed by elevated cardiac enzyme levels and electrocardiogram; peripheral arterial thrombosis diagnosed by arteriography and (iii) intra-abdominal infarctions confirmed by computed tomography and/or magnetic resonance imaging scans.

SLE-related features. Epidemiological, clinical and immunological SLE features were defined as previously reported [1,13]. SLE disease activity was measured using the European Consensus Lupus Activity Measurement (ECLAM) [14]; SLE cumulative damage was measured using the Systemic Lupus International Collaborating Clinics (SLICC) damage index [15].

Genomic DNA and serum samples

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-treated whole-blood samples by using the QIAamp DNA blood mini kit and following manufacturer’s instructions (QIAGEN GmbH, Hilden, Germany) and then stored at −80°C until used.

MBL2 genotyping. Genotyping of the MBL2 gene was done by using a sequencing-based typing (SBT) technique. A 969 bp fragment encompassing the promoter and the exon 1 of the MBL2 was obtained by polymerase chain reaction (PCR) amplification using the sense 5’-GGG GAA TTC CTGCCA GAAAGT-3’ and antisense 5’-CAT ATC CCCAGG CAG TTT CC-3’ primers and the Expand 20kbPLUS PCR System (Roche Diagnostics GmbH, Mannheim, Germany). The cycling conditions were 94°C for 8 min; 35 cycles of 94°C for 45 s, 58°C for 30 s and 72°C for 90 s; and 72°C for 10 min. Five microtitre of the resulting PCR reaction were treated with ExoSAP-IT® (USB Corporation, Cleveland, Ohio) and then subjected to direct cyclic sequencing with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) by following manufacturer’s instructions with the sense and antisense gene-specific primers aforementioned.

Three SNP at codons 52, 54 and 57 (named D, B and C variants, respectively) are major determinants of serum MBL levels [8, 9]. These variants are collectively named O, and A indicates the wild-type variant. Three additional SNP at positions −551 (H/L), −221 (X/L) and +4 (P/Q) in the 5’-flanking region of the MBL2 gene also influence serum MBL levels in individuals with the wild-type genes [8]. The genotypes 0/0, 0/XA and XA/XA were considered as MBL-low genotypes according to previous studies [8, 10, 16].

Statistical analysis

Categorical data were compared using the χ² and Fisher’s exact tests. Continuous variables were analysed with the Student’s t-test in large samples of similar variance and with the non-parametric Mann–Whitney U-test for small samples, with results indicated as mean ± SEM. A two-tailed value of P < 0.05 was taken to indicate statistical significance. When several independent variables appeared to have statistical significance in the univariate analysis, a multiple logistic regression analysis was performed, taking the immunological features (MBL-low) as the dependent variable and those that reached statistical significance in the univariate analysis as independent variables. The results of the analysis of continuous variables are indicated as mean ± SEM.

The statistical analysis was performed using the SPSS program.

Results

Prevalence of MBL genotypes

Of the 114 patients with SLE included in the study, 106 (93%) were female and 8 (7%) male, with a mean age at SLE diagnosis of 31.1 ± 1.2 yrs (range, 8–77). Forty-one (36%) patients had genotype A/A, 30 (26%) genotype A/O, 20 (17%) genotype A/XA, 5 (4%) genotype XA/XA, 10 (9%) genotype 0/XA and 8 (7%) had genotype 0/0. Twenty-three (20%) SLE patients had MBL-low genotypes (0/0, 0/XA, XA/XA) compared with 19 (18%) controls (P > 0.05).

Cardiovascular features

A higher prevalence of cardiovascular disease was observed in patients carrying MBL-low genotypes compared with those carrying MBL-high genotypes (30 vs 9%, P = 0.012) (Table 1). The odds ratio (OR) for cardiovascular disease in patients carrying MBL-low genotypes was 4.54 [95% confidence interval (CI) 1.20–16.46]. Patients with MBL-low genotypes presented a higher frequency of venous thrombotic disease (22 vs 4%, P = 0.016, OR 6.04, CI 95% 1.15–32.91); a higher prevalence of arterial thrombotic disease was also found in patients carrying MBL-low genotypes, although the differences did not reach statistical significance (13 vs 5%, P = 0.20). In an additional statistical analysis, we studied which genetic, clinical and immunological features where associated with the dependent cardiovascular disease. SLE patients with cardiovascular events presented a higher frequency of MBL-deficient genotypes (43 vs 17%, P = 0.035), valvulopathy (73 vs 35%, P = 0.039), associated APS (64 vs 11%, P < 0.001) and a higher mean SLICC score (2.50 ± 1.28, P = 0.008), although only associated APS reached statistical significance in the multivariate analysis (P = 0.001).

We also analysed the association between MBL polymorphisms and the main cardiovascular risk factors. No statistical association was found between MBL-low genotypes and cardiovascular risk factors, except for the serum lipid profile at the time of study. Higher mean values for total cholesterol (228.6 ± 202.3 mg/dl, P = 0.017) and LDL-cholesterol (139.9 ± 121.9 mg/dl, P = 0.045) were found in patients carrying MBL-low genotypes. A higher prevalence of carotid atherosclerotic plaques (38 vs 24%) was also found in patients carrying MBL-low genotypes, although the differences did not reach statistical significance.

SLE-related features

Patients carrying MBL-low genotypes presented a higher prevalence of chronic renal failure (30 vs 4%, P = 0.001), vasculitis (30 vs 11%, P = 0.043), heart valve lesions (71 vs 32%, P = 0.026),
cardiac valve dysfunction (57 vs 7%, \(P = 0.0004\)), associated APS (39 vs 12%, \(P = 0.005\)) and a higher mean SLICC score (2.09 vs 1.26, \(P = 0.024\)). No statistical association was found with the ECLAM activity index at the time of visit. Chronic renal failure (\(P = 0.024\)) and associated APS (\(P = 0.046\)) were significant independent variables in the multivariate analysis (Table 1).

Immunologically, a lower prevalence of low C4 levels (43 vs 71%, \(P = 0.015\)) was found in patients carrying MBL-low genotypes. Analysis of anti-dsDNA levels, anti-Sm antibodies, anti-RNP antibodies, C3 levels, CH50 activity and antiphospholipid antibodies (including LA, IgG-aCL and IgM-aCL) showed no significant differences (Table 2).

**Discussion**

Within the study of genetic susceptibility to SLE, there is growing interest in the clinical significance of MBL variant alleles. Preliminary studies in the UK, Spain, China and Greece suggested a possible association between MBL dysfunctional alleles and SLE [17–20]. In 1999, Garred *et al.* [21] reported a higher frequency of infections in SLE patients carrying the deficient homozygous 0/0 MBL-genotype, although this has not been confirmed in recent studies [22]. More recently, the same authors [11] have reported an association between this genotype and an increased incidence of arterial thrombosis. We have investigated the possible association of MBL polymorphisms with the broader spectrum of clinical manifestations that may be presented by SLE patients and with the main cardiovascular risk factors.

The prevalence of cardiovascular disease in our SLE patients carrying MBL-low genotypes was 3.3 times higher than in patients with other MBL genotypes. Garred *et al.* [21] were the first to describe a trend to a higher frequency of thrombotic disease, although in a subsequent study, Øhlenschlaeger *et al.* [11] limited the association to the development of arterial thrombosis and not to venous thrombosis. We found contrasting results, with MBL-low genotypes showing a lower association with venous rather than arterial thrombosis. This may be due to the different variant alleles analysed (0/XA and XA/XA were also included as deficient alleles) and/or to the varying prevalence of thrombotic events. Øhlenschlaeger *et al.* [11] reported the development of arterial thrombosis in 26% of their SLE patients, a frequency several times higher than that found retrospectively in our patients and in other reported series [23]. In addition, a recent study by Calvo-Alen *et al.* [24] found no association between the deficient homozygous 0/0 MBL-genotype and arterial thrombotic events in 415 patients with SLE.

Antiphospholipid syndrome was independently associated with the MBL genotype, with a prevalence of APS being 3.2 times higher in SLE patients with MBL-low genotypes than in those without. The structural and functional characteristics of MBL may affect the susceptibility to thrombotic events, since MBL is capable of binding to certain phospholipids, oligosaccharide structures and glycosylated IgG [25–27]. Recent clinical studies have suggested a close association between MBL deficiency, thrombosis and antiphospholipid antibodies. Limnell *et al.* [28] described MBL deficiency and IgG-aCL as independent factors for the thrombotic occlusion of venous bypass grafts. In patients with SLE, Seelen *et al.* [29] reported that the presence of aCL was significantly associated with the occurrence of MBL gene polymorphisms. In our study, we found a higher frequency of

### Table 1. Association between MBL-low genotypes and SLE-related clinical features

<table>
<thead>
<tr>
<th></th>
<th>MBL-low genotypes (0/0, XA/0, XA/XA)</th>
<th>Other MBL genotypes (XA/A, 0/A, A/A)</th>
<th>Statistical significance (two-tailed (P)-value &lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>20 (87%)</td>
<td>86 (94%)</td>
<td>–</td>
</tr>
<tr>
<td>Age at SLE diagnosis (yrs)</td>
<td>35.6 ± 3.3</td>
<td>29.6 ± 1.3</td>
<td>–</td>
</tr>
<tr>
<td>Mean time of SLE evolution (months)</td>
<td>136.5 ± 16.9</td>
<td>156.3 ± 11.7</td>
<td>–</td>
</tr>
<tr>
<td>SLE-specific cutaneous features</td>
<td>19 (83%)</td>
<td>69 (76%)</td>
<td>–</td>
</tr>
<tr>
<td>Arthritis</td>
<td>22 (96%)</td>
<td>86 (95%)</td>
<td>–</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>7 (30%)</td>
<td>4 (4%)</td>
<td>–</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>7 (30%)</td>
<td>10 (11%)</td>
<td>–</td>
</tr>
<tr>
<td>Venous thrombotic disease</td>
<td>5 (22%)</td>
<td>4 (4%)</td>
<td>–</td>
</tr>
<tr>
<td>Arterial thrombotic disease</td>
<td>3 (13%)</td>
<td>5 (5%)</td>
<td>–</td>
</tr>
<tr>
<td>Heart valve disease</td>
<td>10/14 (71%)</td>
<td>13/40 (32%)</td>
<td>0.026</td>
</tr>
<tr>
<td>Valvular dysfunction</td>
<td>8/14 (57%)</td>
<td>3/40 (7%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Associated APS</td>
<td>9 (39%)</td>
<td>11 (12%)</td>
<td>0.005*</td>
</tr>
<tr>
<td>SLICC score</td>
<td>2.09 ± 0.44</td>
<td>1.26 ± 0.15</td>
<td>0.029</td>
</tr>
<tr>
<td>ECLAM score</td>
<td>3.6 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td>–</td>
</tr>
</tbody>
</table>

*Statistically significant in the multivariate analysis.

### Table 2. Association between MBL-low genotypes and SLE-related immunological factors

<table>
<thead>
<tr>
<th></th>
<th>MBL-low genotypes (0/0, XA/0, XA/XA)</th>
<th>Other MBL genotypes (XA/A, 0/A, A/A)</th>
<th>Statistical significance (two-tailed (P)-value &lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA values</td>
<td>81.6 ± 17.5</td>
<td>60.4 ± 6.2</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Sm antibodies</td>
<td>1 (4%)</td>
<td>10 (11%)</td>
<td>–</td>
</tr>
<tr>
<td>Anti-RNP antibodies</td>
<td>4 (17%)</td>
<td>21 (24%)</td>
<td>–</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>10 (44%)</td>
<td>28 (31%)</td>
<td>–</td>
</tr>
<tr>
<td>IgG-anticardioplin antibodies</td>
<td>10 (43%)</td>
<td>24 (26%)</td>
<td>–</td>
</tr>
<tr>
<td>IgM-anticardioplin antibodies</td>
<td>2 (9%)</td>
<td>17 (19%)</td>
<td>–</td>
</tr>
<tr>
<td>Low C3 levels</td>
<td>14 (61%)</td>
<td>66 (72%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Low C4 levels</td>
<td>10 (43%)</td>
<td>65 (71%)</td>
<td>–</td>
</tr>
<tr>
<td>Low CH50 activity</td>
<td>15 (65%)</td>
<td>68 (75%)</td>
<td>–</td>
</tr>
</tbody>
</table>
LA and IgG-aCL in patients carrying MBL-deficient genotypes, although the difference was not statistically significant, which is in line with the results found by Ohlenschlaeger et al. [11]. This suggests that MBL-deficient genotypes are mainly associated with aPL-related thrombotic disease (APS) and not with the isolated presence of aPL in asymptomatic SLE patients.

With respect to SLE-related clinical expression, our patients carrying MBL-low genotypes had a higher mean SLICC score, suggesting a higher level of chronic organ damage caused by SLE. This was closely related to the more severe disease observed in these patients, who presented a higher prevalence of thrombotic events, heart valve dysfunction, vasculitis and chronic renal failure. In contrast, MBL polymorphisms were not associated with the main immunological markers of SLE, except for a lower frequency of hypercomplementaemia. Roos et al. [30] found that sera from individuals having mutations in the MBL gene showed significantly less activation of C4 by IgA and mannose than sera from individuals with the wild-type genotype, while Seelen et al. [29] found that SLE patients with MBL variant alleles have an impaired ability to activate exogenous C4 by MBL: MBL-associated seline protease complexes bound to mannose.

In SLE, the pathogenesis of cardiovascular disease is multifactorial including, on the one hand, a high prevalence of conventional risk factors for atherosclerosis [31] and, on the other hand, the existence of thrombotic and autoimmune SLE-related processes [32]. Our results suggest that the association between MBL-low genotypes and cardiovascular disease in SLE might be mediated through multiple mechanisms, including an increased susceptibility to lipid alterations, the cumulated chronic organ damage associated with SLE evolution (mainly related to nephropathy, vasculitis and valvulopathy) and, especially, the association with APS. When we analysed the features associated with cardiovascular events (including MBL genotypes in the analysis), the multivariate model showed that only APS was independently associated with thrombotic events. This possible association was not evaluated by Ohlenschlaeger et al. [11], since their study was not primarily designed to measure cardiovascular outcomes and this information (associated APS) was not systematically recorded. This suggests that the higher risk of developing thrombotic events in SLE patients carrying MBL-deficient genotypes might be due, at least partially, to an associated APS.

In conclusion, the prevalence of cardiovascular disease in our SLE patients carrying MBL-deficient genotypes was 3.3 times higher than in patients with non-deficient genotypes. Patients carrying MBL-low genotypes also had a higher level of chronic organ damage. However, the multivariate analysis showed that only APS was independently associated with cardiovascular disease, suggesting that the higher risk of developing thrombotic events in SLE patients carrying MBL-deficient genotypes might be related to coexisting APS.

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References

Clinical Vignette

Interstitial granulomatous dermatitis (Ackerman’s syndrome) in SLE presenting with the ‘rope sign’

A 50-yr-old man presented with synovitis of the metacarpophalangeal joints, Raynaud’s phenomenon, recurrent pericarditis and a linear palpable rash in both axillae. Investigations: erythrocyte sedimentation rate: 16 mm [normal range (nr) 1–12], C-reactive protein: 18 mg/dl (nr 0–10), rheumatoid factor (RF) and anti nuclear antibody (ANA) negative, double stranded DNA >300 IU/ml (nr <10). Echocardiogram revealed thickened pericardium. He was diagnosed as ANA negative systemic lupus erythematosus [although he did not fulfil the American College of Rheumatology (ACR) criteria]. He responded well to steroids, but not to hydroxychloroquine. The rash was biopsied and methotrexate was commenced.

The biopsy revealed palisading dermal infiltration with histiocytes, with foci of collagen necrosis/necrobiosis along with vasculitis and venulitis. Interstitial granulomatous dermatitis (IGD, Ackerman’s syndrome) was diagnosed.

Ackerman’s syndrome, first described in 1993 [1], is a rare disorder characterized by the combination of arthritis and a pathognomonic rash (linear strands or erythematous, palpable cords, Fig. 1)—‘the rope sign’. Its associations include connective tissue disease (particularly SLE), rheumatoid arthritis, autoimmune thyroiditis, carcinomas and drug reactions. Histology reveals an interstitial and palisading granulomatous dermatitis associated with piecemeal fragmentation of collagen and elastic fibres [2].

Our patient’s symptoms are well-controlled on methotrexate. This is a rare syndrome and recognition of the signs may enable early diagnosis.

The authors have declared no conflicts of interest.

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Fig. 1. Left axillary rash depicting the ‘rope sign’.