Mannose-binding lectin-low genotypes are associated with milder systemic and immunological disease expression in primary Sjögren’s syndrome

M. Ramos-Casals1, P. Brito-Zerón1, N. Soria1, N. Nardi1, A. Vargas1, S. Muñoz1, A. Bove1, B. Suárez2 and F. Lozano2

Objective. To investigate the association of mannose-binding lectin (MBL)-low genotypes with the clinical and immunological expression of primary SS.

Methods. Eighty-one patients with primary SS who fulfilled the 2002 classification criteria were included in the study. MBL2 polymorphisms were investigated by sequence-based DNA typing of the promoter and exon 1. Genotypes 0/0, 0/XA or XA/XA were considered as MBL-low and XA/A, A/0 and A/A as MBL-sufficient. Control groups included 46 patients who exclusively fulfilled the 1993 SS criteria, 114 SLE patients and 104 healthy individuals.

Results. Twelve (15%) SS patients had MBL-low genotypes, of whom six (7%) had genotype 0/XA, five (6%) had genotype 0/0 and one (1%) had genotype XA/XA. A higher prevalence of the XA/A genotype (32 vs 17%, P = 0.01) was found in primary SS patients in comparison with SLE patients. No patient with primary SS carrying MBL-low genotypes had purpura, glomerulonephritis or neurological involvement (0 vs 29%, P = 0.025). Immunologically, patients carrying MBL-low genotypes had a lower frequency of anti-Ro/SS-A antibodies (17 vs 55%, P = 0.014), anti-La/SS-B antibodies (8 vs 48%, P = 0.009) and low C4/C3 levels (0 vs 32%, P = 0.016). No patient with primary SS carrying the homozygous MBL-deficient genotype 0/0 had anti-Ro/SS-A or anti-La/SS-B antibodies, low C3/C4 levels or circulating cryoglobulins.

Conclusion. SS patients with MBL-low genotypes have a less pronounced systemic and immunological disease expression in comparison with those carrying MBL-sufficient genotypes. In primary SS, MBL deficiency may represent a protective factor against the development of more aggressive autoimmune damage.

Key words: Primary Sjögren’s syndrome, Mannose-binding lectin, Gene polymorphism, Anti-Ro/SS-A antibodies, Innate immunity.

Introduction
SS is a systemic autoimmune disease that presents with sicca symptomatology of the main mucosa surfaces [1]. The main sicca features (xerophthalmia and xerostomia) are determined by specific ocular (Rose Bengal staining, Schirmer test) and oral (salivary flow measurement, parotid scintigraphy) tests. The histological hallmark is a focal lymphocytic infiltration of the exocrine glands, determined by a biopsy of the minor labial salivary glands [2]. Patients with SS present a broad spectrum of analytical features (cytopenias, hypergammaglobulinaemia, high ESR) and auto-antibodies, of which ANAs are the most frequently detected, anti-Ro/SS-A the most specific, and cryoglobulins and hypocomplementaemia the main prognostic markers [3]. The disease spectrum extends from sicca syndrome to systemic involvement (extraglandular manifestations) [4].

The complement system is a key component of the innate inflammatory response that mediates tissue damage in autoimmune diseases. In addition to the classical and alternative pathways, the complement system can also be activated through the lectin pathway, which is switched on when the mannose-binding lectin (MBL) forms a complex with MBL-associated serine proteases (MASPs) [5]. This complex binds with some carbohydrates motifs on pathogens, facilitating their opsonophagocytosis and/or lysis [6]. The MBL pathway also contributes to the clearance of apoptotic cells and circulating immune complexes [7], suggesting a possible implication in the aetiopathogenesis of systemic autoimmune diseases (SADs). Accordingly, genetically defined loss-of-function variations of the MBL and MASP-2 molecules have been reported to influence susceptibility and disease expression of both infections and autoimmune diseases [8, 9]. Three single nucleotide polymorphisms (SNPs) at exon 1 of MBL2 introducing non-synonymous amino acid changes at codons 52, 54 and 57 (named D, B and C variants, respectively, and collectively referred as O variants) are major determinants of serum MBL levels [10, 11]. The wild-type variant is referred as A. Additional SNP at positions –551 (H/L), –221 (X/Y) and +4 (P/Q) in the 5’-flanking region also influence serum MBL levels in individuals with the wild-type A variant [12]. Of these, two promoter haplotypes (HY, which is associated with high levels of MBL, and LX, which is associated with low MBL levels) appear to be the most important [12]. The exon 1 and promoter polymorphisms are in linkage disequilibrium and give rise to a limited number (seven) of haplotypes (HYPα, LYPA, LYYQA, LXPA, LYQC, LYPB, HYPD), which correlate well with MBL serum levels [12–16]. Genotypes 0/0, 0/XA and XA/XA were considered as MBL-low genotypes according to previous studies [12, 17–20]. The MBL2 and MASP2 genes have been located to chromosomes 10q11.1-q21 and 1p36.23–31, respectively [21]. Some studies have suggested a higher risk of developing SLE in patients carrying a specific MBL allele in combination with null alleles for other complement genes located within the MHC class III region (C4B) [22].

Hypocomplementaemia is considered one of the key immunological markers of primary SS. Recent studies have described a close association between low complement levels at diagnosis and a higher risk of developing extraglandular features and auto-antibodies, and death [23–25]. It may be hypothesized that genetic variability in proteins involved in the activation of the lectin pathway of the complement system (MBL) could influence the systemic and immunological expression of primary SS. In this

1Department of Autoimmune Diseases, Laboratory of Autoimmune Diseases 'Josep Font', Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and 2Department of Immunology, Hospital Clinic, Barcelona, Spain.

Submitted 8 April 2008; revised version accepted 22 September 2008.

Correspondence to: M. Ramos-Casals, Department of Autoimmune Diseases, Hospital Clinic, Villarroel, 170, 08036-Barcelona, Spain.

E-mail: mramos@clinic.ub.es

© The Author 2008. Published by Oxford University Press on behalf of the British Society for Rheumatology. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
study, we investigated the association between MBL2 and MASP2 gene polymorphisms and the clinical and immunological manifestations of primary SS.

Methods

Patients

We analysed 127 consecutive patients diagnosed before 2002 who fulfilled four or more of the 1993 European Classification Criteria for primary SS [26]. A retrospective application of the 2002 American–European criteria [27] identified 81 patients who fulfilled these criteria (77 women and 4 men, mean age at SS diagnosis of 51.4 yrs). The remaining 46 patients, all with negative anti-Ro/La antibodies, were excluded and analysed as a control group. In all patients, an exhaustive evaluation discarding other causes of sicca syndrome (coexisting systemic autoimmune diseases, chronic viral infections, metabolic disorders and pre-existing lymphoma) was performed. Systemic involvement was defined as the presence of at least one of the following features [28, 29]: lung involvement, cardiovascular involvement, nephropathy, vasculitis, peripheral neuropathy or central nervous system involvement. Clinical and laboratory data were collected and computerized according to our standard department protocol [28, 29]. The study was approved by the ethical committee of the Hospital Clinic (Barcelona, Spain) and all patients gave informed, written consent.

MBL2 and MASP2 genotyping

Genomic DNA was extracted from 1.5 ml EDTA-treated whole blood samples from Caucasian SS patients and controls (114 SLE patients and 104 healthy voluntary blood donors from the Hospital Clinic, Barcelona, Spain, mean age of 42 yrs, female: male ratio of 4:1) using the QIAamp DNA blood mini kit following the manufacturer’s instructions (QIAGEN GmbH, Hilden, Germany) and stored at −20°C until used. Genotyping of MBL2 and MASP2 was done by a PCR–sequencing based typing (SBT) technique, as previously reported [20, 28]. In brief, a 969-bp fragment of MBL2 encompassing a region from the promoter to the end of exon 1 was obtained by PCR amplification using the sense 5’-GGGGAATTCCTGCCAGAAAAGT-3’ and anti-sense 5’-CATATCCGGACGAGTTCTC-3’ primers and the Expand 20kbPLUS PCR System (Roche Diagnostics GmbH, Mannheim, Germany). Similarly, a 354-bp fragment from exon 3 of MASP2 was PCR-amplified using the sense 5’-GGC AGTGACGCTTCTGCAAGG-3’ and anti-sense 5’-CTCGGC TGCATGAAAGGCTC-3’ oligonucleotides and the Expand™ High Fidelity PCR System (Roche Diagnostics GmbH, Mannheim, Germany). Cycling conditions were: 94°C for 8 min; 35 cycles of 94°C for 45 s, 58°C for 30 s, 72°C for 90 s and, finally, 72°C for 10 min. Five microlitres of each PCR was treated with ExoSAP-IT® (USB Corporation, Cleveland, OH, USA) and subjected to direct sequencing with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) following the manufacturer’s instructions with the sense and anti-sense gene-specific primers mentioned above. Sequencing reactions were analysed on an automated capillary DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems).

The SNP D105>G at exon 3 of MASP2 is known to affect the binding of MASP-2 to MBL and subsequently reduces the serum levels of MASP-2 [30]. Individuals were divided into wild-type or mutant categories according to the presence of the D105> variant in either homo- or heterozygosis.

Statistical analysis

Qualitative variables were compared using the χ²-test and Fisher’s exact tests. Quantitative variables were analysed with the Student’s t-test, with results indicated as mean ± s.e.m. A two-tailed value of P < 0.05 was taken to indicate statistical significance. The results of the analysis of quantitative variables are indicated as mean ± s.e.m. The statistical analysis was performed using the SPSS program (Chicago, IL, USA).

Results

MBL2 polymorphisms

Twelve (15%) patients with primary SS had MBL-low genotypes, of whom six (7%) had genotype 0/0, five (6%) had genotype 0/1 and one (1%) had genotype XA/XA. Sixty-nine (85%) had other MBL2 genotypes (XA/A, 0/A, A/A), of whom 19 (23%) had other MBL2 genotypes (XA/A, 24 (30%) had genotype A/O and 26 (32%) had genotype A/XA (Table 1). When we compared the prevalence of MBL2 genotypes and haplotypes between patients with primary SS and controls, a higher prevalence of the XA/A genotype (32 vs 17%, P = 0.01) and a lower frequency of the A/XA genotype (23 vs 36%, P = 0.06) was found in primary SS patients in comparison with SLE patients.

When we analysed the association between MBL2 genotypes and the main epidemiological, clinical and immunological characteristics of SS (Table 2), we found a lower prevalence of systemic manifestations in SS patients with MBL-low genotypes (25 vs 41%). Specifically, no patient carrying MBL-low genotypes had purpura, glomerulonephritis or neurological involvement (0 vs 29%, P = 0.025). Patients carrying MBL-low genotypes had lower mean levels of CRP, erythrosedimentation rate, β2-microglobulin, serum IgG and pro-inflammatory cytokines such as IL-6 and -10 in comparison with patients carrying MBL-sufficient genotypes, although the differences did not reach statistical significance (Table 3).

Table 1. Prevalence of MBL2 gene polymorphisms in patients with primary SS compared with patients with SLE and controls

<table>
<thead>
<tr>
<th>MBL2 genotypes</th>
<th>Primary SS n = 81</th>
<th>SLE n = 114</th>
<th>Healthy controls n = 104</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL-low, n (%)</td>
<td>12 (15)</td>
<td>23 (20)</td>
<td>19 (18)</td>
</tr>
<tr>
<td>0/0, n (%)</td>
<td>5 (6)</td>
<td>8 (7)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>0/XA, n (%)</td>
<td>6 (7)</td>
<td>10 (9)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>XA/XA, n (%)</td>
<td>1 (2)</td>
<td>5 (4)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>MBL-sufficient, n (%)</td>
<td>69 (85)</td>
<td>91 (80)</td>
<td>85 (82)</td>
</tr>
<tr>
<td>XA/A, n (%)</td>
<td>26 (32)*</td>
<td>20 (17)</td>
<td>24 (23)</td>
</tr>
<tr>
<td>0/A, n (%)</td>
<td>24 (30)</td>
<td>30 (26)</td>
<td>28 (27)</td>
</tr>
<tr>
<td>A/A, n (%)</td>
<td>19 (23)**</td>
<td>41 (36)</td>
<td>33 (32)</td>
</tr>
<tr>
<td>MBL2 haplotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, n (%)</td>
<td>40 (25)</td>
<td>56 (24)</td>
<td>52 (25)</td>
</tr>
<tr>
<td>XA, n (%)</td>
<td>34 (21)</td>
<td>40 (18)</td>
<td>38 (18)</td>
</tr>
<tr>
<td>A, n (%)</td>
<td>88 (54)</td>
<td>132 (58)</td>
<td>118 (57)</td>
</tr>
</tbody>
</table>

*P = 0.01 with respect to SLE. **P = 0.06 with respect to SLE.

Table 2. Epidemiological, clinical and immunological features in patients with primary SS according to the presence or absence of MBL-low genotypes (0/0, 0/XA, XA/XA)

<table>
<thead>
<tr>
<th>MBL-sufficient genotypes n = 69</th>
<th>MBL-low genotypes n = 12</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, female, n (%)</td>
<td></td>
<td>0.520</td>
</tr>
<tr>
<td>Age at SS diagnosis, mean ± s.e.m., yrs</td>
<td>50.96 ± 1.77</td>
<td>54.17 ± 2.31</td>
</tr>
<tr>
<td>Parotid scintigraphy grades III or IV, n (%)</td>
<td>45/64 (70)</td>
<td>5/10 (50)</td>
</tr>
<tr>
<td>Parotid enlargement, n (%)</td>
<td>17 (25)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Systemic involvement, n (%)</td>
<td>28 (41)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>Hypergammaglobulinemia &gt; 30%, n (%)</td>
<td>12 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ACLS, n (%)</td>
<td>8/58 (14)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>ANAs ≥ 1/80, n (%)</td>
<td>64 (93)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>Positive antiRo/S-S-A antibodies, n (%)</td>
<td>38 (55)</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Positive antiSS-A antibodies, n (%)</td>
<td>33 (48)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>RF &gt; 25 IU/l, n (%)</td>
<td>40 (58)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Low C3/C4 levels, n (%)</td>
<td>22 (32)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cryoglobulins &gt; 1%, n (%)</td>
<td>14 (20)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
No patient carrying MBL-low genotypes had positive RF, although the differences did not reach statistical significance (18 vs markers including ANA (64 polymorphisms in the 46 patients who only fulfilled the 1993 cryoglobulins.

SS-A or anti-La/SS-B antibodies, low C3/C4 levels or circulating SS carrying the homozygous deficient genotype /C6 0/0, 0/XA, XA/XA deficiency initially centered on a hypothetically increased risk of SLE, investigation of the possible aetiopathogenic role of MBL involvement. Thus, hypothetical blockade of the MBL pathway could reduce chronic autoimmune damage in SS patients, with reduced MBL levels leading to a low degree of complement activation and inflammation, as suggested by the low serum levels of acute-phase reactants and pro-inflammatory cytokines in our patients carrying MBL-low genotypes. Clinical studies have recently described a close association with cardiovascular disease, chronic damage and APS (18, 20, 35).

Regarding the study of genetic susceptibility to primary SS, recent studies suggest that investigation of the clinical significance of MBL variant alleles may be of interest. On the one hand, experimental studies in murine models have demonstrated the up-regulated expression of the MBL-A gene in cornes [36] and lymphoid infiltration of salivary glands in the MBL-null mice model [32]. On the other hand, clinical studies have recently highlighted the key role of hypocomplementaemia in the systemic disease expression and outcomes of patients with primary SS [23–25]. Preliminary studies on the prevalence of MBL genotypes A/A, A/0 and 0/0 in primary SS from Finland, Japan and Australia mainly centered on analysing possible differences in the prevalence of these genotypes with respect to control groups, contrasting results [37–39]. Wang et al [38] found a lower frequency of polymorphisms of the 54 codon in SS patients in comparison with controls, while Aitioineni et al [37] and Mullighan et al [39] found no statistically significant differences. In this study, we included additional MBL genotypes (analysing polymorphic positions in the promoter region, together with those at codons 52, 54 and 57) and found MBL-low genotypes in 15% of patients with primary SS, a similar prevalence to that seen in SLE patients (20%) and healthy controls (18%). This suggests that MBL-deficient polymorphisms have little influence in genetic susceptibility to primary SS.

In contrast, we found that MBL genotypes were associated with the clinical and immunological expression of primary SS. Clinically, our patients carrying MBL-low genotypes were characterized by a less severe involvement, with a statistical trend for a lower frequency of involvement in parotid scintigraphy and a statistically significantly lower prevalence of extraglandular involvement. Some studies have suggested that high levels of MBL are related to tissue damage [40] and that high levels of MBL in patients carrying wild-type MBL genotypes may facilitate autoimmune tissue damage [41]. It may be hypothesized that, in primary SS, MBL-sufficient levels could be necessary to induce the autoimmune-mediated damage, while patients carrying MBL-low genotypes may be protected against severe autoimmune involvement. Thus, hypothetical blockade of the MBL pathway could reduce chronic autoimmune damage in SS patients, with reduced MBL levels leading to a low degree of complement activation and inflammation, as suggested by the low serum levels of acute-phase reactants and pro-inflammatory cytokines in our patients carrying MBL-low genotypes.

Patients with primary SS carrying MBL-low genotypes had a lower frequency of positive autoantibodies, including ANA, RF and aCLs, especially a lower prevalence of anti-Ro/SS-A and anti-La/SS-B antibodies. Recent studies have found that SLE patients carrying MBL-low genotypes have a higher frequency of anti-dsDNA, anti-Ro/SS-A, anti-La/SS-B but a lower frequency of aCLs, anti-RNP and anti-Sm antibodies in comparison with SLE patients carrying non-deficient genotypes [42, 43]. This suggests that MBL genotypes may induce different patterns of autoantibodies in each autoimmune disease. Therefore, high MBL levels may be important in mounting autoimmune response against Ro and La antigens in patients with primary SS.

None of our patients carrying MBL-low genotypes had low C3/C4 levels or positive cryoglobulins, which are key prognostic factors in primary SS. This is consistent with the functional role of MBL in complement activation. MBL pathway dysfunction in variant allele carriers is associated with reduced MBL ligand binding and a relative increase in low-molecular mass MBL. Roos et al [44] found that sera from individuals with mutations in the MBL2 gene showed significantly less activation of C4 by IgA and mannose than sera from individuals with the wild-type genotype.

**Table 3. Analytical features in patients with primary SS according to the presence or absence of MBL-low genotypes (G0, G0/XA, XA/XA)**

<table>
<thead>
<tr>
<th>CRP levels, mg/dl</th>
<th>MBL-sufficient genotypes (G0)</th>
<th>MBL-low genotypes (G0/XA, XA/XA)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.44±0.55</td>
<td>0.63±0.19</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>j2-microglobulin levels, mg/l</td>
<td>2.97±0.31</td>
<td>2.90±0.55</td>
<td>0.934</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>47.70±3.99</td>
<td>36.83±10.71</td>
<td>0.306</td>
</tr>
<tr>
<td>Gamma globulins, %</td>
<td>21.62±0.97</td>
<td>19.83±1.79</td>
<td>0.427</td>
</tr>
<tr>
<td>IgG levels, g/l</td>
<td>15.55±0.06</td>
<td>12.47±2.98</td>
<td>0.304</td>
</tr>
<tr>
<td>IgM levels, g/l</td>
<td>1.96±0.31</td>
<td>4.47±3.00</td>
<td>0.062</td>
</tr>
<tr>
<td>IgA levels, g/l</td>
<td>2.62±0.19</td>
<td>3.23±0.79</td>
<td>0.343</td>
</tr>
<tr>
<td>IL-6 levels, pg/ml</td>
<td>25.36±6.74</td>
<td>22.57±11.80</td>
<td>0.851</td>
</tr>
<tr>
<td>IL-10 levels, pg/ml</td>
<td>4.13±1.22</td>
<td>1.57±0.54</td>
<td>0.323</td>
</tr>
</tbody>
</table>

Results are indicated as mean ± s.e.m. *Measured in 35 patients.

Immunologically, patients carrying MBL-low genotypes had a lower frequency of anti-Ro/SS-A antibodies (17 vs 55%, P = 0.014), anti-La/SS-B antibodies (8 vs 48%, P = 0.009) and low C4/C3 levels (0 vs 32%, P = 0.016) in comparison with patients carrying MBL-sufficient genotypes. Only three (25%) patients carrying MBL-low genotypes presented some of the immunological markers closely associated with the main extraglandular features of primary SS (anti-Ro/SS-A, anti-La/SS-B, low C3/C4 levels, cryoglobulins and monoclonal serum immunoglobulins) in comparison with 49 (71%) of patients carrying MBL-sufficient genotypes. Only three (25%) patients carrying MBL-low genotypes had positive RF, although the differences did not reach statistical significance. No patient carrying primary SS carrying the homozygous deficient genotype 0/0 had anti-Ro/SS-A or anti-La/SS-B antibodies, low C3/C4 levels or circulating cryoglobulins.

We also analysed the clinical significance of the MBL2 polymorphisms in the 46 patients who only fulfilled the 1993 criteria (Ro/La-negative patients). Patients carrying MBL-low genotypes had a lower prevalence of systemic manifestations (9 vs 43%, P = 0.04) and a lower frequency of immunological markers including ANA (64 vs 80%, RF (0 vs 17%), low C3 levels (18 vs 23%), low C4 levels (0 vs 6%) and cryoglobulins (0 vs 9%) in comparison with patients carrying MBL-sufficient genotypes, although the differences did not reach statistical significance. No patient carrying MBL-low genotypes had positive RF, hypocomplementaemia or circulating cryoglobulins.

**MASP2 polymorphism**

Five (6%) patients with primary SS were heterozygous for the MASP2 D105>G variant, a prevalence similar to that found in SLE patients (8%) but higher than that found in the 1993 SS patients (2%) and in healthy controls (3%). No statistically significant association was found between the MASP2 variant and the main epidemiological, clinical and immunological characteristics of primary SS.

**Discussion**

MBL is a liver-derived type C lectin protein that binds to certain carbohydrate motifs on the surface of pathogens, damaged host cells and immune complexes [7]. This activates MASP3 and results in the formation of C3-convertase, which facilitates removal of pathogens by opsonophagocytosis or complement-mediated lysis. Low MBL levels have been associated with an inadequate innate immune response, increasing the risk of infection especially in immunocompromised subjects [31], while its possible involvement in susceptibility to autoimmune diseases is unclear. Experimental studies have shown that the MBL-deficient murine model does not develop a broad spectrum of autoimmune processes (glomerulonephritis, ANA and dsDNA titres) in comparison with age-matched mice controls [32]. In clinical studies of patients with SLE, investigation of the possible aetopathogenic role of MBL deficiency initially centered on a hypothetically increased risk of infection, with controversial results [33, 34]. However, interest has recently turned to the possible influence of MBL deficiency in the clinical expression of SLE, and several studies have described a close association with cardiovascular disease, chronic damage and APS [18, 20, 35].
Moreover, Seelen et al. [45] found that patients with MBL variant alleles have an impaired ability to activate exogenous C4 by MBL/MASP complexes bound to mannanose, as well as an impaired capacity to activate the whole complement cascade upon binding of MBL to mannanose. Histological studies have shown an increase in the level of heterogeneity of the binding sites for mannanose in salivary glands of patients with SS [46], suggesting that genetic MBL heterogeneity may influence the degree of autoimmune damage and inflammation.

This poses the question of whether MBL deficiency could be beneficial in patients with primary SS. Our data suggest that MBL polymorphisms have little influence on genetic susceptibility to primary SS but much more influence on the genetic modification of disease expression, with MBL-deficient genotypes being associated with milder disease. The possible protective role of low MBL levels in inflammatory and autoimmune processes is a hypothesis proposed by Casanova and Abel [47] and supported by recent genetic, experimental and clinical studies. First, the worldwide extension of deleterious MBL2 haplotypes suggests that carrying MBL-low genotypes may be potentially beneficial and not harmful [48]. Secondly, the MBL-deficient murine model has significant protection against skeletal muscle reperfusion injury [49]. Thirdly, patients with low MBL levels seem to be protected against some intracellular infections such as tuberculosis [50], leishmaniasis [51] or HIV [52]. Fourthly, some patients with autoimmune diseases such as primary biliary cirrhosis have a higher frequency of MBL-high genotypes [53]. Taken together, these data suggest that MBL deficiency may provide some form of protection against the development of some inflammatory or autoimmune processes such as primary SS. However, recent studies have shown that MBL deficiency has been associated with an enhanced susceptibility to cardiovascular and thrombotic events [18, 20, 35]. This suggests that MBL deficiency may play a different role in each autoimmune disease.

This is the first study to analyse the prevalence and clinical significance of the genetic variability of MASP-2 in patients with a systemic autoimmune disease. We found a low prevalence of MASP-2 mutations in patients with primary SS, which was similar to that of the control groups. Thus, MASP-2 polymorphisms seem to have little clinical significance in primary SS.

We also analysed the clinical significance of MBL2 polymorphisms in a subset of patients with negative anti-Ro/La antibodies who did not fulfil the 2002 criteria [27] due to the lack of salivary gland biopsy. In these patients, MBL-low genotypes were associated with a lower frequency of systemic involvement and immunological markers, similar to that found in patients fulfilling the 2002 criteria. This suggests that genetically induced low levels of MBL may be protective against severe autoimmunity involvement not only in primary SS patients fulfilling the 2002 criteria, but also in those with negative Ro/La antibodies who only fulfilled the 1993 criteria.

In conclusion, patients with primary SS carrying MBL-deficient genotypes have a less severe systemic and immunological disease expression in comparison with those carrying MBL-sufficient genotypes. In contrast, MASP-2 mutations have little clinical significance. In primary SS, insufficient levels of MBL may represent a protective factor against the development of more aggressive autoimmune damage.

**Acknowledgements**

The authors wish to thank David Buss for his editorial assistance.

**Funding** This study was funded by La Marató de TV3 (grant 071810), Spanish Research Network on Infectious Diseases (REIPI, RD06/008/1013) from Instituto de Salud Carlos III and Fondo de Investigaciones Sanitarias (FIS 080103).

**Disclosure statement** The authors have declared no conflicts of interest.

**References**


