Association of the mannose-binding lectin 2 gene polymorphic variants with susceptibility and clinical progression in systemic lupus erythematosus

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Abstract

Objective

This study investigates the role of mannose-binding lectin (MBL) in susceptibility and clinical expression of systemic lupus erythematosus (SLE), through the analysis of promoter region and exon 1 polymorphisms of the MBL2 gene.

Methods

We analysed 325 SLE patients from the Hospital de Clínicas de Porto Alegre and 344 controls. All individuals were grouped according to ethnic origin. Genotyping of the promoter and exon 1 variants were performed by PCR-SSP and PCR-RFLP, respectively. Polymorphisms frequencies between patients and controls were compared by Chi-square or Fisher's exact tests.

Results

A statistically significant difference was observed among the frequencies of both promoter haplotypes ($p=0.005$) and haplotypic combinations ($p=0.004$) in African-derived patients, with a higher incidence of HY haplotype and LY/HY combination in SLE patients when compared to controls. These results showed a tendency to higher frequencies of genotypes related to high MBL levels in African-derived patients. A joint analysis of data from the promoter and exon 1 polymorphisms showed an increased frequency of genotypes conferring a deficient of MBL levels in European-derived patients ($p<0.001$).

Conclusion

Our data suggest a possible influence of MBL deficiency in SLE European-derived although we did not observe any involvement of MBL2 variants in SLE clinical progression. The conflicting results shown by the analysis of patients grouped by ethnicity emphasise the need for studies considering this variable.

Key words

mannose-binding lectin, systemic lupus erythematosus, polymorphism, complement system proteins

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Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune chronic inflammatory disease that involves multiple organs and exhibits a wide spectrum of clinical manifestations (1, 2). It is characterised by the breakdown of self tolerance and activation of auto-reactive lymphocytes, resulting in immune responses to self antigens (3) and in formation and deposition of immune complexes. This leads to an intense inflammatory response and to tissue damage (4). SLE is a multifactorial disease, with susceptibility associated to genetic, hormonal, immunological and environmental factors (5). For instance, the SLE prevalence is about nine times higher in childbearing women than in men, possibly due to effects of the estrogen hormone (6, 7). A predisposition to the disease may be related to the inheritance of gene variants located in specific chromosomal regions, including genes that encode complement system components and TLR genes (8, 9).

In the recent years, there has been an emerging interest in mannose-binding lectin (MBL) due to its central role as a recognition molecule in the complement system, but also because of the potential clinical implications of genetically determined differences in MBL oligomerisation and serum levels among subjects (10). MBL is an acute phase protein mainly produced in the liver and it is an important constituent of innate immune system. MBL is able to recognise and bind to specific groups of sugars, arranged on the surface of many microorganisms, thus providing one of the first defense lines of the body (11, 12). After connecting to a pathogen, MBL undergoes to a conformational change activating associated molecules such as serine proteases associated with MBL, resulting in activation of complement through the lectin pathway and in a consequent microorganisms phagocytosis (13-15).

The human mannose-binding lectin gene (*MBL2*) is located on chromosome 10 (q11.2–q21) and contains four exons (11, 16). Polymorphisms in *MBL2* have been reported and a wide interindividual variation in serum concentrations of MBL protein is a result of the presence of these allelic variants (17). It is well established that MBL serum levels are influenced by three variants in exon 1 of *MBL2* and are also modulated by polymorphisms in the promoter region (18). The most studied variants in exon 1 are the polymorphisms R52C (Arg52Cys), a CGT to TGT substitution called allele D; G54D (Gly54Asp), generated by a GGC to GAC substitution, determining allele B; and G57E (Gly47Glu), generated by a GGA to GAA substitution and called as allele C. A coding region containing any one of the three polymorphisms is generally called as allele O, while the wild allele is referred to as A (19, 20). In addition to structural mutations, three polymorphisms have been described in the *MBL2* gene promoter (21). These are H/L (G → C mutation), Y/X (G → C mutation) and P/Q (T → C mutation), respectively at positions -550, -221 and +4 of the gene (22). These variants are under strong linkage disequilibrium and only seven haplotypes (HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD) are commonly found (21).

Recent studies suggest a relationship between *MBL2* polymorphic variants and several autoimmune disorders, including SLE (18, 23, 24). The established association between the deficiency of complement system components and the susceptibility to autoimmune diseases suggested that a similar association could also be found in MBL (25). A MBL deficiency promotes retention of apoptotic cells in macrophages and consequent abnormal clearance leads to an excessive self-antigens expression. Moreover, *MBL2* polymorphisms, responsible for changes in protein levels, are related to increased susceptibility to infections. Since infections may be a susceptibility factor for SLE, MBL could also be related to disease development (17). Studies associating SLE with MBL are a promising area of research and these studies imply a role of MBL in infectious, inflammatory and autoimmune diseases. The persistence of these gene polymorphisms in different human populations may be explained by a genetic balance in which some individuals can benefit from high levels of protein, which protects

against infections with extracellular microorganisms, although individuals with low MBL levels are more resistant against infections by intracellular microorganisms (18).

In view of the ethnic variability, both in frequency of MBL allelic variants and in prevalence and incidence of SLE, and considering the unknown etiology of SLE and the lack of consistent information about the influence of MBL polymorphisms in this disease, the objective of this study was the immunogenetic characterisation of SLE individuals, through the analysis of *MBL2* polymorphic variants, seeking a possible association of these data with clinical and laboratory expression of disease.

Materials and methods

Samples

This study included 325 SLE patients followed at the Division of Rheumatology of Hospital de Clínicas de Porto Alegre (HCPA). Patients were classified as European or African-derived according to phenotypic characteristics of individuals and ethnicity data of parents/grandparents reported by the participants. The issue arisen on the skin colour-based classification criteria that is used in Brazil is well documented and has been already assessed by our group in previous studies (26, 27). Although the individuals classified as European-derived or African-derived can present a certain degree of admixture, a recent study published by Santos et al. assessed individual interethnic admixture using a 48-insertion-deletion Ancestry-Informative Marker panel. The authors identified a very high level of European contribution (94%) and fewer Native American (5%) and African (1%) genes in a sample of 81 European-derived individuals from southern Brazil (28). Therefore, the subgrouping of our SLE patients and controls seems to reflect the actual ethnic/genetic background of this human population. Information regarding demographic, clinical and laboratory features of SLE patients were collected from data contained in medical records filled in the Medical Archive Service and Health Information.

The clinical and laboratory features evaluated are presented at Table I and were: presence or absence of malar rash, discoid rash, photosensitivity, oral or nasal ulcers, serositis, arthritis, nephritis, neurological manifestations (psychosis and convulsions), haematologic events (hemolytic anaemia, leukopenia, lymphopenia and thrombocytopenia), positive antinuclear antibodies (ANAs) (titer >1:100) and other autoantibodies (anti-double-stranded DNA, anti-Sm, anti-Ro/SSA, anti-La/SSB, anti-RNP, anti-Scl 70, anticardiolipin, lupus anticoagulant and false positive VDRL). The definition of each variable followed the description from the classification criteria for SLE according to American College of Rheumatology (29). Furthermore, patients were evaluated for the presence of Sjögren's syndrome and secondary antiphospholipid syndrome, according to the classification criteria proposed for both (30, 31). SLICC damage index and SLEDAI disease activity index were also performed for each patient. The control group was formed by 344 unexposed, uninfected healthy blood donors, from the urban population of Porto Alegre, the capital of the southern-most state of Brazil, with ages ranging from 20 to 62 years. All patients and controls participating in this study gave their written informed consent. This study was approved by the ethics committee of HCPA.

Molecular characterisation of promoter and exon 1 polymorphisms

The DNA used for molecular techniques was obtained from 5 mL peripheral blood samples, collected with EDTA**,** and purified through salting-out method described by Lahiri e Nurnberger (32). DNA samples were stored at -20ºC. The *MBL2* promoter regions spanning the -550 (L or H) and -221 (X or Y) polymorphisms were amplified by polymerase chain reaction with sequence-specific primers (PCR-SSP) as described by Neonato *et al*. (33), using the following primers: L forward: 5'-GCTTACCCAGGCAAGCCTGTC-3', X reverse: 5'-GGAAGACTATAAACATGCTTTCG-3', H forward: 5'-GCTTACCCAGGCAAGCCTGTG-3' and Y reverse: 5'-GGAAGACTATAAACATGCTTTCC-3'. Four simultaneous reactions were made for each sample, using different combinations of primers (LX, LY, HX and HY) to identify the haplotypes. The amplified fragments were visualised in 2% agarose gel. In addition, a control gene (cytochrome P450 debrisoquine 4-hydroxylase, *CYP2D6*) was amplified in every reaction with specific primers: D6M2: 5'-TCGCCCTGCAGAGACTCCTC-3' and D6B5: 5'-TGCCGCCTTCGCCAACCACT-3'. Genotyping of exon 1 polymorphisms was already performed by our group and was previously published (4). In the present work, the previously determined exon 1 polymorphisms were added to the newly genotyped promoter polymorphisms in order to haplotypes establishment.

Statistical analysis

MBL genotypic and allelic frequencies were determined by direct counting and compared to Hardy-Weinberg expectations using Chi-Square tests. Polymorphisms frequencies between patients and controls were compared by Chi-square test or Fisher's exact test, if appropriate. The adjusted residuals were also calculated. All the data were analysed with SPSS software version 15.0 and WinPepi 10.0. The quantitative variables were analysed through the calculation of mean and standard deviation values, while the analyses of categorical variables were done by calculating frequencies and percentages. Comparisons were also made correlating clinical and laboratory variables of patients with the frequencies of polymorphisms, through Chi-square test (or Fisher's exact test) for qualitative variables and Kruskal-Wallis test for quantitative variables, using Bonferroni correction to the level of statistical significance. Significance level was established at $p<0.05$ (two-tailed).

Results

Among the 325 SLE patients studied, 248 (76%) were classified as European-derived and 77 (24%) as African-derived and among 344 the controls, 244 (71%) were classified as European-derived and 100 (29%) as African-derived. Classification according to ethnic origin was performed since

Table I. Clinical and laboratory features of SLE patients according to genotypes related to MBL production (%).

Clinical and laboratory features	European-derived n=248			African-derived n=77		
	DF n=54	LP n=74	HP n=120	DF n=13	LP n=19	HP n=45
Malar rash	51.9	52.7	57.1	69.2	31.6	52.3
Discoid rash	14.8	18.9	12.6	7.7	15.8	13.6
Photosensitivity	68.5	79.7	82.4	61.5	36.8	65.9
Oral/nasal ulcers	35.2	40.5	36.1	30.8	36.8	29.5
Arthritis	85.2	85.1	79.8	92.3	84.2	81.8
Serositis	27.8	38.4	25.2	46.2	42.1	36.4
Nephritis	31.5	45.9	43.7	38.5	36.8	52.3
Neurologic disorders	16.7	13.5	9.2	7.7	10.5	11.4
Psychosis	7.4	9.5	4.2	0	10.5	6.8
Convulsions	13.0	4.1	5.9	7.7	0	4.5
Haematologic disorders	83.3	74.3	72.3	69.2	100.0	86.4
Haemolytic anaemia	38.9	31.1	27.7	15.4	31.6	31.8
Leukopenia/Lymphopenia	63.0	50.0	60.5	69.2	89.5	65.9
Thrombocytopenia	25.9	17.6	16.0	7.7	31.6	20.5
Positive ANA	100.0	100.0	97.5	92.3	100.0	100.0
Immunologic disorders	61.1	70.8	62.7	46.2	73.7	70.5
Anti-DNA	37.0	50.0	47.5	30.8	42.1	59.1
Anti-Sm	16.7	208.0	18.6	23.1	26.3	20.5
Anticardiolipin	29.6	29.2	21.4	7.7	42.1	29.5
Lupus anticoagulant	11.1	6.9	3.4	0	0	4.5
False positive VDRL	3.7	4.2	1.7	0	0	2.3
Anti-Ro/SS-A	36.0	33.9	39.6	61.5	64.7	66.7
Anti-La/SS-B	8.0	10.2	13.5	38.5	17.6	20.5
Anti-RNP	30.0	35.6	28.1	30.8	29.4	30.8
Anti-Scl 70	2.0	1.7	3.1	0	0	2.6
Sjögren	5.7	11.6	13.0	7.7	16.7	7.1
APS	3.8	10.1	7.0	0	5.6	4.8
SLICC *	1 (0–8)	1 (0–5)	1 (0–7)	0 (0–5)	1 (0–3)	1 (0–5)
SLEDAI *	2 (0–16)	0 (0–12)	0 (0–16)	2.5 (0–8)	0 (0–10)	1.5 (0–36)

DF: genotypes related to deficient serum MBL levels; LP: genotypes related to low serum MBL levels; HP: genotypes related to high serum MBL levels; ANA: antinuclear antibody; APS: antiphospholipid syndrome; SLEDAI: systemic lupus erythematosus disease activity index; SLICC: systemic lupus international collaborating clinics; VDRL: venereal disease research laboratory test.
* Median (minimum-maximum).
Statistical significance was considered with p-value <0.0024 by Bonferroni correction.

both SLE incidence and frequencies of the *MBL2* polymorphisms vary in different populations (10, 34). Due to the lack of clinical and/or laboratory data from medical records of some patients, a difference between the total number of individuals sampled and the number of individuals analysed may be observed in some cases. The mean age of patients was 42.2±14.3 years and the mean age of disease diagnostic was 32.7±13.6 years. The frequencies of patient's clinical and laboratory features were previously published by our group (4). The values corresponding to *MBL2* promoter polymorphisms frequencies and to the haplotype combinations frequencies related with the MBL production levels in patients and controls are shown in Tables II and III, respectively.

The genotypic frequencies of *MBL2* promoter polymorphisms in European-derived SLE patients and in African-derived controls, for H/L variants, were different from those expected under Hardy-Weinberg equilibrium. This finding can be explained by the strong linkage disequilibrium between H/L and Y/X loci. The H/L and Y/X polymorphisms genotypic frequencies were compared between patients and controls (Table II), showing a statistically significant difference among the H/L polymorphism frequencies in European-derived (p=0.033) and African-derived (p=0.001) groups. There was a higher frequency of H/L genotype (0.56) and a lower frequency of L/L genotype (0.33) in European-derived patients when compared with the controls (0.44 and 0.41, respectively). Among African-derived individuals, 49.4% of patients had H/L genotype and 41.6% had L/L genotype, compared to 24% and 69% of controls, respectively. No significant difference was observed analysing Y/X variants in any of these groups (see Table II).

Haplotypes analysis (Table II) showed a statistically meaningful difference among frequencies of LX, LY and HY haplotypes in African-derived group (p=0.005), with a higher frequency of HY haplotype in patients (0.34) when compared to healthy controls matched by ethnic origin (0.19). However, no statistically significant difference was observed between European-derived group. As expected, HX haplotype was not observed in this study.

Likewise, the frequencies of all possible combinations of haplotypes (Table II) generated by genotypes and haplotypes found were evaluated. A statistically significant difference was observed only between the frequencies of haplotypes' combinations in African-derived individuals (p=0.004), with 40.3% of patients presenting LY/HY combination compared to 18% of controls. A higher frequency of LY/LY genotype was found in African-derived controls (0.39) when compared to patients (0.22). Besides, none of the African-derived patients had LX/LX genotype, while 6% of controls presented it.

The main analysis was made by combining the newly obtained data of promoter polymorphisms with the data of polymorphisms from exon 1 previously published by our group (4). The haplotypic combinations were obtained and they refer to the levels of MBL production (Table III): high serum levels (HYA/A and LYA/A), low serum levels (LXA/LXA, HYA/0 and LYA/0) and deficient serum levels (LXA/0 and 0/0). Our results showed

Table II. H/L and Y/X polymorphisms frequencies of *MBL2* gene in SLE patients and controls.

Genotypes	European-derived		African-derived	
	Controls (%) n=244	Patients (%) n=248	Controls (%) n=100	Patients (%) n=77
L/L	101 (41.4)*	81 (32.7)*	69 (69.0)*	32 (41.6)*
H/L	107 (43.9)*	138 (55.6)*	24 (24.0)*	38 (49.4)*
H/H	36 (14.8)	29 (11.7)	7 (7.0)	7 (9.1)
	χ^2 p=0.033		χ^2 p=0.001	
X/X	10 (4.1)	9 (3.6)	6 (6.0)	0
X/Y	72 (29.5)	75 (30.2)	30 (30.0)	22 (28.6)
Y/Y	162 (66.4)	164 (66.1)	64 (64.0)	55 (71.4)
	χ^2 p=0.954		Fisher p=0.075	
Haplotypes	2n=488	2n=496	2n=200	2n=154
LX	92 (18.8)	93 (18.8)	42 (21.0)	22 (14.3)
LY	217 (44.5)	208 (41.9)	120 (60.0)	80 (51.9)
HY	179 (36.7)	195 (39.3)	38 (19.0)*	52 (33.8)*
	χ^2 p=0.665		χ^2 p=0.005	
Haplotypic combination	n=244	n=248	n=100	n=77
LX/LX	10 (4.1)	9 (3.6)	6 (6.0)*	0*
LX/LY	47 (19.3)	39 (15.7)	24 (24.0)	15 (19.5)
LX/HY	25 (10.2)	36 (14.5)	6 (6.0)	7 (9.1)
LY/LY	44 (18.0)	34 (13.7)	39 (39.0)*	17 (22.1)*
LY/HY	82 (33.6)	101 (40.7)	18 (18.0)*	31 (40.3)*
HY/HY	36 (14.8)	29 (11.7)	7 (7.0)	7 (9.1)
	χ^2 p=0.239		χ^2 p=0.004	

*Significant adjusted residuals (p<0.05).

Table III. Haplotypic combinations frequencies related to serum MBL levels in SLE patients and controls.

Haplotypic combination	European-derived		African-derived	
	Controls (%) n=244	Patients (%) n=248	Controls (%) n=100	Patients (%) n=77
Deficient production	13 (5.3)*	54 (21.8)*	17 (17.0)	13 (16.9)
Low production	91 (37.3)	74 (29.8)	29 (29.0)	19 (24.7)
High production	140 (57.4)*	120 (48.4)*	54 (54.0)	45 (58.4)
	Fisher p<0.001		Fisher p=0.8165	

*Significant adjusted residuals (p<0.05).
Haplotypes: High serum MBL levels (LYA/A, HYA/A); Low serum MBL levels (LXA/LXA, LYA/0, HYA/0); Deficient serum MBL levels (LXA/0, 0/0).

21.8% of European-derived SLE patients with genotypes related to MBL deficient production compared to 5.3% of healthy controls and 48.4% of these patients with genotypes related to high serum MBL levels compared to 57.4% of controls (p<0.001). The African-derived individuals did not present any statistically meaningful difference for such condition.

Clinical, laboratory and demographic features observed in SLE patients were previously described and compared among different ethnic groups (4). We analysed the clinical differences between patients with SLE according to the haplotypic combinations for promoter polymorphisms (data not shown). A statistically significant difference was observed between the haplotypic combinations and SLICC damage index in African-derived SLE patients (p=0.030). The median for SLICC was higher in patients presenting HY/HY haplotypic combination when compared to patients with other combinations. However, when Bonferroni correction was applied, these statistically significant differences were not maintained.

Comparisons between disease features and frequencies of haplotypes combined, related to serum MBL levels, were also performed (Table I). The results showed a prevalence of 100% of haematologic disorders in African-derived patients with genotypes related to low serum MBL levels compared to 86.4% of patients with genotypes related to high serum MBL levels and 69.2% with genotypes corresponding to deficient serum MBL levels (p=0.038), although after Bonferroni correction, the statistically significant differences were lost.

Discussion

In this study we investigated the possible influence of *MBL2* polymorphisms on the SLE development. MBL appears to have a key role in innate immunity due to its central role in the host defense (35). *MBL2* gene polymorphisms have been associated with susceptibility to infectious and auto-immune diseases, highlighting the possible interference of different MBL serum levels, attenuating or aggravating certain pathologies (36, 37). In this view, several studies have suggested that the genetic variability of *MBL2* may be involved in SLE pathogenesis (5, 35, 37, 38). However, genotyping studies have shown inconsistent results.

It is already known that the SLE incidence varies according to different human populations and that certain ethnic groups are more susceptible to develop this disease than others (39). For this reason and since the frequencies of *MBL2* polymorphisms vary in different populations, we analysed patients and controls grouped according to their ethnic origin. The analysis of *MBL2* promoter region variants showed a higher frequency of H/L genotype in SLE patients as compared to their respective controls, both among the European- and African-derived individuals. Conversely, a higher frequency of L/L genotype was observed in controls as compared to SLE patients in both groups. In contrast, no differences were

observed considering Y/X polymorphic variants, which is in agreement with a Hungarian study, which also found no significant differences among the genotypic frequencies of Y/X polymorphism between SLE patients and controls (40).

Analysing the haplotypic frequencies, we found a higher frequency of African-derived SLE patients carrying HY haplotype compared to controls, suggesting that HY haplotype, related to high serum MBL levels (21, 41), is possibly associated with SLE development. This association can be explained based on the fact that high serum levels of MBL could cause excessive complement activation by the lectin pathway, after initial tissue damage. Consequently, this could promote an immune response, resulting in autoimmunity and tissue injury. High activity and high serum levels of MBL have been associated with inflammatory diseases, rejection to transplants and diabetic nephropathy (42). Furthermore, along with other susceptibility, genetic or immunological factors, high MBL levels could influence the susceptibility to infections by intracellular microorganisms, since that such protein promotes phagocytosis, facilitating pathogens that act inside the cell, and thus contributing to the SLE development (13, 42, 43). These findings differ from those found in a study performed with 92 black SLE patients at Philadelphia, which reported that the promoter haplotype related to high MBL serum levels was negatively associated to SLE (44).

In the European-derived group, the HY, LY and LX haplotypes frequencies were similar between patients and controls. In two studies with Chinese populations the LX haplotype frequency was significantly higher in SLE patients than controls, suggesting that LX haplotype is a risk factor for SLE in this ethnic group. According to these studies, LX haplotype, which determines low serum MBL levels, may hamper clearance of immune complexes and thus lead to an overall impairment of the immune system (45, 46). The HX haplotype was not found in these studies nor in our study, but an unusual frequency of this haplotype was observed in a study made by Navarre *et al*. in a population of the Philippines (47). Nevertheless, such HX frequency is controversial since, besides their occurrence in a few other individuals (44, 48) it is a haplotype never before described in literature (49).

We observed a significantly higher frequency of the LY/HY haplotypic combination in African-derived SLE patients when compared to controls and a tendency to increase of LY/HY combination was also observed in European-derived (although not significant), confirming the high frequency of H/L genotype in patients when compared to controls in both ethnic groups. There was a significant increase of the haplotypic combinations LY/LY and LX/LX in African-derived controls compared to patients and a tendency to increase of these combinations in European-derived. This analysis confirmed the increasing L/L genotype frequency in controls, as previously reported. However, these findings contrast with those found by Huang *et al*., which showed an increase of LX/LX haplotypic combination in Chinese SLE patients, indicating a strong association with the disease development ($p<0.01$) (46). Another study performed with African American and Caucasians observed that LX haplotype was over-represented in African American SLE patients and that an increase of LX/LX combination was observed in African American SLE patients when those were compared to controls ($p<0.0001$) (50). Our study failed to show association between LX haplotype and SLE and, instead, it seemed to act as a protective factor against the development of the disease. The marked interethnic differences in the frequencies of promoter haplotypes and the fact that African-derived population in Brazil is highly admixed may have influenced the results.

As previously stated, the high frequency of *MBL2* allelic variants that alter protein function in different populations suggests that MBL deficiency may protect against some infectious diseases (51). Actually, MBL deficiency confers selective advantages against intracellular pathogens and it can be associated with resistance against leprosy and leishmaniasis (52, 53). Conversely, a study with Australian indigenous suggested that very high MBL levels may, in part, explain the devastating consequences caused by tuberculosis in 19th and 20th centuries (54).

Variants effects of promoter region explain much of the ethnic differences that are not explainable by structural variants alone. Exon 1 mutations are believed to interfere in oligomerisation of protein causing a decrease of functional MBL in the circulation while the promoter polymorphisms influence MBL expression. Interplay between promoter and structural variants of *MBL2* gene control basal serum levels of this protein and can vary among individuals (21, 46, 51). The common haplotypes established, due to linkage disequilibrium, are correlated with different serum levels of MBL. In European-derived individuals, there was an increase of haplotypic combinations related to deficient MBL levels (LXA/O, O/O) in SLE patients when compared to controls, suggesting a possible influence of deficiency of protein in the disease. These results are in agreement with a study that analysed Icelandic SLE families where genotypes determining low MBL production were suggested as a contributing factor in SLE (3). Our data corroborate the results of both Sandrin-Garcia *et al*. and Villarreal *et al*., that also reported a higher frequency of haplotypic combinations related to high MBL production in controls. According to the authors, MBL haplotypes encoding for high protein serum levels are protective against the development of SLE (55, 56) and the protective nature of these haplotypes may become more apparent during an acute-phase response, when baseline levels of protein can increase up to 4-fold (55).

Besides the possible influence on SLE development, there is growing interest in the clinical significance of *MBL2* variant alleles and several studies have reported their association with different clinical and laboratory features of disease (38, 40, 57). For example, a recent analysis from Southern Brazil found a significant association between *MBL2* A/O genotype and nephritic disorders as well as for *MBL2* promoter X/Y

genotype and antiphospholipid syndrome. Moreover, SLE patients carrying combined haplotypes determining low MBL levels presented an increased risk to develop nephritis (56). A study with SLE children from Taiwan showed that high MBL expression genotype was associated with renal disorders and it had a protective role against bacterial infections, suggesting the influence of serum MBL levels in SLE activity (58). An association was also observed between 0/0 genotype and the development of arterial thrombosis in SLE patients (59). According to other studies, patients carrying genotypes related to low MBL levels presented a higher prevalence of chronic renal failure, vasculitis, heart valve lesions, cardiac valve dysfunction, associated APS and a higher mean SLICC score (38) and MBL deficient SLE patients had more renal involvement, increased infection, strongly increased risk for arterial thrombosis and increased levels of autoantibodies against molecules associated with apoptotic cells, such as C1q and cardiolipin (42). But studies involving the clinical implications of MBL in the SLE development are still very contradictory. These genes *versus* disease associations studies are controversial since the allelic frequencies are different among ethnic groups and one must be cautious in extrapolating results to other populations.

The results presented in the present manuscript are conflicting with the previous data, when these same patients were analysed only in relation to polymorphisms of exon 1 (4). This shows the need for a joint analysis of variants at both structural and promoter regions of *MBL2* in order to determine the influence of MBL in SLE.

In conclusion, the results of this study suggest an association of the genotypes related to deficient serum MBL levels with the SLE development in European-derived patients, indicating that *MBL2* structural and promoter polymorphisms can have a possible role in disease susceptibility in this ethnic group. The opposite was seen in African-derived SLE patients who showed a tendency to higher frequencies of genotypes related to high serum MBL levels. Our data showed a large contrast in the frequencies of *MBL2* SNPs according to ethnicity, emphasising the importance of interethnic genetic variability and the need to include more population groups in the analysis before making any association with the disease. Therefore, further studies should be made to support our findings and also to reveal more clearly the molecular mechanisms involved in this association.

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References

1. COOPER GS, DOOLEY MA, TREADWELL EL, ST CLAIR EW, PARKS CG, GILKESON GS: Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. *Arthritis Rheum* 1998; 41: 1714-24.
2. JONSEN A, GULLSTRAND B, GUNER N *et al*.: Genetically determined mannan-binding lectin deficiency is of minor importance in determining susceptibility to severe infections and vascular organ damage in systemic lupus erythematosus. *Lupus* 2007; 16: 245-53.
3. KRISTJANSDOTTIR H, SAEVARSDOTTIR S, GRONDAL G *et al*.: Association of three systemic lupus erythematosus susceptibility factors, PD-1.3A, C4AQ0, and low levels of mannan-binding lectin, with autoimmune manifestations in Icelandic multicase systemic lupus erythematosus families. *Arthritis Rheum* 2008; 58: 3865-72.
4. MONTICIELO OA, CHIES JA, MUCENIC T *et al*.: Mannose-binding lectin gene polymorphisms in Brazilian patients with systemic lupus erythematosus. *Lupus* 2010; 19: 280-7.
5. GARRED P, VOSS A, MADSEN HO, JUNKER P: Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes Immun* 2001; 2: 442-50.
6. SEKIGAWA I, FUJISHIRO M, YAMAGUCHI A *et al*.: A new hypothesis of the possible mechanisms of gender differences in systemic lupus erythematosus. *Clin Exp Rheumatol* 2010; 28: 419-23.
7. MANZI S: Epidemiology of systemic lupus erythematosus. *Am J Manag Care* 2001; 7: S474-9.
8. SANCHEZ E, CALLEJAS-RUBIO JL, SABIO JM *et al*.: Investigation of TLR5 and TLR7 as candidate genes for susceptibility to systemic lupus erythematosus. *Clin Exp Rheumatol* 2009; 27: 267-71.
9. SAEVARSDOTTIR S, KRISTJANSDOTTIR H, GRONDAL G, VIKINGSDOTTIR T, STEINSSON K, VALDIMARSSON H: Mannan-binding lectin and complement C4A in Icelandic multicase families with systemic lupus erythematosus. *Ann Rheum Dis* 2006; 65: 1462-7.
10. GARRED P: Mannose-binding lectin genetics: from A to Z. *Biochem Soc Trans* 2008; 36: 1461-6.
11. DOMMETT RM, KLEIN N, TURNER MW: Mannose-binding lectin in innate immunity: past, present and future. *Tissue Antigens* 2006; 68: 193-209.
12. MATSUSHITA M, FUJITA T: Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med* 1992; 176: 1497-502.
13. FIANE AE, UELAND T, SIMONSEN S *et al*.: Low mannose-binding lectin and increased complement activation correlate to allograft vasculopathy, ischaemia, and rejection after human heart transplantation. *Eur Heart J* 2005; 26: 1660-5.
14. SELANDER B, MARTENSSON U, WEINTRAUB A *et al*.: Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2. *J Clin Invest* 2006; 116: 1425-34.
15. IP WK, TO YF, CHENG SK, LAU YL: Serum mannose-binding lectin levels and mbl2 gene polymorphisms in different age and gender groups of southern Chinese adults. *Scand J Immunol* 2004; 59: 310-4.
16. MADSEN HO, GARRED P, KURTZHALS JA *et al*.: A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 1994; 40: 37-44.
17. TAKAHASHI R, TSUTSUMI A, OHTANI K *et al*.: Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. *Ann Rheum Dis* 2005; 64: 311-4.
18. TURNER MW, HAMVAS RM: Mannose-binding lectin: structure, function, genetics and disease associations. *Rev Immunogenet* 2000; 2: 305-22.
19. MULLER S, KEIL T, GRUBER C *et al*.: MBL2 variants in relation to common childhood infections and atopy-related phenotypes in a large German birth cohort. *Pediatr Allergy Immunol* 2007; 18: 665-70.
20. WIERTSEMA SP, HERPERS BL, VEENHOVEN RH *et al*.: Functional polymorphisms in the mannan-binding lectin 2 gene: effect on MBL levels and otitis media. *J Allergy Clin Immunol* 2006; 117: 1344-50.
21. MADSEN HO, GARRED P, THIEL S *et al*.: Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J Immunol* 1995; 155: 3013-20.
22. MADSEN HO, SATZ ML, HOGH B, SVEJGAARD A, GARRED P: Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. *J Immunol* 1998; 161: 3169-75.
23. LEE YH, WITTE T, MOMOT T *et al*.: The mannose-binding lectin gene polymorphisms and systemic lupus erythematosus: two case-control studies and a meta-analysis. *Arthritis Rheum* 2005; 52: 3966-74.

24. MONTICIELO OA, MUCENIC T, XAVIER RM, BRENOL JC, CHIES JA: The role of mannose-binding lectin in systemic lupus erythematosus. *Clin Rheumatol* 2008; 27: 413-9.
25. TURNER MW: The role of mannose-binding lectin in health and disease. *Mol Immunol* 2003; 40: 423-9.
26. VEIT TD, CORDERO EA, MUCENIC T *et al*.: Association of the HLA-G 14 bp polymorphism with systemic lupus erythematosus. *Lupus* 2009; 18: 424-30.
27. VARGAS AE, MARRERO AR, SALZANO FM, BORTOLINI MC, CHIES JA: Frequency of CCR5delta32 in Brazilian populations. *Braz J Med Biol Res* 2006; 39: 321-5.
28. SANTOS NP, RIBEIRO-RODRIGUES EM, RIBEIRO-DOS-SANTOS AK *et al*.: Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat* 2010; 31: 184-90.
29. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
30. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
31. MIYAKIS S, LOCKSHIN MD, ATSUMI T *et al*.: International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295-306.
32. LAHIRI DK, NURNBERGER JI JR.: A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; 19: 5444.
33. NEONATO MG, LU CY, GUILLOUD-BATAILLE M *et al*.: Genetic polymorphism of the mannose-binding protein gene in children with sickle cell disease: identification of three new variant alleles and relationship to infections. *Eur J Hum Genet* 1999; 7: 679-86.
34. MOLOKHIA M, MCKEIGUE P: Systemic lupus erythematosus: genes versus environment in high risk populations. *Lupus* 2006; 15: 827-32.
35. VERDU P, BARREIRO LB, PATIN E *et al*.: Evolutionary insights into the high worldwide prevalence of MBL2 deficiency alleles. *Hum Mol Genet* 2006; 15: 2650-8.
36. RECTOR A, LEMEY P, LAFFUT W *et al*.: Mannan-binding lectin (MBL) gene polymorphisms in ulcerative colitis and Crohn's disease. *Genes Immun* 2001; 2: 323-8.
37. BERNIG T, TAYLOR JG, FOSTER CB, STAATS B, YEAGER M, CHANOCK SJ: Sequence analysis of the mannose-binding lectin (MBL2) gene reveals a high degree of heterozygosity with evidence of selection. *Genes Immun* 2004; 5: 461-76.
38. FONT J, RAMOS-CASALS M, BRITO-ZERON P *et al*.: Association of mannose-binding lectin gene polymorphisms with antiphospholipid syndrome, cardiovascular disease and chronic damage in patients with systemic lupus erythematosus. *Rheumatology* (Oxford) 2007; 46: 76-80.
39. LAU CS, YIN G, MOK MY: Ethnic and geographical differences in systemic lupus erythematosus: an overview. *Lupus* 2006; 15: 715-9.
40. JAKAB L, LAKI J, SALLAI K *et al*.: Association between early onset and organ manifestations of systemic lupus erythematosus (SLE) and a down-regulating promoter polymorphism in the MBL2 gene. *Clin Immunol* 2007; 125: 230-6.
41. TURNER MW: Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol Today* 1996; 17: 532-40.
42. BOUWMAN LH, ROEP BO, ROOS A: Mannose-binding lectin: clinical implications for infection, transplantation, and autoimmunity. *Hum Immunol* 2006; 67: 247-56.
43. EZEKOWITZ RA: Role of the mannose-binding lectin in innate immunity. *J Infect Dis* 2003; 187 (Suppl. 2): S335-9.
44. SULLIVAN KE, WOOTEN C, GOLDMAN D, PETRI M: Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus. *Arthritis Rheum* 1996; 39: 2046-51.
45. IP WK, CHAN SY, LAU CS, LAU YL: Association of systemic lupus erythematosus with promoter polymorphisms of the mannose-binding lectin gene. *Arthritis Rheum* 1998; 41: 1663-8.
46. HUANG YF, WANG W, HAN JY *et al*.: Increased frequency of the mannose-binding lectin LX haplotype in Chinese systemic lupus erythematosus patients. *Eur J Immunogenet* 2003; 30: 121-4.
47. NAVARRA SV, VILLAMIN CA, BAES RP, PIMENTA L, NICDAO JL, BERNAS GD: Increased frequency of mannose-binding lectin promoter LX haplotype among Filipinos with systemic lupus erythematosus. *Lupus* 2007; 16: 147-8.
48. STEFFENSEN R, THIEL S, VARMING K, JERSILD C, JENSENIUS JC: Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. *J Immunol Methods* 2000; 241: 33-42.
49. CHIES JA: On the haplotypic frequencies of the MBL2 gene among human populations. *Lupus* 2007; 16: 838.
50. SULLIVAN KE, JAWAD AF, PILIERO LM *et al*.: Analysis of polymorphisms affecting immune complex handling in systemic lupus erythematosus. *Rheumatology* (Oxford) 2003; 42: 446-52.
51. PETERSEN SV, THIEL S, JENSENIUS JC: The mannan-binding lectin pathway of complement activation: biology and disease association. *Mol Immunol* 2001; 38: 133-49.
52. GARRED P, HARBOE M, OETTINGER T, KOCH C, SVEJGAARD A: Dual role of mannan-binding protein in infections: another case of heterosis? *Eur J Immunogenet* 1994; 21: 125-31.
53. SANTOS IK, COSTA CH, KRIEGER H *et al*.: Mannan-binding lectin enhances susceptibility to visceral leishmaniasis. *Infect Immun* 2001; 69: 5212-5.
54. TURNER MW, DINAN L, HEATLEY S *et al*.: Restricted polymorphism of the mannose-binding lectin gene of indigenous Australians. *Hum Mol Genet* 2000; 9: 1481-6.
55. VILLARREAL J, CROSDALE D, OLLIER W *et al*.: Mannose binding lectin and FcgammaRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. *Rheumatology* (Oxford) 2001; 40: 1009-12.
56. SANDRIN-GARCIA P, BRANDAO LA, COELHO AV *et al*.: Mannose binding lectin gene (MBL2) functional polymorphisms are associated with systemic lupus erythematosus in southern Brazilians. *Hum Immunol* 2011; 72: 516-21.
57. PIAO W, LIU CC, KAO AH *et al*.: Mannose-binding lectin is a disease-modifying factor in North American patients with systemic lupus erythematosus. *J Rheumatol* 2007; 34: 1506-13.
58. TSAI YC, YEH KW, YAO TC, HUANG YL, KUO ML, HUANG JL: Mannose-binding lectin expression genotype in pediatric-onset systemic lupus erythematosus: associations with susceptibility to renal disease and protection against infections. *J Rheumatol* 2011; 38: 1429-35.
59. OHLENSCHLAEGER T, GARRED P, MADSEN HO, JACOBSEN S: Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. *N Engl J Med* 2004; 351: 260-7.