

RESEARCH ARTICLE

The -2518 A/G polymorphism in the monocyte chemoattractant protein 1 gene is associated with the risk of developing systemic lupus erythematosus in Argentinean patients: a multicenter study

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Systemic lupus erythematosus (SLE) is a systemic, autoimmune disorder. Monocyte chemoattractant protein 1 (MCP-1), a chemokine involved in the recruitment and migration of monocytes/macrophages, has been shown to be increased in the plasma of SLE patients. The aim of our study was to evaluate the possible association of the polymorphism -2518 of the MCP-1 gene with the risk of developing SLE, manifesting lupus nephritis (LN) and with other clinical features of SLE in an Argentinean population. A group of 171 SLE patients and 120 control subjects were examined. Genotypic and allelic frequencies of the MCP-1 -2518 A/G polymorphism showed significant differences between the SLE and the control groups ($p=0.001$ and $p=0.01$, respectively). However, the polymorphism showed no association with LN or with the other clinical variables studied. Our results suggest that the presence of the MCP-1 -2518 A/G polymorphism might be a risk factor for developing SLE in genetically predisposed individuals, but it does not seem to have a role in the evolution of the disease in the Argentinean population.

Key words: systemic lupus erythematosus, lupus nephritis, MCP-1 -2518 A/G polymorphism

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic, autoimmune disorder characterized by the presence of auto-antibodies to nuclear antigens, immune complex deposition and subsequent tissue destruction. SLE has a broad range of clinical manifestations, including photosensitive skin rashes, discoid lesions, arthritis/arthralgia, nephritis, cardiac and pulmonary diseases and central nervous system disorders [1]. Lupus nephritis (LN) is one of the most common and severe complications of SLE and is an important predictor of poor outcome that substantially contributes to patient morbidity and mortality [2, 3].

The etiology of SLE and the resulting pathological features of the disease, such as LN, are multifactorial and involve multiple genes, sex hormones and environmental

factors [4]. Studies of twins and families with SLE provide evidence that a genetic component contributes to disease susceptibility [5-7].

SLE is characterized by deregulation of the immune system with consequent chronic inflammation and an increased expression of cytokines such as IL1, IL2, IL6, IFN γ , TNF α , and monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), among others [4]. These cytokines play a critical role in the differentiation, maturation and activation of immune cells and also participate in the local inflammatory processes that mediate tissue damage in SLE [8].

MCP-1 is the most important chemokine involved in the recruitment and migration of monocytes/macrophages to sites of inflammation. It may also be responsible for inflammation in tissues affected by autoimmune diseases

[9]. In fact, lupus patients with both active and inactive disease have elevated plasma levels of MCP-1 [10]. Specifically in kidneys, MCP-1 is produced by renal mesangial cells, endothelial cells, tubular epithelial cells and smooth muscle cells, in response to IL-1 β , TNF α or low-density lipoprotein [11, 12]. Urine levels of MCP-1 are increased in patients with active LN compared to those with inactive renal disease or healthy controls. These increased MCP-1 levels are associated with lupus renal disease and lupus flares [13, 14].

A polymorphism in the distal regulatory region of the MCP-1 gene, located at position -2518 A/G (rs 1024611), was originally reported to affect MCP-1 transcriptional activity in response to inflammatory stimuli [15]. Subsequently, this polymorphism has been associated with susceptibility to SLE or its clinical characteristics, particularly LN. However, these associations are still controversial, and differences have been reported in a variety of studies [16-25].

The aim of our study was to evaluate the possible association of the -2518 A/G polymorphism in the MCP-1 gene with the risk of developing SLE, manifesting LN and with other clinical features of SLE within an Argentinean cohort.

MATERIALS AND METHODS

Patients

A non-family-based, case-control multicenter study was designed. We studied a total of 171 SLE patients who fulfilled the revised classificatory criteria of the American College of Rheumatology (ACR) for SLE [26].

The control group consisted of 120, unrelated subjects that were considered to be "metabolically healthy" individuals. The control group subjects had no personal history of chronic inflammatory diseases. We considered metabolically healthy individuals to be those who were clinically healthy, with a normal waist circumference. Furthermore, all healthy individuals had no direct family or personal histories of obesity or dyslipidemia.

All subjects were of Argentine descent and had the same ethno-geographic and social origin. The Argentinean population is the result of genetic admixture processes involving three continental contributors: Native Americans, Western Africans and Europeans (mainly Spaniards and Italians), although the African component detected in different studies was very low (less than 4%) [27, 28]. We selected a representative sample of the admixed, urban Argentinean population.

The study was approved by the ethics committee of the respective medical centers and was performed according to the principles of the Declaration of Helsinki. Informed consent was obtained from all of the participants.

Demographic, clinical and laboratory data

The following data were collected for all of the study participants: sex, age, disease evolution, Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLICC/ACR) Damage Index, lupus flares number, renal disease, lupus anticoagulant (LA) and anti-cardiolipin antibodies (ACA) [29].

Antiphospholipid syndrome (APS) was defined according to the International Consensus Statement of the classification criteria for definitive APS [30].

Blood collection and sample preparation

Venous blood was drawn without stasis from 291 patients and collected in tubes containing EDTA. Subsequently, DNA from peripheral leukocytes was extracted using a fully automated technique from the QIAamp DNA Blood Mini Kit (Qiagen).

The MCP-1 -2518 A/G polymorphism was detected by the polymerase chain reaction (PCR) followed by restriction enzyme-fragment (RF) analysis. A 251-bp fragment including the distal regulatory segment of the MCP-1 5' flanking region (nucleotides -2608 to -2357) was analyzed. The following primers were used: forward, 5'-TTTCCACTCACTTCTCTCACG-3' and reverse, 5'-TGGCTGAGTGTTTCACATAGGC-3'. DNA was amplified by cycling at 95°C for 5 min and then at 95°C for 60 s, 57°C for 40 s and 72°C for 90 s for 38 cycles, followed by 10 min at 72°C. The product was digested with Pvu II restriction enzyme (Fermentas, Life Sciences, USA) for 4 h at 37°C. The cleaved fragments were separated by electrophoresis in a 3% agarose gel stained with SybrSafe. Samples with a single 251-bp band were identified as A/A. Samples with three bands of 251, 167 and 84 bp were typed as A/G, and samples with two bands of 167 and 84 bp were typed as G/G.

Statistical analysis

Statistical analysis was performed with the SPSS statistical package (version 15.0 for windows; SPSS, Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium was tested for the polymorphisms studied using a Chi-squared test (χ^2). The data were not normally distributed and are presented as median and percentiles (25 and 75) or as a percentage. Non-parametric tests (Mann-Whitney U-test) were used to compare quantitative data, and the Chi-squared test was used to compare proportions. A $p < 0.05$ was considered statistically significant. The Odds Ratio (OR) was estimated to assess the strength of the association between the G allele carriers and the risk of developing SLE. A conditional logistic regression analysis was performed to test the association between the -2518 polymorphism of the MCP-1 gene and the variables studied adjusted for sex, age and duration of the disease (years).

RESULTS

This study included 171 SLE patients (159 women and 12 men), with a median age of 35 years (28-45) and 120 metabolically healthy controls (99 women and 21 men), with a median age of 31 years (26-37). The SLE patients and control subjects differed regarding sex ($p = 0.009$) and age ($p = 0.001$). Consequently, statistical analyses were adjusted for these variables. *Table 1* describes the main clinical and biochemical features of the SLE patients.

All study participants (171 SLE patients and 120 controls) were genotyped for the MCP-1 -2518 A/G polymorphism. The frequencies of the MCP-1 genotypes observed in the control group were in Hardy-Weinberg equilibrium ($\chi^2 = 0.61$; $p = 0.43$).

Table 1
Clinical and biochemical features of the SLE group.

| | SLE Group n=171 |
|-------------------------------|--------------------|
| SLICC Score (%) | |
| 0 | 47.3 |
| 1-2 | 31.7 |
| >2 | 21.0 |
| Lupus nephritis (%) | |
| Negative | 50.3 |
| Positive | 49.7 |
| Lupus flares (%) | |
| 0 | 35.3 |
| 1-2 | 53.9 |
| >2 | 10.8 |
| Course of disease (%) | |
| Mild/Moderate | 62.9 |
| Severe | 37.1 |
| ACA IgG and/or IgM (%) | |
| Titer > 30 GPL/MPL | 18.1 |
| LA (%) | |
| Negative | 76.6 |
| Positive | 23.4 |
| APA (%) | |
| Negative | 64.6 |
| Positive | 35.4 |

ACA: Anti-cardiolipin antibodies; LA: lupus anticoagulant; APA: anti-phospholipid antibodies

The aim of this study was to investigate the possible impact of the -2518 polymorphism on the development of SLE. We first analyzed the distribution of this polymorphism in SLE patients and controls subjects. We found that there were significant differences for both genotypic ($p=0.001$) and allelic frequencies ($p=0.01$) between the two groups (table 2). Conditional logistic regression analysis, adjusted for age and sex, showed an association between the presence of the G allele and the risk of developing SLE ($p=0.001$; OR=2.466 [1.479-4.113]).

We also examined the association of the MCP-1 -2518 A/G polymorphism with different clinical features of SLE patients. One of the most problematic clinical characteristics of this disease is the development of LN. Consequently, we analyzed the distribution of genotypic and allelic frequencies of the -2518 A/G polymorphism within the SLE group and found that there were no differences between patients with or without LN (table 3). Regression analysis adjusted for age, sex, and years

Table 2
Genotypic and allelic frequencies of the MCP-1 -2518 A/G polymorphism in the SLE and control groups.

| Variables studied | Control group n=120 | SLE group n=171 | <i>p</i> |
|--------------------|------------------------|--------------------|----------|
| Genotype | | | 0.001 |
| A/A (%) | 57 (47.5) | 50 (29.2) | |
| G/A (%) | 54 (45.0) | 86 (50.3) | |
| G/G (%) | 9 (7.5) | 35 (20.5) | |
| G allele frequency | 0.30 | 0.46 | 0.01 |

MCP-1: monocyte chemoattractant protein-1

Table 3
Genotypic and allelic frequencies of the MCP-1 -2518 A/G polymorphism in SLE patients with regard to the presence of lupus nephritis.

| Variables studied | SLE patients without LN n=86 | SLE patients with LN n=85 | <i>p</i> |
|--------------------|------------------------------------|---------------------------------|----------|
| Genotype | | | 0.591 |
| A/A (%) | 27 (31.4) | 22 (25.9) | |
| G/A (%) | 41 (47.7) | 47 (55.3) | |
| G/G (%) | 18 (20.9) | 16 (18.8) | |
| G allele frequency | 0.45 | 0.46 | 0.944 |

LN: Lupus nephritis

of disease duration showed no association between the presence of the G allele and the development of LN ($p=0.424$). Similar analyses showed no association between the G allele and the presence ($p=0.339$) or number (more than two) of lupus flares ($p=0.803$). However, the number of lupus flares was associated with the years of disease duration, as might have been expected ($p=0.016$, OR=1.14, 95% CI=[1.07-1.22]). When we compared mild and moderate disease cases to severe cases, no association with the presence of the G allele was observed ($p=0.262$). Finally, we found no association between the presence of the G allele and antiphospholipid antibodies (APA) ($p=0.631$), ACA ($p=0.706$) or LA ($p=0.058$).

DISCUSSION

SLE is a multifactorial, autoimmune disease with an important inflammatory component. Because MCP-1 is involved in the recruitment and migration of monocytes/macrophages to sites of inflammation, it has been proposed to be the main chemokine responsible for initiating autoimmune tissue damage [9]. The MCP-1 -2518 A/G polymorphism affects MCP-1 transcriptional activity in response to inflammatory stimuli. Individuals carrying a G allele at this position express more MCP-1 protein in response to pro-inflammatory cytokines than individuals with the A/A genotype [15].

Our data suggest that there are significant differences in the allelic and genotypic frequencies of the MCP-1 -2518 A/G polymorphism between SLE and control patients. This could reflect the possible role of this single nucleotide polymorphism in the development of SLE. Previous studies in both humans and mice showed that MCP-1 might be responsible for autoimmune tissue injury. MCP-1-deficient mice are partially protected from autoimmune disease and injury to the kidney, lung and skin, which results in a prolonged life span [31-33]. Thus, the increase in MCP-1 expression as a consequence of the G allele might over-stimulate leukocyte recruitment under inflammatory conditions. This increased stimulation could enable the clinical development of SLE in genetically predisposed individuals under the influence of environmental factors that trigger an immune response.

It is known that the incidence and prevalence of LN is higher in Hispanic populations (43%) than Caucasians

(14%). Furthermore, Hispanic ethnicity is associated with more severe renal involvement [34, 35]. In this study, we found no significant differences in the allelic and genotypic distributions between SLE patients with or without nephropathy. Several studies using mouse models have ascribed an essential role for MCP-1 in the development of LN and it has been demonstrated that this chemokine is overexpressed in patients with LN pathology. However, we found no association between the MCP-1 -2518 polymorphism and the risk of developing LN [36-40]. We also did not find any association between this polymorphism and the presence of other clinical features of SLE such as the number of lupus flares, the course of disease or the presence and levels of APA, ACA and LA.

Previous studies on the MCP -2518 A/G polymorphism in SLE have yielded controversial results [16-25]. These discrepancies could be explained by clinical heterogeneity, ethnic differences, real genetic heterogeneity, small sample sizes or low statistical power. Lee *et al.* performed a meta-analysis to clarify the possible role of this polymorphism in the development of SLE and LN [41]. They found no evidence of an association between the MCP -2518 A/G polymorphism and SLE or LN. Nevertheless, their analysis had several limitations, including the inability to detect slight associations in ethnic groups.

Our study was designed to include rigorous patient selection criteria. We acknowledge that the ethno-geographic origin of the population studied is a key factor regarding the incidence of SLE. We also recognize that the population of our country is an admixture of two main ethnic groups: Native Americans and Europeans (mostly Spaniards and Italians). Thus, we selected representative samples of the admixed, urban Argentinean population for both SLE and control groups. Besides, all of the subjects in the control group were carefully selected and included only "metabolically healthy" individuals who were clinically healthy, had a normal waist circumference and with no dyslipidemia. Additionally, our control subjects had no direct family or personal history of obesity. MCP-1 might play a role in the pathogenesis of obesity and diabetes [42, 43]. The selection of biased control groups might be a key factor in explaining the discrepancies found between previous studies. Although encouraging, our study has limitations. One limitation may be the small sample size used to compare the development of LN in SLE patients.

In conclusion, these results suggest that the MCP-1 -2518 A/G polymorphism might influence the risk of developing SLE in genetically predisposed individuals within an Argentinean cohort. SLE is a complex disease that involves a large number of genes. Each gene may provide a small contribution to disease development. Additionally, the influence of a given gene variant is dependent on environmental influences and genetic background. Therefore, further association studies are needed to elucidate clearly the role of other genes in the pathogenesis of SLE.

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Disclosure. The authors declare that they have no conflicts of interest.

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