

Research Article

Monocyte Chemoattractant Protein-1 –2518 A/G Single Nucleotide Polymorphism Might Be Associated with Renal Disease and Thrombocytopenia of SLE

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There is conflicting evidence on the contribution of the MCP-1 –2518 A>G (rs 1024611) polymorphism to SLE incidence and clinical manifestations. We examined the prevalence of the MCP-1 –2518 A>G polymorphism in SLE patients ($n = 199$) and controls ($n = 250$) in Poland. We did not observe a significant difference in the distribution of MCP-1 –2518 A>G polymorphic variants in patients with SLE and healthy individuals. However, we found an association between the GG versus AG and AA genotypes as well as the AG and GG versus AA genotypes with renal manifestations of SLE OR = 3.614 (1.123–11.631, $P = 0.0345$) and OR = 2.297 (1.301–4.057, $P = 0.0046$), respectively. We also observed that the MCP-1 AG and GG -genotypes contribute to the occurrence of thrombocytopenia in SLE patients OR = 2.618 (1.280–5.352, $P = 0.0089$). Our observations indicate that either MCP-1 –2518 G variant can be associated with some clinical findings in patients with SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic and progressive multisystem autoimmune connective tissue disorder, which is characterized by immune-mediated host tissue destruction [1]. Occupational exposure, chemicals, drugs, food, as well as viral and bacterial infectious agents can contribute significantly to changes in the immune system [2, 3].

Immune cells from patients with SLE exhibit various defects including skewed cytokine production, a decrease in cytotoxic T cell function, and an increase in the humoral response [4–7]. The defective function of CD4⁺ T cells associated with abnormal stimulation of B cells causes abundant autoantibody production [4, 6]. These autoantibodies form immune complexes with antigens on the patient's own cells and are accumulated in the skin, synovium, lungs, renal

glomeruli, and other organs and tissues. This results in the clinical presentation of SLE [1, 8].

The pathogenesis of SLE remains unclear, though there is much evidence demonstrating the involvement of genetic factors in the incidence of this autoimmune disease [9–13]. The genetic background contributing to SLE development includes, in particular, genes encoding disparate proteins that control immune system pathways [9, 10, 13].

The monocyte chemoattractant protein 1 (MCP-1), currently also designated CCL2, belongs to the family of chemotactic cytokines. MCP-1 functions as a potent agonist for monocytes, memory T cells, and basophils [14]. Evidence in animal and human studies suggests a significant role of MCP-1 in the progression of glomerular and tubulointerstitial injuries and glomerulonephritis in patients with SLE [15–19].

It has been demonstrated that the MCP-1 –2518 A>G (rs 1024611) transition in the promoter region may modulate

the levels of MCP-1 expression [20, 21]. The MCP-1 –2518 G allele, compared to the –2518 A allele, is linked with increased production of both MCP-1 transcript and protein [20, 21]. Involvement of the MCP-1 –2518 A>G promoter polymorphism in SLE development and its contribution to some clinical manifestations of SLE remains controversial [22–28]. We analysed the distribution of functional MCP-1 –2518 A>G polymorphic variants in SLE patients ($n = 199$) and controls ($n = 250$).

2. Patients and Methods

2.1. Patients and Controls. One hundred ninety nine (women only) patients fulfilling the American College of Rheumatology Classification criteria for SLE [29, 30] were chosen for investigation at Institute of Rheumatology in Warsaw, Poland (Table 1). Two hundred and fifty healthy women were included as controls. The protocol of the study was approved by the Local Ethical Committee of Poznań University of Medical Sciences. Written agreement was obtained from patients and controls. All participating subjects were of Polish and Caucasian origin. The mean age of SLE patients at diagnosis was 36 ± 12 years, and of controls, 35 ± 11 years.

2.2. Genotyping. DNA was isolated from peripheral white blood cells employing a standard salting out procedure. The presence of the MCP-1 –2518 A>G (rs 1024611) polymorphic variant was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was carried out using primer pair 5'-CTTTCCCTTGTGTGTCCTCC-3' and 5'-TTACTCCTTTCTCCCAACC-3'. The PCR-amplified fragments of MCP-1 that were 940 bp in length were isolated and subjected to digestion with endonuclease PvuII (CAG/CTG) (New England Biolabs, Ipswich, USA). The MCP-1 –2518 G allele was cleaved into 650 bp and 290 bp fragments; whereas the MCP-1 –2518 A allele remained uncut. DNA fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. Presence of the MCP-1 polymorphism was additionally confirmed by commercial sequencing analysis.

2.3. Statistical Analysis. The prevalence of genotypes in patients and controls was examined for deviation from Hardy-Weinberg equilibrium. Uncorrected χ^2 test was employed to examine differences in genotypic and allelic distribution between patients and controls. Moreover, the Odds Ratio (OR) and 95% Confidence Intervals (95% CI) were calculated. A P -value < 0.05 was considered statistically significant. Associations between clinical manifestations, production of autoantibodies, and polymorphism distribution in patients with SLE were determined by Fisher exact test. The Mann-Whitney test for nonparametric data was used to determine the statistical difference in SLE disease activity index (SLEDAI) [31] between MCP-1 GG, AG, and AA genotype groups. Power analysis was performed using uncorrected χ^2 test, which is available at an online internet service: <http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>.

3. Results

3.1. Distribution of MCP-1 –2518 A>G Genotypes and Alleles in SLE Patients and Healthy Individuals. Genotype analysis of the MCP-1 –2518 A>G polymorphism did not reveal a significant deviation from Hardy-Weinberg equilibrium in any group. We did not observe a significant difference in the prevalence of the MCP-1 –2518 A>G polymorphic variant in patients with SLE and healthy individuals (Table 2). OR for SLE patients with the GG genotype was 0.8629 (95% CI = 0.4428–1.682, $P = 0.6647$), and OR of the GG and AG genotypes was 0.9320 (95% CI = 0.6421–1.353, $P = 0.7111$) (Table 2). The power of this study amounted to 7.0% for the GG genotype and 6.6% for the GG and GA genotypes. We also did not observe a difference in the distribution of alleles between patients and controls. OR for the MCP-1 –2518 G allele frequency was 0.9314 (95% CI = 0.6963–1.246, $P = 0.6320$).

3.2. Association of MCP-1 –2518 A>G Genotypes with Clinical Symptoms of SLE. Since the previous studies indicated the contribution of either MCP-1 –2518 GG or AG genotypes to some clinical SLE manifestations [22, 23, 28], we assessed the association of these genotypes to clinical findings in our investigated group. We observed the association between the GG versus AG and AA genotypes and AG and GG versus AA genotypes with renal manifestations of SLE OR = 3.614 (1.123–11.631, $P = 0.0345$) and OR = 2.297 (1.301–4.057, $P = 0.0046$), respectively (Table 1). However, these P -values did not remain statistically significant after Bonferroni correction ($P_{\text{corr}} = 0.621$; $P_{\text{corr}} = 0.0828$, resp.). We observed an association between the GG versus AA genotypes with renal manifestations OR = 4.923 (1.483–16.343, $P = 0.0067$) ($P_{\text{corr}} = 0.1206$) (Table 1). There was also an association of the MCP-1 (AG and GG versus AA) genotypes with occurrence of thrombocytopenia OR = 2.618 (1.280–5.352, $P = 0.0089$) ($P_{\text{corr}} = 0.1602$) in SLE patients (Table 1). We did not find a significant association between the GG genotype or G allele with the presence of anti-dsDNA, anti-Smith, anti-snRNP, anti-Ro, anti-Scl-70, or antiphospholipid antibodies. There were also no significant differences in SLEDAI at diagnosis between MCP-1 GG, AG, and AA genotype groups.

4. Discussion

Completion of the human genome project revealed the existence of approximately ten million single nucleotide polymorphisms (SNPs). However, the role of SNPs in states of either health or sickness remains under investigation [32].

To date, the MCP-1 –2518 A>G polymorphism has been associated with coronary artery disease, nonfamilial idiopathic dilated cardiomyopathy, carotid atherosclerosis in patients with type 2 diabetes, myocardial infarction, ischemic heart disease, and hypertension [33–38]. Moreover, the MCP-1 –2518 G variant may also contribute to ocular Behçet's disease, adult and juvenile type of rheumatoid arthritis, systemic sclerosis, and psoriasis [39–43].

TABLE 1: Association of the MCP-1 –2518 A>G polymorphism with clinical manifestation in patients with SLE.

Characteristic	Genotypes distribution			Odds ratio (95% CI), <i>P</i> ^(e)
	A/A (103) ^(a)	A/G (80) ^(a)	G/G (16) ^(a)	
Malar rash	46	39	6	
Discoid rash	32	23	5	
Photosensitivity	47	38	7	
Oral ulcers	41	32	6	
Arthritis	59	42	8	
Serositis	18	13	3	
Renal	39	44	12	3.614 (1.123–11.631, <i>P</i> = 0.0345) ^(b) 2.297 (1.301–4.057, <i>P</i> = 0.0046) ^(c) 4.923 (1.483–16.343, <i>P</i> = 0.0067) ^(d)
Neurologic symptoms	19	16	4	
Hematologic symptoms	28	23	6	
Thrombocytopenia	14	23	5	2.618 (1.280–5.352, <i>P</i> = 0.0089) ^(c)
Leukopenia	21	16	3	
Immunologic symptoms	51	43	7	
ANA	103	80	16	

^(a)represents the absolute number of positive patients for A/A, A/G, A/G genotypes, respectively.

Comparison genotype ^(b)(G/G versus A/A and A/G); ^(c)(G/G and A/G versus A/A); ^(d)(G/G versus A/A), between patients with and patients without a manifestation was performed by ^(e)Fisher exact test.

TABLE 2: Association of the MCP-1 –2518 A>G polymorphisms in SLE patients and controls.

MCP-1 –2518 A>G (rs1024611)	SLE <i>n</i> = 199 (%)	Controls <i>n</i> = 250 (%)	OR	95%CI	^(d) <i>P</i> -value
Genotype frequency					
A/A	103 (51.8)	125 (50.0)			
A/G	80 (40.2)	102 (40.8)			
G/G	16 (8.0)	23 (9.2)	0.8629 ^(a)	0.4428–1.682	0.6647
A/G+ G/G	96 (48.2)	125 (50.0)	0.9320 ^(b)	0.6421–1.353	0.7111
Allele frequency					
A	286 (71.9)	352 (70.4)			
G	112 (28.1)	148 (29.6)	0.9314 ^(c)	0.6963–1.246	0.6320

The Odds ratio was calculated for patients ^(a)(G/G genotype versus A/G and A/A genotypes); ^(b)(G/G and A/G genotype versus A/A genotype).

We also determined the OR for the patients' minor allele ^(c)(G versus A allele); ^(d)uncorrected Chi².

Reports on the contribution of the MCP-1 –2518 A>G polymorphism to SLE incidence and clinical manifestations have been inconsistent [22–28, 44, 45]. We did not observe differences in the distribution of the MCP-1 –2518 A>G polymorphic variant between patients with SLE and healthy individuals; however this may be due to the low power of our investigation.

There have been no reports of a contribution of either the MCP-1 AG or GG genotype to SLE incidence in African American, Spanish, Korean, and Mexican cohorts [23–26, 44, 45]. By contrast, Tucci et al. showed a significant association of the MCP-1 AG and GG genotypes to SLE incidence in patients from North America [22]. Moreover, Brown et al. found that Caucasians bearing the MCP-1 –2518 G allele exhibited a significantly increased risk of developing SLE [26].

These conflicting reports on the effect of the MCP-1 –2518 A>G polymorphism to the incidence of SLE in various populations may be due to differences in the racial

heterogeneity of the examined groups. This disparity may also have resulted from each population's exposure to distinct environmental factors, which may act in synergy with the MCP-1 –2518 A>G polymorphism to change the risk of SLE incidence among the studied populations [3].

We observed that the MCP-1 G/G and A/G genotypes contribute to renal manifestations of the disease. A correlation between the MCP-1 –2518 G allele and nephritis was also observed in a North American SLE cohort [22]. Furthermore, the MCP-1 –2518 G allele was associated with cutaneous vasculitis in a Spanish SLE group [23], arthritis in Chinese adults with SLE [28] and the presence of anti-dsDNA and antiphospholipid antibodies in Mexican patients with SLE [45].

The MCP-1 –2518 A>G polymorphism is located at a relatively proximal position to the major transcriptional start site of the MCP-1 gene. Rovin et al. cloned the distal regulatory region of a luciferase reporter gene, including the MCP-1 –2518 A>G polymorphic variant upstream of

the reporter gene [20]. They indicated that peripheral blood mononuclear cells with either the MCP-1 AG or GG genotype demonstrated increased interleukin 1 beta-induced MCP-1 transcription compared to cells with genotype AA [20]. Moreover, Fenoglio et al. also showed that Alzheimer's disease patients bearing at least one MCP-1-2518 G allele exhibited a significant increase of MCP-1 levels in blood plasma [21].

It has been reported that different renal cells, including glomerular endothelial, mesangial, and tubular epithelial cells, are able to biosynthesize MCP-1 in response to immune complexes or some proinflammatory cytokines [46–48]. This MCP-1 production by renal cells can be enhanced by the MCP-1 –2518 G variant leading to the renal manifestations of SLE observed in both our investigation and other studies [22].

We also found that the MCP-1 GG and AG genotypes are associated with thrombocytopenia. An increase in serum levels of MCP-1 has previously been linked to elevated soluble CD40L in patients with autoimmune thrombocytopenic purpura [49].

The platelets of SLE patients are perpetually activated, and these patients exhibit an increase of soluble CD40L levels in the blood plasma [50, 51]. The interaction of CD40L from activated platelets with CD40 on endothelial cells induces an inflammatory reaction in the endothelium leading to the secretion of chemokines, including MCP-1 [52]. This may produce signals for the recruitment and extravasations of macrophages, which are able to interact with anti-platelet antibodies complexed with the platelets, which may lead to platelet destruction [49].

Our genetic investigation suggests that the MCP-1 –2518 G variant can contribute to some clinical findings in patients with SLE. However, to more precisely evaluate the effect of the MCP-1 –2518 G variant on SLE manifestations, further examination of this variant's distribution in other sample populations is required.

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