MCP-1 Gene Haplotype Association in Biopsy Proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** Giant cell arteritis (GCA) is the most frequent vasculitis in European and North American countries. Increased expression of monocyte chemoattractant protein 1 (MCP-1) has been observed within the inflammatory infiltrates of blood vessels and serum of patients with GCA and in other autoimmune and inflammatory conditions. MCP-1 gene polymorphisms have been reported to contribute to susceptibility to several immune and inflammatory conditions. To investigate the clinical implication of MCP-1 polymorphisms in GCA, we examined the association of 3 single nucleotide polymorphisms (SNP) in a series of patients with GCA from Northwest Spain.

Methods. Seventy-nine patients with biopsy proven GCA and 99 ethnically matched controls were studied. Patients and controls were genotyped for MCP-1 polymorphisms. SNP included in this study (rs2857657, rs4586, rs139000) were located in intron 1(G/C), exon 2(T/C), and 3'UTR(C/T) region of MCP-1 gene.

Results. The distribution of the alleles and genotypes for each MCP-1 polymorphism showed no significant differences between GCA patients and controls. When we compared the overall distribution of haplotype frequencies between GCA cases and controls a significant difference was observed ($p = 0.005$, by chi-square test from 4×2 contingency table). In addition, haplotype C-C was significantly increased in GCA patients compared with controls ($p = 0.03$, OR 2.09, 95% CI 1.09–4.02). Similarly, haplotype T-T was overrepresented in GCA patients ($p = 0.005$).

Conclusion. Significant differences in haplotype frequencies between GCA patients and controls may indicate a role for MCP-1 gene in susceptibility to GCA. (J Rheumatol 2005;32:507–10)

Key Indexing Terms:
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Giant cell arteritis (GCA, temporal arteritis) is the most frequent vasculitic syndrome in European and North American countries[1,2]. It involves large and middle-size blood vessels with a predisposition to the cranial arteries in people generally over 50 years of age[1,2]. Granuloma inflammation with presence of multinucleated giant cells is one of the characteristic histological features of vascular lesions in GCA. Macrophages and T cells appear to play an important role in the pathogenic mechanism of this condition[3].

Monocyte chemoattractant protein-1 (MCP-1) is a CC chemokine that attracts and activates monocytes and T cells in acute inflammatory conditions, and also can act as an important mediator in chronic inflammation[4,5]. MCP-1 has been purified from various components of vessel walls, which indicates its possible role in recruiting monocytes and T cells to inflammatory lesions in vessels[6].

Increased expression of MCP-1 has been observed in patients with atherosclerosis[7], idiopathic pulmonary fibrosis[8], rheumatoid arthritis[9], and delayed hypersensitivity reactions[10] as well as in patients with systemic lupus erythematosus (SLE) nephritis[11] and Kawasaki disease[4]. Increased expression of MCP-1 has also been reported in arterial lesions of patients with GCA, and increased plasma concentration of MCP-1 has been reported in patients with GCA and polymyalgia rheumatica[6].

Several single nucleotide polymorphisms (SNP) have been described within the MCP-1 gene and have been associated with a number of immune and inflammatory conditions[12-14].

In this study 3 common SNP located in intron 1(G/C), exon 2(T/C), and 3'UTR(C/T) region of the MCP-1 gene in a region about 2–5 kb across the gene were selected from the database and examined in patients with GCA and controls from Northwest Spain.

MATERIALS AND METHODS

Study population. The study group comprised patients diagnosed with biopsy proven GCA (n = 79) in the Division of Rheumatology of the Hospital Xeral-Calde, Lugo, Spain. Ethnically matched controls (n = 99) were recruited from the Lugo area. The main characteristics of the Lugo population have been reported[15].

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Patients were included for study if they had a positive temporal artery biopsy showing disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells. Visual ischemic complications were considered to be present if patients had at least one of the following: (1) permanent visual loss (partial or complete permanent visual loss related to GCA despite any possible improvement related to corticosteroid therapy); (2) amaurosis fugax (transient visual loss that was followed by complete recovery of normal vision); or (3) diplopia (related to palsy of extrinsic ocular muscles).

Patients gave informed consent prior to participation in the genetic study, which was approved by the local institutional committee.

Genotyping. DNA was extracted from anticoagulated blood collected in EDTA using a phenol-chloroform extraction method.

Three SNP (rs2857657, rs4586, rs13900) were selected from the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP/).

Genotyping for MCP-1 polymorphisms was performed using the Taqman system (Applied Biosystems, Foster City, CA, USA). All reactions were performed using 2.5 µl Taqman® universal PCR master mix, and 20 ng genomic DNA were used for each reaction.

SNP1 (rs2857657) and SNP2 (rs4586) genotyping was performed using ABI Assays-by-Design™ with 0.125 µl of 40× mix of unlabeled polymerase chain reaction (PCR) primers and Taqman® MGB probes (FAM and VIC dye-labeled) for each reaction. Primers and probes used for each SNP are as follows: SNP1 (rs2857657, intron 1 G/C): forward primer GTA TAG GCA GAA GCA CTG GGA TTT A; reverse primer CAG AAA AGA GTC ATG AGG AAA AAG CA; probes: VIC, ATG AGC TCT TTG TCT TCT; FAM, ATG AGC TCT TTC TCT TCT. SNP2 (rs4586, exon 2 T/C): forward primer TGC AAT CAA TGC CCC AGT CA; reverse primer GAG CCT CTG CAC TGA GAT CTT C; probes: VIC, TTG GTG AAG TTA TAA CAG CA (reverse probe); FAM, ATT GGT GAA GTT ATA GCA GCA (reverse probe). SNP3 (rs13900, 3'UTR C/T): genotyping was performed by ABI Assays-on-Demand™ using 0.25 µl of a 20× mix of primers and probes for each reaction.

Thermal cycling conditions were 95°C for 10 min then 50 cycles of 95°C for 15 s and 60°C for 1 min. Genotypes were obtained by reading fluorescent signals of the reaction end product. Taqman PCR products were read directly in an ABI 7700 analyzer (Applied Biosystems). DNA were grouped according to genotype. The PCR reaction was initially validated by real-time PCR. Subsequently genotypes were obtained by reading the fluorescent signal from the PCR reaction end product.

HLA-DRB1 typing of biopsy proven GCA was as described[16].

Statistical analysis. Strength of association between alleles or genotypes was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square or Fisher exact analysis. Statistical significance was defined as $p < 0.05$. Testing for linkage disequilibrium between pairs of polymorphisms in cases and controls was calculated using the EHPLUS and HelixTree program. EHPLUS provides log-likelihood, chi-square, and number of degrees of freedom. To test for heterogeneity in haplotype frequencies between cases and controls, the likelihood ratio test was used. The program was used on the cases to find estimated haplotype frequencies, and the corresponding log-likelihood (In L_{case}). It was then repeated on the controls and combination of case and control data to obtain log-likelihood (In $L_{controls}$) and (In $L_{combined}$), respectively. The test statistic $-2(\text{In } L_{case} + \text{In } L_{controls} - \text{In } L_{combined})$ is a chi-square with n – 1 degrees of freedom (when n is the number of haplotypes). The SNPHAP program, which assigns haplotypes to unrelated individuals, was used to estimate haplotype frequencies for SNP2, SNP3.

RESULTS

Visual ischemic manifestations were observed in 22 (28%) of the 79 patients with biopsy proven GCA. Allele and genotype frequencies were found to conform to Hardy-Weinberg equilibrium for all 3 SNP tested in both cases and controls.

MCP-1 SNP allele and genotype frequencies in patients and controls. When each individual polymorphism was examined, equal distributions of allele and genotype frequencies were observed for all 3 SNP in GCA patients and controls (Table 1). We also examined the allele and genotype frequency for all 3 SNP in GCA patients who developed visual manifestations during the course of disease and compared this to patients without visual manifestations and controls. No significant difference was observed (data not shown).

MCP-1 SNP allele and genotype frequencies in GCA patients stratified according to HLA-DR4+ vs HLA-DR4–. Patients were stratified as HLA-DR4+ versus DR4–, and allele and genotype frequencies for MCP-1 polymorphisms were determined in each group. It was observed that allele and genotype frequencies for all 3 MCP-1 polymorphisms were equally distributed among HLA-DR4+ and HLA-DR4– individuals, and frequencies were similar compared to the healthy controls (data not shown).

Linkage disequilibrium test for MCP-1 polymorphisms in patients and controls. Haplotype frequencies for the 3 MCP-1 polymorphisms were estimated. Chi-square analysis indicated significant linkage disequilibrium among the 3 MCP-1 polymorphisms in both GCA and controls ($p < 0.00001$).

Table 1. Allele and genotype frequencies of MCP-1 polymorphisms in patients with biopsy proven GCA and in controls*.

MCP-1	GCA, (%) n = 79	Controls, (%) n = 95
SNP1 (rs2857657)		
Genotype		
GG	4 (5)	4 (4)
GC	25 (32)	27 (28)
CC	50 (63)	64 (67)
Allele	2N = 158	2N = 190
G	33 (21)	35 (18)
C	125 (79)	155 (82)
SNP2 (rs4586)	n = 79	n = 99
Genotype		
TT	30 (38)	40 (40)
TC	39 (49)	44 (45)
CC	10 (13)	15 (15)
Allele	2N = 158	2N = 198
T	99 (63)	124 (63)
C	59 (37)	74 (37)
SNP3 (rs13900)	n = 77	n = 96
Genotype		
CC	42 (55)	49 (51)
CT	30 (39)	43 (45)
TT	5 (6)	4 (4)
Allele	2N = 154	2N = 192
C	114 (74)	141 (73)
T	40 (26)	51 (27)

* No statistically significant differences between GCA patients and controls were found.

When heterogeneity in haplotype frequencies was examined in patients and controls using a log-likelihood analysis, a significant difference was observed between GCA and controls (p = 0.04). Also, a significant difference for pairwise linkage disequilibrium was observed for SNP2/SNP3 two-locus linkage disequilibrium between cases and controls (p = 0.01).

Haplotype frequencies in patients and controls. Haplotype frequencies for the 3 MCP-1 polymorphisms were estimated. Chi-square analysis indicated linkage disequilibrium among the 3 MCP-1 polymorphisms in both GCA and controls (p < 0.00001); pairwise linkage disequilibrium measure r^2 (SNP 1 + 2, $r^2 = 0.32$; SNP 1 + 3, $r^2 = 0.24$; SNP 2 + 3, $r^2 = 0.1$). When heterogeneity in haplotype frequencies was examined in patients and controls using a log-likelihood analysis, a significant difference was observed between GCA and controls (p = 0.04). As well, a significant difference for pairwise linkage disequilibrium was observed for SNP2/SNP3 two-locus linkage disequilibrium between cases and controls (p = 0.01); haplotype frequencies for the SNP in highest linkage disequilibrium (i.e., SNP 2 + 3) are given in Table 2. SNP2/SNP3 two-locus haplotype frequencies were determined in cases and control populations (Table 2).

Haplotype T-T was not found in controls, and both C-C and T-T haplotypes were observed to be increased in GCA patients compared to the controls. When we compared the overall distribution of SNP2/SNP3 haplotype frequencies between cases and controls, a significant difference was observed (p = 0.005, chi-square test from 4 × 2 contingency table). In addition, haplotype C-C was significantly increased in GCA patients compared with controls (p = 0.03, OR 2.09, 95% CI 1.09–4.02). Similarly, haplotype T-T was overrepresented in GCA patients compared with controls (p = 0.005, Fisher exact test; RR 1.3, Haldane method).

DISCUSSION

Tissue expression of MCP-1 has been observed in human and experimental autoimmune models of glomerulonephritis and several other autoimmune diseases, suggesting a role for MCP-1 in autoimmune conditions[4,11,17].

Increased expression of MCP-1 in both arterial biopsy specimens and serum of patients with GCA has been observed[6], and together with its known role in monocyte and T lymphocyte activation and chemotaxis this evidence suggests that MCP-1 should be considered as a potential candidate gene contributing to GCA susceptibility. A functional polymorphism in the regulatory region of MCP-1 has been shown to have association with the cutaneous vasculitis in patients with SLE[14].

We examined 3 common SNP within the MCP-1 gene in patients with biopsy proven GCA. Although no significant differences were observed for allele and genotype frequencies between cases and controls, significant difference in MCP-1 SNP haplotype frequencies between patients and controls was observed. This suggests that genetic markers within MCP-1 gene are associated with GCA, although this association emerges at the level of haplotypes rather than individual SNP. Pairwise haplotype analysis showed that this difference was more significant for SNP2/SNP3 haplotype compared to other haplotype combinations between SNP tested. It seems that 2 of 4 common haplotypes for SNP2 and SNP3 are increased in GCA patients compared to healthy controls (haplotypes C-C and T-T). This could be due to functional interactions between these SNP that influence MCP-1 production in favor of GCA susceptibility. This hypothesis needs further investigation. It is also possible that another marker somewhere in the MCP-1 gene region may be associated with GCA susceptibility that is in linkage disequilibrium with these haplotypes. This will require testing of other known SNP within MCP-1 gene, to ascertain their possible association with GCA. SNP within the promoter region that possibly affect levels of MCP-1 expression should also be examined, and these may well be contained within GCA-associated haplotypes. Further replication studies in populations of different genetic background are required to determine the role of MCP-1 gene polymorphism in GCA.

Table 2. Estimated haplotype frequencies in patients with biopsy proven GCA and controls*.

Haplotype Frequency			
Haplotype		Controls (%)	GCA (%)
SNP2	SNP3	2N = 192	2N = 154
T	C	119 (62)	89 (58)
C	T	56 (28)	33 (21)
C	C	17 (9)	26 (17)
T	T	0 (0)	6 (4)

* Overall SNP2/SNP3 haplotype frequencies comparison, p = 0.005 by chi-square test from 4 × 2 contingency table. Haplotype C-C versus other haplotypes in GCA patients compared with controls: p = 0.03, OR = 2.09, 95% CI 1.09–4.02. Haplotype T–T versus the rest of haplotypes in GCA patients compared with controls: p = 0.005 (Fisher exact test), RR = 1.3 (Haldane method).

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