Lack of Association Between Macrophage Migration Inhibitory Factor Gene Polymorphism and Giant Cell Arteritis

MAHSA M. AMOLI, CARLOS GARCIA-PORRUA, WILLIAM E.R. OLLIER, and MIGUEL A. GONZALEZ-GAY

ABSTRACT. ***Objective.*** To investigate the role of macrophage migration inhibitory factor (MIF) gene polymorphism in giant cell arteritis (GCA).

Methods. Eighty-three patients with biopsy-proven GCA, 20 of them with visual ischemic complications, and 122 healthy matched controls from the Lugo region of Northwest Spain were studied. Patients and controls were genotyped for a single nucleotide polymorphism in the 5'-flanking region at position –173 of the MIF gene, using SNapshot ddNTP primer extension, followed by capillary electrophoresis (ABI 3100).

Results. No significant differences in MIF gene polymorphism were observed in patients with biopsy-proven GCA compared to controls. This was also the case when GCA patients with or without visual ischemic complications were compared.

Conclusion. Polymorphism in MIF gene promoter –173 G/C does not appear to be a genetic risk factor for GCA in Northwest Spain. (J Rheumatol 2005;32:74–6)

Key Indexing Terms:
GIANT CELL ARTERITIS TEMPORAL ARTERITIS
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Giant cell arteritis (GCA; temporal arteritis) is the most frequent vasculitic syndrome in Europe and North America[1,2]. It involves large and medium-size blood vessels with a predisposition to the cranial arteries in people generally over 50 years of age[1-3]. 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 mechanisms of this condition[1,4-7].

Macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine, originally identified as a T cell derived factor. MIF is a potent activator of macrophages, inhibiting the random migration of macrophages and concentrating macrophages at the inflammatory site, and is thought to play an important role in cell mediated immunity[8,9].

Association between functional polymorphisms in the promoter region of the MIF gene has been reported in both juvenile and adult inflammatory arthritis[10-12]. The finding of polymorphism in the MIF promoter in association with inflammatory illnesses has led to the postulate that the overproduction of MIF that has been documented in these inflammatory conditions is a consequence of genetically predetermined dysregulated (excessive) MIF production.

We reported an association of the MIF –173 (G/C) polymorphism in a group of patients with erythema nodosum secondary to sarcoidosis[13]. Since sarcoidosis is an inflammatory disease characterized by tissue infiltration of mononuclear phagocytes with associated granuloma formation, we further investigated the role of MIF gene polymorphism in a series of unselected biopsy-proven patients with GCA.

MATERIALS AND METHODS

Patients and controls. The study group comprised patients diagnosed with biopsy-proven GCA (n = 83) in the Department of Medicine of the Hospital Xeral-Calde (Lugo, Spain) and ethnically matched controls (n = 122) from the Lugo region, in Galicia, Northwest Spain. The main characteristics of the Lugo population have been reported[14,15].

Patients were included in this study if they had a positive temporal artery biopsy showing 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 followed by complete recovery of normal vision); or (3) diplopia (related to palsy of extrinsic ocular muscles). Patients with biopsy-proven GCA were considered to have an associated diagnosis of polymyalgia rheumatica (PMR) if they also had marked aching and stiffness bilaterally without other apparent cause in at least 2 of 3 regions: neck, shoulder girdle, and pelvic girdle[14,15].

From the Centre for Integrated Genomic Medical Research, School of Epidemiology and Health Sciences, the University of Manchester, Manchester, UK, and the Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain.

M.M. Amoli, MD, PhD; W.E.R. Ollier, PhD, FRCPath, Centre for Integrated Genomic Medical Research, School of Epidemiology and Health Sciences, University of Manchester; C. Garcia-Porrua, MD, PhD; M.A. Gonzalez-Gay, MD, PhD, Division of Rheumatology, Hospital Xeral-Calde.

Address reprint requests to Dr. M.A. Gonzalez-Gay, Rheumatology Division, Hospital Xeral-Calde, c) Dr. Ochoa s/n, 27004 Lugo, Spain. E-mail: miguelaggay@hotmail.com

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Genotyping. SNapshot ddNTP primer extension was used for genotyping the –173 G/C polymorphism of the MIF gene. DNA from patients and controls was extracted from anticoagulated blood collected in EDTA using a commercial DNA extraction kit (Bioline ™, London, UK). The following primers were used for PCR: forward 5' ACT AAG AAA GAC CCG AGG C 3'; reverse 5' GGG GCA CGT TGG TGT TTA C 3'. A total of 20 ng genomic DNA was amplified in a 10 µl final polymerase chain reaction (PCR) volume containing 5 pmoles of each primer, 0.08 nmoles of dNTP, 10× KCl buffer, and 0.6 units of Taq polymerase (Bioline). All the reactions were performed in 384-well microtiter plates on a Tetrad thermal cycler (MJ Research, Waltham, MA, USA). The DNA was denatured at 95°C for 5 min followed by 40 cycles of 95°C for 45 s, 60°C for 45 s, and 72°C for 45 s. The final extension was carried out at 72°C for 5 min. The presence of the 364-bp PCR product was visualized on a 2% agarose gel stained with ethidium bromide. The probe used for the single nucleotide extension in the primer extension kit was 5'-AGC CGC CAA GTG GAG AAC AG-3'. After extension and purification, the products were electrophoresed on a 3100 ABI analyzer and the results analyzed with Genescan software.

Statistical analysis. Strength of association between patient groups and controls and alleles or genotypes of the –173 G/C polymorphism 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's exact analysis. Statistical significance was defined as $p \leq 0.05$. Corrections of p values for multiple comparisons were made by Bonferroni adjustment. Calculations were performed with the statistical package Stata V6.

RESULTS

No significant differences for MIF gene polymorphism were observed in patients with biopsy-proven GCA compared to controls. Allele and genotype frequencies for MIF polymorphism in patients with GCA and controls are shown in Tables 1 and 2. The allele and genotype frequencies were also examined in patients stratified by the presence of visual manifestations during the disease course. However, no statistically significant differences between patients with GCA with or without visual ischemic complications were observed. This was also the case when biopsy-proven GCA patients with associated PMR manifestations were compared with those without PMR (data not shown).

DISCUSSION

Macrophages and T cells secrete MIF in response to low physiologic steroid concentration. The dose-response curve is bimodal. Not all doses of steroids induce MIF. In studies by Bucala, *et al*[16] physiologic glucocorticoids in higher levels did not induce MIF. For this reason, it has been postulated that MIF might counter-regulate glucocorticoid effects in many inflammatory and autoimmune conditions, and it has been considered as a potential candidate gene for susceptibility to autoimmune inflammatory disorders[17]. Increased MIF expression has also been reported in several inflammatory diseases[17].

There is evidence that the inflammatory reaction in GCA is mediated by TH1 CD4+ T cells that recognize an antigen residing in the arterial wall, resulting in granulomatous inflammation[4-7]. However, in contrast to patients with erythema nodosum associated with sarcoidosis, our results show that polymorphism in MIF gene promoter –173 G/C is not associated with GCA susceptibility or severity, indicating that different pathogenic mechanisms may be implicated in the development of granulomatous inflammatory disease in GCA.

It is possible that the MIF gene may play a role in an impaired inflammatory response in patients with sarcoidosis and other inflammatory conditions such as rheumatoid arthritis and juvenile idiopathic arthritis, which show association with MIF gene polymorphisms[10-12]. However, this does not seem to be the case in GCA.

Further studies to assess the role of MIF –173 (G/C) polymorphism in other diseases with granuloma formation are needed.

Table 1. Allele frequencies of the MIF –173 (G/C) polymorphism in patients with GCA and controls. No statistically significant differences between patients and controls were observed.

	Controls n = 122 (%)	GCA n = 83 (%)	GCA With Visual Manifestations n = 20 (%)	GCA Without Visual Manifestations n = 63 (%)
Allele (2N)				
G	206 (84)	137 (83)	33 (83)	104 (83)
C	38 (16)	29 (17)	7 (17)	22 (17)

Table 2. Genotype distribution of the MIF –173 (G/C) polymorphism in patients with GCA and controls. No statistically significant differences between patients and controls were observed.

	Controls n = 122 (%)	GCA n = 83 (%)	GCA With Visual Manifestations n = 20 (%)	GCA Without Visual Manifestations n = 63 (%)
Genotype				
GG	85 (70)	55 (66)	13 (65)	42 (67)
GC	36 (29)	27 (33)	7 (35)	20 (32)
CC	1 (1)	1 (1)	0 (0)	1 (2)

REFERENCES

1. Salvarani C, Cantini F, Boiardi L, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. N Engl J Med 2002;347:261-71.
2. Gonzalez-Gay MA, Garcia-Porrua C. Epidemiology of the vasculitides. Rheum Dis Clin North Am 2001;27:729-49.
3. Healey LA, Wilske KR. Manifestations of giant cell arteritis. Med Clin North Am 1977;61:261-70.
4. Sneller MC, Fauci AS. Pathogenesis of vasculitis syndromes. Med Clin North Am 1997;81:221-42.
5. Blain H, Abdelmouttaleb I, Belmin J, et al. Arterial wall production of cytokines in giant cell arteritis: results of a pilot study using human temporal artery cultures. J Gerontol A Biol Sci Med Sci 2002;57:M241-5.
6. Weyand CM, Goronzy JJ. Pathogenic principles in giant cell arteritis. Int J Cardiol 2000;75 Suppl 1:S9-S15.
7. Weyand CM, Ma-Krupa W, Goronzy JJ. Immunopathways in giant cell arteritis and polymyalgia rheumatica. Autoimmun Rev 2004;3:46-53.
8. Shimizu T, Abe R, Nishihira J, et al. Impaired contact hypersensitivity in macrophage migration inhibitory factor-deficient mice. Eur J Immunol 2003;33:1478-87.
9. Leech M, Metz C, Hall P, et al. Macrophage migration inhibitory factor in rheumatoid arthritis: evidence of proinflammatory function and regulation by glucocorticoids. Arthritis Rheum 1999;42:1601-8.
10. Donn RP, Shelley E, Ollier WE, Thomson W. A novel 5'-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2001;44:1782-5.
11. Baugh JA, Chitnis S, Donnelly SC, et al. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun 2002;3:170-6.
12. Donn R, Alourfi Z, De Benedetti F, et al. Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum 2002;46:2402-9.
13. Amoli MM, Donn RP, Thomson W, et al. Macrophage migration inhibitory factor gene polymorphism is associated with sarcoidosis in biopsy proven erythema nodosum. J Rheumatol 2002;29:1671-3.
14. Gonzalez-Gay MA, Garcia-Porrua C, Llorca J, et al. Visual manifestations of giant cell arteritis. Trends and clinical spectrum in 161 patients. Medicine (Baltimore) 2000;79:283-92.
15. Gonzalez-Gay MA, Garcia-Porrua C, Vazquez-Caruncho M, Dababneh A, Hajeer A, Ollier WER. The spectrum of polymyalgia rheumatica in Northwestern Spain: Incidence and analysis of variables associated with relapse in a ten year-study. J Rheumatol 1999;26:1326-32.
16. Bucala R. Neuroimmunomodulation by macrophage migration inhibitory factor (MIF). Ann NY Acad Sci 1998;840:74-82.
17. Gregersen PK, Bucala R. Macrophage migration inhibitory factor, MIF alleles, and the genetics of inflammatory disorders: incorporating disease outcome into the definition of phenotype. Arthritis Rheum 2003;48:1171-6.