Lack of association between macrophage migration inhibitory factor-173 gene polymorphism with disease susceptibility and cardiovascular risk in rheumatoid arthritis patients from northwestern Spain

R. Palomino-Morales[1], C. Gonzalez-Juanatey[2], T.R. Vazquez-Rodriguez[3], O. Torres[1], J.A. Miranda-Filloy[3], J. Llorca[4], J. Martin[1], M.A. Gonzalez-Gay[3]

[1]Instituto de Parasitología y Biomedicina, López Neyra, Consejo Superior de Investigaciones Cientificas (CSIC), Granada; Spain; [2]Cardiology; [3]Rheumatology Divisions, Hospital Xeral-Calde, Lugo, Spain; [4]Division of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, Santander, Spain.

Abstract

Objective

To assess whether the polymorphism of the macrophage migration inhibitory factor (MIF) gene at the position -173 is implicated in the disease susceptibility, risk of cardiovascular (CV) events and presence of subclinical atherosclerosis in patients with rheumatoid arthritis (RA).

Patients and methods

A series of 293 unselected patients fulfilling the 1987 American College of Rheumatology classification criteria for RA seen at the rheumatology outpatient clinic of Hospital Xeral-Calde, Lugo, Spain and 526 matched controls were studied for differences in the MIF-173 G/C gene biallelic polymorphism. A total of 182 consecutive patients that had been periodically followed between March 1996 and September 1996 until patient's death or January 1, 2008 were assessed for the presence of CV events. Moreover, between March and December 2007, a subgroup of unselected RA patients with no history of CV events was studied for the presence of subclinical atherosclerosis by the assessment of the endothelial function (n=107) and the carotid artery intima-media thickness (IMT) (n=91) by ultrasonography studies. Patients and controls were genotyped for the MIF-173 G/C gene polymorphism using a PCR system with pre-developed TaqMan allelic discrimination assay.

Results

No significant differences in allele or genotype frequencies for the MIF-173 gene polymorphism between RA patients and controls were found. Forty-four of the 182 patients followed between 1996 and January 2008 experienced CV events. Although the frequency of MIF-173 GG homozygous was increased in those who had CV events (88.6%) compared to those who did not suffer these complication (73.2%), the difference was not statistically significant. It was also the case when we analyzed the potential influence of MIF-173 genotypes in the presence of endothelial dysfunction or increased carotid IMT of patients with RA.

Conclusions

Our results do not show that MIF-173 gene polymorphism may infer a direct risk for disease susceptibility or CV disease in patients with RA.

Key words

Rheumatoid arthritis, atherosclerosis, cardiovascular disease, genetics, MIF-173

Rogelio Palomino-Morales, PhD
Orlando Torres, PhD
Javier Martin, MD, PhD
Carlos Gonzalez-Juanatey, MD, PhD
Tomás Vazquez -Rodriguez, MD
Jose A. Miranda-Filloy MD
Miguel A. Gonzalez-Gay, MD, PhD
Carlos Gonzalez-Juanatey, MD, PhD
Javier Llorca, MD, PhD

** Drs Palomino-Morales and Gonzalez-Juanatey made an equal contribution.*
***Drs Gonzalez-Gay and Martin share senior authorship.*

This study was supported by a grant from Fondo de Investigaciones Sanitarias PI06-0024 (Spain), and was also partially supported by RETICS Program, RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII).

Please address correspondence and reprint requests to:
Miguel A. Gonzalez-Gay, MD, PhD,
Rheumatology Division,
Hospital Xeral-Calde,
c) Dr. Ochoa s/n,
27004 Lugo, Spain.
E-mail: miguelaggay@hotmail.com

Received on May 30, 2009; accepted in revised form on October 12, 2009.

Competing interests: none declared.

Introduction

Patients with rheumatoid arthritis (RA) have increased risk of cardiovascular (CV) disease due to accelerated atherosclerosis (1). Besides classic CV risk factors, a number of non-traditional CV risk factors have also been implicated in the elevated CV mortality observed in these patients (2).

Chronic inflammation and the genetic background increase the risk of CV events in RA (3). Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine of the innate immunity, which has been reported to contribute to the development of CV disease (4). MIF is a predominantly macrophage-derived cytokine associated with a number of proinflammatory actions. It induces secretion of tumor necrosis factor (TNF)-α and T cell activation that are implicated in the pathogenesis of RA (5). In RA MIF has been identified as a key upstream regulator of local inflammatory profile by induction of TNF-α production (6). Interestingly, TNF-α is a pivotal proinflammatory cytokine implicated in the pathogenesis of RA and the increased CV mortality associated to this autoimmune disease. Moreover, by activating CXC chemokine receptors, MIF displays chemokine-like functions and acts as a major regulator of inflammatory cell recruitment and atherogenesis (7).

A biallelic polymorphism (G-to-C transition) in the 5'-flanking region at position -173 of the MIF gene creates an activating enhancer binding protein 4 transcription factor binding site and it has been associated with RA (8), RA severity (9), systemic-onset juvenile idiopathic arthritis (10) and other autoimmune diseases such as systemic lupus erythematosus (11).

In the present study we aimed to determine whether the polymorphism of MIF-173 gene polymorphism is implicated in the disease susceptibility and the increased risk of CV events and subclinical atherosclerosis in patients with RA from a well-characterised population of northwestern Spain.

Methods

Patients and controls

Two hundred and ninety-three unselected patients (213 (73%) women and 80 (27%) men; 234 (80%) rheumatoid factor positive) who fulfilled the 1987 American College of Rheumatology classification criteria for RA (12), seen at the Rheumatology outpatient clinic of Hospital Xeral-Calde, Lugo (Northwestern Spain) between March 1996 and December 2007 and 526 controls, matched by age, sex and ethnicity, from the same region, were assessed for differences in the MIF-173 G/C gene biallelic polymorphism. Information on the characteristics of this Caucasian population has previously been reported (13). The mean age ± standard deviation (SD) of this series of RA patients at the time of disease diagnosis was 50.1±15.1 years; median 51 years (interquartile range-IQ: 40–61). The mean± SD disease duration from the onset of the disease until patient's death or until December 2007 was 15.1±9.6 years; median 15 years (IQ range: 8–20). Other clinical features are shown in Table I.

Assessment of influence of the MIF-173 gene polymorphisms in the susceptibility to CV events

A total of 182 consecutive patients with RA seen at the Rheumatology outpatient clinic of Hospital Xeral-Calde between March and September 1996 were prospectively followed and clinical records were examined until patient's death or January 1, 2008 to determine the potential implication of the MIF-173 G/C gene biallelic polymorphism in the development of CV events. At the end of the study 44 (24%) patients had experienced CV events. Clinical information on classic CV risks factors and CV events in these 182 RA patients is shown in Table II.

Brachial artery reactivity and carotid artery ultrasonographic studies

To determine the potential association between the MIF-173 gene polymorphism and the presence of subclinical atherosclerosis, between March and December 2007 a subgroup of unselected RA patients with no history of CV events was studied by the assessment of the endothelial function and the carotid artery intima-media thickness (IMT). The percentage of flow-mediated-endothelium-dependent-vasodilatation

(FMD%) (post-ischemia) and endothelium independent-NTG% (post-nitroglycerin) vasodilatation were measured in 107 patients by brachial ultrasonography as previously reported (14). Also, in 91 patients assessment of the carotid IMT in the right common carotid artery was performed using high-resolution B-mode ultrasound as previously reported (14).

The subject's written consent was obtained and the design of the work was approved by the Ethical Committee of Galicia (Spain).

Genotyping

DNA from patients and controls was obtained from peripheral blood, using standard methods. Patients and controls were genotyped for the MIF-173 G/C gene polymorphism using a PCR system with pre-developed TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA) as previously reported (11). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

Figure 1 shows the composition of the different subgroups of patients included in each study.

Statistical analysis

Strength of association between RA and alleles or genotypes of the MIF-173 gene polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by Chi-square analysis. Strength of association between CV events in RA and alleles or genotypes of polymorphisms in the MIF-173 gene was estimated using OR and 95% CI, via multiple logistic regression; estimates were adjusted by age at diagnosis of the disease (continuous), gender, rheumatoid factor status and traditional CV risk factors as potential confounders.

The association between genotypes of the MIF-173 gene polymorphism and FMD%-endothelium dependent vasodilatation, NTG%-endothelium independent vasodilatation and carotid IMT was tested using analysis of covariance (ancova) adjusting by gender, age and duration of the disease at the time of the ultrasonographic studies (continuous), and traditional (classic) CV risk factors (presence/ absence). Statistical significance was defined as $p \leq 0.05$. Calculations were performed with the statistical package Stata 10/SE.

Table I. Phenotype disease characteristics of RA patients with or without the MIF genetic variants*.

Main characteristics	*MIF -173* G>C		*p*-value
	G/G genotype	C carrier	
Age at disease onset, years	49.7 ± 15.4	50.9 ± 13.9	NS
Follow-up, years	14.8 ± 9.5	14.5 ± 11.0	NS
Women (%)	73	72	NS
Rheumatoid factor positive (%)	81	80	NS
Anti-CCP antibodies positive (%)	71	65	NS
Shared epitope positive (%)	68	68	NS
Extraarticular manifestations (%)	26	29	NS
Erosions (%)	66	66	NS
Patients receiving DMARD (%)	90	92	NS
DMARD therapies used (%)			
Methotrexate	82	90	NS
TNF inhibitors	13	27	NS
Other medications administered during follow-up (%)	53	49	NS
CRP, mg/liter	9.1 ± 6.0	9.3 ± 7.1	NS
ESR, mm/hour	25.2 ± 12.98	26.2 ± 14.6	NS

*Except where indicated otherwise, values are the mean ±SD.
NS: not significant; DMARD: disease-modifying antirheumatic drug.

Table II. Classic cardiovascular (CV) risk factors and CV events of the second step patient group composed of 182 individuals diagnosed with RA.*

Risk factors	88 (48.4%)
Hypercholesterolemia and/or hypertriglyceridemia	48 (26.4%)
Hypertension n (%)	47 (25.8%)
Diabetes mellitus n (%)	23 (12.6%)
Obesity n (%)	12 (6.6%)
Current smoking	21 (11.5%)
Number of patients who suffered CV events	44 (24.2%)
Before disease diagnosis	4 (2.2%)
After disease diagnosis	40 (22%)
Type of CV event†	
Ischemic heart disease	23 (12.6%)
Acute myocardial infarction	17 (9.3%)
Heart failure	8 (4.4%)
Cerebrovascular accidents	16 (8.8%)
Peripheral arteriopathy	2 (1.1%)

*Values are the number (percentage).
†Three patients had acute myocardial infarction and later new admissions due to heart failure. Another two suffered ischemic heart disease and then stroke.

Results

Allele and genotype frequencies of the MIF-173 polymorphism in RA patients and controls

Allele and genotype frequencies for the gene polymorphisms in 293 RA patients and 526 controls are shown in Table III. Genotype frequencies were in Hardy-Weinberg equilibrium in patients and controls. No significant differences in the allele or genotype frequency between RA patients and controls were found (Table III). Similarly, no significant differences in the allele or genotype frequency were found when we stratified RA patients according to the different clinical features (Table I). Likewise, no significant allele or genotype differences were found when we compared anti-CCP positive or anti-CCP negative RA patients with controls (data not shown).

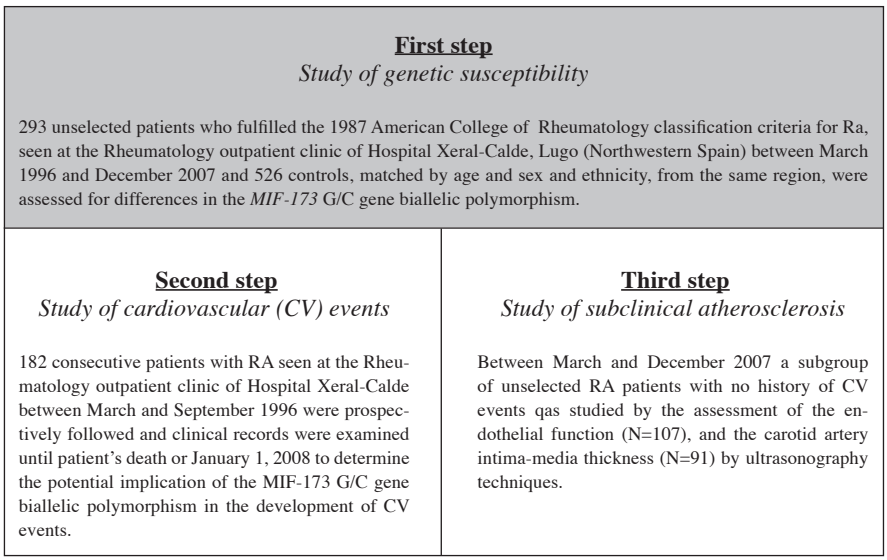

Fig. 1. Flow chart showing the different steps of the present study

Table III. Genotype and allele frequencies of *MIF-173* gene polymorphism in RA patients and controls.

No. Individuals	RA patients 293 (%)	Controls 526 (%)	OR (95%CI)	*p*-value
Genotype				
G/G	209 (71)	398 (76)	1 (reference)	–
G/C	76 (26)	121 (23)	1.20 (0.84 – 1.69)	0.29
C/C	8 (3)	7 (1)	2.18 (0.68 – 7.15)	0.13
Allele (2N)	586 (%)	1052 (%)		
G	494 (84)	917 (87)	1 (reference)	–
C	92 (16)	135 (13)	1.27 (0.94 – 1.70)	0.11

Table IV. Genotype and allele frequencies of the *MIF-173* gene polymorphism in a cohort of 182 RA patients stratified according to the presence (with) or the absence (without) of cardiovascular (CV) events (adjusted by age at disease diagnosis, gender, rheumatoid factor status and classic CV risk factors).

Gene polymorphism	Genotype	Without CV events n (%)	With CV events n (%)	OR (95% CI)	*p*-value
MIF (-173)	G/G	101 (73.2)	39 (88.6)	1 (reference)	–
	G/C	33 (23.9)	4 (9.1)	0.67 (0.29 - 1.51)	0.33
	C/C	4 (2.9)	1 (2.3)	0.44 (0.05 - 4.09)	0.47
	Total	138	44		
	Allele (2N)				
	G	235 (85.1)	82 (93.2)	1 (reference)	–
	C	41 (14.9)	6 (6.8)	0.45 (0.16 – 1.21)	0.10

MIF-173 polymorphism and CV events

MIF gene polymorphisms were examined in the subgroup of 182 RA patients followed between 1996 and January 2008. Patients from this cohort were assessed for the presence of CV events over the extended follow-up. However, no significant differences in the allele and genotype frequencies were observed between the group of patients who experienced CV or not. Table IV shows the genotype distribution in this series of RA patients stratified by the presence of CV events. The frequency of MIF-173 GG homozygous was increased in the subgroup of patients who had CV events (88.6%) compared to those who did not suffer these complications (73.2%). In this regard, RA patients carrying the MIF-173 allele C had reduced incidence of CV events (OR: 0.45; 95% CI: 0.16–1.21). However, the difference did not achieve statistical significance (p=0.10) (Table IV).

MIF-173 gene polymorphism and endothelial function and carotid IMT

Endothelial function was studied in 107 RA patients that were stratified according to the MIF-173 genotypes. The mean value of FMD% in this series of RA patients was 5.4%. Values lower than 7% are considered abnormal in our echocardiography laboratory (14). Therefore, these results confirm the presence of endothelial dysfunction in long-standing RA patients from northwestern Spain.

However, the mean values of FMD%-endothelium dependent and NTG%-endothelium independent vasodilatation stratified according to the MIF-173 genotypes did not show statistically significant differences (Table VA). Likewise, no significant differences according to the MIF-173 genotypes were observed in the 91 patients assessed for the carotid IMT (mean carotid IMT in the whole series 0.75 mm) (Table VB).

Discussion

Rheumatoid arthritis is a polygenic disease. Therefore, due to the implication of MIF-173 gene polymorphism in the susceptibility to autoimmune diseases, in the present study we assessed the potential influence of this gene polymorphism in the susceptibility to RA in northwestern Spain. However, our results do not confirm an association between this biallelic polymorphism and the risk of susceptibility to RA in our population. It was also the case when we previously analysed the influence of this biallelic polymorphism in patients from northwestern Spain with cutaneous vasculitis (15).

There is unanimous consensus on the increased incidence of CV events in patients with RA (16). This is the result of the influence of both classic and new CV risk factors including the

Table VA. Lack of association between *MIF*-173 genotypes and endothelial function in patients with RA.

	GG (n=77)	G/C (n=27)	C/C (n=3)	*p*-value
FMD%	5.4 ± 4.7	5.5 ± 3.8	5.3 ± 2.2	0.99
NTG%	16.2 ± 7.2	16.7 ± 6.6	14.7 ± 1.4	0.92

Table VB. Lack of association between *MIF-173* genotypes and carotid IMT in patients with RA.

Genotype	No. patients	Carotid IMT	*p*-value
G/G	65	0.78 ± 0.15	
G/C	23	0.75 ± 0.19	0.72
C/C	3	0.74 ± 0.42	

presence of chronic inflammation (1). Genetic susceptibility may also of major importance. In this regard, besides an increased association with HLA-DRB1*04-shared epitope positive alleles (3), we have recently reported a contribution of inducible and endothelial nitric oxide synthase (NOS2A and NOS3) gene polymorphisms to CV event risk in patients with RA (17).

Endothelial function and the carotid IMT are two useful non-invasive surrogate markers of subclinical atherosclerosis that have been proved to be predictors of future CV events in asymptomatic stages of the atherosclerotic disease (14). With respect to this, the results described in the present study confirm the presence of endothelial dysfunction in patients with RA. However, no association between MIF-173 genotypes and these two markers of subclinical atherosclerosis were found in this series of RA patients.

MIF has been reported to contribute to the development of CV disease and this cytokine is over expressed in atherosclerotic lesions in humans (18). These data together with the increased serum MIF values in C allele carrier RA patients reported by Radstake *et al*. (9) support a role of this variant in the development of CV disease in RA patients. Nevertheless, taken together all the results discussed above, our results do not confirm the implication of the MIF-173 biallelic polymorphism in the susceptibility to the accelerated atherosclerosis found in patients with RA. These results are in concordance with the lack of association of MIF-173 with myocardial infarction recently reported by Tereshchenko *et al*. (19). However, further studies in individuals with RA and different genetic backgrounds are needed to fully exclude the role of this biallelic polymorphism in the susceptibility and severity, manifested by accelerated atherogenesis of RA.

References

1. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005; 35: 8-17.
2. DESSEIN PH, JOFFE BI, VELLER MG *et al*.: Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2005; 32: 435-42.
3. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, LOPEZ-DIAZ MJ *et al*.: HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. *Arthritis Rheum* 2007; 57: 125-32.
4. ZERNECKE A, BERNHAGEN J, WEBER C: Macrophage migration inhibitory factor in cardiovascular disease. *Circulation* 2008; 117: 1594-602.
5. LIEW FY: Regulation of nitric oxide synthesis in infectious and autoimmune diseases. *Immunol Lett* 1994; 43: 95-8.
6. 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.
7. BERNHAGEN J, KROHN R, LUE H *et al*.: MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med* 2007; 13: 587-96.
8. MARTINEZ A, OROZCO G, VARADE J *et al*.: Macrophage migration inhibitory factor gene: influence on rheumatoid arthritis susceptibility. *Hum Immunol* 2007; 68:744-7.
9. RADSTAKE TR, SWEEP FC, WELSING P *et al*.: Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor. *Arthritis Rheum* 2005; 52: 3020-9.
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. SÁNCHEZ E, GÓMEZ LM, LOPEZ-NEVOT MA *et al*.: Evidence of association of macrophage migration inhibitory factor gene polymorphisms with systemic lupus erythematosus. *Genes Immun* 2006; 7:433-6.
12. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
13. GONZALEZ-GAY MA, GARCIA-PORRUA C, GUERRERO J *et al*.: The epidemiology of the primary systemic vasculitides in northwest Spain: implications of the Chapel Hill Consensus Conference definitions. *Arthritis Rheum* 2003; 49: 388-93.
14. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, VAZQUEZ-RODRIGUEZ TR *et al*.: Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis. *Semin Arthritis Rheum* 2008; 38: 67-70.
15. AMOLI MM, MARTIN J, MIRANDA-FILLOY JA *et al*.: Lack of association between macrophage migration inhibitory factor gene (-173 G/C) polymorphism and cutaneous vasculitis. *Clin Exp Rheumatol* 2006; 24: 576-9.
16. LÉVY L, FAUTREL B, BARNETCHE T *et al*.: Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis patients. A systematic review of the literature. *Clin Exp Rheumatol* 2008; 26: 673-9.
17. GONZALEZ-GAY MA, LLORCA J, PALOMINO-MORALES R *et al*.: Influence of nitric oxide synthase gene polymorphisms on the risk of cardiovascular events in rheumatoid arthritis. *Clin Exp Rheumatol* 2009; 27: 116-9.
18. HERDER C, ILLIG T, BAUMERT J *et al*.: Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Atherosclerosis* 2008; 200: 380-8.
19. TERESHCHENKO IP, PETRKOVA J, MRAZEK F *et al*.: The macrophage migration inhibitory factor (MIF) gene polymorphism in Czech and Russian patients with myocardial infarction. *Clin Chim Acta* 2009; 402: 199-202.