Influence of *MHCIITA* rs3087456 and rs4774 polymorphisms in the susceptibility to cardiovascular disease of patients with rheumatoid arthritis

M. García-Bermúdez[1], C. González-Juanatey[2], R. Lopez-Mejias[3], L. Rodriguez-Rodriguez[1,4], S. Pérez-Esteban[5], S. Castañeda[5], E. Urcelay[6], J.A. Miranda-Filloy[7], C. Gómez-Vaquero[8], B. Fernández-Gutierrez[4], A. Balsa[9], I. González-Alvaro[5], R. Blanco[3], J. Llorca[10], J. Martín[1], M.A. González-Gay[3]

[1]Instituto de Parasitología y Biomedicina López-Neyra, C.S.I.C., Granada; [2]Cardiology Division, Hospital Xeral-Calde, Lugo; [3]Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander; [4]Department of Rheumatology, Hospital Clinico San Carlos, Madrid; [5]Rheumatology Department, Hospital Universitario la Princesa, IIS-Princesa, Madrid; [6]Clinical Immunology Department, Hospital Universitario Clínico San Carlos, Madrid; [7]Department of Rheumatology, Hospital Xeral-Calde, Lugo;[8]Department of Rheumatology, Hospital Universitario Bellvitge, Barcelona; [9]Rheumatology Unit, Hospital Universitario La Paz, Madrid; [10]Department of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, and CIBER Epidemiología y Salud Pública (CIBERESP), IFIMAV, Santander, Spain.

Abstract

Objectives

MHCIITA *is a major regulator of MHC expression that has been reported to be involved in the susceptibility to rheumatoid arthritis (RA) and myocardial infarction. In this study we investigated the potential association of two* MHCIITA *gene polymorphisms with cardiovascular (CV) risk in patients with RA.*

Methods

1302 patients fulfilling the 1987 ACR classification criteria for RA were genotyped for the MHCIITA *rs3087456 and rs4774 gene polymorphisms to determine the influence of* MHCIITA *variants in the development of CV events. The potential influence of these polymorphisms in the development of subclinical atherosclerosis was also analysed in a subgroup of patients with no history of CV events by the assessment of two surrogate markers of atherosclerosis; brachial and carotid ultrasonography to determine endothelial function and carotid artery intima-media thickness, respectively.*

Results

No statistically significant differences in the allele or genotype frequencies for each individual MHCIITA *gene polymorphism between RA patients who experienced CV events, or not, were found. This was also the case when each polymorphism was assessed according to results obtained from surrogate markers of atherosclerosis. Also, in assessing the combined influence of both* MHCIITA *gene polymorphisms in the risk of CV disease after adjustment for gender, age at time of disease diagnosis, follow-up time, traditional CV risk factors, and shared epitope status, patients with CV events only showed a marginally decreased frequency of the* MHCIITA *rs3087456-rs4774 G-G allele combination (p=0.08; odds ratio: 0.63 [95% confidence interval: 0.37–1.05]).*

Conclusion

Our data do not support an influence of MHCIITA *rs3087456 and rs4774 polymorphisms in the increased risk of CV events of patients with RA.*

Key words

rheumatoid arthritis, atherosclerosis, cardiovascular disease, genetics, *MHCIITA* rs3087456 (-168A>G), rs4774 (+1614G>C)

*Mercedes García-Bermúdez, PhD**
*Carlos González-Juanatey, MD, PhD**
Raquel Lopez-Mejias, PhD
Luis Rodriguez-Rodriguez, MD
Silvia Pérez-Esteban, MD
Santos Castañeda, MD, PhD
Elena Urcelay, PhD
José A. Miranda-Filloy, MD
Carmen Gómez-Vaquero, MD, PhD
Benjamín Fernández-Gutiérrez MD, PhD
Alejandro Balsa, MD, PhD
Isidoro González-Alvaro, MD, PhD
Ricardo Blanco, MD, PhD
Javier Llorca, MD, PhD
*Javier Martín, MD, PhD***
*Miguel A. González-Gay, MD, PhD***

**These authors made an equal contribution to the study.*
***These authors share senior authorship in this study.*

Please address correspondence and reprint requests to:
Dr Miguel A. González-Gay, MD, PhD, Rheumatology Department, Hospital Universitario Marqués de Valdecilla, IFIMAV, Avenida de Valdecilla, s/n, 39008 Santander, Spain.
E-mail: miguelaggay@hotmail.com

Received on March 27, 2011; accepted in revised form on September 6, 2011.

Funding: This study was supported by two grants from Fondo de Investigaciones Sanitarias PI06-0024 and PS09/00748 (Spain). This work was partially supported by RETICS Program, RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII), within the VI PN de I+D+i 2008-2011 (FEDER). MGB is a recipient of a grant from Fundación Española de Reumatología (FER).

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA), a chronic autoimmune disease affecting up to 1% of the Caucasian population, is associated with accelerated atherosclerosis (1, 2), which is responsible for an increased cardiovascular (CV) morbidity and mortality risk (3). Chronic systemic inflammatory status plays a pivotal role in the development of the accelerated atherogenesis observed in RA patients, independently of the traditional CV risk factors (1, 4-10), promoting development of unstable plaque morphology with an increased likelihood of plaque rupture resulting in acute vascular events.

The multifactor etiology of RA is well established through linkage studies (11-14). The role of the *MHC* locus on chromosome 6p21 as a RA susceptibility factor has been consistently replicated in different populations. The shared epitope-containing human leukocyte antigen (HLA)-DRB1 alleles represents the most important genetic RA risk factor (15). *MHC class II* (*MHCII*) gene expression is regulated mainly at the level of transcription (16). The class II transactivator, encoded by the *MHCIITA* gene, is essential for the expression of the genes encoding *MHCII* (17, 18). The human *CIITA* gene, *MHCIITA*, is a 42-kb gene mapping to chromosome 16p13, which encodes the non-DNA binding coactivator (19). The activity of this gene is also regulated primarily at the transcriptional level, which is under the control of four different promoters in humans, acting independently of one another with no apparent cross talk (18, 19). The *MHCIITA* gene exhibits a strict cell type-specific, cytokine inducible and differentiation stage-specific pattern of expression that parallels that of *MHCII* genes.

Interestingly, results from whole-genome scans of UK, US, and European families with RA (12, 14, 20) have identified linkage to the chromosome 16p13 locus and, consequently, it has been proposed as a strong candidate susceptibility locus. A study in Swedish population showed evidence for association with RA of a functional polymorphism mapping in the promoter region of the *MHCIITA* gene, as well as lower expression of *MHCIITA* after stimulation of leucocytes with interferon-gamma (21). Another study in Japanese population (22) confirmed an association with this variant. The effect sizes were small but of the order expected for complex diseases. However, different studies in other populations have yielded controversial results. In this regard, in British (23, 24), German (25), Austrian (26), Argentinean or Swedish (27) populations, results were negative for association between *MHCIITA* rs3087456 polymorphism and RA. Also, studies in Spanish cohorts showed negative association results (27, 28); and a more recent meta-analysis revealed no evidence of association (29). On the other hand, a haplotype analysis between rs3087456 and rs 4774 *MHCIITA* variants in Spanish population disclosed association between a risk/protection haplotype and RA (30, 31). In keeping with these findings, another haplotype analysis performed in Northern Irish individuals suggested a weak protective effect of the -168A/+1614C haplotype in patients with chronic inflammatory diseases and also specifically in RA patients compared with controls (32).

Interestingly, rs3087456 *MHCIITA* polymorphism was associated with CV mortality after myocardial infarction and predictors of CV mortality, microalbuminuria in nondiabetic individuals and metabolic syndrome (33). Downregulation of *MHCIITA* expression mediated by statins has also been observed. In this regard, these drugs used for prevention of CV disease (34) have anti-inflammatory effects (35, 36).

Given the reported association with RA and the risk of myocardial infarction mediated by *MHCIITA* gene polymorphisms along with the elevated CV mortality observed in patients with RA, the main goal of the present study was to determine the potential implication of the rs3087456 and rs4774 *MHC2TA* gene variants in the CV risk of patients with RA.

Materials and methods

Patients

Between March 1996 and September 2008, 1302 consecutive patients that fulfilled the 1987 American College of

Rheumatology classification criteria for RA (37) were recruited from the Rheumatology Outpatient Clinics of Hospital Xeral-Calde (Lugo), Hospital Clínico San Carlos (Madrid), Hospital Universitario La Paz (Madrid), Hospital Universitario La Princesa (Madrid), Hospital Universitario Bellvitge (Barcelona), and Hospital Universitario Marqués de Valdecilla (Santander), Spain. Then, they were assessed for differences in the *MHCIITA* rs3087456 (-168A/G) and rs4774 (1614G/C, Gly500Ala) gene polymorphisms. A DNA sample was extracted from these patients.

Table I. Demographic characteristics of the patients with rheumatoid arthritis included in the study.

Variables	n=1302
Females	949 (72.9)
Age of patients at the time of disease diagnosis, years, median (IQR)	55 (44–65)
Time follow-up, years, median (IQR)	10 (5–16)
Anti-CCP positive (n=999)	583 (58.4)
Rheumatoid factor positive (n=1273)	893 (70.2)
Shared epitope positive (n=777)	490 (63.1)
Cardiovascular events	233 (17.9)
Ischaemic heart disease	125 (9.6)
Cerebrovascular accidents	62 (4.8)
Heart failure	62 (4.8)
Peripheral arteriopathy	25 (1.9)
Hypertension (n=1290)	507 (39.3)
Diabetes mellitus (n=1282)	169 (13.2)
Dyslipidemia (n=1196)	493 (41.2)
Obesity (n=1155)	138 (11.9)
Smoking habit (n=1247)	302 (24.2)

Values are expressed as n (%), except where indicated otherwise. IQR: interquartile range. Anti-CCP: anti-cyclic citrullinated peptide antibodies.

Study protocol

Between December 2009 and January 2010 patients' clinical records were examined until patient's death, loss of follow-up or December 1st, 2009. Information on the main demographical data, clinical characteristics of the patients enrolled in the study, CV risk factors and CV events of patients is shown in Table I. Clinical definitions for CV events (ischaemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy) and classic CV risk factors were established as previously described (4,38). Two hundred and thirty-three (17.9%) of these 1302 patients with RA experienced clinically evident CV events. The majority of them occurred after the diagnosis of RA. Also, 91 patients died during follow-up, 29 of them as a direct consequence of CV events.

To determine the potential association between the *MHCIITA* rs3087456 and rs4774 polymorphisms and the presence of subclinical atherosclerosis, between March 2007 and September 2009 a random subgroup of patients from the Lugo cohort with no previous history of CV events was selected. Presence of endothelial dysfunction was assessed by a brachial artery reactivity study in 138 patients. Flow-mediated endothelium-dependent vasodilatation-FMD (post-ischaemia) and endothelium-independent-NTG (post-nitroglycerin) vasodilatation were measured by brachial ultrasonography as previously reported (9, 39). Also, carotid ultrasonography studies were performed in 114 patients to determine the carotid artery intima-media wall thickness (IMT). It was assessed in the right common carotid artery as previously reported (9, 40).

A subject's written consent was obtained according to the declaration of Helsinki, and the design of the work was approved by the Ethics Committee of Galicia (Spain). The Ethics Committees of the Hospital Clinico San Carlos (Madrid), Hospital La Paz (Madrid), Hospital de la Princesa (Madrid), Hospital Universitario Bellvitge (Barcelona) and Hospital Universitario Marqués de Valdecilla (Santander) also approved the study.

Genotyping

MHCIITA genotyping. DNA was obtained from peripheral blood, using standard methods. Subjects were genotyped to determine *MHCIITA* rs3087456 (-168A>G, located within the promoter region) and rs4774 (+1614G>C, Gly500Ala, located in exon 11, which is predicted to have a tolerable effect on protein function) (41) polymorphisms status using TaqMan Assays-on-Demand, and analysed using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Shared Epitope determination. Several *HLA-DRB1* alleles (*HLA-DRB1**0401, *0404, *0405, *0408, *0101, *0102, *1001, *1402) are associated with susceptibility to RA. These alleles encode a conserved amino acid sequence (QKRAA, QRRAA, or RRRAA), called the shared epitope, at position 70–74 in the third hypervariable region of the HLA-DRβ1 molecule (15). *HLA-DRB1*-shared epitope alleles are also implicated in the severity of the disease (42), including increased risk of CV events (4) and presence of endothelial dysfunction (39). *HLA-DRB1* typing was carried out using a reverse dot-blot kit with sequence-specific oligonucleotide (SSO) probes (Dynal RELITM SSO HLA-DRB1 typing kit; Dynal Biotech, Bromborough, UK).

Statistical analysis

All genotype data were checked for deviation from Hardy-Weinberg equilibrium (HWE) using http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Comparison of proportions was performed using χ^2 test or Fisher test, when required. Strength of associations between CV events and genotypes or alleles of *MHCIITA* polymorphisms were estimated using odds ratios (OR) and 95% confidence intervals (CI), via multiple logistic regression; estimates were further adjusted for gender, age at RA diagnosis, time of follow-up, classic CV risk factors (hypertension, diabe-

tes mellitus, dyslipidemia, obesity and smoking habit) and presence or absence of the rheumatoid shared epitope as potential confounders.

The association between genotypes of the *MHCIITA* polymorphisms and surrogate markers of subclinical atherosclerosis: carotid IMT, FMD-endothelium dependent or NTG-endothelium independent vasodilatation were tested using unpaired *t*-test to compare between 2 groups, and one-way analysis of variance (ANOVA) to compare among more than two groups. Moreover, we also tested the association between these parameters and alleles using analysis of covariance (ANCOVA) adjusting for gender, age and duration of the disease at the time of the ultrasonography study, traditional CV risk factors, and presence or absence of shared epitope. Statistical significance was defined as $p<0.05$. All analyses were performed with STATA statistical software 9.1 (Stata Corp., College Station, TX, USA).

Results

Influence of the MHCIITA rs3087456 (-168A>G) and rs4774 (+1614G>C) polymorphisms in the risk of clinically evident CV disease in RA patients

The study reached a genotyping success >97%. The population under study had no deviation from Hardy-Weinberg equilibrium for both *MHCIITA* polymorphisms.

The power for the study was calculated using "CaTS – Power Calculator for Two Stage Association Studies" (http://www.sph.umich.edu/csg/abecasis/CaTS/) (43). The study had 80% power to detect allelic OR greater than 1.4 at the stated significance level (α=0.05), with a minor allele frequency of 0.23 and a prevalence of the disease in Spanish population 0.005 (44).

We analysed the genotype and allele distribution of both *MHCIITA* variants regarding the presence or absence of clinically evident CV disease in patients with RA (Table II). No significant differences in the genotype and allele frequencies were found for the *MHCIITA* -168A>G variant (p=0.46). It was also the case for the *MHCIITA* +1614G>C polymorphism (p=0.55), although a slight decrease in the frequency of the minor alleles in both polymorphisms was observed in RA patients who experienced CV events (23.36% *versus* 25.0% for rs3087456, and 24.23% *versus* 25.58% for rs4774, respectively).

No association between *MHCIITA* variants alone or in combination with first CV event during the follow-up period was found. Likewise, Cox proportional hazard regression analyses to estimate the level of risk that the *MHCIITA* polymorphisms (or particular allele combinations) did not disclose association of *MHCIITA* polymorphisms with mortality – all cause or CV related (data not shown).

Since the *MHC2TA* polymorphism was previously reported to be associated with ischaemic heart disease (21, 33), we specifically analysed the influence of this polymorphism in the occurrence of cardiac or cerebrovascular ischaemic events – ischaemic heart disease including myocardial infarction or angina and/or stroke – in patients with RA. However, the results of these analyses did not show any significant association with any of these two polymorphisms both in the unadjusted or the in the adjusted analyses (data not shown).

In a further step, we constructed a logistic regression model to explain the presence of CV disease according to the *MHCIITA* rs3087456 (-168A>G) and rs4774 (+1614G>C) allele distribution (Table IIIA and B). However, neither of these two polymorphisms had a sta-

Table II. Differences in the genotype and allele frequencies of *MHCIITA* rs3087456 and rs4774 variants between rheumatoid arthritis patients with and without cardiovascular (CV) events.

MHCIITA	With CV events (%)	Without CV events (%)	*p*-value	OR [95% CI]
rs3087456				
AA	135 (58.95)	594 (56.68)	–	Ref.
AG	81 (35.37)	384 (36.64)	0.63	0.93 [0.68–1.27]
GG	13 (5.68)	70 (6.68)	0.52	0.82 [0.42–1.57]
AG+GG	94 (41.05)	454 (43.32)	0.53	0.91 [0.67–1.23]
A	351 (76.64)	1572 (75.0)	–	Ref.
G	107 (23.36)	524 (25.0)	0.46	0.91 [0.72-1.17]
rs4774				
GG	135 (58.95)	583 (56.06)	–	Ref.
GC	77 (33.62)	382 (36.73)	0.38	0.87 [0.63–1.20]
CC	17 (7.42)	75 (7.21)	0.94	0.98 [0.54–1.76]
GC+CC	94 (41.04)	457 (43.94)	0.42	0.89 [0.66–1.20]
G	347 (75.56)	1548 (74.42)	–	Ref.
C	111 (24.23)	532 (25.58)	0.55	0.93 [0.73–1.19]

OR [95% CI]: Odds Ratio with 95% Confidence Interval.

Table III A. Logistic regression model to explain the presence of cardiovascular disease in rheumatoid arthritis patients according to *MHCIITA* rs3087456 and rs4774 allele distribution. **B.** Conditional analysis of *MHCIITA* rs3087456 and rs4774 polymorphisms and the risk of cardiovascular events in rheumatoid arthritis patients.

A. *MHCIITA*	*p*-value	OR [95% CI]	*p*-value*	OR [95% CI]*
rs3087456				
G *vs*. A	0.47	0.92 [0.72–1.16]	0.39	0.84 [0.56–1.26]
rs4774				
C *vs*. G	0.56	0.93 [0.74–1.18]	0.94	1.01 [0.69–1.49]
B.				
rs3087456	0.42	0.91 [0.72–1.15]	0.33	0.82 [0.54–1.23]
rs4774	0.59	0.94 [0.74–1.18]	0.88	1.03 [0.70–1.51]

*Analyses adjusted for gender, age at rheumatoid arthritis diagnosis, follow-up time, hypertension, diabetes mellitus, dyslipidemia, obesity, smoking habit and presence or absence of shared epitope.
OR [95% CI]: Odds ratio with 95% confidence interval.

Table IV. Distribution of allelic combinations of *MHCIITA* rs3087456 and rs4774 in rheumatoid arthritis patients with and without cardiovascular disease.

MHCIITA rs3087456-rs4774	with CV events	without CV events	*p*-value	OR [95% CI]	*p*-value*	OR [95% CI]*
A-G	284 (62.56)	1211 (58.73)	–	Ref.	–	Ref.
A-C	64 (14.10)	333 (16.15)	0.19	0.82 [0.61–1.10]	0.49	0.84 [0.50–1.39]
G-G	61 (13.44)	332 (16.10)	0.11	0.78 [0.58–1.06]	0.08	0.63 [0.37–1.05]
G-C	45 (9.91)	186 (9.02)	0.86	1.03 [0.73–1.46]	0.68	1.13 [0.64–1.99]

*Analyses adjusted for gender, age at rheumatoid arthritis diagnosis, follow-up time, traditional cardiovascular risk factors and presence or absence of share epitope.

tistically significant association before (rs3087456 p=0.47; rs4774 p=0.56) or after adjustment (rs3087456 adjusted p=0.39; rs4774 adjusted p=0.94) with clinically evident CV disease, alone or in combination.

Afterwards, we also analysed the combined influence of both *MHCIITA* gene polymorphisms in the risk of CV disease comparing the frequency of their estimated allelic combinations (Table IV). However, after adjustment for gender, age at the time of RA diagnosis, follow-up time, traditional CV risk factors, and presence or absence of share epitope, we only observed that RA patients with CV events had a marginally decreased frequency of the *MHCIITA* rs3087456-rs4774 G-G allele combination (p=0.08; OR: 0.63 [95% CI 0.37–1.05]).

Influence of MHCIITA polymorphisms in the risk of CV events, according to the presence or absence of the shared epitope

Subsequently, we stratified our sample according to the presence of absence of the shared epitope. To do so, in the logistic regression model described above we established a statistical interaction between the presence or the absence of the shared epitope and the fact of carrying or not at least one copy of the minor allele *MHCIITA* polymorphisms. No interaction effect was found for patients carrying the shared epitope and the minor allele of rs4774; nor was any effect seen for the rs3087456 polymorphism (data not shown).

Influence of the MHCIITA rs3087456 (-168A>G) and rs4774 (+1614 G>C) variants in the risk of subclinical CV disease in RA patients

Previous studies shown an increased frequency of subclinical atherosclerosis in RA patients without clinically evident CV disease (10, 39), so we also aimed to establish the possible influence of these two *MHCIITA* polymorphisms in the development of subclinical atherosclerosis using two well-defined surrogate markers of atherosclerosis, endothelial function and the carotid IMT (9, 40), which have been proved to be predictors of future CV events in asymptomatic stages of the atherosclerotic disease (38).

This study had 95% power to detect a difference in carotid IMT of 0.1 mm or higher, a difference of 2.5% or higher in FMD-endothelium-dependent vasodilatation and a difference of 4.0% or higher in NTG-endothelium-independent vasodilatation.

Results described in the present study confirmed the presence of endothelial dysfunction in patients with RA, although no association between *MHCIITA* rs3087456 and rs4774 polymorphisms and markers of subclinical atherosclerosis were found in this series of RA patients (Table VA and B).

In the ANCOVA model adjusted for

Table V A. Comparison of carotid artery IMT according to *MHCIITA* rs3087456 and rs4774 polymorphisms. **B**. Comparison of brachial flow-mediated endothelium dependent (post-ischemia) vasodilatation (FMD) and endothelial independent (post-nitroglycerin) vasodilatation (NTG) according to *MHCIITA* rs3087456 and rs4774 polymorphisms. **C**. Comparison of carotid artery IMT, FMD and NTG vasodilatation, according to *MHCIITA* rs3087456 and rs4774 alleles in an adjusted ANCOVA model.

A.

MHCIITA rs3087456	IMT mm, mean (SD)	*p*-value	*MHCIITA* rs4774	IMT mm, mean (SD)	*p*-value
AA (n=70)	0.72 (0.19)		GG (n=62)	0.74 (0.16)	
AG (n=35)	0.74 (0.17)		GC (n=43)	0.70 (0.21)	
GG (n=9)	0.71 (0.15)		CC (n=8)	0.80 (0.17)	
Model		0.86			0.26
A (n=175)	0.73 (0.19)		G (n=167)	0.73 (0.17)	
G (n=53)	0.73 (0.16)	0.96	C (n=59)	0.73 (0.19)	0.94

B.

MHCIITA rs3087456	FMD%, mean (SD)	*p*	NTG%, mean (SD)	*p*	*MHCIITA* rs4774	FMD%, mean (SD)	*p*	NTG% mean (SD)	*p*
AA (n=86)	5.88 (4.93)		16.69 (8.68)		GG (n=76)	5.92 (5.25)		16.87 (8.18)	
AG (n=42)	4.94 (4.80)		16.26 (7.42)		GC (n=53)	5.57 (4.52)		15.93 (8.40)	
GG (n=10)	6.33 (4.84)		15.4 (5.51)		CC (n=8)	3.95 (1.44)		17.51 (4.18)	
Model		0.53		0.88	Model		0.55		0.76
A (n=214)	5.69 (4.90)		16.61 (8.41)		G (n=205)	5.83 (5.07)		16.63 (8.20)	
G (n=62)	5.39 (4.78)	0.66	15.98 (6.79)	0.59	C (n=69)	5.19 (4.15)	0.35	16.30 (7.62)	0.77

C.

	IMT	FMD	NTG
MHCIITA rs3087456, p G *vs*. T*	0.40	0.39	0.66
MHCIITA rs4774, p T *vs*. G*	0.46	0.55	0.59
MHCIITA rs3087456, p G *vs*. T**	0.53	0.35	0.56
MHCIITA rs4774, p T *vs*. G**	0.49	0.64	0.65

*Analyses adjusted for gender, age at the time of ultrasonography study, follow-up time, classic (traditional) CV risk factors and presence or absence of shared epitope.
**Conditional analysis of the *MHCIITA* rs3087456 and rs4774 polymorphisms.

gender, age at the time of the ultrasonography assessment, follow-up time, traditional CV risk factors and presence or absence of shared epitope, no significant differences were found according to *MHCIITA* rs3087456 or rs4774 alleles (Table VC). Moreover, no association between allelic combinations and any of these surrogate markers of subclinical atherosclerosis was found. Finally, no association between the *MHCIITA* variants, analysed simultaneously in a conditional analysis, and any of the surrogate markers of subclinical atherosclerosis were found (Table VC).

Discussion

It is now well established that there is an increased incidence of CV events in patients with RA (45) as the result of the influence of both classic and new CV risk factors including the presence of chronic inflammation (4-6), leading to enhanced atherosclerotic burden in these patients (3). Our group has performed a series of studies to determine the potential influence of genetic factors in the accelerated atherosclerosis observed in RA. In this regard, we confirmed an influence of *MHC-HLA-DRB1* alleles in the development of endothelial dysfunction (39) and CV events (4, 46). It was not the case for other gene polymorphisms associated with increased susceptibility to RA (47) or with immunity and inflammation (48).

The *MHCIITA* -168A/G promoter polymorphism (rs3087456) was found to be associated with reduced transcription levels of *CIITA ex vivo* in human leukocytes stimulated with interferon-gamma, and experiments in rats showed a strong correlation between lowered levels of *CIITA* transcripts and reduced expression of *MHC* class II molecules (21). Also, evidence for association between the *MHCIITA* type III promoter rs3087456 (-168A/G) variant and RA, myocardial infarction and multiple sclerosis was described (21). Because of this reported association with RA, several replication studies were conducted in different populations (20, 22, 24-32). However, only two of them (22, 27) confirmed the association between RA and *MHCIITA* rs3087456. Moreover, a recent meta-analysis revealed no evidence of association (29). In keeping with these data, more recent studies have found no association of the *MHCIITA* gene with other autoimmune inflammatory disorders, such as myasthenia gravis (49), celiac disease (50) or systemic lupus erythematosus (51, 52).

To further establish the potential role of *MHCIITA* gene polymorphisms in the development of CV disease, in the present study we assessed for the first time the influence of two *MHCIITA* variants in the risk of both clinically evident and subclinical CV disease in a large series of Spanish RA patients. However, only a marginally a decreased frequency of CV events was observed in patients carrying the *MHCIITA* rs3087456-rs4774 G-G allele combination.

Conclusion

In conclusion, our data do not support an influence of *MHCIITA* rs3087456 and rs4774 polymorphisms in the increased risk of CV events of patients with RA. However, further replication studies in individuals with RA and different genetic backgrounds are needed to fully elucidate a role of the *MHCIITA* region in the susceptibility to the accelerated atherogenesis observed in RA.

Acknowledgements

We are indebted to all the patients for their essential collaboration. We thank Sofia Vargas, Sonia Garcia and Gema Robledo for their excellent technical assistance. We also thank Mr Rodrigo Ochoa for his valuable contribution to the recruitment of patients.

References

1. DEL RINCÓN I, ESCALANTE A: Atherosclerotic cardiovascular disease in rheumatoid arthritis. *Curr Rheumatol Rep* 2003; 5: 278-86.
2. GAZI IF, BOUMPAS DT, MIKHAILIDIS DP, GANOTAKIS ES: Clustering of cardiovascular risk factors in rheumatoid arthritis: the rationale for using statins. *Clin Exp Rheumatol* 2007; 25: 102-11.
3. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005; 35: 8-17.
4. 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.
5. SATTAR N, MCCAREY DW, CAPELL H, MCINNES IB: Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 2003; 108: 2957-63.
6. 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.
7. SATTAR N, MCINNES IB: Vascular comorbidity in rheumatoid arthritis: potential mechanisms and solutions. *Curr Opin Rheumatol* 2005; 17: 286-92.
8. TURESSON C, JARENROS A, JACOBSSON L: Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study. *Ann Rheum Dis* 2004; 63: 952-5.
9. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, VAZQUEZ-RODRIGUEZ TR, MARTIN J, LLORCA J: Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis. *Semin Arthritis Rheum* 2008; 38: 67-70.
10. GONZALEZ-JUANATEY C, LLORCA J, TESTA A, REVUELTA J, GARCIA-PORRUA C, GONZALEZ-GAY MA: Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease. *Medicine* (Baltimore) 2003; 82: 407-13.
11. CORNELIS F, FAURE S, MARTINEZ M *et al*.: New susceptibility locus for rheumatoid arthritis suggested by a genome-wide linkage study. *Proc Natl Acad Sci USA* 1998; 95: 10746-50.
12. MACKAY K, EYRE S, MYERSCOUGH A *et al*.: Whole genome linkage analysis of rheumatoid arthritis susceptibility loci in 252 affected sibling pairs in the United Kingdom. *Arthritis Rheum* 2002; 46: 632-9.
13. JAWAHEER D, SELDIN MF, AMOS CI *et al*.: Screening the genome for rheumatoid arthritis susceptibility genes: a replication study and combined analysis of 512 multicase families. *Arthritis Rheum* 2003; 48: 906-16.
14. JOHN S, SHEPHARD N, LIU G *et al*.: Whole-genome scan, in a complex disease, using 11,245 single-nucleotide polymorphisms: comparison with microsatellites. *Am J Hum Genet* 2004; 75: 54-64.
15. GREGERSEN PK, SILVER J, WINCHESTER RJ: The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987; 30: 1205-13.
16. REITH W, LEIBUNDGUT-LANDMANN S, WALDBURGER JM: Regulation of MHC class II gene expression by the class II transactivator. *Nat Rev Immunol* 2005; 5: 793-806.
17. LEIBUNDGUT-LANDMANN S, WALDBURGER JM, KRAWCZYK M *et al*.: Mini-review: specificity and expression of CIITA, the master regulator of MHC class II genes. *Eur J Immunol* 2004; 34: 1513-25.
18. TING JP, TROWSDALE J: Genetic control of MHC class II expression. *Cell* 2002; 109: 21-33.

19. MUHLETHALER-MOTTET A, OTTEN LA, STEIMLE V, MACH B: Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J* 1997; 16: 2851-60.
20. EYRE S, BARTON A, SHEPHARD N *et al*.: Investigation of susceptibility loci identified in the UK rheumatoid arthritis whole-genome scan in a further series of 217 UK affected sibling pairs. *Arthritis Rheum* 2004; 50: 729-35.
21. SWANBERG M, LIDMAN O, PADYUKOV L *et al*.: MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet* 2005; 37: 486-94.
22. IIKUNI N, IKARI K, MOMOHARA S *et al*.: MHC2TA is associated with rheumatoid arthritis in Japanese patients. *Ann Rheum Dis* 2007; 66: 274-5.
23. EYRE S, BOWES J, SPRECKLEY K *et al*.: Investigation of the MHC2TA gene, associated with rheumatoid arthritis in a Swedish population, in a UK rheumatoid arthritis cohort.*Arthritis Rheum* 2006; 54: 3417-22.
24. HARRISON P, POINTON JJ, FARRAR C, HARIN A, WORDSWORTH BP: MHC2TA promoter polymorphism (-168*G/A, rs3087456) is not associated with susceptibility to rheumatoid arthritis in British Caucasian rheumatoid arthritis patients. *Rheumatology* (Oxford) 2007; 46: 409-11.
25. AKKAD DA, JAGIELLO P, SZYLD P *et al*.: Promoter polymorphism rs3087456 in the MHC class II transactivator gene is not associated with susceptibility for selected autoimmune diseases in German patient groups. *Int J Immunogenet* 2006; 33: 59-61.
26. YAZDANI-BIUKI B, BRICKMANN K, WOHLFAHRT K *et al*.: The *MHC2TA* -168A>G gene polymorphism is not associated with rheumatoid arthritis in Austrian patients *Arthritis Res Ther* 2006; 8: R97.
27. OROZCO G, ROBLEDO G, LINGA REDDY MVP *et al*.: Study of the role of a functional polymorphism of MHC2TA in rheumatoid arthritis in three ethnically different populations. *Rheumatology* (Oxford) 2006; 45: 1442-4.
28. DIEGUEZ-GONZÁLEZ R, AKAR S, CALAZA M *et al*.: Lack of association with rheumatoid arthritis of selected polymorphisms in 4 candidate genes: CFH, CD209, eotaxin-3, and MHC2TA. *J Rheumatol* 2009; 36: 1590-5.
29. BRONSON PG, CRISWELL LA, BARCELLOS LF: The MHC2TA -168A/G polymorphism and risk for rheumatoid arthritis: a meta-analysis of 6861 patients and 9270 controls reveals no evidence for association. *Ann Rheum Dis* 2008; 67: 933-6.
30. MARTÍNEZ A, SÁNCHEZ-LÓPEZ M, VARADÉ J *et al*.: Role of *MHC2TA* gene in autoimmune diseases. *Ann Rheum Dis* 2007; 66: 325-9.
31. MARTÍNEZ A, PERDIGONES N, CÉNIT MC *et al*.: Chromosomal region 16p13: further evidence of increased predisposition to immune diseases. *Ann Rheum Dis* 2010; 69: 309-11.
32. O'DOHERTY C, HAWKINS S, ROONEY M, VANDENBROECK K: The MHC2TA –168A/G and +1614G/C polymorphisms and risk for multiple sclerosis or chronic inflammatory arthropathies. *Tissue Antigens* 2007; 70: 247-51.
33. LINDHOLM E, MELANDER O, ALMGREN P *et al*.: Polymorphism in the MHC2TA Gene is Associated with Features of the Metabolic Syndrome and Cardiovascular Mortality. *PLoS ONE* 2006; 1: e64. doi:10.1371/journal.pone.0000064.
34. LEE JMS, CHOUDHURY RP: Rheumatoid arthritis: RA lowering cardiovascular risk with statins. *Nat Rev Rheumatol* 2010; 6: 123-4.
35. KWAK B, MULHAUPT F, MYIT S, MACH F: Statins as a newly recognized type of immunomodulator. *Nat Med* 2000; 6:1399-402.
36. CHARLES-SCHOEMAN C, KHANNA D, FURST DE *et al*.: Effects of high-dose atorvastatin on anti-inflammatory properties of high density lipoprotein in patients with rheumatoid arthritis: a pilot study. *J Rheumatol* 2007; 34: 1459-64.
37. 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.
38. GONZALEZ-JUANATEY C, LLORCA J, MARTIN J, GONZALEZ-GAY MA: Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. *Semin Arthritis Rheum* 2009; 38: 366-71.
39. GONZALEZ-JUANATEY C, TESTA A, GARCIA-CASTELO A *et al*.: HLA-DRB1 status affects endothelial function in treated patients with rheumatoid arthritis. *Am J Med* 2003; 114: 647-52.
40. GONZALEZ-JUANATEY C, LLORCA J, GARCIA-PORRUA C, MARTIN J, GONZALEZ-GAY MA: Effect of anti-tumor necrosis factor alpha therapy on the progression of subclinical atherosclerosis in severe rheumatoid arthritis. *Arthritis Rheum* 2006; 55: 150-3.
41. NG PC, HENIKOFF S: Predicting deleterious amino acid substitutions. *Genome Res* 2001; 11: 863-74.
42. GONZALEZ-GAY MA, GARCIA-PORRUA C, HAJEER AH: Influence of human leukocyte antigen-DRB1 on the susceptibility and severity of rheumatoid arthritis. *Semin Arthritis Rheum* 2002; 31: 355-60.
43. SKOL AD, SCOTT LJ, ABECASIS GR, BOEHNKE M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006; 38: 209-13.
44. CARMONA L, VILLAVERDE V, HERNÁNDEZ-GARCÍA C *et al*.: The prevalence of rheumatoid arthritis in the general population of Spain. *Rheumatology* (Oxford) 2002; 41: 88-95.
45. LÉVY L, FAUTREL B, BARNETCHE T, SCHAEVERBEKE T: 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.
46. RODRIGUEZ-RODRÍGUEZ L, GONZALEZ-JUANATEY C, PALOMINO-MORALES R *et al*.: TNFA -308 (rs1800629) polymorphism is associated with a higher risk of cardiovascular disease in patients with rheumatoid arthritis. *Atherosclerosis* 2011, 216: 125-30.
47. PALOMINO-MORALES R, GONZALEZ-JUANATEY C, VAZQUEZ-RODRIGUEZ TR *et al*.: Lack of association of PTPN22, STAT4 and TRAF1/C5 gene polymorphisms with cardiovascular risk in rheumatoid arthritis. *Clin Exp Rheumatol* 2010; 28: 695-701.
48. PALOMINO-MORALES R, GONZALEZ-JUANATEY C, VAZQUEZ-RODRIGUEZ TR *et al*.: Lack of association between macrophage migration inhibitory factor-173 gene polymorphism with disease susceptibility and cardiovascular risk in rheumatoid arthritis patients from Northwestern Spain. *Clin Exp Rheumatol* 2010; 28: 68-72.
49. RAMANUJAM R, ZHAO Y, PIRSKANEN R, HAMMARSTRÖM L: Lack of association of the CIITA -168A*G promoter SNP with myasthenia gravis and its role in autoimmunity. *BMC Medical Genetics* 2010; 11: 147.
50. DEMA B, MARTINEZ A, FERNANDEZ-ARQUERO M *et al*.: Autoimmune disease association signals in CIITA and KIAA0350 are not involved in celiac disease susceptibility. *Tissue Antigens* 2009; 73: 326-9.
51. SÁNCHEZ E, J. SABIO JM, JIMENEZ-ALONSO J *et al*.: Study of two polymorphisms of the *MHC2TA* gene with systemic lupus erythematosus. *Rheumatology* (Oxford) 2008; 47: 102-3.
52. LINGA-REDDY MV, GUNNARSSON I, SVENUNGSSON E *et al*.: A polymorphic variant in the MHC2TA gene is not associated with systemic lupus erythematosus. *Tissue Antigens* 2007; 70: 412-4.