Analysis of the influence of the ghrelin receptor rs509035, rs512692 and rs2922126 polymorphisms in the risk of cardiovascular disease in patients with rheumatoid arthritis

Sirs,

Cardiovascular (CV) events due to accelerated atherosclerosis constitute the leading cause of mortality in patients with rheumatoid arthritis (RA) (1). Chronic systemic inflammation predicts the progression of atherosclerosis and contributes to the increased incidence of CV events observed in RA (2). The mechanisms involved in inflammation related CV disease in RA require further study.

Ghrelin is a 28-amino-acid gastric peptide that was discovered in 1999 and was identified as the endogenous ligand for the growth hormone secretagogue receptor (3). Ghrelin receptor (GHSR) is a seven-transmembrane domain G protein-coupled receptor located both in the central nervous system (the pituitary and hypothalamus) and in a wide variety of peripheral tissues including the heart, blood vessels and endothelial cells (4). The ghrelin/GHSR system exerts antiinflammatory effects, both inhibiting proinflammatory cytokine release (IL-1beta, IL-6, and TNF-alpha) in monocytes, T cells and endothelial cells and increasing the production of antiinflammatory cytokines and chemokines (5). This system also appears to exert a cardioprotective effect, protecting myocites against ischaemia and having cardiotropic actions (6).

In RA the ghrelin/GHSR system also seems to exert a protective effect in the vascular system. We reported that anti-TNF-α therapy increased serum levels of ghrelin (7), which, in turn, was associated with a reductions in soluble P-selectin serum level, a biomarker of endothelial activation that predicts CV event rates.

Polymorphisms located in the *GHSR* gene have been associated with CV disease and classic CV risk factors (6) In the present study we assessed for first time the potential the effect of tree polymorphisms the *GHSR* gene (8) on the risk of clinically evident CV disease in patients with RA.

Six hundred and fifty-nine consecutive patients, fulfilling the 1987 American College of Rheumatology classification criteria for RA (9), seen at the rheumatology outpatient clinics of Hospital Xeral-Calde, Lugo and Hospital Clínico San Carlos, Madrid, Spain, between March 1996 and March 2008 were assessed for the *GHSR* rs509035, rs512692 and rs2922126 polymorphisms. Patients were genotyped for the *GHSR* polymorphisms using predesigned TaqMan single nucleotide polymorphism genotyping assay as previously reported (8). Also, *HLA-DRB1* genotyping was performed using molecular based methods. A CV event was considered to be present if the patient had ischaemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy.

The local institutional committees approved the study.

No deviation from Hardy-Weinberg equilibrium for any *GHSR* polymorphisms was found in patients with or without CV events. Statistical significance was defined as $p \leq 0.05$. Calculations were performed with STATA 10 (STATA Corporation, College Station, Texas).

None of these tree polymorphisms were associated with susceptibility to RA (8).

No significant differences in the allele or genotype frequencies of the *GHSR* variants between RA patients with or without CV disease were found (Table I). In the unadjusted logistic regression model patients homozygotes for the minor allele of the rs509035 and rs2922126 polymorphisms showed a non-significant trend towards a protective effect against clinically evident CV disease (odds ratio - OR 0.37 [95% confidence interval - CI 0.09–1.04], p=0.05 and OR 0.55 [95% CI 0.28–1.08], p=0.06, respectively). However, when an adjustment for gender, age at RA diagnosis, time of follow-up, presence or absence of the rheumatoid shared epitope and classic CV risk factors was made, this protective trend remained out of the range of significance. Likewise, the haplotype analysis showed no statistically significant differences in the CV risk after adjustment for classic CV risk factors.

Table I. Differences in genotype and allele frequencies of *GHSR* rs509035, rs512692 and rs2922126 polymorphisms between RA patients with CV disease or without CV disease.

	With CV disease	Without CV disease	*p*-value	OR [95% CI]
GHSR rs509035				
GG	70 (60.34)	331 (60.96)		1
GA	42 (36.21)	164 (30.20)	0.38	1.21 [0.77–1.89]
AA	4 (3.45)	48 (8.84)	0.07	0.39 [0.10–1.13]
AA *vs*. GG+GA			0.05	0.37 [0.09–1.04]
Alleles 2n (%)				
G	182 (78.45)	826 (76.06)		1
A	50 (21.55)	260 (23.94)	0.44	0.87 [0.61–1.25]
GHSR rs512692				
AA	77 (66.38)	354 (65.19)		1
AT	34 (29.31)	162 (29.83)	0.87	0.96 [0.60–1.54]
TT	5 (4.31)	27 (4.97)	0.75	0.85 [0.25–2.34]
Alleles 2n (%)				
A	188 (81.03)	870 (80.11)		1
T	44 (18.97)	216 (19.89)	0.75	0.94 [0.65–1.37]
GHSR rs2922126				
TT	55 (47.41)	235 (43.28)		1
TA	49 (42.24)	214 (39.41)	0.92	0.98 [0.62–1.53]
AA	12 (10.34)	94 (17.31)	0.07	0.55 [0.26–1.11]
AA *vs*. TT+TA			0.06	0.55 [0.28–1.08]
Alleles 2n (%)				
T	159 (68.53)	684 (62.98)		1
A	73 (31.47)	402 (37.02)	0.11	0.78 [0.57–1.07]

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

RA is a polygenic disease. Previous studies on gene polymorphisms associated with susceptibility to the disease have shown contradictory results in terms of gene association with increased risk of CV disease in RA. In this regard, an association of HLA-DRB1*04 shared epitope alleles with increased incidence of CV events has been reported in Spanish individuals with RA (2). Also, an association of endothelial dysfunction with genes implicated in the inflammatory response such as *IL6* was observed in patients with RA (10). In contrast, in the same cohort, no association with clinically evident CV disease was observed when other gene variants located outside the MHC region (*PTPN22*, *STAT4* and *TRAF1/C5*) which are also associated with increased disease susceptibility were studied (11). Likewise, no association between CV events and RA with other gene polymorphisms such as *MIF-173* was found (12).

The results found in the present study indicate that *GHSR* rs509035, rs512692 and rs2922126 polymorphisms are not risk factors for clinically evident CV disease in patients with RA. Therefore, the genetic influence in the development of CV disease in patients with RA is still far from being understood. It is possible that complex gene interactions might influence the development of accelerated atherosclerosis observed in these patients. The search for the potential influence of other genes associated with the inflammatory response in the development of CV disease in patients with RA is warranted.

L. RODRÍGUEZ-RODRÍGUEZ, *MD*[1*]
M. GARCÍA-BERMUDEZ, *PhD*[1*]
C. GONZALEZ-JUANATEY, *MD*, *PhD*[2*]
T.R. VAZQUEZ-RODRIGUEZ, *MD*[3]
J.A. MIRANDA-FILLOY, *MD*[3]
B. FERNANDEZ-GUTIERREZ, *MD*, *PhD*[4]
J. LLORCA, *MD*, *PhD*[5]
J. MARTIN, *MD*, *PhD*[1**]
M.A. GONZALEZ-GAY, *MD*, *PhD*[6**]

[1]Instituto de Parasitología y Biomedicina López-Neyra, C.S.I.C., Granada, Spain; [2]Cardiology Division, Hospital Xeral-Calde, Lugo, Spain; [3]Dept. of Rheumatology, Hospital Xeral-Calde, Lugo, Spain; [4]Dept. of Rheumatology, Hospital Clinico San Carlos, Madrid, Spain; [5]Dept. of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, and CIBER Epidemiología y Salud Pública (CIBERESP), Santander, Spain; [6]Dept. of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander, Spain.

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Address correspondence to:
Miguel A. Gonzalez-Gay, MD, PhD, Rheumatology Service, Hospital Universitario Marqués de Valdecilla, IFIMAV, Avenida de Valdecilla s/n, 39008 Santander, Spain.
E-mail: miguelaggay@hotmail.com

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