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Lack of association between *IL6* single nucleotide polymorphisms and cardiovascular disease in Spanish patients with rheumatoid arthritis

Raquel López-Mejías[a,*], Mercedes García-Bermúdez[b], Carlos González-Juanatey[c], Santos Castañeda[d], Silvia Pérez-Esteban[d], José A. Miranda-Filloy[e], Carmen Gómez-Vaquero[f], Benjamín Fernández-Gutiérrez[g], Alejandro Balsa[h], Dora Pascual-Salcedo[h], Ricardo Blanco[a], Isidoro González-Álvaro[d], Javier Llorca[i], Javier Martín[b,1], Miguel A. González-Gay[a,1]

[a] *Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, s/n, 39008 Santander, Spain*
[b] *Instituto de Parasitología y Biomedicina López-Neyra, C.S.I.C., Granada, Spain*
[c] *Cardiology Division, Hospital Xeral-Calde, Lugo, Spain*
[d] *Rheumatology Department, Hospital Universitario la Princesa, IIS-Princesa, Madrid, Spain*
[e] *Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain*
[f] *Department of Rheumatology, Hospital Universitario Bellvitge, Barcelona, Spain*
[g] *Department of Rheumatology, Hospital Clinico San Carlos, Madrid, Spain*
[h] *Department of Rheumatology, Hospital Universitario La Paz, Madrid, Spain*
[i] *Department of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, and CIBER Epidemiología y Salud Pública (CIBERESP), IFIMAV, Santander, Spain*

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a complex polygenic inflammatory disease associated with accelerated atherosclerosis. IL-6 is a key mediator of inflammation in RA. A recent study showed an association between *IL6*-174 G/C gene polymorphism and cardiovascular (CV) disease in UK individuals with RA. To confirm this association we assessed the influence of three *IL6* gene polymorphisms in the risk of CV disease in a large series of patients with RA.
Material and methods: We studied 1250 Spanish patients with RA. Besides genotyping the traditional single nucleotide polymorphism (SNP) promoter -174G/C (rs1800795), we assessed another two SNPs (rs2069827 and rs2069840) located in the *IL6* gene that were selected by SNP-tagging.
Results: Two-hundred and twenty (17.6%) of the 1250 patients experienced CV events. No significant differences in the genotype, allele and haplotype frequencies between RA patients with and without CV events were observed.
Conclusion: Our results do not confirm in a Spanish population the association of *IL6* gene with CV disease in RA previously reported in the UK.

1. Introduction

Rheumatoid arthritis (RA) is a disease associated with accelerated atherosclerosis [1,2]. The increased risk of cardiovascular (CV) events observed in patients with RA cannot be completely explained by the presence of classic CV risk factors [3]. Both chronic inflammatory response [4] and genetic factors [4–6] have been implicated in the augmented CV mortality observed in patients with RA.

IL-6, a key mediator of inflammation in RA [7], is directly implicated in the production of serum C-reactive protein (CRP), which was reported to be a predictor of CV mortality in patients with inflammatory polyarthritis [8]. Also, an association between the magnitude and chronicity of the inflammatory response measured by CRP levels and the presence of both subclinical atherosclerosis [9] and the development of CV events was found in patients with RA [4].

Panoulas et al. assessed the influence of the *IL6*-174 G/C (rs1800795) gene polymorphism in a series of 383 UK patients with RA [10]. Carriers of the *IL6*-174C-allele demonstrated increased prevalence of CV disease, even after adjustment for traditional CV disease risk factors [10]. Since replication studies are of major importance to define strategies on the management of patients with autoimmune diseases, in the present study we aimed to replicate the results reported by Panoulas et al. [10]. For this purpose we assessed the *IL6*-174 G/C (rs1800795) along with another two

* Corresponding author. Tel.: +34 942 20 25 10; fax: +34 942 20 16 95.
E-mail address: rlopezmejias78@gmail.com (R. López-Mejías).
[1] Shared senior authorship in this study.

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IL6 polymorphisms (rs2069827 and rs2069840) in a large series of Spanish patients with RA stratified by the presence or absence of CV events.

2. Patients and methods

2.1. Patients and study protocol

A set of 1250 Spanish patients with RA were included in the present study. Blood samples were obtained from patients recruited from Hospital Xeral-Calde (Lugo), Hospital Universitario Marqués de Valdecilla (Santander), Hospital Universitario Bellvitge (Barcelona), and Hospital La Paz, Hospital de La Princesa and Hospital Clínico San Carlos (Madrid). The study was approved by the ethics committee of the corresponding hospitals. All the patients fulfilled the 1987 American College of Rheumatology (ACR) criteria for the classification of RA [11]. Also, a control group constituted by blood donors from National DNA Bank Repository (Salamanca, Spain) was assessed.

Clinical definitions for CV events and classic (traditional) CV risk factors were established as previously described [4,12].

A CV event was considered to be present if the patient had ischemic heart disease (including acute coronary syndromes with or without persistent ST-segment elevation and chronic coronary heart disease), heart failure (based on the Framingham criteria), cerebrovascular accident (when the patient had a stroke and/or transient ischemic attacks), or peripheral arteriopathy (considered to be present if it was confirmed by Doppler and arteriography) [4,12].

Definition of classic CV risk factors: hypertension (if before the diagnosis of RA patients had been diagnosed as having hypertension by their family physicians, or if at the time of disease diagnosis or over the extended follow-up they had blood pressure >150/90 mm Hg in 2 different examinations performed on different days), diabetes mellitus (if before disease diagnosis they had been diagnosed as having diabetes mellitus by their family physicians or if 2 fasting plasma glucose levels on different days at the time of disease diagnosis or over the extended follow-up were >125 mg/dl), dyslipidemia (if they had hypercholesterolemia and/or hypertriglyceridemia, defined as diagnosis of hypercholesterolemia or hypertriglyceridemia by the patients' family physicians prior to the diagnosis of RA, or total cholesterol and/or triglyceride levels in fasting plasma was >240 mg/dl and 160 mg/dl, respectively, at the time of disease diagnosis or over the extended follow-up), obesity (if body mass index calculated as weight in kg divided by height in m^2 was >30 kg/m^2 at enrollment or over the extended follow-up) and smoking habit (considered to be present in those patients who smoked at the time of disease diagnosis, during the follow-up or who had smoked within the 10 years before the onset of RA symptoms or the disease diagnosis) [4].

2.2. SNPs selection and genotyping

DNA from patients was obtained using standard methods.

We performed a SNP tagging by Haploview software with the aim of covering the major variability in the *IL6* gene. For this purpose we genotyped 3 SNPs (rs2069827, rs1800795 and 2069840) in linkage disequilibrium block of $D' = 1$ and r^2 coefficient 0.097 between rs2069827 and rs1800795, 0.051 between rs2069827 and rs2069840 and 0.476 between rs1800795 and rs2069840 (data obtained from HapMap).

All SNPs were genotyped with TaqMan SNP genotyping assays in a 7900 HT real-time polymerase chain reaction (PCR) system, according to the conditions recommended by the manufacturer (Applied Biosystem, Foster City, CA, USA). Negative controls and duplicate samples were included to check the accuracy of genotyping.

Table 1
Demographic characteristics of the patients with RA included in the study.

Clinical feature	% (*n*/*N*)
Patients	1250
Main characteristics	
Age at the time of disease onset, years, mean ± SE)	54.8 ± 14.8
Time follow-up, years, mean ± SE	11 ± 7.6
Percentage of women	73
Rheumatoid factor positive	70.0 (875/1250)
Shared epitope positive	63.1 (492/779)
Anti-CCP antibodies positive	59.8 (603/1008)
Cardiovascular risk factors	
Hypertension	39.3 (492/1250)
Diabetes mellitus	12.8 (165/1250)
Dyslipidemia	41.2 (495/1202)
Obesity	12.0 (139/1161)
Smoking habit	24.5 (306/1250)
Patients with cardiovascular events	17.6 (220/1250)
Ischemic heart disease	9.6 (120/1250)
Heart failure	4.8 (60/1250)
Cerebrovascular accident	4.8 (60/1250)
Peripheral arteriopathy	2.0 (25/1250)

SE, standard error; Anti-CCP antibodies, anti-cyclic citrullinated peptide antibodies.

2.3. Statistical analysis

Both allelic and genotypic frequencies were calculated and compared by χ^2 or Fisher tests using the StatsDirect software V2.6.6 (StatsDirect http://www.statsdirect.com, England: StatsDirect 2008). Strength of associations between CV events and genotypes or alleles were estimated using odds ratios (OR) and 95% confidence intervals (CI), via multiple logistic regression; estimates were further adjusted for sex, age at RA diagnosis, time of follow-up, classic CV risk factors and the presence or absence of the rheumatoid shared epitope as potential confounders. Statistical significance was defined as $p < 0.05$ (p-values were two-tailed). All analyses were performed with STATA statistical software 9.1 (Stata Corp., College Station, TX, USA).

3. Results

Information on classic CV risk factors and the main clinical features of the whole series of 1250 RA patients is shown in Table 1. Two-hundred and twenty patients with RA experienced CV events (17.6%; 220/1250).

Genotyping success in our study was greater than 95%. With this sample size, the study had 89% statistical power to detect an OR equal to or higher than 1.4 in alleles present in 40% of the patients and 78% power to detect that OR in alleles present in 20% of the patients. Of note, the less prevalent the allele the lower the statistical power; nevertheless, even for alleles as rare as 5%, the study had a 92% power to detect an OR higher than 2.

No statistical significant association of *IL6* allele and/or genotypes with the general characteristics of the study participants was observed (Supplementary table s1A and s1B).

3.1. Cases versus controls

No significant differences in the allele or genotype frequencies for the *IL6*-174 G/C (rs1800795) gene polymorphism between this series of RA patients and controls were seen. It was also the case when differences in the allele or genotype frequencies between patients and controls were assessed for the other two polymorphisms (data not shown).

Table 2
Genotype and allele frequencies for *IL6*-tagging single nucleotide polymorphisms (SNPs) in RA patients with and without cardiovascular (CV) events.

SNP	Change 1/2	Samples set	*N*	Genotype, no. (%)			Allele test	
				1/1	1/2	2/2	P-adj*	OR [95% CI]*
rs2069827	G/T	RA without CV	1030	885 (85.9%)	139 (13.5%)	6 (0.6%)	0.922	1.04 [0.43–2.50]
		RA with CV	220	177 (80.5%)	39 (17.7%)	4 (1.8%)		
rs1800795	G/C	RA without CV	1030	432 (42.0%)	468 (45.4%)	130 (12.6%)	0.259	1.34 [0.81–2.22]
		RA with CV	220	89 (40.4%)	101 (46.0%)	30 (13.6%)		
rs2069840	C/G	RA without CV	1030	467 (45.3%)	435 (42.2%)	128 (12.4%)	0.407	0.83 [0.49–1.43]
		RA with CV	220	109 (49.6%)	78 (35.4%)	33 (15.0%)		

CV, cardiovascular; OR [95% CI], odds ratio with 95% confidence interval.
* Multiple regression adjusted for sex, age at RA diagnosis, time of follow-up, classic CV risk factors (hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit) and presence or absence of the rheumatoid shared epitope as potential confounders.

3.2. Patients with and without CV events

Table 2 describes the distribution of *IL-6* polymorphisms in RA patients with and without CV events. As shown in this table, the genotype frequencies were similar in patients with and without CV events.

In keeping with this observation, no significant differences in the allele frequency between RA patients with CV and those without CV events were found (Table 2).

3.3. Haplotype analysis

In a further step, we assessed these polymorphisms together to create haplotypes but again the haplotype analysis did not yield additional information as the frequencies did not differ between RA patients with CV events and those without CV events (Supplementary table s2).

4. Discussion

Association studies offer a potentially powerful approach to identify genetic variants that influence susceptibility to common diseases such as cardiac phenotypes, but are plagued by the impression that they are not consistently reproducible. Inconsistency between studies is frequently observed. It may be due to false positive studies, false negative studies or true variability in association among different populations.

IL-6 is a pivotal proinflammatory cytokine implicated in the pathogenesis of RA [7]. Since RA and atherosclerosis are inflammatory diseases, the search for proinflammatory markers that may explain the development of the accelerated atherogenesis observed in RA is of major interest. In this regard, Panoulas et al. studied the *IL6*-174 G/C (rs1800795) gene polymorphism in 383 UK patients diagnosed with RA [10]. These authors found an association between the *IL6*-174C carriers and CV disease in this population [10].

In the present study we aimed to replicate the results reported by Panoulas et al. [10]. For this purpose we assessed the *IL6*-174 G/C (rs1800795) along with another two *IL6* polymorphisms (rs2069827 and rs2069840) in a large series of Spanish patients with RA stratified by the presence or absence of CV events. However, our results did not confirm an allele or genotype association of these three *IL6* gene polymorphisms with CV disease in RA. It was also the case when a haplotype analysis was performed.

The discordance in the results between our study and that by Panoulas et al. may be due to the genetic variability in the different populations. However, our negative results in terms of genetic association in our ethnically homogenous population are in keeping with former reports that showed no significant differences for the *IL6* rs1800795 gene polymorphism between RA patients and controls in the UK and Spain [10,13]. Also, in accordance with our data, no significant differences in the *IL6*-174 (rs1800795) genotype distribution were observed when a large series of German individuals with myocardial infarction ($n = 1322$) were compared with population-based matched controls ($n = 1023$) [14].

There are some potential limitations to our study. First, our sample was ethnically homogenous and we did not measure *IL6* levels. Therefore, we cannot exclude that circulating *IL6* levels may have a role in the risk of CV disease of RA patients. In this regard, Dessein et al. disclosed a relationship between circulating IL-6 concentrations and endothelial dysfunction and the improvement of IL-6 related endothelial dysfunction upon disease activity suppression in RA [15]. On the other hand, other factors different from genetics may influence *IL6* levels.

5. Conclusions

Our data do not support an association of the *IL6* rs1800795, rs2069827, and rs2069840 gene polymorphisms with the risk of developing CV events in patients with RA. Further studies aimed to determine the potential influence of other polymorphisms located within the *IL6* gene are required to shed light on the actual role of this gene in the increased risk of CV disease observed in RA.

Competing interest

The authors declare that they have no competing interest.

Author's contributions

RLM carried out genotyping, participated in the design of the study, data analysis and helped to draft the manuscript. MGB participated genotyping and data analysis. CGJ, SPE, DPS, AB, CGV, JMF, and RB participated in the acquisition and interpretation of data. SC, IGA, and BFG, has been involved in the acquisition and interpretation of data and in revising it critically for important intellectual content. JL carried out the analysis and interpretation of the data. JM has made substantial contributions to conception and design of the study, acquisition of data, coordination and helped to draft the manuscript and has given final approval of the version to be published. MAG-G has made substantial contributions to conception and design of the study, acquisition of data, coordination and helped to draft the manuscript and has given final approval of the version to be published.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.07.124.

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