Association of acid phosphatase locus 1*C allele with the risk of cardiovascular events in rheumatoid arthritis patients

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Abstract

Introduction: Acid phosphatase locus 1 (ACP1) encodes a low molecular weight phosphotyrosine phosphatase implicated in a number of different biological functions in the cell. The aim of this study was to determine the contribution of ACP1 polymorphisms to susceptibility to rheumatoid arthritis (RA), as well as the potential contribution of these polymorphisms to the increased risk of cardiovascular disease (CV) observed in RA patients.

Methods: A set of 1,603 Spanish RA patients and 1,877 healthy controls were included in the study. Information related to the presence/absence of CV events was obtained from 1,284 of these participants. All individuals were genotyped for four ACP1 single-nucleotide polymorphisms (SNPs), rs10167992, rs11553742, rs7576247, and rs3828329, using a predesigned TaqMan SNP genotyping assay. Classical ACP1 alleles (*A, *B and *C) were imputed with SNP data.

Results: No association between ACP1 gene polymorphisms and susceptibility to RA was observed. However, when RA patients were stratified according to the presence or absence of CV events, an association between rs11553742*T and CV events was found (P = 0.012, odds ratio (OR) = 2.62 (1.24 to 5.53)). Likewise, the ACP1*C allele showed evidence of association with CV events in patients with RA (P = 0.024, OR = 2.43).

Conclusions: Our data show that the ACP1*C allele influences the risk of CV events in patients with RA.

Introduction

Rheumatoid arthritis (RA) is a complex polygenic autoimmune inflammatory disease characterized by persistent synovitis and joint damage. Several genetic polymorphisms, such as HLA-DRB1, PTPN22, STAT4, TRAF1/C5 and TNFAIP3, have been implicated in the susceptibility to RA [1]. On the other hand, increased cardiovascular (CV) mortality is observed in patients with RA. This is the result of accelerated atherogenesis [2-4].

Acid phosphatase locus 1 (ACP1) is a gene located on chromosome 2p25 that encodes a low molecular weight phosphotyrosine phosphatase (LMW-PTP), which presents two main enzymatic activities: phosphoprotein tyrosine phosphatase and flavin mononucleotide phosphatase [5]. Two different isoforms of LMW-PTP have been described: ‘fast’ (also noted as ACP1-F (fast), isoform 1, IF1, HCPTP-A) and ‘slow’ (also noted as ACP1-S(slow), isoform 2, IF2, HCPTP-B), that arise through alternative splicing mechanisms, in which either exon 3 or exon 4 is excised and the other retained respectively [5,6]. These two LMW-PTP isoforms have different molecular and catalytic properties, suggesting that they may be implicated in different biological functions in the cell [5,7]. In Caucasian populations there are three common codominant alleles of ACP1, ACP1*A, ACP1*B, ACP1*C. ACP1 allelic differences on single-nucleotide polymorphisms (SNPs), which affect both
the total enzymatic activity and the ratio between iso-
forms F/S, being the ratio F/S 2:1 in ACP1*A, 4:1 in
ACP1*B and 1:4 in ACP1*C [5,7,8].

LMW-PTP is considered to play a key role as regula-
tor of signaling pathways in receptor-stimulated immune
cells [9]. LMW-PTP has also been involved in the regu-
lation of many growth factors such as platelet-derived
growth factor receptor (PDGFR) [10], fibroblast growth
factor receptor (FGFR) [11], insulin receptor (IR) [12,13]
and EphA2 receptor, a ligand that binds to the Ephrin
family of signaling molecules [14]. LMW-PTP has also
been implicated in the regulation of ZAP70 Kinase (ζ-
chain- associated protein kinase of 70 kDa) [15] playing
a role in T-cell development and lymphocyte activation,
enhancing signaling from the T cell antigen receptor
[15]. Additionally, LMW-PTP has been found to be a
key mediator in the integrin signaling during cellular
adhesion [9].

Allelic polymorphisms of the ACP1 gene have been
associated with susceptibility to several human diseases,
including inflammatory and autoimmune diseases [5,16].
Interestingly, the ACP1 gene was also associated with
susceptibility to coronary atherosclerotic artery disease
(CAD) [17].

Taking into account the possible influence that ACP1
may have in the susceptibility to immune-mediated dis-
orders and in the pathogenesis of the CV disease, in the
present study we aimed to investigate the possible asso-
ciation of ACP1 alleles with the susceptibility to RA as
well as whether ACP1 gene polymorphism may contribu-
te to the increased risk of CV complications observed
in patients with RA.

Materials and methods

Material

A set of 1,603 RA Spanish patients and 1,877 healthy
individuals were included in the present study. Blood
samples were obtained from RA patients recruited from
the Hospital Xeral-Calde (Lugo), Hospital Universitario
Marqués de Valdecilla (Santander), Hospital Universi-
tario Bellvitge (Barcelona), and Hospital La Paz, Hospital
de La Princesa and Hospital Clínico San Carlos
(Madrid). All the patients fulfilled the 1987 American
College of Rheumatology (ACR) criteria for the classifi-
cation of RA [18].

Information related to the presence or absence of CV
events was obtained in 1,284 RA patients (80.1%, 1284/
1,603). Among them, 229 experienced CV events
(17.8%, 229/1,284). Information on traditional CV risk
factors was also collected.

Clinical features of the whole series of 1,603 RA
patients are shown in Table 1.

A CV event was considered to be present if the
patient had ischemic heart disease, heart failure, a
cerebrovascular accident or peripheral arterio-
pathy. Clinical definitions for CV events and classic CV risk
factors were established as previously described [4,19].
The study was approved by local ethics committees
from all the participating centers and all subjects pro-
vided informed consent according to the Declaration of
Helsinki.

SNPs selection and genotyping

DNA from patients and controls was obtained using
standard methods. We selected four ACP1 SNPs for the
present study. rs11553742 and rs7576247 were selected
because of their ability to tag classical ACP1 alleles (that
is, ACP1*A, ACP1*B, ACP1*C) [5]. rs11553742 is a
synonymous polymorphism located in the codon 44
(exon 3) and rs7576247 encodes an aminoacid change
in the codon 105 (exon 6) from arginine, present in
ACP1*A allele, to glutamine in ACP1*B and *C alleles.
Hence, ACP1*A allele differs from ACP1*C allele in two
base substitutions in those positions, so the CG allele
combination is responsible for the ACP1*A allele and
TA for the ACP1*C allele. In addition, ACP1*B allele is
defined as not *A, not *C, that is, for the allelic combi-
nation CA. Another two polymorphisms, rs10167992
and rs3828329, were also selected because they showed
association with quantitative traits related to type 2 dia-
betes mellitus [17]. All SNPs were genotyped with Taq-
Man SNP genotyping assays in a 7900 HT Real-Time

| Clinical feature | % (n/N) |
|-----------------|--------|
| Patients        | 1,603  |
| Main characteristics |
| Age at disease onset (years, means ± SE) | 54.1 ± 14.8 |
| Follow up (years, means ± SE)             | 11 ± 7.5   |
| Female          | 73.5   |
| Hypertension    | 39.4 (516/1,310) |
| Diabetes mellitus | 13.2 (171/1,300) |
| Dyslipidemia    | 41.3 (540/1,307) |
| Obesity         | 12.4 (142/1,146) |
| Smoking habit   | 24.0 (303/1,261) |
| Patients with cardiovascular events       |
| Ischemic heart disease          | 9.5 (122/1,284) |
| Heart failure                  | 4.8 (62/1,284) |
| Cerebrovascular accidents       | 4.6 (59/1,284) |
| Peripheral arterioopathy        | 1.9 (25/1,284) |

SE, Standard error

Anti-CCP antibodies, anti-cyclic citrullinated peptide antibodies

Table 1 Demographic characteristics of the patients with rheumatoid arthritis included in the study
polymerase chain reaction (PCR) system, according to the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). All samples were genotyped at the same center.

Statistical analysis
Controls were tested for significant differences in their genotype distribution and Hardy-Weinberg equilibrium (HWE) theoretical distribution by means of a χ² test. The case-control association study was performed by 2 × 2 contingency tables with χ² to obtain P-values, odds ratios (OR) and 95% confidence intervals (CI), according to Woolf’s methods. The same procedure was applied in the subgroups stratified according to the presence or absence of anti-cyclic citrullinated peptide antibodies (ACPA). Association analysis for CV events in RA patients was performed via multiple logistic regression; estimates were adjusted for age at the time of disease diagnosis, gender, rheumatoid shared epitope status and traditional CV risk factors (hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit) as potential confounders.

All P-values < 0.05 were considered as statistically significant. All statistical analyses were carried out with Plink [20] and haplotype analysis with Haploview [21]. The estimation of the statistical power of the study to detect an effect of a polymorphism in disease susceptibility was performed using the CaTS Power Calculator software (Center for Statistical Genetics, University of Michigan, Michigan, USA) [22]. The study had between 98 and 100% power to detect the OR of 1.50 at the 5% significance level, assuming a RA Spanish prevalence of this disease relative risk, with an OR of 1.50 at the 5% significance level. The estimation of the statistical power of the study to detect an effect of a polymorphism in disease susceptibility was performed using the CaTS Power Calculator software (Center for Statistical Genetics, University of Michigan, Michigan, USA) [22]. The study had between 98 and 100% power to detect the relative risk, with an OR of 1.50 at the 5% significance level, assuming a RA Spanish prevalence of this disease of 0.5% and considering a minor allele frequency (MAF) between 0.05 and 0.25 respectively. Under the same conditions described above, our study had between 98 and 100% power to detect the OR of 1.50 at the 5% significance level. The estimation of the statistical power of the study to detect an effect of a polymorphism in disease susceptibility was performed using the CaTS Power Calculator software (Center for Statistical Genetics, University of Michigan, Michigan, USA) [22]. The study had between 98 and 100% power to detect the relative risk, with an OR of 1.50 at the 5% significance level, assuming a RA Spanish prevalence of this disease of 0.5% and considering a minor allele frequency (MAF) between 0.05 and 0.25 respectively. Under the same conditions described above, our study had between 98 and 100% power to detect the OR of 1.50 at the 5% significance level.

Table 2 Differences between RA patients with and without CV events according to ACP1 polymorphisms

| SNP         | Change | Samples Set | Genotype, no. (frequency) | Minor allele, no. (frequency) | Allele test |
|-------------|--------|-------------|---------------------------|-------------------------------|-------------|
| rs10167992  | C/T    | RA with CV  | 215                       | 171 (0.826) 35 (0.169) 1 (0.005) | 37 (0.089) 0.321 | 0.72 (0.38 to 1.37) |
| rs11553742  | C/T    | RA with CV  | 221                       | 200 (0.966) 21 (0.101) 0 (0.000) | 21 (0.048) 0.012 | 2.62 (1.24 to 5.53) |
| rs7576247   | A/G    | RA with CV  | 207                       | 112 (0.541) 76 (0.367) 18 (0.087) | 112 (0.272) 0.203 | 0.76 (0.50 to 1.16) |
| rs3828329   | C/T    | RA with CV  | 221                       | 88 (0.425) 103 (0.498) 28 (0.135) | 641 (0.319) 0.079 | 1.38 (0.96 to 1.97) |

CV, cardiovascular; RA, rheumatoid arthritis

* multiple regression adjusted by age at diagnosis of the disease, gender, shared epitope status and traditional CV risk factors, that is, hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit, as potential confounders.
Table 3 Distribution of ACP1 alleles in RA patients with and without CV events

| ACP1 allele | Haplotype | RA with CV (no. (frequency)) | RA without CV (no. (frequency)) | P-adj* | OR* |
|-------------|-----------|-------------------------------|---------------------------------|--------|-----|
| ACP1*A     | CG        | 110 (0.276)                  | 525 (0.281)                    | 0.217  | 0.76|
| ACP1*B     | CA        | 270 (0.678)                  | 1,263 (0.676)                  | 0.859  | 1.04|
| ACP1*C     | TA        | 18 (0.045)                   | 80 (0.043)                     | 0.024  | 2.43|

CV, cardiovascular; RA, rheumatoid arthritis. The order of the SNPs is rs11553742|rs7576247.

* multiple regression adjusted by age at diagnosis of the disease, gender, shared epitope status, hypertension, diabetes mellitus, dyslipidemia, obesity and smoking habit.

Discussion
Since the association of ACP1 gene with autoimmunity has previously been described [5], in the present study we sought to investigate the possible association of ACP1 polymorphisms with RA. Furthermore, taking into account that this gene has been involved in the susceptibility to CAD [17], we also assessed whether ACP1 variations could be involved in the risk of CV events in patients with RA. Our result revealed that ACP1 polymorphisms do not influence the susceptibility to RA. However, these polymorphisms seem to influence the risk of CV events in these patients. In this regard, both rs11553742*T and ACP1*C alleles increased the risk of CV complications in patients with RA. Interestingly, rs11553742*T has been observed to decrease the F/S ratio of the LMW-PTP isoenzymes [5]; in this regard the ACP1*C allele, carrier of the minor allele of rs11553742, was found to produce a major amount of S isoforms and is also associated with the highest total LMW-PTP activity [8,23].

Our results are in accordance with the findings by Banci et al. [17], who observed that high S isoform genotypes were associated with increased risk to develop CAD. Moreover, patients with hypertrophic cardiomyopathy, an autosomal dominant disease, were found to have the highest frequencies for ACP1*C allele and showed a linear relationship between maximum wall thickness and the amount of total LMW-PTP activity [16].

The effect of the ACP1*C allele in the development of CV events could be explained by its possible role in the regulation of the energy metabolism and oxidative stress through its flavin mononucleotide phosphatase activity [8]. With respect to this, a negative interaction between LMW-PTP and the enzyme glutathione reductase (GSR), which affects the cellular concentration of their cofactor flavin adenosine dinucleotide (FAD), has been described [8]. GSR is a flavoenzyme involved in the cellular antioxidant mechanism that reduces oxidized glutathione disulfide (GSSG) to the sulphydryl form glutathione (GSH) that is an important cellular antioxidant. Low LMW-PTP activity increases the levels of cofactor flavin adenine dinucleotide (FAD) in the cytosol leading to increased activity of GSR; while higher LMW-PTP activity yields low GSR activity. Accordingly, low activity of GSR has also been found to be significantly associated with hypertension [24], and it has also been considered to be a risk factor for CV by influencing cholesterol levels [25]. Furthermore, Bottini et al. [26] reported that the ACP1*A allele, the opposite allelic combination of ACP1*C, is a protective factor for hypertriglyceridemia and hypercholesterolemia in obese women.

RA is a complex polygenic disease and, besides the association of HLA-DRB1*04 shared epitope alleles with CV disease [4,27], recent reports have also emphasized the potential implication of other gene polymorphisms in the increased risk of CV events observed in patients with RA. In this regard, interactions between NOS gene polymorphisms and HLA-DRB1*04 shared epitope alleles seem to confer an increased risk of developing CV events in these patients [28]. Also, the A1298C polymorphism in the MTHFR gene was found to predispose to CV risk in RA [29]. More recently, an association of the TNFA rs1800629 gene polymorphism with predisposition to CV complications in RA patients carrying the rheumatoid shared epitope was also described [30].

Conclusions
Our data show for first time the association of the ACP1*C allele with increased susceptibility to CV events in patients with RA. This effect may be based on the major production of the S isoform of LMW-PTP by this allele, which may influence the regulation of energy metabolism and the response to oxidative stress.

Additional material

Additional file 1: Genotype and allele distribution of ACP1 polymorphisms in Spanish RA patients and healthy subjects
Supplementary table S1 shows the genotype and allele frequencies of ACP1 polymorphisms in Spanish RA patients and healthy controls. That table also shows the lack of association among cases and controls.

Additional file 2: Distribution of ACP1 alleles in Spanish RA patients and healthy controls
Supplementary table S2 shows the frequencies of ACP1 alleles in Spanish RA patients and individuals controls. No association was observed.

Abbreviations
ACP1: acid phosphatase locus 1; ACPA: anti-cyclic citrullinated peptide antibodies; ACR: American College of Rheumatology; CAD: coronary atherosclerotic artery disease; CI: confidence intervals; CV: cardiovascular;
FAD: flavin adenine dinucleotide; FGFR: fibroblast growth factor receptor; GSH: glutathione; GSR: glutathione reductase; GSSR: glutathione disulfide; HWE: Hardy-Weinberg equilibrium; IR: insulin receptor; LMW-PTP: low molecular weight phosphotyrosine phosphatase; MAF: minor allele frequency; OR: Odds ratio; PCR: polymerase chain reaction; PDGFR: platelet-derived growth factor receptor; RA: rheumatoid arthritis; SNP: single-nucleotide polymorphism.

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Authors’ contributions
MT, JEM, NB and JM made substantial contributions to the conception and design of the study, and the interpretation of data. MT carried out genotyping, analysis of data and drafted the manuscript. JEM carried out genotyping. CGG, RLM, JAMF, RB, AB, DPS, LRR, BFG, AMO, IGA and CGV were involved in the acquisition of cardiovascular data in the different Spanish hospitals included in this study. JL carried out the analysis and interpretation of the data. JM and MAGE were involved in revising the manuscript and gave final approval of the version to be published.

Competing interests
The authors declare that they have no competing interests.

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