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Vitamin D receptor GATG haplotype association with atherosclerotic disease in patients with rheumatoid arthritis

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ABSTRACT

Introduction: An association between the *vitamin D receptor* (*VDR*) GAT haplotype and coronary artery disease (CAD) in type-2 diabetes has recently been described. Since cardiovascular mortality in rheumatoid arthritis (RA) is comparable to that observed for patients with type-2 diabetes, we aimed to determine if *VDR* GAT haplotype is also associated with atherosclerotic disease in RA.
Material and Methods: 591 Northern Spanish RA patients were genotyped for 4 *VDR* polymorphisms (rs731236 A/G; rs7975232 A/C; rs1544410C/T; rs2228570 G/A). Atherosclerotic disease was established by the presence of carotid plaques in carotid ultrasound.
Results: VDR rs7975232 AA genotype was increased in RA patients with plaques ($p = 0.045$, OR = 1.46 [1.01–2.18]). More importantly, the frequency of carotid plaques was significantly increased in RA patients who carried the GATG haplotype ($p = 0.009$, OR = 1.56 [1.09–2.42]).
Conclusion: Our results suggest a potential *VDR* GATG haplotype association with atherosclerotic disease in RA patients.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with augmented atherogenic burden and elevated risk of cardiovascular (CV) disease [1]. CV mortality in RA is comparable to that observed for type-2 diabetes mellitus (DM) [2–5]. Vitamin D deficiency is common in RA patients [6]. In this respect, low levels of vitamin D may influence the risk and severity of autoimmune diseases [7]. An inverse association between 25-hydroxyvitamin D levels and activity parameters of RA has been found [8]. This link between low levels for Vitamin D and disease severity is of potential relevance as the persistence of a chronic inflammatory burden has been associated with an increased risk of CV disease in RA [9]. Besides inflammation and traditional CV risk factors [10], a genetic component is also related to the increased risk of CV disease in RA [9,11–13]. Interestingly, an association of a *vitamin D receptor* (*VDR*) haplotype GAT (composed of the minor allele of *VDR* rs731236, major allele of *VDR* rs7975232 and minor allele of *VDR*

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rs1544410) with increased risk of coronary artery disease (CAD) in patients with type-2 DM has been disclosed [14].

Besides an increased incidence of CV events, RA patients have increased frequency of atherosclerosis manifested by higher frequency of carotid plaques when compared with the general population [15,16].

Since the presence of carotid plaques reflects subclinical atherosclerosis and associates closely with CAD [15], we aimed to establish, for the first time, if the *VDR* haplotype association with CAD observed in type-2 DM is also related to increased frequency of carotid plaques in RA.

2. Patients and methods

2.1. Patients and study protocol

A set of 591 unrelated RA Spanish patients, fulfilling the 2010 American College of Rheumatology classification criteria for RA [17], were included in the present study. Blood samples were obtained from patients recruited from Hospital Universitario Marqués de Valdecilla (Santander) and Hospital Lucus Augusti (Lugo) in Northern Spain. A subject's written consent was obtained and propose of the work was approved by Ethics Committees. Patients were assessed for *VDR* rs731236, *VDR* rs7975232, *VDR* rs1544410 and *VDR* rs2228570. Presence/absence of carotid plaques was evaluated by carotid ultrasound (US).

Epidemiological and clinical characteristics of these patients as well as information on carotid plaques are shown in Table 1. Definitions of traditional CV risk factors and CV events were established as previously described [9,15]. The study was conducted following the STREGA (guidelines for reporting genetic associations) recommendations [18].

2.2. Genotyping

Deoxyribonucleic-acid from peripheral blood was stored at Hospital Universitario Marqués de Valdecilla where the genotyping was performed.

We analyzed 3 polymorphisms located in *VDR* (rs731236, rs7975232 and rs1544410), that conform the GAT haplotype associated with CAD in type-2 diabetes [14]. Since *VDR* rs2228570 has been related to immune-mediated diseases, we decided to include the analysis of this polymorphism in our study. The linkage disequilibrium of *VDR* polymorphisms is shown as a Supplementary Figure.

Polymorphisms were genotyped with TaqMan genotyping assays in a 7900 HT Real-Time polymerase chain reaction system (Applied Biosystem, Foster City, CA, USA). Negative controls and duplicate samples were included to check the accuracy of genotyping. The genotype distribution of *VDR* polymorphisms was in Hardy–Weinberg equilibrium (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Genotyping success was >97% (<3% of the samples failed the genotyping).

2.3. Carotid US examination

Presence/absence of carotid plaques was established by carotid US. Patients from Santander were assessed using a commercially available scanner, Mylab 70, Esaote (Genoa, Italy) [19]. Patients from Lugo were assessed using high-resolution B-mode ultrasound, Hewlett Packard SONOS 5500 [20]. The plaque criteria in the accessible extracranial carotid tree were focal protrusion in the lumen at least carotid intima-media (IMT) thickness >1.5 mm, protrusion at least 50% greater than the surrounding carotid IMT, or arterial lumen encroaching >0.5 mm, according to Mannheim consensus criteria [21]. Agreement between the two US methods in RA patients was previously reported [22].

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

Clinical feature	RA patients (n = 591)
	% (n/N)
Main characteristics	
Age at the time of disease onset (years, mean ± SD)	50.9 ± 15.3
Follow-up (years, mean ± SD)	9.5 ± 8.2
Female gender	76.5 (452/591)
Rheumatoid factor positive[a]	61.5 (361/587)
Anti-CCP antibodies positive	51.6 (279/541)
Shared epitope positive	61.9 (200/323)
CRP (mg/l, mean ± SD)	
at the time of disease diagnosis	14.2 ± 24.4 (564/591)
at the time of the study	6.8 ± 10.3 (591/591)
Traditional CV risk factors	
Hypertension	38.7 (227/586)
Diabetes mellitus	10.6 (62/586)
BMI (kg/m^2, mean ± SD)[b]	27.8 ± 5.3
Obesity	22.0 (129/586)
Smoking habit	41.1 (241/586)
Total cholesterol (mg/dl, mean ± SD)[b]	205.9 ± 39.0
HDL cholesterol (mg/dl, mean ± SD)[b]	62.3 ± 17.6
LDL cholesterol (mg/dl, mean ± SD)[b]	121.4 ± 32.4
Dyslipidemia	39.4 (231/586)
Family history of premature CV events	13.8 (82/591)
Patients with CV events	16.8 (99/591)
Ischemic heart disease	6.4 (38/591)
Heart failure	6.4 (38/591)
Cerebrovascular accident	5.2 (31/591)
Peripheral arteriopathy	2.2 (13/591)
Patients with carotid plaques	53.6 (317/591)

RA: Rheumatoid arthritis; SD: Standard deviation; Anti-CCP antibodies: Anti-cyclic citrullinated peptide antibodies; BMI: Body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CV: cardiovascular. CRP: C-reactive protein.
[a] At least two determinations at different times were required.
[b] At the time of the study.

2.4. Statistical analysis

Power for the study was calculated using "CaTS -Power Calculator for Two Stage Association Studies" (http://www.sph.umich.edu/csg/abecasis/CaTS/).

Differences in the genotypic and allelic frequencies according to the presence/absence of carotid plaques were calculated by $\chi 2$ or Fisher tests. Haplotypes were constructed using Haploview v4.2 software. Strength of associations were estimated using odds ratios (OR) and 95% confidence intervals (CI). Results were adjusted by sex, age at the time of US study, follow-up time and traditional CV risk factors by logistic regression. To obtain an internal validation, we performed a bootstrap with 1000 replications. The association between *VDR* rs7975232 genotypes and *VDR* haplotypes and inflammatory markers of RA disease, serological markers of the disease as well as according to presence of extra-articular manifestations, bone erosions and rheumatoid shared epitope, was tested using $\chi 2$ and Student-t tests for dichotomous and continuous variables, respectively.

All analyses were performed with STATA statistical software 12/SE (Stata Corp., College Station, TX, USA).

3. Results

The study has statistical power ≥82% to detect OR≥1.4.

First, we analyzed the *VDR* rs731236, *VDR* rs7975232, *VDR* rs1544410 and *VDR* rs2228570 polymorphisms independently. A statistically significant association between *VDR* rs7975232 and