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The angiotensin-like protein 4, apolipoprotein C3, and lipoprotein lipase axis is disrupted in patients with rheumatoid arthritis

Laura de Armas-Rillo¹, Juan Carlos Quevedo-Abeledo², Vanesa Hernández-Hernández³,
Antonia de Vera-González⁴, Alejandra González-Delgado⁴, José A. García-Dopico⁴,
Miguel Á. González-Gay^{5,6,7†} and Iván Ferraz-Amaro^{3*†} 

Abstract

Background: Modulators of triglyceride metabolism include lipoprotein lipase (LPL), angiotensin-like protein 4 (ANGPTL4), and apolipoprotein C-3 (ApoC3). There is evidence on the influence of this triangle of molecules on an increased risk of atherosclerotic cardiovascular disease (CV) in the general population. Patients with rheumatoid arthritis (RA) present changes in lipid profiles and accelerated CV disease. In the present study, we set out to study whether the ANGPTL4, ApoC3, and LPL axis differs in subjects with RA compared to controls. In a further step, we investigated the relationship of this axis with subclinical atherosclerosis in patients with RA.

Methods: Cross-sectional study that included 569 individuals, 323 patients with RA and 246 age-matched controls. ANGPTL4, ApoC3 and LPL, and standard lipid profiles were analyzed in patients and controls. Carotid intima-media thickness (cIMT) and carotid plaques were assessed in RA patients. A multivariable analysis was performed to assess whether the ANGPTL4, ApoC3, and LPL axis was altered in RA and to study its relationship with RA dyslipidemia and subclinical carotid atherosclerosis.

Results: Most lipid profile molecules did not differ between patients and controls. Despite this, and after fully multivariable analysis including CV risk factors, use of statins, and changes in the lipid profile caused by the disease itself, patients with RA showed higher serum levels of ANGPTL4 (beta coef. 295 [95% CI 213–376] ng/ml, $p < 0.001$) and ApoC3 (beta coef. 2.9 [95% CI 1.7–4.0] mg/dl, $p < 0.001$), but lower circulating LPL (beta coef. –174 [95% CI –213 to –135] ng/ml, $p < 0.001$). ANGPTL4 serum levels were positively and independently associated with a higher cIMT in patients with RA after fully multivariable adjustment.

Conclusion: The axis consisting in ANGPTL4, ApoC3, and LPL is disrupted in patients with RA. ANGPTL4 serum levels are positively and independently associated with a higher cIMT in RA patients.

Keywords: Rheumatoid arthritis, Dyslipidemia, Inflammation, Lipoprotein lipase, Angiotensin-like protein 4, Apolipoprotein C3

*Correspondence: iferrazamaro@hotmail.com

†Miguel Á. González-Gay and Iván Ferraz-Amaro shared senior authorship.

³ Division of Rheumatology, Hospital Universitario de Canarias, Tenerife,

Spain

Full list of author information is available at the end of the article

Background

Key molecules involved in the metabolism of triglycerides include lipoprotein lipase (LPL), angiotensin-like protein 4 (ANGPTL4), and apolipoprotein C-3 (ApoC3).



LPL is the primary enzyme that hydrolyzes lipoprotein triglycerides releasing free fatty acids for utilization by and clearance from tissues [1]. Among the endogenous modulators of LPL are ANGPTL4 that inhibits LPL and modulates the uptake of free fatty acids in fasting and fed states [2] and ApoC3 that also inhibits the lipolysis of triglyceride-rich lipoproteins by LPL [3].

There is evidence on the influence of this axis constituted by ANGPTL4-LPL-ApoC3 on an increased risk of atherosclerotic cardiovascular disease (CV) in the general population. It has emerged not only from genetic studies, but also from the relationship of circulating levels of these molecules with CV disease and CV events. For example, loss-of-function mutations in APOC3 were associated with low levels of triglycerides and a reduced risk of ischemic CV disease, and elevated ApoC3 levels are associated with increased triglyceride levels and elevated risk of atherosclerotic CV disease [4]. Besides, truncating mutations that increase LPL activity decrease serum triglyceride levels and the risk of CV disease, whereas mutations that diminish LPL function have been shown to increase serum triglyceride levels [5]. Similarly, carriers of inactivating mutations in ANGPTL4 exhibit lower triglyceride levels and a lower risk of coronary artery disease than non-carriers [6].

Rheumatoid arthritis (RA) has been associated with a modified lipid profile that is considered a consequence of the inflammatory state that accompanies the disease [7]. RA is also known to be associated with a higher incidence of CV events [8]. In the present study, we set out to study whether the ANGPTL4, ApoC3, and LPL axis differs in subjects with RA compared to controls. In a further step, we investigated the relationship of this axis with subclinical atherosclerosis in patients with RA.

Material and methods

Study participants

This was a cross-sectional study that included 569 individuals, 323 patients with RA and 246 age-matched controls. All RA patients were 18 years old or older and fulfilled the 2010 ACR/EULAR diagnostic criteria [9]. They had been diagnosed by rheumatologists and were periodically followed up at rheumatology outpatient clinics. For the purposes of inclusion in the present study, the duration of RA disease was required to be ≥ 1 year. Although anti-tumor necrosis factor-alpha (TNF) treatment has been associated with changes in lipid profiles [10], RA patients undergoing TNF-alpha antagonists or other biologic therapies were not excluded from the present study. Apart from possible statin use, age-matched controls included in the study were required not to have conditions or drug treatment that could influence lipids and were not taking any other lipid-lowering

medications. The controls were community-based, recruited by general practitioners in primary health centers. Moreover, controls with a history of any inflammatory rheumatic diseases were excluded, as well as those with a history of CV disease. None of the controls was receiving glucocorticoids. However, since they are often used in the management of RA, patients taking prednisone, or an equivalent dose ≤ 10 mg/day, were not excluded. As previously mentioned, both patients and controls under statin treatment were allowed to participate in the study. Patients and controls were excluded if they had a history of myocardial infarction, angina, stroke, a glomerular filtration rate < 60 ml/min/1.73 m², a history of cancer, or any other chronic disease, or evidence of active infection. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent.

Data collection and laboratory assessments

Individuals included in the study completed a CV risk factor and medication use questionnaire and underwent a physical examination. Weight, height, body mass index, abdominal circumference, and systolic and diastolic blood pressure (measured with the participant in a supine position) were assessed under standardized conditions. Information regarding smoking status (current smoker versus non-smoker) and hypertension was obtained from the questionnaire. Medical records were reviewed to ascertain specific diagnoses and medications. Disease activity in patients with RA was measured using the Disease Activity Score (DAS28) in 28 joints [11], the Clinical Disease Activity Index (CDAI) [12], and the Simple Disease Activity Index (SDAI) [13]. Disease disability was measured through the Health Assessment Questionnaire (HAQ) score [14].

Serum LPL mass was measured using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) (Biomatik, Cambridge, Canada). The assay sensitivity (minimum detectable concentration) for LPL was 0.58 ng/ml. Precision was estimated as an inter-assay $< 15\%$ and an intra-assay $< 10\%$ coefficients of variability. ANGPTL4 was assessed through R&D Duoset ELISA (Abingdon, UK). ANGPTL4 minimum detectable values were 1.3 ng/ml and both inter- and intra-assay coefficients of variability were $< 10\%$. For the detection of ApoC3, an ELISA kit was used (Elabscience, USA). No significant cross-reactivity or interference between human ApoC3 and analogues is observed with this kit. Both intra- and inter-coefficients of variability are $< 10\%$ for this assay. Cholesterol, triglycerides, and HDL cholesterol were measured using the enzymatic colorimetric

assay. LDL cholesterol was calculated using the Friedewald formula. A standard technique was used to measure the erythrocyte sedimentation rate (ESR) and high-sensitivity C-reactive protein (CRP).

Carotid ultrasound assessment

Carotid ultrasound examination was used to assess cIMT in the common carotid artery and to detect focal plaques in the extracranial carotid tree in patients with RA [15]. A commercially available scanner, the Esaote Mylab 70 (Genoa, Italy), equipped with a 7–12-MHz linear transducer and an automated software-guided radiofrequency technique, Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland), was used for this purpose. As previously reported [15], based on the Mannheim consensus, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb and internal carotid artery) were defined as follows: a focal protrusion in the lumen measuring at least cIMT >1.5 mm, a protrusion at least 50% greater than the surrounding cIMT, or arterial lumen encroaching >0.5 mm [16].

Statistical analysis

Demographic and clinical characteristics in patients with RA and controls were described as mean (standard deviation) or percentages for categorical variables. For non-normally distributed continuous variables, data were expressed as median and interquartile range (IQR). Univariable differences between patients and controls were assessed through the Student *T*, Mann–Whitney *U*, chi-square, or Fisher exact tests according to normal distribution or number of subjects. Differences between patients and controls regarding their lipid profiles were assessed through multivariable regression analysis. Confounding variables in this analysis were those with a statistical *p* value <0.20 for those differences in traditional CV risk factors between patients and controls. To neutralize the effect of other modifications on the lipid profile, an additional multivariable analysis was constructed, adding to the model those differences in lipid-related molecules between patients and controls with a *p* value <0.20. Demographic- and disease-related data associations with ANGPTL4, LPL, and ApoC3 are shown using univariable linear regression. Mediation analysis [17] was used to further understand the associations of RA with changes in ANGPTL4, LPL, and ApoC3. Therefore, an attempt was made to assess whether any of these molecules was responsible for the change of the others. Therefore, in these significant relationships, it was ruled out that there was no mediation of another molecule. In cases where the mediation was not significant, it was established that the relationship is direct and not mediated by another molecule. Mediation analysis estimated two models as

previously described [18]: a model for the mediator conditional on exposure and covariates, and another model for the outcome conditional on exposure, the mediator and covariates. All the analyses used a 5% two-sided significance level and were performed using SPSS software, version 25 (IBM, Armonk, NY, USA), and Stata software, version 17/SE (StataCorp, College Station, TX, USA). *p* values <0.05 were considered statistically significant.

Results

Demographic and disease-related data

A total of 569 participants, 323 patients with RA and 246 controls, were included in this study. Demographic- and disease-related characteristics of the participants are shown in Table 1. Patients and controls showed no differences in age (54 ± 16 vs. 55 ± 10 years, $p=0.62$) nor in the frequency of the CV risk factors smoking, obesity, hypertension, and type 2 diabetes mellitus. Similarly, the use of statins did not differ between patients and controls (32 vs. 27%, $p=0.19$). Contrary, patients with RA were more frequently female and had a lower BMI and abdominal circumference. However, for these differences, the size effect was found to be small.

The median duration of the disease in RA patients was 8 (IQR 4–15) years. Sixty-seven percent of patients were positive for rheumatoid factor and 55% for ACPA. Disease activity measured by DAS28-ESR showed a value of 2.32 ± 1.19 . Thirty-eight percent of the patients were being treated with prednisone and 86% were taking at least one conventional DMARD in any of its types, being methotrexate the most widely used (74%). The frequency of use of other treatments is shown in Table 1. Additionally, the mean values of CRP and ESR at the time of the study were respectively 2.6 (IQR 1.3–6.1) mg/l and 25 (IQR 12–45) mm/1st hour. The cIMT of patients with RA was 698 ± 137 , and 38% of these presented carotid plaque on ultrasound examination. Additional information on patients and controls is shown in Table 1.

Multivariable analysis of the differences in lipid profiles between RA patients and controls

In general, lipid profile did not differ between RA patients and controls in the univariable analysis. Only HDL cholesterol was found to be significantly higher in RA patients compared to controls (56 ± 15 mg/dl vs. 52 ± 15 mg/dl, $p=0.001$). Despite this, ANGPTL4, ApoC3, and LPL were found to be different in patients with RA compared to controls. In this sense, in the univariable analysis, ANGPTL4 (151 [IQR 90–290] ng/ml vs. 73 [IQR 47–121] ng/ml, $p<0.001$) and ApoC3 (8.8 ± 5.2 mg/dl vs. 6.2 ± 5.6 , $p<0.001$) were found to be significantly higher in patients with RA. Contrary, in this univariable analysis, LPL was found to be lower in RA when compared to

Table 1 Demographics, cardiovascular risk factors, and disease-related data in subjects

| | Controls (n=246) | RA (n=323) | p |
|---|---------------------|---------------------|--------|
| Age, years | 54 ± 16 | 55 ± 10 | 0.62 |
| Female, n (%) | 162 (66) | 263 (81) | <0.001 |
| BMI, kg/m ² | 30 ± 3 | 28 ± 5 | <0.001 |
| Abdominal circumference, cm | 100 ± 6 | 97 ± 13 | <0.001 |
| Cardiovascular data | | | |
| CV risk factors, n (%) | | | |
| Current smoker | 47 (19) | 64 (20) | 0.83 |
| Obesity | 67 (27) | 104 (32) | 0.20 |
| Hypertension | 92 (37) | 101 (31) | 0.13 |
| Diabetes mellitus | 42 (17) | 42 (13) | 0.16 |
| Blood pressure, mm Hg | | | |
| Systolic | 139 ± 8 | 133 ± 19 | <0.001 |
| Diastolic | 84 ± 5 | 81 ± 12 | 0.001 |
| Statins, n (%) | 66 (27) | 104 (32) | 0.19 |
| Disease-related data | | | |
| CRP at time of study, mg/l | 2.0 (1.1–4.6) | 2.6 (1.3–6.1) | 0.023 |
| Disease duration, years | | 8 (4–15) | |
| ESR at time of study, mm/1° hour | | 25 (12–45) | |
| Rheumatoid factor, n (%) | | 218 (67) | |
| ACPA, n (%) | | 179 (55) | |
| DAS28-ESR | | 2.32 ± 1.19 | |
| DAS28-PCR | | 2.54 ± 1.05 | |
| SDAI | | 12 (6–20) | |
| CDAI | | 8 (4–14) | |
| HAQ | | 0.750 (0.250–1.250) | |
| Current drugs, n (%) | | | |
| Prednisone | | 123 (38) | |
| Prednisone doses, mg/day | | 5 (3–5) | |
| NSAIDs | | 154 (48) | |
| DMARDs | | 277 (86) | |
| Methotrexate | | 238 (74) | |
| Leflunomide | | 72 (22) | |
| Hydroxychloroquine | | 37 (11) | |
| Sulfasalazine | | 25 (8) | |
| Anti-TNF therapy | | 68 (21) | |
| Tocilizumab | | 17 (5) | |
| Rituximab | | 6 (2) | |
| Abatacept | | 6 (2) | |
| JAK inhibitors | | 7 (2) | |
| Historical disease-related data | | | |
| History of extraarticular manifestations, n (%) | | 24 (7) | |
| Erosions, n (%) | | 113 (35) | |
| CRP at time of disease diagnosis, mg/l | | 6.4 (2.5–17.1) | |
| CRP >3 at time of disease diagnosis, n (%) | | 152 (47) | |
| ESR at disease diagnosis, mm/1° hour | | 31 ± 19 | |
| Subclinical atherosclerosis | | | |
| Carotid IMT, microns | | 698 ± 137 | |
| Carotid plaques, n (%) | | 124 (38) | |

Data represent means ± SD or median (IQR) when data were not normally distributed

CV cardiovascular, LDL low-density lipoprotein, HDL high-density lipoprotein, CRP C-reactive protein, NSAID nonsteroidal anti-inflammatory drugs, DMARD disease-modifying antirheumatic drug, TNF tumor necrosis factor, Obesity, ESR erythrocyte sedimentation rate, BMI body mass index, DAS28 Disease Activity Score in 28 joints, ACPA anti-citrullinated protein antibodies, CDAI Clinical Disease Activity Index, SDAI Simple Disease Activity Index, HAQ Health Assessment Questionnaire

controls (99 [IQR 60–156] ng/ml vs. 230 [IQR 183–328] ng/ml, $p < 0.001$).

In the full adjustment model (model 1 in Table 2), most of these differences between the two populations were maintained with some exceptions. In this sense, apolipoprotein A1 was found to be lower in patients with RA (beta coef. -9 [95% CI -15 to -4] mg/dl, $p = 0.001$) and the difference in HDL cholesterol serum levels between populations was lost. Remarkably, ANGPTL4 and ApoC3 remain significantly upregulated and LPL decreased in patients with RA compared to controls.

Because lipid-related molecules are interrelated (they share metabolic pathways and it is not easy to separate the effect of one from the others), we performed a multivariable analysis adjusting for demographics and CV risk factors plus all the lipid-related molecules that were found to be different between patients and controls in the univariable analysis (model 2 in Table 2). Because of collinearity, lipid molecules derived from a formula were excluded from the regression models (LDL cholesterol, LDL:HDL ratio, non-HDL cholesterol, apoB:apoA, and atherogenic index). In this final multivariable model, ANGPTL4, ApoC3, and LPL were found to be different in RA patients compared to controls: ANGPTL4 and ApoC3 were found to be higher and LPL lower.

Figure 1 shows a graphical representation of the differences in main lipid molecules between patients and controls.

Disease-related data relation with angiotensin-like protein 4, apolipoprotein C3, and lipoprotein lipase axis

Disease activity scores were not related to the ANGPTL4, ApoC3, and LPL molecules (Table 3). However, some associations were found with acute phase reactants. In this sense, the ESR was negatively correlated with ANGPTL4 (beta coef. -3 [95% CI -6 to -1] ng/ml, 0.020) but positively correlated with LPL (beta coef. 1 [95% CI $0-3$] ng/ml, $p = 0.033$). Besides, CRP was positively and significantly associated with ApoC3 (beta coef. 0.05 [95% CI $0.01-0.10$] mg/dl, $p = 0.013$). Similarly, patients on hydroxychloroquine, sulfasalazine, and anti-TNF therapies showed higher and significant serum levels of LPL (Table 3).

Relationship of angiotensin-like protein 4, apolipoprotein C3, and the lipoprotein lipase axis with subclinical atherosclerosis in patients with RA

Age, male gender, traditional CV risk factors, the use of statins, and some lipid profile-related molecules like triglycerides, LDL cholesterol, and lipoprotein (a) were

Table 2 Multivariable analysis of the differences in lipid profile and angiotensin-like protein 4, apolipoprotein C3, and lipoprotein lipase serum levels between RA patients and controls

| | Controls (n=246) | RA patients (n=323) | Univariable model <i>p</i> | Model #1 beta coef. (95% CI), <i>p</i> | Model #2 beta coef. (95% CI), <i>p</i> |
|--------------------------------------|------------------|---------------------|-------------------------------|---|---|
| Lipid profile | | | <i>p</i> | | |
| Cholesterol, mg/dl | 198 ± 45 | 203 ± 38 | 0.12 | 5 (−2–12), 0.18 | 14 (6–23), 0.001 |
| Triglycerides, mg/dl | 144 ± 68 | 149 ± 88 | 0.44 | | |
| HDL cholesterol, mg/dl | 52 ± 15 | 56 ± 15 | 0.001 | 1 (−1–4), 0.29 | |
| LDL cholesterol, mg/dl | 117 ± 37 | 117 ± 33 | 0.96 | | |
| LDL:HDL cholesterol ratio | 2.38 ± 0.89 | 2.25 ± 0.94 | 0.10 | 0.01 (−0.15–0.17), 0.95 | |
| Non-HDL cholesterol, mg/dl | 146 ± 40 | 147 ± 38 | 0.72 | | |
| Lipoprotein (a), mg/dl | 38 (14–101) | 33 (11–111) | 0.99 | | |
| Apolipoprotein A1, mg/dl | 174 ± 39 | 170 ± 29 | 0.12 | −9 (−15 to −4), 0.001 | −9 (−16 to −3), 0.006 |
| Apolipoprotein B, mg/dl | 104 ± 29 | 107 ± 47 | 0.38 | | |
| Apo B:Apo A ratio | 0.61 ± 0.18 | 0.64 ± 0.25 | 0.097 | 0.05 (0.01–0.09), 0.008 | – |
| Atherogenic index | 4.01 ± 1.12 | 3.88 ± 1.33 | 0.22 | | |
| Angiotensin-like protein 4, ng/ml | 73 (47–121) | 151 (90–290) | <0.001 | 215 (147–284), <0.001 | 295 (213–376), <0.001 |
| Apolipoprotein C3, mg/dl | 6.2 ± 5.6 | 8.8 ± 5.2 | <0.001 | 2.9 (1.9–3.9), <0.001 | 2.9 (1.7–4.0), <0.001 |
| Lipoprotein lipase, ng/ml | 230 (183–328) | 99 (60–156) | <0.001 | −121 (−160 to −82), <0.001 | −174 (−213 to −135), <0.001 |

Data represent means ± standard deviation or median (interquartile range) when data were not normally distributed

HDL high-density lipoprotein, LDL low-density lipoprotein

Model #1: adjusted for sex, body mass index, abdominal circumference, hypertension, diabetes, C-reactive protein, and statins (variables with a *p* value < 20 difference between patients and controls)

Model #2: adjusted for model #1 + rest of lipid molecules (with a *p* value < 0.20 in the univariate analysis) other than the one that is compared

Because collinearity LDL cholesterol, LDL:HDL ratio, non-HDL cholesterol, apoB:apoA, and atherogenic index were excluded of the multivariable analyses in model 2

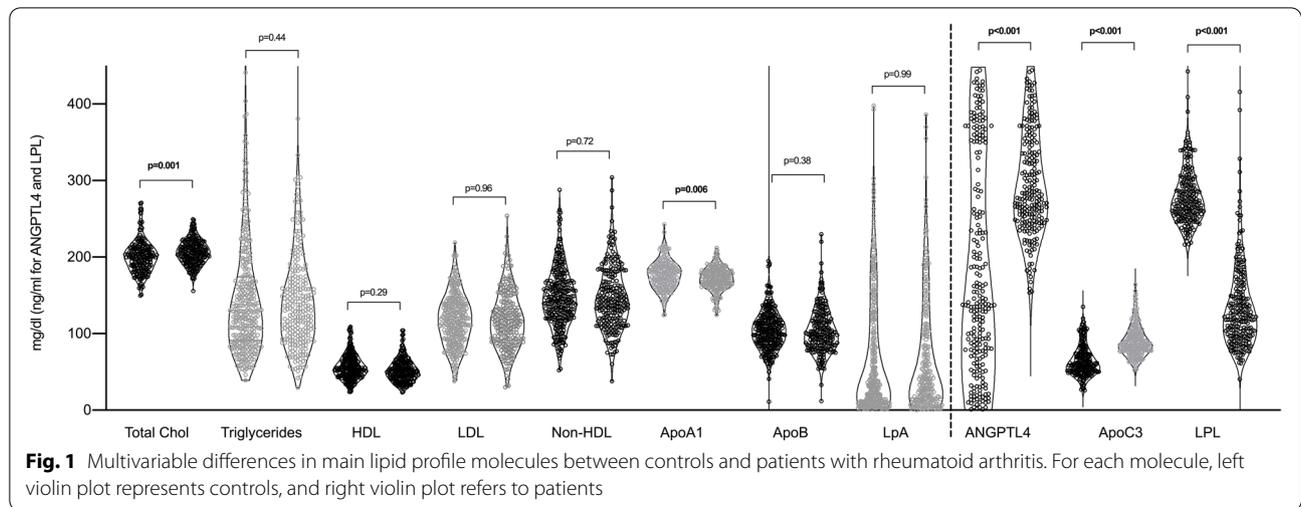


Fig. 1 Multivariable differences in main lipid profile molecules between controls and patients with rheumatoid arthritis. For each molecule, left violin plot represents controls, and right violin plot refers to patients

Table 3 Disease-related data relation with angiotensin-like protein 4, apolipoprotein C3, and lipoprotein lipase axis

| | ANGPTL4, ng/ml beta coef. (95% CI), p | Apo C3, mg/dl | LPL, ng/ml |
|--|--|--------------------------------|----------------------------|
| Disease-related data | | | |
| Disease duration, years | -7 (-14-0), 0.058 | 0.04 (-0.04-0.12), 0.32 | 4 (0-7), 0.025 |
| CRP, mg/l | 1 (-3-5), 0.67 | 0.05 (0.01-0.10), 0.013 | 1 (-1-3), 0.46 |
| ESR, mm/1st hour | -3 (-6 to -1), 0.020 | -0.00 (-0.03-0.03), 0.93 | 1 (0-3), 0.033 |
| Rheumatoid factor | 18 (-116-153), 0.79 | 0.2 (-1.2-1.6), 0.77 | 35 (-28-99), 0.27 |
| ACPA | 117 (-12-247), 0.075 | 0.2 (-1.2-1.5), 0.80 | 61 (-5-128), 0.068 |
| DAS28-ESR | -19 (-93-16), 0.17 | -0.4 (-1.0-0.2), 0.18 | 7 (-18-32), 0.58 |
| DAS28-PCR | -15 (-75-45), 0.63 | -0.4 (-1.0-0.3), 0.25 | 2 (-26-30), 0.88 |
| SDAI | 0 (-3-4), 0.88 | 0.03 (-0.01-0.06), 0.17 | 0 (-1-2), 0.70 |
| CDAI | -2 (-10-6), 0.66 | -0.08 (-0.16-0.01), 0.069 | 0 (-3-4), 0.90 |
| HAQ | -25 (-128-77), 0.62 | -0.3 (-1.4-0.8), 0.60 | 52 (9-94), 0.017 |
| Current drugs | | | |
| Prednisone | 22 (-105-149), 0.73 | -0.1 (-1.4-1.3), 0.94 | -10 (-69-49), 0.74 |
| Prednisone, mg/day | 16 (-28-59), 0.47 | -0.2 (-0.5-0.1), 0.15 | 1 (-6-7), 0.87 |
| NSAIDs | 21 (-101-144), 0.73 | -0.4 (-1.7-0.8), 0.50 | 9 (-48-66), 0.77 |
| DMARDs | -20 (-181-140), 0.80 | -0.4 (-2.1-1.3), 0.63 | 29 (-53-110), 0.49 |
| Methotrexate | 25 (-105-155), 0.71 | 0.4 (-0.9-1.8), 0.53 | 41 (-24-106), 0.21 |
| Leflunomide | -50 (-218-118), 0.56 | 0.4 (-1.4-2.2), 0.67 | 51 (-17-119), 0.14 |
| Hydroxychloroquine | 257 (-229-744), 0.30 | -3.5 (-8.7-1.6), 0.18 | 116 (30-202), 0.008 |
| Salazopyrin | 150 (-819-1120), 0.76 | -3.6 (-13.9-6.6), 0.49 | 143 (41-245), 0.006 |
| Anti-TNF therapy | -11 (-182-159), 0.90 | -0.2 (-2.1-1.6), 0.79 | 101 (33-169), 0.004 |
| Tocilizumab | 53 (-204-311), 0.68 | -1.3 (-4.0-1.4), 0.35 | 9 (-118-137), 0.89 |
| Rituximab | -202 (-601-197), 0.32 | 0.0 (-4.3-4.2), 0.99 | -23 (-228-181), 0.82 |
| Abatacept | -182 (-581-217), 0.37 | 3.9 (-0.3-8.1), 0.069 | -58 (-263-146), 0.58 |
| JAK inhibitors | 347 (-22-715), 0.065 | -3.6 (-7.5-0.3), 0.072 | -39 (-216-139), 0.67 |
| History of extraarticular manifestations | -136 (-349-76), 0.21 | 0.4 (-1.9-2.7), 0.76 | 37 (-54-127), 0.42 |
| Erosions | 17 (-122-157), 0.81 | -1.0 (-2.4-0.4), 0.16 | 57 (-7-121), 0.081 |

NSAID nonsteroidal anti-inflammatory drugs, DMARD disease-modifying antirheumatic drug, TNF tumor necrosis factor, Obesity, ESR erythrocyte sedimentation rate, DAS28 Disease Activity Score in 28 joints, CRP C-reactive protein, ACPA anti-citrullinated protein antibodies, CDAI Clinical Disease Activity Index, SDAI Simple Disease Activity Index, HAQ Health Assessment Questionnaire

significantly associated with the presence of carotid plaque or a higher cIMT (Table 4). Although ANGPTL4 and LPL were not related with carotid plaque, ApoC3 was associated with the presence of carotid plaque in patients with RA in the univariable analysis. However, this association was lost after multivariable analysis.

Regarding cIMT, ApoC3 and LPL were not associated with cIMT. Contrary, a significant association between ANGPTL4 and cIMT was found after full multivariable

regression analysis (beta coef. 0.05 [95% CI 0.02–0.08] microns, $p < 0.001$) (Table 4).

Mediation analysis and hypothetical pathways of disruption of the axis

As previously mentioned, patients with RA had higher serum levels of ANGPTL4 and ApoC3 but lower circulating LPL (Fig. 2). ApoC3 and LPL (Rho

Table 4 Relation of angiotensin-like protein 4, apolipoprotein C3, and lipoprotein lipase axis to subclinical atherosclerosis in RA patients

| | Carotid plaque | | | cIMT, microns | | | | |
|-----------------------------------|----------------|---------------|----------|------------------|----------|---------------------------|-------------------------------|--|
| | Univariable | | <i>p</i> | Adjusted | | Univariable | Adjusted | |
| | No=199 | Yes=124 | | OR (95% CI) | <i>p</i> | | beta coef. (95% CI), <i>p</i> | |
| Age, years | 51 ± 10 | 61 ± 8 | <0.001 | | | 7 (6–8), <0.001 | | |
| Female, <i>n</i> (%) | 175 (88) | 88 (71) | <0.001 | | | –88 (–126 to –50), <0.001 | | |
| BMI, kg/m ² | 28 ± 5 | 28 ± 5 | 0.51 | | | 2 (–8–5), 0.14 | | |
| Abdominal circumference, cm | 97 ± 14 | 97 ± 11 | 0.71 | | | 2 (1–3), 0.004 | | |
| Cardiovascular data | | | | | | | | |
| CV risk factors, <i>n</i> (%) | | | | | | | | |
| Current smoker | 37 (19) | 27 (22) | 0.49 | | | 4 (–34–42), 0.84 | | |
| Obesity | 67 (34) | 37 (30) | 0.47 | | | 21 (–10–54), 0.18 | | |
| Hypertension | 49 (25) | 52 (42) | 0.001 | | | 61 (29–93), <0.001 | | |
| Diabetes mellitus | 17 (9) | 25 (20) | 0.003 | | | 87 (43–132), <0.001 | | |
| Blood pressure, mm Hg | | | | | | | | |
| Systolic | 130 ± 18 | 138 ± 19 | 0.001 | | | 2 (1–3), <0.001 | | |
| Diastolic | 81 ± 12 | 83 ± 11 | 0.077 | | | 1 (0.3), 0.061 | | |
| Statins, <i>n</i> (%) | 46 (23) | 57 (46) | <0.001 | | | 51 (20–83), 0.002 | | |
| Lipid profile | | | | | | | | |
| Cholesterol, mg/dl | 205 ± 37 | 201 ± 39 | 0.42 | | | 0.2 (–0.2–0.6), 0.24 | | |
| Triglycerides, mg/dl | 138 ± 82 | 168 ± 93 | 0.003 | | | 0.1 (–0.0–0.3), 0.13 | | |
| HDL cholesterol, mg/dl | 57 ± 15 | 55 ± 16 | 0.50 | | | –0.7 (–1.7–0.3), 0.17 | | |
| LDL cholesterol, mg/dl | 121 ± 32 | 112 ± 35 | 0.030 | | | 0.3 (–0.2–0.7), 0.24 | | |
| LDL:HDL cholesterol ratio | 2.30 ± 0.97 | 2.18 ± 0.90 | 0.24 | | | 12 (–4–28), 0.14 | | |
| Non-HDL cholesterol, mg/dl | 148 ± 39 | 146 ± 38 | 0.61 | | | 0.3 (–0.0–0.7), 0.085 | | |
| Lipoprotein (a), mg/dl | 29 (10–87) | 38 (14–132) | 0.018 | | | 0.0 (–0.2–0.2), 0.74 | | |
| Apolipoprotein A1, mg/dl | 170 ± 29 | 171 ± 30 | 0.70 | | | 0.0 (–0.5–0.5), 0.98 | | |
| Apolipoprotein B, mg/dl | 108 ± 57 | 104 ± 24 | 0.51 | | | 0.2 (–0.2–0.5), 0.35 | | |
| Apo B:Apo A ratio | 0.65 ± 0.28 | 0.63 ± 0.17 | 0.42 | | | 36 (–25–97), 0.25 | | |
| Atherogenic index | 3.87 ± 1.38 | 3.89 ± 1.25 | 0.92 | | | 9 (–3–20), 0.13 | | |
| Axis | | | | | | | | |
| Angiotensin-like protein 4, ng/ml | 143 (85–281) | 161 (104–451) | 0.15 | 1.00 (0.99–1.00) | 0.14 | 0.07 (0.03–0.10), <0.001 | 0.05 (0.02–0.08), <0.001 | |
| Apolipoprotein C3, mg/dl | 8.3 ± 4.7 | 9.8 ± 5.9 | 0.028 | 1.03 (0.97–1.09) | 0.41 | 2.9 (–0.3–6.2), 0.077 | 0.4 (–2.6–3.4), 0.78 | |
| Lipoprotein lipase, ng/ml | 91 (53–149) | 108 (76–166) | 0.34 | | | 0.03 (–0.03–0.09), 0.26 | 0.01 (–0.04–0.6), 0.73 | |

BMI body mass index, CV cardiovascular, HDL high-density lipoprotein, LDL low-density lipoprotein, OR odds ratio, cIMT carotid intima-media thickness

Carotid plaque and cIMT are dependent variables. Multivariable logistic regression—odds ratios—is adjusted for age, gender, hypertension, diabetes mellitus, statins, triglycerides, LDL cholesterol and lipoprotein (a). Multivariable linear regression is adjusted for age, gender, abdominal circumference, hypertension, diabetes mellitus, statins, triglycerides, and HDL cholesterol

Spearman=0.188, $p=0.003$) and ANGPTL4 and LPL (Rho Spearman=0.242, $p<0.001$) serum levels correlated between them (discontinued arrows). This was not the case of the correlation between ApoC3 and ANGPTL4, which did not show a statistical significance (Rho Spearman=-0.050, $p=0.44$). Figure 2 shows a hypothetical representation of the relationship of these molecules.

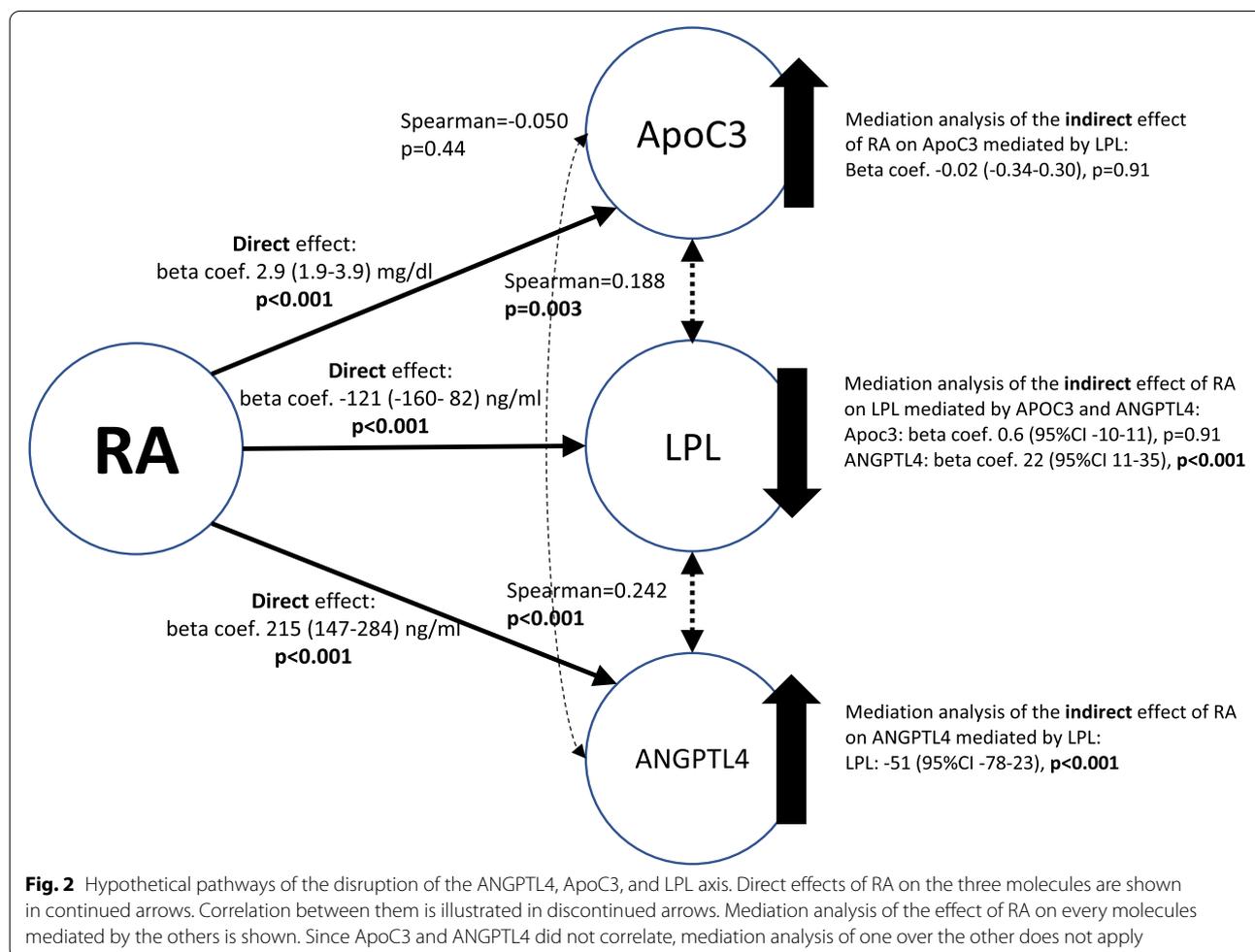
As we were interested in evaluating whether the disturbance of the axis molecules was interrelated, or the disruption of one molecule was the consequence of the modification of another, we performed a mediation analysis. In this sense, direct and indirect effects of RA over each molecule were analyzed studying the mediation effect of the other two. Since ApoC3 and ANGPTL4 were not correlated, the study of the mediation effect of RA on each of them mediated for the other did not apply. The mediation analyses of the indirect effect of RA on ApoC3 mediated by LPL and on LPL mediated by ApoC3 were not significant (Fig. 2). In contrast, the indirect effects of RA on ANGPTL4 mediated by LPL and on LPL

mediated by ANGPTL4 were significant but small in size. Furthermore, the analysis of the direct effect of RA over the three molecules remain statistically significant when mediation analysis variables were included in the multi-variable models (Fig. 2). All the mediation analyses were evaluated adjusting for the same variables of model 1 of Table 2.

Discussion

Our study is the first to analyze the key molecules related to triglyceride metabolism in RA. According to our results, the axis constituted by ANGPTL4, ApoC3, and LPL is disrupted in RA and related to subclinical CV disease in these patients. Our approach focuses on how the inflammatory state produced by RA modifies the lipid profile and how, in turn, it can influence the atherosclerotic burden observed in patients with RA.

In the current work, most of the lipid profile molecules did not differ between patients and controls. This



means that inflammatory dyslipidemia, which has been described in patients with RA [7], was not present in our cohort. It may be because most of the patients recruited in our study had low or moderate disease activity. This reinforces our hypothesis, since, for this reason, the disruption in the ANGPTL4, ApoC3, and LPL axis found in our study cannot be attributed to differences in the lipid profile between patients and controls. In this regard, such a disruption of the ANGPTL4-LPL-ApoC3 axis in RA patients compared to controls was found to be significant after a fully multivariable analysis that included traditional CV risk factors and other lipid molecules.

The positive regulation of ApoC3 in RA found in our study has been previously described. Regarding this, a study in 94 RA patients and 79 controls showed that the serum concentration of ApoC3 was found to be higher in patients compared to controls [19]. This is of potential relevance since ApoC3 has been recognized as a link between atherogenic and inflammatory processes not only in the general population [20] but also in subjects with RA. In this sense, in a study of 152 patients with RA who had a coronary artery calcium score evaluated at baseline and at year 3, ApoC3 was found to be significantly elevated in progressors compared to non-progressors [21]. In our study, ApoC3 was associated with carotid plaque and cIMT in the univariable analysis, but this association did not reach statistical significance after multivariable adjustment.

In a small study of 17 women with RA and 16 age- and sex-matched controls, LPL mass and activity levels were significantly lower in RA patients [22]. This is consistent with our study, which also found a decrease in LPL. Moreover, in our work, we found a positive relationship between LPL and ESR but not with disease activity scores. However, the RA patients in our series showed reduced LPL levels after multivariate adjustment, reinforcing the claim that the disease itself may be responsible for the LPL decrease.

ANGPTL4 was positively associated with cIMT in our work which is consistent with previous studies that showed a link between ANGPTL4 serum levels and atherosclerosis. In this regard, serum ANGPTL4 levels in a series of 712 patients with stroke due to large artery atherosclerosis disease were significantly higher than those in 828 controls after adjustment for other risk factors [23]. Pathological studies indicate that cIMT mainly represents hypertensive medial hypertrophy or thickening of smooth muscles in the media. In contrast, carotid plaques probably represent a later stage of atherogenesis related to inflammation, endothelial dysfunction, oxidative stress, and smooth muscle cell proliferation [24]. Therefore, since cIMT is biologically distinct from plaque and represents a different process, it is possible that in

RA patients ANGPTL4 may have more influence on cIMT than on plaque development.

Given that the three axis molecules evaluated in our study are interrelated and mutually modified, we performed a mediation analysis to clarify whether the effect of the disease in each of them was mediated by the alteration of the others. However, it did not yield statistically significant results or, when significant, the indirect (mediated) effect was small. This means that the modification in the three molecules may be because of the disease itself and does not seem to be produced from the modification that each of the three molecules can exert on the others.

We recognize as a potential limitation of our study that we measured LPL serum levels and not its enzymatic activity. However, although serum LPL is catalytically inactive, its mass reflects the level of systemic LPL biosynthesis and there is an excellent correlation between mass and LPL activity as reported elsewhere [25].

Conclusion

In conclusion, the axis related to triglyceride metabolism constituted by ANGPTL4, ApoC3, and LPL is different in patients with RA and healthy controls. Since the serum levels of ANGPTL4 are related to cIMT, this molecule may represent a biomarker of subclinical atherosclerosis in these patients. Our findings may contribute to improving the understanding of the relationship between inflammatory dyslipidemia and CV disease in patients with RA.

Abbreviations

ACPA: Anti-citrullinated peptide/protein antibody; ANGPTL4: Angiopoietin-like protein 4; ApoC3: Apolipoprotein C-3; BMI: Body mass index; CDAI: Clinical Disease Activity Index; CI: Confidence of interval; cIMT: Carotid intima-media thickness; CRP: C-reactive protein; CV: Cardiovascular; DAS28: Disease Activity Score in 28 joints; DMARD: Disease-modifying antirheumatic drug; ELISA: Enzyme-linked immunosorbent assay; ESR: Erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire; HDL: High-density lipoprotein; IQR: Interquartile range; LDL: Low-density lipoprotein; LPL: Lipoprotein lipase; NSAID: Nonsteroidal anti-inflammatory drug; OR: Odds ratio; RA: Rheumatoid arthritis; SD: Standard deviation; SDAI: Simplified Disease Activity Index; TNF- α : Tumor necrosis factor- α .

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Authors' contributions

IFA, MAGG: conception, design, and interpretation of the data; LAR, JCQA, VHH, AVG, AGD, JAGD: acquisition of the data. All the authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. The authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed in the present study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the institutional review committees at Hospital Universitario de Canarias and Hospital Universitario Doctor Negrín, and all subjects provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Nevertheless, Professor MA Gonzalez-Gay and Dr. Iván Ferraz-Amaro would like to acknowledge that they received grants/research supports from Abbott, MSD, Jansen, and Roche and received consultation fees from company-sponsored speakers bureaus associated with Abbott, Pfizer, Roche, Sanofi, Sobi, Amgen, Celgene, and MSD.

Author details

¹Division of Health Sciences, Universidad Europea de Canarias, Tenerife, Spain. ²Division of Rheumatology, Hospital Doctor Negrín, Las Palmas de Gran Canaria, Spain. ³Division of Rheumatology, Hospital Universitario de Canarias, Tenerife, Spain. ⁴Division of Central Laboratory, Hospital Universitario de Canarias, Tenerife, Spain. ⁵Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, Hospital Universitario Marqués de Valdecilla, IDIVAL, Santander, Spain. ⁶Division of Rheumatology, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, Santander, Spain. ⁷Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

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References

- Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity [Internet]. *Am J Physiol Endocrinol Metab*. 2009; [cited 2021 Jun 13]. Available from: <https://pubmed.ncbi.nlm.nih.gov/19318514/>.
- Köster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, et al. Transgenic angiotensin-like (Angptl)4 overexpression and targeted disruption of Angptl4 and Angptl3: regulation of triglyceride metabolism. *Endocrinology*. 2005; [cited 2021 Jun 13];146:4943–50. Available from: <https://pubmed.ncbi.nlm.nih.gov/16081640/>.
- Bobik A. Editorial: Apolipoprotein CIII and atherosclerosis; beyond effects on lipid metabolism [Internet]. *Circulation*. 2008; [cited 2021 Jun 13]. p. 702–4. Available from: <https://pubmed.ncbi.nlm.nih.gov/18695202/>.
- Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32–41 [cited 2021 Jun 28]. Available from: <https://pubmed.ncbi.nlm.nih.gov/24941082/>.
- Jensen MK, Rimm EB, Rader D, Schmidt EB, Sørensen TIA, Vogel U, et al. S447X variant of the lipoprotein lipase gene, lipids, and risk of coronary heart disease in 3 prospective cohort studies. *Am Heart J*. 2009;157:384–90 [cited 2021 Jun 28]. Available from: <https://pubmed.ncbi.nlm.nih.gov/19185650/>.
- Dewey FE, Gusarova V, O'Dushlaine C, Gottesman O, Trejos J, Hunt C, et al. Inactivating variants in ANGPTL4 and risk of coronary artery disease. *N Engl J Med*. 2016;374:1123–33. [cited 2021 Jun 28]. Available from: <https://doi.org/10.1056/nejmoa1510926>.
- Quevedo-Abeledo JC, Sánchez-Pérez H, Tejera-Segura B, de Armas-Rillo L, Armas-González E, Machado JD, et al. Differences in HDL-cholesterol efflux capacity between patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Care Res (Hoboken)*. 2020; [cited 2021 Jun 28]; Available from: <https://pubmed.ncbi.nlm.nih.gov/32770725/>.
- Corrales A, Vegas-Revenga N, Rueda-Gotor J, Portilla V, Atienza-Mateo B, Blanco R, et al. Carotid plaques as predictors of cardiovascular events in patients with rheumatoid arthritis. Results from a 5-year-prospective follow-up study. *Semin Arthritis Rheum* [Internet]. 2020;50:1333–8 [cited 2021 Jun 28]. Available from: <https://pubmed.ncbi.nlm.nih.gov/32416998/>.
- Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative [Internet]. *Arthritis Rheum*. 2010. 2010:2569–81 [cited 2018 Nov 10]. p. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20872595>.
- Daien CI, Dunny Y, Barnette T, Daurès JP, Combe B, Morel J. Effect of TNF inhibitors on lipid profile in rheumatoid arthritis: a systematic review with meta-analysis. *Ann Rheum Dis* [Internet]. *Ann Rheum Dis*. 2012;71:862–8 [cited 2021 Jun 14]. Available from: <https://pubmed.ncbi.nlm.nih.gov/22267329/>.
- Prevo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL, et al. *Arthritis Rheum*. 1995;38:44–8 [cited 2019 Jul 3]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7818570>.
- Smolen JS, Breedveld FC, Schiff MH, Kalden JR, Emery P, Eberl G, et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology (Oxford)*. 2003;42:244–57 [cited 2018 Nov 10]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12595618>.
- Aletaha D, Smolen J. The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI): a review of their usefulness and validity in rheumatoid arthritis. *Clin Exp Rheumatol*. [cited 2019 Aug 19];23:S100–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16273793>.
- Pincus T, Swearingen C, Wolfe F. Toward a multidimensional Health Assessment Questionnaire (MDHAQ): assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. *Arthritis Rheum*. 1999;42:2220–30 [cited 2019 Jul 3]. Available from: <http://doi.wiley.com/10.1002/1529-0131%28199910%2942%3A10%3C2220%3A%3AAID-ANR26%3E3.0.CO%3B2-5>.
- Corrales A, González-Juanatey C, Peiró ME, Blanco R, Llorca J, González-Gay MA. Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: results of a population-based study. *Ann Rheum Dis*. 2014;73:722–7 [cited 2020 Nov 20]. Available from: <https://pubmed.ncbi.nlm.nih.gov/23505241/>.
- Touboul P-J, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness consensus (2004–2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis*. 2007;23:75–80 [cited 2019 Aug 2]. Available from: <https://www.karger.com/Article/FullText/97034>.
- Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research. Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* [Internet]. *J Pers Soc Psychol*. 1986;51:1173–82 [cited 2021 Jul 8]. Available from: <https://pubmed.ncbi.nlm.nih.gov/3806354/>.
- Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods*. 2013;18:137–50.
- Knowlton N, Wages JA, Centola MB, Alaupovic P. Apolipoprotein-defined lipoprotein abnormalities in rheumatoid arthritis patients and their potential impact on cardiovascular disease. *Scand J Rheumatol*. 2012;41:165–9 [cited 2021 Jul 9]. Available from: <https://pubmed.ncbi.nlm.nih.gov/22401593/>.
- Kawakami A, Yoshida M. Apolipoprotein CIII links dyslipidemia with atherosclerosis [Internet]. *J. Atheroscler. Thromb*. 2009;6–11 [cited 2021 Jul 9]. Available from: <https://pubmed.ncbi.nlm.nih.gov/19262004/>.
- Knowlton N, Wages JA, Centola MB, Giles J, Bathon J, Quiroga C, et al. Apolipoprotein B-containing lipoprotein subclasses as risk factors for cardiovascular disease in patients with rheumatoid arthritis. *Arthritis Care Res*. 2012;64:993–1000 [cited 2021 Jul 9]. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/acr.21646>.

22. Wällberg-Jonsson S, Dahlén G, Johnson O, Olivecrona G, Rantapää-Dahlqvist S. Lipoprotein lipase in relation to inflammatory activity in rheumatoid arthritis. *J Intern Med*. 1996;240:373–80 [cited 2021 Jul 11]. Available from: <https://pubmed.ncbi.nlm.nih.gov/9010384/>.
23. He XW, Shen YG, Zhu M, Hu XF, Zheng Z, Liu P, et al. Angiopoietin-like protein 4 serum levels and gene polymorphisms are associated with large artery atherosclerotic stroke. *J Neurol Sci*. 2016;362:333–8 [cited 2021 Jul 5]. Available from: <https://pubmed.ncbi.nlm.nih.gov/26944173/>.
24. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. *Atherosclerosis*. 2012;220:128–33 [cited 2021 Jul 12]. Available from: <https://pubmed.ncbi.nlm.nih.gov/21764060/>.
25. Hirano T, Nishioka F, Murakami T. Measurement of the serum lipoprotein lipase concentration is useful for studying triglyceride metabolism: comparison with postheparin plasma. *Metabolism*. 2004;53:526–31 [cited 2021 Jun 28]. Available from: <https://pubmed.ncbi.nlm.nih.gov/15045703/>.

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