

Apolipoprotein E Gene Polymorphisms Are Strong Predictors of Inflammation and Dyslipidemia in Rheumatoid Arthritis

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ABSTRACT. *Objective.* Rheumatoid arthritis (RA), a condition with a strong genetic etiology, is associated with excess cardiovascular disease (CVD). Dyslipidemia in RA may be driven by inflammation and genetic factors. Apolipoprotein E (ApoE) is important for the regulation of lipid levels and CVD risk and immune function in the general population. We compared the frequency of 2 ApoE single-nucleotide polymorphisms (SNP) in patients with RA and controls, and studied the relationship of ApoE genotypes with lipids and inflammation in RA.

Methods. A total of 387 patients with well-characterized RA and 420 non-RA controls were studied. Two ApoE SNP, rs7412 (ApoE2) and rs429358 (ApoE4), were identified.

Results. Genotypic ($p = 0.908$) and allelic ($p = 0.894$) frequencies did not differ between RA and controls. Within RA, the E2 allele was associated with the lowest and E4 allele with the highest levels of total cholesterol ($p = 0.007$), low-density lipoproteins ($p = 0.004$), and apolipoprotein B ($p = 0.009$). The E4 allele was also associated with lower C-reactive protein ($p = 0.007$), erythrocyte sedimentation rate ($p = 0.001$), and Disease Activity Score ($p = 0.015$) compared to the E3 allele. E2 or E4 alleles were not associated with CVD in RA, although a trend was observed ($p = 0.074$).

Conclusion. The frequency of ApoE polymorphisms did not differ between patients with RA and controls. ApoE genotypes are strongly linked to inflammation and lipid levels in RA, raising interest in the prognostic implications of ApoE genotypes. (First Release Dec 15 2011; J Rheumatol 2012;39:218–25; doi:10.3899/jrheum.110683)

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Rheumatoid arthritis (RA) is a complex autoimmune disease that primarily presents with peripheral joint pain and swelling;

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it is also associated with excess cardiovascular morbidity and mortality, which account for up to half of all RA deaths^{1,2,3}. The pathogenesis of cardiovascular disease (CVD) in RA is multifactorial, involving traditional risk factors, such as hypertension^{4,5}, dyslipidemia⁶, obesity^{7,8}, and physical inactivity⁹, as well as novel factors, including systemic inflammation¹⁰.

The lipid profile in RA is characterized by low levels of total cholesterol (TC), high-density lipoproteins (HDL), and low-density lipoproteins (LDL)^{11,12,13}, and an increased atherogenic index (TC:HDL ratio)^{12,14,15}. Systemic inflammation is thought to drive many of these changes through alterations in enzymatic activity and cytokine/acute-phase protein-mediated effects on lipid metabolism⁶. However, multiple other factors may also contribute. For example, in the general population genetic polymorphisms have been shown to be fundamental for both the regulation of lipid levels and the development of CVD^{16,17,18,19,20,21,22}. In RA, a condition with a strong genetic etiology^{23,24,25,26,27}, the frequency and role of genetic polymorphisms regulating lipid metabolism are unknown.

Apolipoprotein E (ApoE) is synthesized by the liver, in brain tissue, and by macrophages resident in atherosclerotic plaques, and is essential for the catabolism of triglyceride (TG)-rich lipoproteins^{28,29} and plays an important role in car-

divascular disease³⁰ and immunoregulation^{31,32,33}. Of particular interest, ApoE has been shown to affect macrophage oxidative status³⁴ and inhibits T lymphocyte proliferation³¹. There are 3 different isoforms of ApoE (ApoE2, ApoE3, and ApoE4). ApoE3 is the parent form, while ApoE2 and ApoE4 occur as a result of 2 single-nucleotide polymorphisms (SNP) at residue 158 and 112, respectively³⁵. These isoforms exhibit different pathophysiological/phenotypic properties in the general population: ApoE2 associates with lower serum ApoE, TC, and LDL levels, while ApoE4 produces the opposite effect, increasing serum ApoE, TC, and LDL levels, as well as CVD risk³⁰. Although the alterations in the lipid profile can partly explain the link between ApoE and CVD, other mechanisms may also contribute. Serum ApoE levels are inversely associated with levels of high sensitivity C-reactive protein (hsCRP) in the general population^{36,37}, a known independent risk factor for CVD³⁸. The E2 allele associates with higher levels and the E4 allele with lower levels of CRP³⁷.

In our study we aimed to assess the frequency of the ApoE genotypes in patients with RA versus healthy controls, and to assess the relationship of ApoE genotypes with lipid levels and systemic inflammation in patients with RA.

MATERIALS AND METHODS

Study populations. The study was approved by the local Research Ethics Committee and Research and Development Directorate and all participants gave written informed consent, according to The Declaration of Helsinki³⁹.

For the purposes of this cross-sectional study, 2 populations were assessed. The first included 387 patients fulfilling the 1987 American College of Rheumatology criteria for RA⁴⁰, recruited from routine rheumatology outpatient clinics held at the Dudley Group of Hospitals National Health Service Foundation Trust, between August 1, 2004, and July 31, 2006. The comparator population consisted of 420 local general population non-RA controls identified from a previously collected anonymized DNA biobank.

Data recorded on the patients with RA included basic demographics (age, sex, height, weight, waist circumference), a full medical history (including specific details regarding RA and CVD), current disease activity using the 28-joint Disease Activity Score (DAS28)⁴¹ and physical function using the Health Assessment Questionnaire (HAQ)⁴². All current medications were documented, including antirheumatic drugs, analgesics, and cardiovascular drugs such as statins and antihypertensives. All patients with RA also gave fasting blood samples for routine tests including full blood count, urea and electrolytes, liver function, CRP, erythrocyte sedimentation rate (ESR), lipid profile, and thyroid function tests. Estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease equation⁴³. Detailed descriptions of the RA population have been published by our group^{44,45}. CVD was defined as a positive history of any of the following: myocardial infarction, angioplasty, coronary artery bypass grafting, peripheral vascular disease, stroke or transient ischemic attack, or positive Rose questionnaire⁴⁶ on assessment. Dyslipidemia was defined by the US National Cholesterol Education Program guidelines: i.e., TC \geq 6.2 mmol/l or HDL $<$ 1.03 mmol/l or LDL \geq 4.13 mmol/l or TG \geq 1.7 mmol/l⁴⁷. Data available on the local population controls were limited to age and sex. All subjects provided DNA samples stored for subsequent genetic analysis.

Genotyping. The 2 ApoE SNP were identified simultaneously using the Roche LightCycler 480 System and a Roche ApoE mutation detection kit (cat. no. 03004716001). Polymerase chain reaction was performed with a reaction volume of 20 μ l containing 2 μ l DNA, 12 μ l sterile water, 2 μ l Lightcycler reaction mix, and 4 μ l Lightcycler ApoE mutation detection mix containing an anchor probe specific for both codons 112 and 158, labeled at the 3' end with

fluorescein, and 2 sensor probes specific for codons 112 and 158 labeled at the 5' end with Lightcycler Red 640 and Lightcycler Red 705, respectively.

The cycle conditions were as follows: 1 cycle of 95°C for 10 min, programmed ramp rate of 4.4°C/s; 35 cycles of 95°C for 6 s, 53°C for 15 s, and 72°C for 10 s each with a temperature transition rate of 4.4°C/s, 2.2°C/s, and 4.4°C/s, respectively; then melting curve analysis for 1 cycle of 95°C for 5 s and 40°C for 30 s, each with a transition temperature rate of 4.4°C/s and 2.2°C/s, respectively, and then rising to 75°C at 0.11°C/s, continuous with 5 acquisitions per 1°C. By combining the melting curve analysis from the genotyping of codon 112 and codon 158 the allelic structure of the analyzed samples was determined, e.g., E2E2 (Table 1).

A negative (water) control and a positive heterozygous control were included in each run.

Statistical analysis. Data analysis was carried out using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Genotype and allele frequencies were compared using the chi-square test in the total populations, before and after adjustment for age and sex. The Kolmogorov-Smirnov test was used to establish the distribution of each parameter. The variation of clinical characteristics across the genotypes was analyzed using chi-square, ANOVA, and Kruskal-Wallis tests for categorical, normally distributed, and non-normally distributed variables, respectively. ANCOVA was used to assess the association between lipid and inflammatory variables with the different genotypes and all results were adjusted for multiple comparisons.

When analyzing the association of ApoE genotypes with lipid levels and inflammatory markers, patients receiving lipid-lowering therapy, e.g., statins, were excluded due to the confounding effects of such treatment on lipid levels and inflammatory markers. In addition, the E2E4 genotype was excluded due to its potential to exert mixed phenotypic effects relating to the E2 and E4 allele.

The study had 80% power at the 5% significance level to detect a 6% difference in the prevalence of the rs7412 genotype and a 7% difference in the prevalence of the rs429358 genotype between RA and controls. Within patients with RA, the study had 80% power at the 5% significance level to detect a difference of at least 0.52 mmol/l in TC and 0.53 mmol/l in LDL between the E2 and E3 alleles, and a difference of 0.42 mmol/l in TC and 0.41 mmol/l in LDL between the E4 and E3 alleles. For non-normally distributed variables the values were logged; this demonstrated that the study had 80% power at the 5% significance level to detect a difference of 13.2% in HDL, 21.3% in TG, 52.1% in CRP, and 53.5% in ESR between the E2 and E3 alleles, and a difference of 10.2% in HDL, 16.1% in TG, 38% in CRP, and 39.3% in ESR between the E4 and E3 alleles.

RESULTS

Genotypic and allelic frequencies of the ApoE gene. Patients with RA were significantly older than the controls ($p < 0.001$), with a median age of 62.2 years [interquartile range (IQR) 53–69.1] compared to 51 years (IQR 39.75–61), respectively.

Table 1. Assignment of genotypes following combination of the melting curves from rs7412 (ApoE2) and rs429358 (ApoE4).

Genotype of Codon 112 (ApoE2)	Genotype of Codon 158 (ApoE4)	Allelic Setup
TGC/TGC	TGC/TGC	E2/E2
TGC/TGC	CGC/CGC	E3/E3
CGC/CGC	CGC/CGC	E4/E4
TGC/TGC	CGC/TGC	E2/E3
CGC/TGC	CGC/TGC	E2/E4
CGC/TGC	CGC/CGC	E3/E4

ApoE: apolipoprotein E.

Sex distribution also differed, with a higher proportion of females among patients with RA (73% vs 60.1%; $p < 0.001$). The ethnic split of the 2 populations was comparable, with 97% of patients with RA and 97.2% of controls being white.

The genotypic ($p = 0.908$; Table 2) and allelic frequencies (E2, 59 vs 64; E3, 604 vs 659; E4, 111 vs 117; $p = 0.894$) were not different between patients with RA and controls, and remained so following adjustment for age and sex ($p = 0.910$ and $p = 0.900$, respectively). Genotype frequencies were found to be in Hardy-Weinberg equilibrium.

Differences in clinical characteristics of patients with RA according to ApoE allele (Table 3). Age ($p = 0.061$) and sex ($p = 0.331$) did not significantly differ according to the presence of the E2 or E4 allele. Methotrexate use was significantly lower in patients with the E2 allele compared to those with the E3 or E4 allele (39.5% vs 62.5% vs 60.3%, respectively; $p = 0.021$). Dyslipidemia was least prevalent in patients with the E2 allele and highest in patients with the E4 allele (E2, 33% vs E3, 61.2% vs E4, 73.1%; $p < 0.001$). No significant differences were observed in the prevalence of other CVD risk factors such as hypertension ($p = 0.537$), diabetes mellitus ($p = 0.962$), body mass index (BMI; $p = 0.739$), or smoking ($p = 0.596$). The metabolic syndrome (defined by the 2009 joint consensus criteria⁴⁸) was lower in patients with 1 or more E2 or E4 alleles than in the parent form, E3E3 [E2, 18 (10.5%), E4, 54 (31.4%) vs E3E3, 100 (58.1%); $p = 0.035$]. Prevalent CVD did not differ across the genotypes ($p = 0.970$). However, if high-risk patients (those receiving lipid-lowering therapy) were included in the analysis, a trend was observed with the prevalence of CVD lowest among patients with the E2 allele and highest among patients with the E4 allele (E2, 16.3% vs E3, 20.4% vs E4, 28.1%; $p = 0.074$).

Association between ApoE alleles and lipid levels in RA. Univariate analyses demonstrated significant differences in lipid levels according to the ApoE allele present (Figure 1). TC ($p < 0.001$), LDL ($p < 0.001$), and ApoB ($p = 0.004$) were all lower in patients with the E2 allele (E2E3, E2E2) compared to those with the parent E3 allele (E3E3). ApoB levels were significantly higher in patients with the E4 compared to the E3 allele ($p = 0.034$), with a trend for LDL also to be higher among patients with the E4 compared to the E3 allele ($p = 0.066$; Table 3).

Table 2. Frequency of apolipoprotein E (ApoE) genotypes in patients with rheumatoid arthritis (RA) and healthy controls.

ApoE Genotype	RA Patients, n = 387	Healthy Controls, n = 420	p
E2E2, n (%)	3 (0.8)	5 (1.2)	0.908
E2E3, n (%)	46 (11.9)	48 (11.4)	
E2E4, n (%)	7 (1.8)	6 (1.4)	
E3E3, n (%)	235 (60.7)	262 (62.4)	
E3E4, n (%)	88 (22.7)	87 (20.7)	
E4E4, n (%)	8 (2.1)	12 (2.9)	

After adjustment for potential confounders [age, sex, RA characteristics (CRP, HAQ, rheumatoid factor status), BMI, glucose levels, and antirheumatic medication (methotrexate, sulfasalazine, hydroxychloroquine, leflunomide, anti-tumor necrosis factor therapy, prednisolone)] in a general linear model, TC ($p = 0.001$), LDL ($p = 0.009$), and ApoB ($p = 0.001$) levels remained significantly lower in patients with the E2 allele (E2E3, E2E2), while LDL ($p = 0.009$) and ApoB ($p = 0.009$) remained significantly higher in patients with the E4 allele (E3E4, E4E4) compared to those with the E3 (E3E3) allele (Table 4).

These findings were replicated in allele dose analyses.

Associations between ApoE alleles and inflammatory markers. Univariate analyses demonstrated that E4 heterozygotes (E3E4) or homozygotes (E4E4) had significantly lower levels of CRP ($p = 0.005$), ESR ($p = 0.002$), and DAS28 scores ($p = 0.024$) compared to patients with the E3 (E3E3) allele. No significant differences in CRP, ESR, or DAS were found between the E2 and E3 alleles. However, allele dose analyses not only confirmed the above findings for the E4 allele but also demonstrated CRP to be significantly higher among patients with 1 or more E2 alleles compared to patients with no E2 alleles ($p = 0.009$).

After adjustment for potential confounders [age, sex, RA characteristics (HAQ, rheumatoid factor status), and medication (methotrexate, sulfasalazine, hydroxychloroquine, leflunomide, anti-tumor necrosis factor therapy, prednisolone)] in a general linear model, lower CRP ($p = 0.007$), ESR ($p = 0.001$), and DAS28 scores ($p = 0.015$) remained significantly associated with the E4 allele (Table 4). The association of the E2 allele with CRP in the allele dose-dependent analysis remained significant following adjustment for potential confounders ($p = 0.044$). No significant differences in ESR or DAS28 were found between the E2 and E3 allele.

Analysis of the data was repeated following the exclusion of data for ethnic minorities; however, the results remained unchanged (data not shown).

DISCUSSION

To our knowledge, this is the first study to assess the frequency and role of ApoE genotypes in patients with RA. We did not find significant differences in the frequency of ApoE genotypes between patients with RA and local population controls, but as expected from general population studies, ApoE genotypes were major determinants of lipid levels in patients with RA, too. We also demonstrate for the first time in a "high-grade" inflammatory condition, i.e., RA, that ApoE genotypes associate with the intensity of inflammation. The overall influence of ApoE genotypes on both articular and cardiovascular outcomes in RA requires further study.

For decades, lipid levels have been shown to be predictive of CVD risk^{49,50,51} and are widely used in CVD risk algorithms^{47,52,53}. Our study demonstrates that ApoE genotypes are strongly linked to lipid levels in RA. The effects of the

Table 3. Clinical characteristics across the ApoE alleles (excluding patients taking statins). Results expressed as percentages, median (25th-75th percentile values), or mean \pm SD as appropriate.

Characteristics	Total, n = 303	E2 allele (E2E2, E2E3), n = 43	E3 allele (E3E3), n = 192	E4 allele (E3E4, E4E4), n = 68	p
General demographics					
Age, yrs	62.2 (53.0–69.1)	63.2 (57–72.6)	62.5 (53.8–69.1)	58 (51.6–66.1)	0.061
Female sex, n (%)	225 (74.8)	28 (65.1)	145 (75.5)	52 (76.5)	0.331
Smoking, never/ex/current smokers, %	137 (46.1)/107 (36.0)/ 53 (17.8)	17 (40.5)/18 (42.9)/ 7 (16.7)	86 (45.7)/70 (37.2)/ 32 (17)	34 (50.7)/19 (28.4)/ 14 (20.9)	0.596
BMI, kg/m ²	27.5 \pm 5.0	26.1 (24.1–30.1)	27 (24.2–30.9)	26.5 (23.6–29.3)	0.739
RA characteristics					
General characteristics					
RF-positive, n (%)	221 (74.9)	34 (85)	138 (73)	49 (74.2)	0.280
ACPA-positive, n (%)	198 (68.3)	30 (73.2)	124 (67.4)	44 (67.7)	0.767
Disease duration, yrs	10 (4–18)	10 (3.5–17.5)	11 (5–17)	10 (4–16)	0.838
Disease activity					
CRP, mg/l	9 (5–20)	14 (6.5–23.5)	10 (5–23)	7 (4–11)	0.001
ESR, mm/h	20 (9–36)	19 (10–36.5)	21 (9–37)	13 (5–26)	0.007
DAS28	4.2 \pm 1.4	4.3 \pm 1.4	4.3 \pm 1.4	3.8 \pm 1.5	0.023
Disease severity					
HAQ, 0–3	1.5 (0.5–2.1)	1.5 (0.3–2.1)	1.5 (0.5–2.1)	1.4 (0.34–2)	0.502
Nodules, n (%)	93 (30.7)	11 (25.6)	60 (31.3)	22 (32.4)	0.725
EAD, n (%)	200 (66.0)	29 (67.4)	123 (64.1)	48 (70.6)	0.607
Joint replacement surgery, n (%)	88 (29.0)	13 (30.2)	59 (30.7)	16 (23.5)	0.523
Medications					
DMARD, n (%)	264 (87.1)	37 (86)	167 (87)	60 (88.2)	0.940
Anti-TNF, n (%)	33 (10.9)	4 (9.3)	19 (9.9)	10 (14.7)	0.515
Sulfasalazine, n (%)	94 (31.0)	14 (32.6)	56 (29.2)	24 (35.3)	0.626
Leflunomide, n (%)	10 (3.3)	2 (4.7)	4 (2.1)	4 (5.9)	0.278
Methotrexate, n (%)	178 (58.7)	17 (39.5)	120 (62.5)	41 (60.3)	0.021
Hydroxychloroquine, n (%)	62 (20.5)	12 (27.9)	35 (18.2)	15 (22.1)	0.340
Prednisolone, n (%)	94 (31.0)	14 (32.6)	60 (31.3)	20 (29.4)	0.935
Antihypertensives, n (%)	107 (35.3)	16 (37.2)	68 (35.4)	23 (33.8)	0.935
Lipids					
Total cholesterol, mmol/l	5.5 \pm 1.1	4.8 \pm 0.9	5.6 \pm 1.0	5.6 \pm 1.4	< 0.001
High-density lipoproteins, mmol/l	1.6 (1.3–1.9)	1.6 (1.2–1.9)	1.6 (1.3–1.9)	1.5 (1.2–1.8)	0.060
Low-density lipoproteins, mmol/l	3.25 \pm 1.1	2.52 \pm 1.0	3.3 \pm 1.1	3.6 \pm 1.3	< 0.001
Triglycerides, mmol/l	1.2 (0.9–1.6)	1.2 (1–1.4)	1.2 (0.9–1.6)	1.3 (1–1.8)	0.278
Apolipoprotein A, mmol/l	1.7 \pm 0.5	1.7 \pm 0.4	1.7 \pm 0.5	1.6 \pm 0.4	0.397
Apolipoprotein B, mmol/l	1.0 \pm 0.8	0.8 \pm 0.2	1.0 \pm 0.3	1.1 \pm 0.3	< 0.001
Lipoprotein (a), mmol/l	0.09 (0.03–0.22)	0.95 (0.04–0.22)	0.09 (0.03–0.21)	0.12 (0.03–0.25)	0.848
Comorbidities					
Cardiovascular disease, n (%)	32 (10.6)	5 (11.6)	20 (10.4)	7 (10.3)	0.970
Diabetes mellitus, n (%)	9 (2.9)	1 (11.1)	6 (66.7)	2 (22.2)	0.962
Hypertension, n (%)	200 (66.0)	26 (60.5)	131 (68.2)	43 (63.2)	0.537
Full MDRD	83.0 (70.8–95.1)	85.3 (66.4–95.6)	80.1 (69.4–93.5)	87.2 (74.6–97.9)	0.074
Metabolic syndrome 2009, n (%)	95 (31.5)	12 (27.9)	57 (29.8)	26 (38.2)	0.381
NCEP-defined dyslipidemia, n (%)*	139 (45.9)	8 (18.6)	91 (47.4)	40 (58.8)	< 0.001
Serum glucose, mmol/l	4.9 (4.6–5.3)	4.9 (4.5–5.5)	4.9 (4.6–5.3)	4.8 (4.5–5.4)	0.999

ApoE: apolipoprotein E; RA: rheumatoid arthritis; BMI: body mass index; RF: rheumatoid factor; ACPA: anticitrullinated protein antibodies; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: Disease Activity Score; HAQ: Health Assessment Questionnaire; EAD: extraarticular disease; DMARD: disease-modifying antirheumatic drugs; anti-TNF: anti-tumor necrosis factor; Full MDRD: modification of diet in renal disease; NCEP: US National Cholesterol Education Program. * Total cholesterol \geq 6.2 or triglycerides \geq 1.7 mmol/l or low-density lipoprotein \geq 4.13 mmol/l or high-density lipoprotein < 1.03 mmol/l.

genotypes are similar to those seen in the general population, with the E2 allele associated with low levels of TC and LDL and the E4 allele associated with high levels of TC and LDL. Interestingly, one study has reported that the effects of ApoE on regression of atherosclerosis occurs independently of

changes in lipid levels⁵⁴. Irrespective of this we found that RA patients harboring the E4 allele are twice as likely to have dyslipidemia as those with the E2 allele, thus potentially increasing their risk for CVD.

Inflammation is fundamental to all stages of atherosclerotic

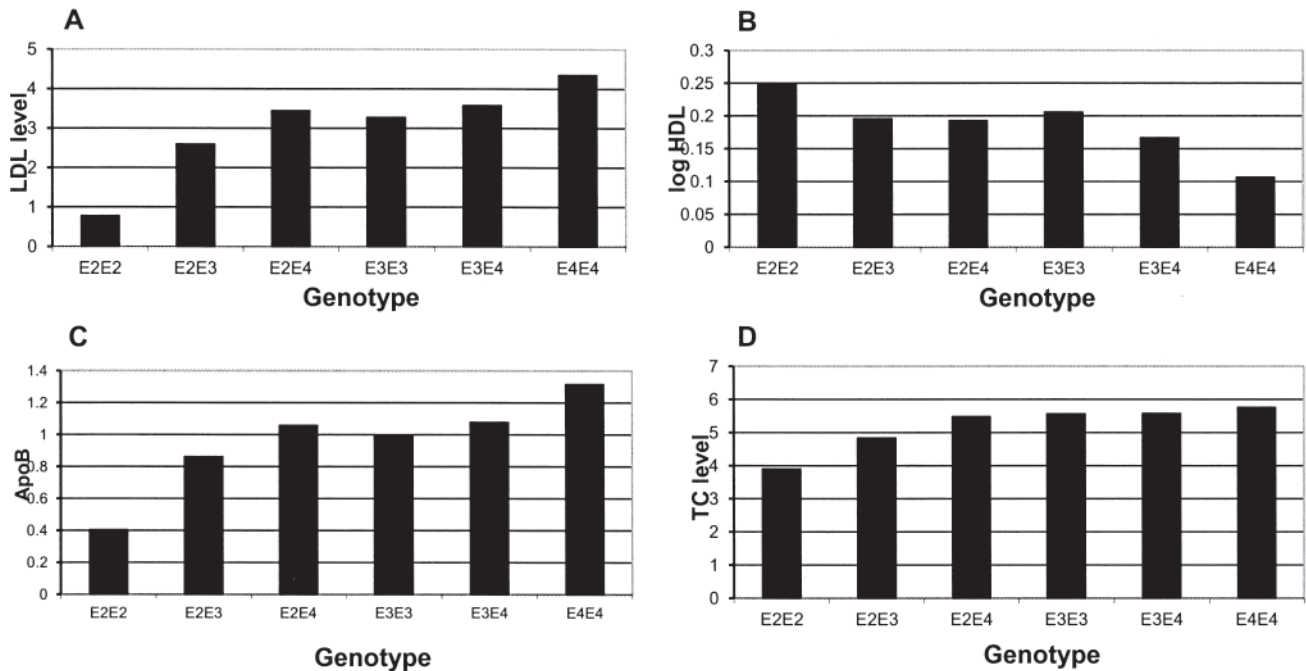


Figure 1. Lipid levels across the apolipoprotein E (ApoE) genotypes in patients with rheumatoid arthritis (excluding patients receiving statins). A. Low-density lipoprotein levels according to genotype. B. High-density lipoprotein levels according to genotype. C. ApoB levels according to genotype. D. Total cholesterol levels according to genotype.

ic plaque formation¹⁰ and is now recognized as an independent CVD risk factor³⁸. ApoE is known to play a key role in “low-grade” subclinical inflammation^{55,56,57}; however, its role in the context of high-grade systemic inflammation had not previously been studied. The association between ApoE genotypes and inflammatory markers appears to be amplified in RA. However, the pattern of allelic association mirrors that observed in the general population, with the E2 allele predicting the highest and the E4 allele the lowest CRP levels⁵⁷. In RA, CRP is an important marker of disease activity and severity, thus it is possible that these ApoE genotypes may also have prognostic value for arthritis and functional outcomes. DAS28 and ESR were also significantly lower in patients with the E4 allele compared to the E2 or E3 allele, but there were no significant associations between ApoE genotypes and other markers of disease severity (HAQ scores or number of joints replaced). This may be due to lack of power to detect such associations. Further specifically designed studies are required to assess the effects of the ApoE genotypes on markers of disease severity and progression, for example, progression of erosive damage.

In the general population, it is well described that the E4 allele increases CVD risk³⁰; whether the E2 allele is “protective” is less clear¹⁶. ApoE genotypes did not significantly associate with prevalent CVD in this RA cohort, probably due to insufficient power, but a trend was observed ($p = 0.074$). It is still feasible that polymorphisms of the ApoE gene contribute to atherosclerotic plaque formation in RA through

inflammation-mediated suppression of gene transcription. At the level of the atherosclerotic plaque, ApoE is primarily produced by macrophages and it exerts antiatherogenic properties by facilitating reverse cholesterol transport. Interestingly, a recent study using lipopolysaccharide (LPS) treatment to mimic inflammatory stress has demonstrated that LPS levels repress ApoE gene expression in macrophages through its effects on inflammatory signaling pathways⁵⁸. Such mechanisms are likely to be escalated in RA as a consequence of high levels of systemic inflammation, and this should be addressed in specifically designed studies.

The strengths of our study include the detailed clinical and laboratory characterization of patients with RA and use of a local control group for the genetic comparisons. The most important limitation of the study is its cross-sectional design, which does not allow definitive interpretation of the causality and directionality of the associations found. Due to the single timepoint data collection, we were unable to take into account biological variations in inflammatory or lipid measures that may also have affected the results. In addition, although this is by far the largest single study to assess the effects of ApoE genotypes on lipid levels and inflammatory markers, it may lack statistical power to detect associations with CVD or RA disease severity. Specifically designed large-scale longitudinal studies are required to confirm/refute our findings (e.g., longitudinal data collection, measurement of postprandial lipid levels⁵⁹).

We found no differences in the genotypic or allelic fre-

Table 4. Multivariate analysis demonstrating associations of lipid measures and inflammatory markers with the ApoE genotypes. Bold text indicates statistically significant result ($p < 0.05$).

	E2 (E2E2, E2E3)		E4 (E3E4, E4E4)	
	Beta (95% CI)	p	Beta (95% CI)	p
Total Cholesterol				
Crude	-0.77 (-1.13 to -0.41)	< 0.001	0.03 (-0.27 to 0.33)	0.853
Model a	-0.76 (-1.12 to -0.42)	< 0.001	0.06 (-0.22 to 0.35)	0.661
Model b	-0.76 (-1.13 to 0.39)	< 0.001	0.02 (-0.29 to 0.32)	0.912
Model c	-0.69 (-1.07 to -0.33)	< 0.001	0.05 (-0.25 to 0.35)	0.737
LDL				
Crude	-0.76 (-1.13 to -0.39)	< 0.001	0.36 (0.05 to 0.67)	0.022
Model a	-0.73 (-1.43 to -0.40)	< 0.001	0.37 (0.06 to 0.68)	0.018
Model b	-0.78 (-1.18 to -0.38)	< 0.001	0.39 (0.06 to 0.72)	0.020
Model c	-0.73 (-1.13 to -0.34)	< 0.001	0.42 (0.10 to 0.74)	0.009
ApoB				
Crude	-0.15 (-0.25 to -0.06)	0.001	0.10 (0.02 to 0.18)	0.011
Model a	-0.16 (-0.26 to -0.07)	0.001	0.11 (0.03 to 0.19)	0.006
Model b	-0.18 (-0.28 to -0.08)	0.001	0.10 (0.02 to 0.18)	0.020
Model c	-0.17 (-0.26 to 0.07)	0.001	0.11 (0.03 to 0.19)	0.009
CRP (log)				
Crude	0.11 (-0.02 to 0.24)	0.101	-0.18 (-0.29 to -0.07)	0.002
Model a	0.10 (-0.03 to 0.23)	0.143	-0.17 (-0.29 to -0.06)	0.002
Model b	0.09 (-0.05 to 0.23)	0.194	-0.16 (-0.27 to -0.04)	0.007
Model c	0.11 (-0.03 to 0.25)	0.138	-0.15 (-0.27 to -0.04)	0.007
ESR (log)				
Crude	0.011 (-0.13 to 0.15)	0.873	-0.21 (-0.33 to -0.09)	0.001
Model a	0.01 (-0.13 to 0.14)	0.928	-0.19 (-0.31 to -0.08)	0.001
Model b	-0.02 (-0.16 to 0.12)	0.774	-0.19 (-0.30 to -0.08)	0.001
Model c	-0.01 (-0.14 to 0.14)	0.966	-0.18 (-0.29 to -0.72)	0.001
DAS				
Crude	-0.03 (-0.44 to 0.51)	0.890	-0.55 (-0.96 to 0.14)	0.008
Model a	-0.01 (-0.48 to 0.46)	0.971	-0.49 (-0.89 to -0.08)	0.019
Model b	-0.11 (-0.55 to 0.33)	0.620	-0.45 (-0.82 to -0.07)	0.019
Model c	-0.01 (-0.45 to 0.43)	0.957	-0.46 (-0.83 to -0.09)	0.015

Crude: uncorrected data. Model a: Crude data plus adjustment for age and sex. Model b: Model a plus adjustment for demographics (BMI/smoking status) and markers of disease activity/severity (rheumatoid factor positivity \pm CRP; adjusted for only in lipid models not ESR, CRP, or DAS. Model c: Model b plus adjustment for medications (methotrexate, sulfasalazine, hydroxychloroquine, prednisolone, anti-tumor necrosis factor therapy); BMI: body mass index; LDL: low-density lipoprotein; ApoB: apolipoprotein B; ESR: erythrocyte sedimentation rate; DAS: Disease Activity Score; CRP: C-reactive protein.

quencies of ApoE between patients with RA and local population controls. However, ApoE genotypes appeared to be strong predictors of TC, LDL, CRP, ESR, and DAS in RA. The ApoE gene may prove to be a useful prognostic marker for arthritis and vascular outcomes in RA, and this needs to be elucidated in further longitudinal studies.

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