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Original article

# Association of rheumatoid arthritis susceptibility gene with lipid profiles in patients with rheumatoid arthritis

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## Abstract

**Objective.** RA patients have an increased risk of cardiovascular (CV) disease, although the mechanisms are unclear. As RA and CV disease may be associated through lipid profiles, we examined whether single nucleotide polymorphisms (SNPs) associated with RA susceptibility were associated with low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG) levels in RA subjects.

**Methods.** Patients ($n = 763$) enrolled in the Veterans Affairs RA registry who were not on hydroxymethyl-glutaryl-CoA reductase inhibitor were genotyped for human leukocyte antigen shared epitope (HLA-DRB1-SE) and SNPs in the following genes: *CTLA-4* (cytotoxic T-lymphocyte antigen 4), *IL-10*, *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), *REL* (c-Rel), *STAT4* (signal transducer and activator of transcription protein), *TNF-α* and *TRAF1* (TNF receptor-associated factor 1). Other covariates included patient characteristics (age, gender, race, smoking status, education, BMI, modified Charlson–Deyo co-morbidity index), CV characteristics (hypertension, diabetes, alcohol abuse), pharmacologic exposures (MTX, anti-TNF, glucocorticoids) and RA severity/activity markers (RA disease duration, mean DAS, CRP, RF positivity, anti-CCP positivity). Multivariate linear regression was performed to determine the factors associated with LDL, HDL and TG levels.

**Results.** The *REL* SNP rs9309331 homozygous minor allele was associated with higher LDL levels. Caucasian race and increasing BMI were associated with lower HDL. Factors associated with higher TG were diabetes, Caucasian race and higher BMI.

**Conclusion.** The *REL* SNP rs9309331 was associated with LDL levels in our study. This association is a possible explanation of the increased risk of RA patients for CV disease and requires further inquiry.

**Key words:** arthritis, rheumatoid, polymorphism, single nucleotide, cholesterol, HDL, LDL, triglycerides.

BASIC SCIENCE

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## Introduction

Patients with RA have an increased risk of cardiovascular (CV) disease compared with those with non-inflammatory states [1, 2] and CV disease is the leading cause of mortality in RA patients [2, 3]. However, mechanisms underpinning the associations between RA and CV disease have not been fully elucidated, as traditional risk factors such as hypertension and obesity appear to be only partial contributors [4, 5].

Dyslipidaemia is one of the strongest predictors of CV disease in the general population [6] and is prevalent in patients with RA [7]. In the general population, lipoprotein and lipid abnormalities account for 30–50% of the population attributable risk for coronary heart disease [8] and lipid levels themselves are highly heritable, with the estimated contribution of genetics to the lipid level ranging

from 40 to 60% [9]. Prior data indicate that patients who later develop RA have a considerably more atherogenic lipid profile than controls at least 10 years prior to the diagnosis of RA [7], indicating a possible genetic or inflammatory cytokine-based link between RA itself and lipid profiles.

As certain genetic polymorphisms have been associated with increased risk for RA, we sought to determine through cholesterol profiles whether these polymorphisms may be indirectly related to CV risk. Given this possible link between the atherogenic lipid profile and RA, we utilized a cross-sectional study to examine the association of RA susceptibility genes, specifically *CTLA-4* (cytotoxic T-lymphocyte antigen 4), *IL-10*, *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), *REL* (c-Rel), *STAT4* (signal transducer and activator of transcription protein), *TNF-α* and *TRAF1* (TNF receptor-associated factor 1), with low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG) levels in RA patients.

## Methods

### Patients

The study cohort was a subset of the Veterans Affairs RA (VARA) prospective registry and included data from six US Department of Veterans Affairs Medical Centers: Denver, CO; Omaha, NE; Salt Lake City, UT; Washington, DC; Dallas, TX; and Jackson, MS. VARA is a prospective longitudinal multicentre observational registry and biologic repository, currently with 11 participating centres, which has been fully described elsewhere [10]. Briefly, all patients meeting the ACR 1987 criteria for RA at participating centres are invited to enrol. Potential subjects are approached as to their interest in VARA by their medical care provider at their regularly scheduled rheumatology visits. Once an interested individual is identified, a trained research assistant takes the potential participant to a quiet setting and explains the study and the study goals and consents the potential subject for participation in the study. Potential subjects are assured that their care is in no way affected by their decision to participate or not in the registry. Participants are provided a copy of the signed and dated authorization document and are apprised that they may withdraw at any time. Patients who consent to enrol allow collection of demographic and longitudinal clinical data and biologic samples (serum, plasma and DNA). For this substudy, patients were included if they had genetic data available (see the Genetic analysis section) and had lipid measures drawn after enrolment into VARA (see the Outcome variable section); patients were excluded if they were taking a hydroxymethylglutaryl-CoA reductase inhibitor (statin) at the time of the lipid panel of interest. This study was approved by the Internal Review Board of the Denver Veterans Affairs Medical Center and University of Colorado Denver, as well as the VARA Scientific Ethics Advisory Committee.

### Genetic analysis

Our cross-sectional study was limited to subjects enrolled in VARA who had whole blood available at the time of genetic analysis (hence the inclusion of only six VARA sites). DNA samples were derived from whole blood and quantitative PCR was used to genotype patient DNA using a tag single nucleotide polymorphism (tagSNP) approach. The markers were chosen using the following parameters: linkage disequilibrium (LD) blocks were defined using a Caucasian LD map of tagSNPs with $r^2 = 0.8$, minor allele frequency (MAF) $> 0.1$, range $-1500$ bp from the initiation codon to +1500 bp from the termination codon and one SNP/LD bin. The RA susceptibility genes of interest were genotyped for the following SNPs: *CTLA-4* (rs10197010, rs231777, rs231779, rs16840252, rs3087243), *IL-10* (rs1800896, rs1800872, rs3024493), *PTPN22* (rs1217418, rs2476602, rs1217414, rs1217395, rs2476601, rs3765598, rs3789609, rs17510162), *REL* (rs10203477, rs13031237, rs842647, rs9309331), *STAT4* (rs1031508, rs16833215, rs16833260, rs3024861, rs3024904, rs4341966, rs4555370, rs4853540, rs4853546, rs6434435, rs7572482, rs7574608, rs7599504, rs925847, rs10189819, rs11685878, rs12327969, rs13389408, rs1517352, rs16833220, rs16833239, rs3024886, rs3024896, rs3024912, rs6752770, rs7582694), *TNF-α* (rs1800629, rs3093662, rs3093668) and *TRAF1* (rs1014529, rs1014530). Genotyping was performed using either a BeadExpress platform (Illumina, San Diego, CA, USA) (SNPs for *CTLA-4*, *PTPN22*, *IL-10*, *TNF-α*, *TRAF1* and all SNPs for *STAT4* with the exception of rs16833220 and rs4341966) or by TaqMan assay (SNPs for *REL* and *STAT4* rs16833220 and rs4341966) using a GeneAmp 9700 PCR machine (Applied Biosystems, Foster City, CA, USA) with endpoint analysis on a PRISM 7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA).

Additionally the patients were genotyped for the human leukocyte antigen shared epitope (HLA-DRB1-SE) containing alleles as previously described [11]. HLA-DRB1-SE containing alleles were considered to be *0101, *0102, *0104, *0105, *0401, *0404, *0405, *0408, *0409, *1001, *1402 and *1406.

### Clinical variables

Clinical variables were divided into four domains consisting of the following:

#### RA activity and severity characteristics

RF, anti-CCP and high-sensitivity CRP (hsCRP) were derived from the blood and serum samples banked upon enrolment into the VARA registry. Anti-CCP (IgG) was measured using a second-generation ELISA (Diastat, Axis-Shield Diagnostics, Dundee, Scotland) and was considered positive with values $\geqslant 5$ U/ml. RF and hsCRP were determined by nephelometry (Siemens Healthcare Diagnostics, Munich, Germany). RF was considered positive with values $\geqslant 15$ IU/ml [11]. ESRs were performed at the study sites and were used to calculate the participant's mean DAS [28-joint DAS (DAS28)] [12]

based on data derived from all observations within the VARA registry. RA disease duration prior to enrolment (in years) was determined from the time of diagnosis until enrolment into VARA.

Patient characteristics

Variables included age, gender, ethnicity (Caucasian *vs* non-Caucasians), smoking status (never, former, current), education (years), BMI (kg/m$^2$) and modified Charlson–Deyo co-morbidity index (diabetes mellitus was evaluated separately as an independent risk factor and thus was not included in the co-morbidity index) [13].

CV characteristics

International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic codes for personal history (prevalent diagnosis) of the following conditions were obtained: hypertension (401.x–405.x, 437.2) [14], diabetes (250.x) [14] and alcohol abuse (265.2, 291.1–291.3, 291.5, 291.8x, 291.9, 303.0, 303.9, 357.5, 425.5, 535.3, 571.0–571.3, 980.x, v11.3) [15, 16].

Pharmacology characteristics

We included a binary (yes/no) variable indicating whether the patient was on MTX, a TNF inhibitor (anti-TNF, including adalimumab, etanercept, infliximab, golimumab and certolizumab) or a glucocorticoid at the time of the clinical visit closest to the time of the lipid panel of interest (see the Outcome variable section). We excluded the data if the clinical visit was $>1$ year prior or following the lipid panel. Data were derived from the VARA database.

### *Outcome variable*

Components of the first available lipid profile obtained after enrolment into VARA were the outcomes of interest. These components, ordered as part of routine care, included LDL, HDL and TG levels. These values were initially measured in milligrams per decilitre and were converted to millimoles per litre for this report. Clinical laboratories at each of the participating medical centres determined the concentration of lipid components.

### *Statistics*

Due to the large number of independent variables, the models associated with each of the three outcomes (LDL, HDL and TG) were constructed based on the five initial domains described above: patient characteristics, CV characteristics, pharmacology characteristics, RA activity and severity characteristics, and genetic characteristics. Each of the resulting 15 domains (five domains per each of the three outcomes) was evaluated for significant correlations and interactions. For each of these domains, backwards stepwise linear regression was performed to determine factors associated with the outcome variable of interest (HDL, LDL or TG). Specifically for the genetic domain, as the type of expression of the polymorphisms in each of the SNPs examined was unknown (dominant, incomplete dominance or recessive), we first performed a power analysis [using proc glmpower in SAS 9.2 (SAS Institute, Cary, NC, USA)] to determine the comparisons within each SNP that had the appropriate effect size difference to determine a real difference given a sample size of 688 (the sample size, once all missing values were removed) and 80% power. The power analysis also took into account the standard deviation of the respective dependent variables. The comparisons explored within each SNP included minor allele homozygous *vs* major allele homozygous, minor allele homozygous *vs* heterozygous and heterozygous *vs* major allele homozygous. Due to the large number of independent variables, the three models for the genetic characteristics in HDL, LDL and TG required intermediate models. In these intermediate models, a variable from the initial model was retained if it had an alpha level of 0.30, and was then included in the intermediate model. This intermediate model was then re-run and those variables with an alpha level of 0.10 were included in the final model. In those initial models that did not require intermediate models (all domain models aside from the genetic characteristics), variables with an alpha level of 0.05 were considered significant and were included in the final models. Bonferroni correction was used due to the multiple comparisons ($0.05/36 = 0.0014$), and a $P$-value $\leqslant 0.002$ was considered significant in the final model. Hardy–Weinberg $P$-values, stratified by race, and comparisons between our cohort MAF and those reported in the 1000 Genomes Project [17] were calculated using chi-square tests. All analyses were conducted using SAS 9.2 or STATA 12 (StataCorp, College Station, TX, USA) software.

## Results

Of the 1354 subjects enrolled in VARA at the time the genetic analysis was performed, 1176 had genetic samples available for analysis. Eighty-six per cent ($n = 1011$) of enrolees with genetic samples also had lipid measures following enrolment into VARA. Of these 1011 with both genetic and lipid data, 763 individuals had serum lipid panels drawn while not on a statin (248 were excluded due to the use of statins). Of these, 761 individuals had results for HDL (mean 1.21 mmol/l), 760 individuals had results for LDL (mean 2.87 mmol/l) and 748 individuals had results for TG (mean 1.53 mmol/l). As we enrolled individuals from three VA sites, we evaluated the variance of the reference values for the different labs over the years and at the different sites. The maximum variance of the HDL, LDL and TG labs were 2.6%, 9.3% and 1.9%, respectively.

The demographics of the cohort are shown in Table 1. Overall, our cohort was largely older (mean age 62.83 years), male (90.43%), Caucasian (75.23%) and overweight (average BMI 28.38 kg/m$^2$), with 19.40% diagnosed with diabetes mellitus, 54.65% diagnosed with hypertension and 29.53% current smokers. With regard to their RA, $>80$% were positive for anti-CCP and/or RF, half were taking MTX and the mean DAS28 score was 3.72, indicating moderate disease activity. The population had well-established disease, with a mean disease duration of almost 15 years. The average length of time between enrolment (when the sample used for the hsCRP

**Table 1** Cohort demographics

| Variable | Patients ($n = 763$) | % | Mean | 95% CI | |
|---|---|---|---|---|---|
| Age, years | 763 | | 62.8 | 62.0 | 63.6 |
| Gender, male | 763 | 90.4 | | | |
| Caucasian | 763 | 75.2 | | | |
| Tobacco use | | | | | |
| Never | 762 | 20.7 | | | |
| Former | 762 | 49.7 | | | |
| Current | 762 | 29.5 | | | |
| Education, years | 763 | | 12.8 | 12.6 | 12.9 |
| BMI, kg/m$^2$ | 763 | | 28.4 | 28.0 | 28.8 |
| Modified Charlson–Deyo index | 761 | | 1.3 | 1.2 | 1.4 |
| Prevalent hypertension | 763 | 54.7 | | | |
| Prevalent diabetes | 763 | 19.4 | | | |
| Prevalent alcohol abuse | 763 | 6.2 | | | |
| MTX use | 763 | 49.8 | | | |
| Anti-TNF use | 710 | 26.9 | | | |
| Steroid use | 710 | 41.4 | | | |
| Average disease duration, years | 685 | | 14.9 | 14.0 | 15.8 |
| Average DAS28 | 754 | | 3.7 | 0.8 | 7.5 |
| hsCRP, mg/l | 762 | | 13.1 | 11.7 | 14.5 |
| Anti-CCP positive | 673 | 82.2 | | | |
| RF positive | 760 | 89.1 | | | |
| HDL, mmol/l | 761 | | 1.2 | 1.2 | 1.2 |
| LDL, mmol/l | 760 | | 2.8 | 2.8 | 2.9 |
| TG, mmol/l | 748 | | 1.5 | 1.5 | 1.6 |

DAS28: 28-joint disease activity score, using the DAS28 four variable/ESR equation; hsCRP: high-sensitivity CRP; HDL; high-density lipoprotein; LDL: low-density lipoprotein; TG: triglyceride.

was banked) and the time of the lipid panel was 625.4 days (S.D. 475.8).

The prevalences of the MAFs of the examined SNPs and the HLA-DRB1-SE may be found in supplementary Table S1, available at *Rheumatology* Online. Population MAFs derived from the 1000 Genome Project phase 1 population [18] may be found in supplementary Table S1, available at *Rheumatology* Online, as are the *P*-values comparing our cohort's MAFs *vs* the population MAFs. The most frequent significant differences in MAFs between our cohort and those of the population reported in the 1000 Genome Project were in *IL-10*, *REL*, and *STAT4*, though there were also significant differences in *PTPN22* and *TNF-α*. These significant differences in MAF between our cohort and that of the 1000 Genome Project in these RA susceptibility genes lends further credence that these SNPs are distinctive between those with RA and the general population. Surprisingly, there were no significant differences in the MAF in the *CTLA-4* and *TRAF1* SNPs. The cohort, stratified by ethnicity [19], was in allelic Hardy–Weinberg equilibrium with the exception of rs3789609 in *PTPN22*.

The results of the initial and intermediate models may be found online (supplementary Tables S2–S4, available at *Rheumatology* Online). The results of the final multivariate models for HDL, LDL and TG appear in Table 2. In the final HDL model there was a significant interaction between alcohol abuse and hypertension; no other models had significant interactions. Factors associated with a lower HDL were Caucasian ethnicity and increasing BMI. In the final LDL model the *REL* SNP rs9309331 homozygous minor allele *vs* the homozygous major allele was associated with a higher LDL. After adjusting for the other variables in the model, those with the *REL* rs9309331 homozygous minor allele had an LDL value that was on average 0.38 mmol/l higher than those with a homozygous major allele. In the final TG model, diabetes, Caucasian race and increasing BMI were associated with increased TGs. No other genetic polymorphisms were considered significant in our analysis.

## Discussion

In this study we examined demographic factors and genetic polymorphisms that have been implicated in RA susceptibility in order to determine their association with LDL, HDL and TG levels. Since RA disease activity and severity factors have been associated with atherosclerosis [4], we questioned whether risk factors for RA may contribute to the elevated CV risk [4, 20] through dyslipidaemia. We found that the homozygous minor allele in *REL* SNP rs9309331 was associated with a 0.38 mmol/l increase in LDL compared with the homozygous major allele. Homozygous minor alleles in this SNP were associated with an increase in LDL level approximately one-fifth of

**Table 2** Results of the final regressions predicting HDL, LDL and TG levels

| Variable | Parameter estimate, mmol/l | 95% CI | | *P*-value |
|---|---|---|---|---|
| HDL model | | | | |
| Intercept | **1.92** | **1.73** | **2.11** | **<0.0001** |
| Presence of diabetes | −0.08 | −0.15 | 0.00 | 0.0464 |
| Presence of alcohol abuse | 0.23 | 0.04 | 0.42 | 0.0169 |
| Alcohol and hypertension interaction term | −0.32 | −0.56 | −0.08 | 0.009 |
| Caucasian | **−0.36** | **−0.46** | **−0.25** | **<0.0001** |
| BMI | **−0.01** | **−0.02** | **−0.01** | **<0.0001** |
| *PTPN22* rs1217414 min. *vs* maj. | −0.08 | −0.18 | 0.03 | 0.1409 |
| *PTPN22* rs1217395 min. *vs* maj. | 0.08 | −0.02 | 0.18 | 0.112 |
| *STAT4* rs4853540 min. *vs* het. | 0.06 | −0.03 | 0.16 | 0.1659 |
| *STAT4* rs3024861 min. *vs* het. | −0.08 | −0.16 | 0.01 | 0.0725 |
| *STAT4* rs1031508 min. *vs* het. | 0.06 | −0.03 | 0.15 | 0.1693 |
| LDL model | | | | |
| Intercept | **2.45** | **2.13** | **2.78** | **<0.0001** |
| BMI | 0.01 | 0.00 | 0.02 | 0.0278 |
| Charlson–Deyo index | −0.04 | −0.08 | 0.00 | 0.0269 |
| *PTPN22* rs1217395 min. *vs* maj. | −0.19 | −0.38 | 0.00 | 0.046 |
| *TRAF1* rs1014530 het. *vs* maj. | 0.22 | 0.07 | 0.37 | 0.0043 |
| *STAT4* rs12327969 min. *vs* het. | −0.27 | −0.48 | −0.05 | 0.0143 |
| *REL* rs9309331 min. *vs* maj. | **0.38** | **0.17** | **0.59** | **0.0005** |
| STAT4 rs925847 min. *vs* het. | 0.18 | −0.01 | 0.38 | 0.0615 |
| TG model | | | | |
| Intercept | 0.39 | 0.08 | 0.70 | 0.0141 |
| Presence of diabetes | **0.29** | **0.15** | **0.44** | **<0.0001** |
| Caucasian | **0.29** | **0.15** | **0.43** | **<0.0001** |
| Tobacco use: never *vs* current | −0.22 | −0.38 | −0.05 | 0.009 |
| BMI | **0.03** | **0.02** | **0.04** | **<0.0001** |
| hsCRP, mg/l | 0.00 | −0.01 | 0.00 | 0.0588 |
| *PTPN22* rs1217414 min. *vs* maj. | 0.10 | −0.08 | 0.28 | 0.2856 |
| *TRAF1* rs1014530 min. *vs* het. | 0.14 | 0.01 | 0.28 | 0.0386 |
| *STAT4* rs1031508 min. *vs* het. | 0.16 | −0.01 | 0.34 | 0.0625 |

The final models only include variables achieving an alpha level of 0.05 in the intermediate models. The initial univariate regression results are presented in supplementary data, available at *Rheumatology* Online. Variables achieving the required *P*-value $\leqslant$ 0.0014 (adjusted for multiple comparisons) appear in bold. min.: homozygous minor; maj.: homozygous major; het.: heterozygous; HDL: high-density lipoprotein; LDL: low-density lipoprotein; REL: c-Rel protein; hsCRP: high-sensitivity CRP.

the amount that the average statin lowers LDL (0.38 mmol/l compared with an average decrease of 1.8 mmol/l) [21]. The fact that this genetic association was discernible within the inflammatory milieu characterizing RA suggests that this association may be of significant clinical interest.

The *REL* rs9309331 homozygous minor allele may be associated with increased production of LDL, decreased clearance of LDL or through linkage disequilibrium with another variant, as the specific function of this SNP is not yet known. *REL* encodes the c-Rel protein, which is a component of the nuclear factor-kappa B (NF-κB) family of transcription factor subunits [22]. Activation of the NF-κB pathway has been established in chronic inflammatory diseases, including RA, as well as in patients with unstable angina [23]. In RA, perpetuation of synovitis is mediated at least in part through the NF-κB pathway [24], and c-Rel has been associated with RA through a genome-wide association study [25]. Additionally the NF-κB pathway has been associated with atherosclerosis, as activation of endothelial cells and proteins involved in leucocyte adhesion and chemotaxis are regulated by NF-κB signalling [26].

c-Rel is thought to have functions in the immune and other systems. It has been found that c-Rel affects B cell proliferation, differentiation and apoptosis [27, 28]; Foxp3+ regulatory T cells; the regulation of numerous cytokines (specifically IL-12 and IL-23) [29, 30]; the proliferation of hepatocytes, cardiocytes and neurons; and the cell cycle [22]. c-Rel knockout mice have minor immunological defects, including compromised lymphocyte proliferation and activation [31]. In humans, polymorphisms in *REL* have been linked not only to RA [25], but also to other autoimmune diseases such as SLE [32], IBD [32] and celiac disease [33]. Interestingly, c-Rel deficiency has been associated with cardiac fibrosis and hypertrophy [34] and NF-κB has been associated with atherosclerosis, inflammation, angiogenesis and thrombosis [23, 35]. To our knowledge, no prior report has linked *REL*

polymorphisms and LDL levels. This possible connection between rs9309331 and LDL levels should be investigated further through replication in other populations and through mechanistic studies.

With regards to our TG and HDL models, our findings are consistent with prior studies. We found that increasing BMI, Caucasian race and the presence of diabetes were associated with increasing TG levels, as previously identified [36–38]. Our work reconfirms these previously described associations in a different population. Increasing BMI and Caucasian race were associated with lower levels of HDL in our study, recapitulating the results of other investigators [36, 39–41].

Ours is not the first study to investigate associations between cholesterol and RA susceptibility genes. Toms *et al.* [42] investigated the association of specific SNPs in *PTPN22* (rs2476601), *TRAF1* (rs3761847), *STAT4* (rs7574865) and *HLA-DRB1* with total cholesterol, HDL, LDL, TG, apolipoproteins A and B and lipoprotein (a). In that study the authors found associations between *TRAF1* rs3761847 and total cholesterol, LDL and apolipoprotein B (apoB). Additionally, they found an association between *STAT4* rs7574865 and lipoprotein (a) and *HLA-DRB1-SE* with apolipoprotein A (apoA) and apoB:apoA ratios. Santos *et al.* [43] found an association of lymphotoxin-$\alpha$ (LTA) SNP rs909253 homozygous major allele (A/A) with dyslipidaemia and the homozygous minor allele (G/G) with earlier development of RA and higher levels of CRP. Vallve *et al.* [44] found an association between TNF-$\alpha$ SNP T1031C minor allele carriers and significantly smaller LDL particles, which have a greater affinity for extracellular matrix and higher susceptibility to oxidation and a greater risk of atherosclerosis. Park *et al.* [45] also found an association between the apolipoprotein M (apoM) SNP rs805297 minor allele (apoM genes have been associated with RA susceptibility [46]) and low HDL. While we did not find the same associations as these studies, it must be observed that our studies did not investigate the same SNPs, nor did we investigate all of the same outcomes. Still, these studies all found associations between dyslipidaemia and RA susceptibility genes. While our studies are not conclusive regarding direct causal effects of RA susceptibility genes and dyslipidaemia, they are intriguing and call for further study.

While RA has been linked with lipid levels [47], the association between lipid levels and RA is not straightforward [48, 49], likely due to the complex interplay between disease activity and medications. Additionally, dyslipidaemia has not always been associated with CV death in RA patients [50], making the associations between RA, lipid levels, atherosclerosis and CV death complex and worthy of further exploration.

Our study has several strengths, including the use of detailed clinical, pharmacy and laboratory information, and a moderately large number of participants. Additionally, our detailed look at the individual SNPs making up each susceptibility gene using a tagSNP approach would indicate that if an association with a gene were there, we would be more likely to find it.

Limitations include the cross-sectional design, which is appropriate for the generation of hypotheses. Additionally, we did not begin with specifically targeted SNPs suspected of influencing the lipid levels based on a biological theory, but rather examined multiple SNPs and utilized Bonferroni correction for multiple comparisons as a means of hypothesis generation. Thus further work should be pursued in which the biology of the interaction between *REL* rs9309331 polymorphisms and LDL levels are further explored. The make-up of our cohort, which is largely male, Caucasian and with a history of tobacco exposure, may have affected the results of our study, and thus the same results may not be found in a dissimilar population. Over-the-counter medication use, including NSAID use, was not available to us for this analysis. Due to the make-up of our registry, we relied on the cholesterol levels drawn for clinical reasons, and thus they were not performed on the same equipment and we cannot guarantee that the participants were fasting. The average 2-year gap between the hsCRP (blood banked at enrolment) and the lipid panel being drawn probably contributed to hsCRP not being statistically significant in the model. Further work on this subject should include, if possible, inflammatory markers being drawn at the same time as the lipid panel. The gap in time between enrolment in the VARA and the lipid draw, 625 days on average, may also have biased the population included in our study toward those individuals with hyperlipidaemia not associated with CV mortality. Lastly, our studies may not reflect the physiologic processes at play in subjects with recent-onset RA.

In conclusion, the findings of this study suggest a potential association between *REL* rs9309331 polymorphisms and LDL levels. Our findings are hypothesis generating and require validation. However, they provide a basis for the evaluation of additional mechanisms in RA-associated atherosclerosis.

**Rheumatology key messages**

- RA patients have an increased risk of cardiovascular (CV) disease through as yet unclear mechanisms.
- We found an association between the *REL* rs9309331 homozygous minor allele and higher low-density lipoprotein (LDL) in RA patients.
- The association between RA susceptibility genes and LDL may contribute to CV disease.

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## Supplementary data

Supplementary data are available at *Rheumatology* Online.

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