Association of Polymorphisms Modulating Low-density Lipoprotein Cholesterol with Susceptibility, Severity, and Progression of Rheumatoid Arthritis

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ABSTRACT. *Objective.* Dyslipidemia, a risk factor for cardiovascular diseases, is more prevalent in patients with rheumatoid arthritis (RA) than in the general population. We investigated whether single-nucleotide polymorphisms (SNP) modulating low-density lipoprotein (LDL) cholesterol affect susceptibility, severity, and progression of RA.

Methods. We enrolled 302 patients with RA and 1636 healthy controls, and investigated the SNP modulating LDL cholesterol. Clinical characteristics of RA, serum adipocytokine concentrations, and radiographic severity were analyzed according to genotype score based on the number of unfavorable alleles. The influence of genotype score on radiographic progression was also investigated using multivariable logistic models.

Results. We identified 3 SNP (rs688, rs693, and rs4420638) modulating LDL cholesterol in Koreans, which correlated well with LDL cholesterol levels in both patients with RA and controls. Among them, 2 SNP, rs688 and rs4420638, were more prevalent in patients with RA than in controls. In patients with RA carrying more unfavorable alleles (genotype score ≥ 3), disease activity measures, serum adipocytokine levels, and radiographic severity were all increased. The genotype score was an independent risk factor for radiographic progression of RA over 2 years, and its effect was greater than the influence of conventional risk factors.

Conclusion. SNP modulating LDL cholesterol influence the risk, activity, and severity of RA. These results provide the first evidence that genetic mechanisms linked to dyslipidemia may directly contribute to the susceptibility and prognosis of RA, a representative of chronic inflammatory diseases, explaining the high incidence of dyslipidemia in RA. (First Release April 15 2013; J Rheumatol 2013;40:798–808; doi:10.3899/jrheum.120954)

Key Indexing Terms:
RHEUMATOID ARTHRITIS   DYSLIPIDEMIA
POLYMORPHISMS   RADIOGRAPHIC PROGRESSION

The main cause of death for patients with rheumatoid arthritis (RA) is cardiovascular disease (CVD)[1]. Dyslipidemia, one of the most important risk factors for CVD, is more prevalent in patients with RA than in the general population[2]. Since RA is a chronic inflammatory disease, it has been suggested that the high inflammatory state associated with RA may induce dyslipidemia[2]. For example, high disease activity is related to unfavorable lipid profile[3], and control of inflammation by antirheumatic drugs conversely improves dyslipidemia in patients with RA[4]. However, evidence is emerging that dyslipidemia is already present years before the advent of arthritis[5]. This suggests that dyslipidemia in patients with RA cannot be explained simply by hyperinflammation *per se*, and that

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other factors also contribute to development of dyslipidemia in RA.

It is estimated that up to 50% of plasma levels of low-density lipoprotein (LDL) cholesterol is heritable in the general population[6]. Because RA is a disease with a strong genetic etiology[7,8], increased LDL concentration in patients with RA, which is critical for accelerated atherosclerosis, might be dependent on a genetic mechanism(s). Indeed, it is reported that the RA susceptibility gene is related to dyslipidemia[9]. The lymphotoxin-α gene, a proinflammatory cytokine gene, is an independent risk factor for dyslipidemia as well as for susceptibility to RA[10]. It has been also demonstrated that apolipoprotein E genotypes are strongly associated with inflammation and lipid levels in RA[11]. Recently, we reported the association of the *APOM* C-1065A polymorphism with an increased risk for developing RA and dyslipidemia in patients with RA[12], and suggested that genetic risk factors for dyslipidemia might play a causal role in RA susceptibility. However, no evidence has been found that such polymorphisms directly affect the risk and phenotype of RA.

Interestingly, lipids have a direct modulating effect on inflammation. Several animal studies have reported that hypercholesterolemia induces inflammation by increasing circulating inflammatory cells[13,14]. Additionally, it is suggested that dyslipidemia, which is noted frequently in patients with the metabolic syndrome or obesity, should be considered a chronic inflammatory state[15]. Similar evidence has also been found in arthritic conditions. For example, patients with osteoarthritis with large infrapatellar fat pad show an inflammatory phenotype[16]. It has been documented that RA patients who have a higher level of circulating adipocytokine, a proinflammatory fat-derived hormone, exhibit more severe joint destruction than patients with lower serum adipocytokines[17,18]. Therefore, it is possible that dyslipidemia itself, such as hyper-LDL cholesterolemia, may be causally associated with inflammatory activity of RA, culminating in joint destruction.

We formulated the following hypotheses. First, there may be common genetic mechanisms that can simultaneously increase the susceptibility of both dyslipidemia and RA. Second, because cholesterol itself can directly influence inflammation[14,15], LDL cholesterol-associated polymorphisms might affect the clinical features of RA, including disease activity. Third, given that a certain gene may influence a disease phenotype throughout life, these polymorphisms could affect the severity of RA as a result of cumulative inflammatory injury. To address these issues, we investigated LDL cholesterol-associated polymorphisms in patients with RA and their association with susceptibility, activity, and radiographic severity of RA.

MATERIALS AND METHODS

Patients and study samples. The Vincent Arthritis Study is a hospital-based prospective cohort of consecutive patients with RA. All participants fulfilled the 1987 American College of Rheumatology criteria for the classification of RA[19]. From this cohort, 302 patients with RA were recruited; all provided written informed consent and received baseline examinations between 2007 and 2010. The non-RA healthy controls consisted of subjects from the Ansung and Ansan cohort. This cohort, representing rural (Ansung) and urban (Ansan) communities, was established in 2001 as a part of the Korean Genome Epidemiology Study (KoGES)[20]. From this cohort, 1636 age- and sex-matched subjects were selected randomly.

All subjects underwent a clinical and laboratory evaluation. Information was collected for the following: age, sex, body mass index (BMI), smoking status, hypertension, and diabetes. Cigarette smoking status was elicited by a self-administered questionnaire and current smoking was defined as any smoking within the past year. Hypertension was defined as average systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive medications. Diabetes was defined as a fasting glucose level ≥ 126 mg/dl, nonfasting glucose level ≥ 200 mg/dl, or the use of glucose-lowering medications.

In patients with RA, the following information was also collected: disease duration, positivity for rheumatoid factor (RF), positivity for anti-citrullinated protein antibody (ACPA), disease activity, and disease severity. Disease activity was evaluated with a Disease Activity Score 28-joint assessment (DAS28)[21]. Disease severity was assessed by evaluating radiographic damage on hand and foot radiographs. The study protocol was approved by the Institutional Review Board of the Catholic Medical Center (XC09TIMI0070).

Genotyping. Genomic DNA from patients was prepared from whole blood samples using the DNeasy Blood & Tissue Kit (Qiagen Sample & Assay Technologies). The genotypes of 3 single nucleotide polymorphisms (SNP; rs688, rs693, and rs4420638) modulating LDL cholesterol were ascertained by PCR amplification of genomic DNA using sequence-specific forward and reverse primers, determined using a BigDye® Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer's protocol, and analyzed on an ABI-3730 apparatus (Applied Biosystems). Genotyping data for control non-RA subjects were obtained from the Korean Association Resource KARE project, which included genotypes of 8842 of 10,038 participants using Affymetrix Genome-Wide Human SNP Arrays 5.0.

Selection of SNP. We wanted to identify SNP modulating LDL cholesterol in Korean patients with RA on the basis of the literature as well as from a genome-wide association study performed in a Korean population. We first selected 7 SNP (in 5 genes) established in previous studies[22,23,24,25,26], all of which showed a close association with plasma LDL cholesterol levels in subjects of European ancestry. These include *APOB* (apolipoprotein B, rs693 and rs7575840), *APOE* cluster (apolipoprotein E, rs4420638), *HMGCR* (3-hydroxy-3-methylglutaryl-coenzyme A reductase, rs12654264), *LDLR* (low-density lipoprotein receptor, rs688 and rs1529729), and *PCSK9* (proprotein convertase subtilisin/kexin type 9, rs11591147). To select SNP modulating LDL cholesterol applicable to the Korean population, we next analyzed a genotype database constructed as part of the KARE project[20], which included genotypes of 10,038 participants using Affymetrix Genome-Wide Human SNP Arrays 5.0. As a result, only 3 (rs693, rs688, and rs4420638) out of the 7 SNP were identified as having a good correlation with plasma concentrations of LDL cholesterol in Koreans[27], and were subjected to further analysis for our study.

Radiographic assessment. Radiographs of the hands and feet were taken at baseline and biannually thereafter. Radiographic severity was scored in chronologic order for erosions and joint space narrowing according to the Sharp-van der Heijde (SvdH) method[28], and was determined by 2 board-certified physicians who were blinded to each patient's identity and clinical status. The interobserver variability described by the interclass correlation coefficient was 0.91. Joint space narrowing and erosion scores were summed to give the total radiographic progression score. Erosion and narrowing progression scores were calculated by subtracting the initial

score from the score after a 2-year followup. Radiographic progression was defined as progression score ≥ 4[17].

Measurement of plasma lipid levels and adipocytokine levels. Lipid variables were measured in fasting venous blood samples according to standard procedures at the Department of Clinical Chemistry, St. Vincent's Hospital, The Catholic University of Korea. Plasma total cholesterol (TC) and triglyceride were assayed by enzymatic methods. High-density lipoprotein (HDL) cholesterol was measured with a homogenous enzymatic colorimetric test. LDL cholesterol was calculated by the Friedewald formula, with assignment of missing values to subjects with a triglyceride level < 400 mg/dl. We considered patients to have dyslipidemia if their fasting plasma HDL cholesterol was < 40 mg/dl in men and < 50 mg/dl in women, or LDL cholesterol was ≥ 160 mg/dl, or triglyceride was ≥ 200 mg/dl, or TC ≥ 200 mg/dl. Serum samples were obtained at baseline and stored at –70°C. The concentrations of adipocytokines in supernatants [leptin (ng/ml), adiponectin (µg/ml), and interleukin 6 (IL-6; pg/ml)] were measured using a commercial ELISA kit (R&D Systems) according to the manufacturer's recommendations.

Statistical analyses. The distributions of all variables were examined. Comparisons were performed by independent t test or chi-square tests for means and proportions between patients and controls. Consistent with a previous study[25], for each subject levels of LDL cholesterol were adjusted for age, sex, and the presence of diabetes to create a multivariable-adjusted residual LDL cholesterol level. Control subjects who were treated with lipid-lowering medication were excluded. We used a logistic regression model to estimate OR for RA susceptibility after adjusting for age, sex, presence of autoantibody, and smoking status. To evaluate the ability of the genotype score to predict risk of radiographic progression, we plotted receiver-operating characteristic (ROC) curves for the genotype score with covariates. We used radiographic progression events (any increase in SvdH score ≥ 4) as the outcome after determining the presence of radiographic progression (any increase in SvdH score ≥ 4) at 2 years. All p values are 2-tailed, with a p value of 0.05 indicating statistical significance. Based on 90% power (1 – β) at a 2-sided error α = 0.05 to detect the difference (rs4420638, 5.3 mg/dl; rs688, 6.3 mg/dl; and rs693, 15.3 mg/dl), 275 patients with RA and 1382 control subjects were needed. Allowing for 10% attrition over the study period, at least 303 RA and 1520 healthy subjects needed to be recruited. Analyses were performed with the use of SPSS software, version 13.0 (SPSS) or R software.

RESULTS

Baseline characteristics. Demographic and clinical characteristics in the study participants are summarized in Table 1. We compared demographic data of patients with RA (n = 302) and healthy controls (n = 1636). The mean age of patients with RA was 53.5 ± 12.3 years and 78.5% were women. Patients with RA had more dyslipidemia but lower BMI than controls (p < 0.001, respectively; Table 1). One hundred sixty-eight patients (55.6%) received methotrexate, 177 (58.6%) hydroxychloroquine, 28 (9.3%) anti-tumor necrosis factor-α (TNF-α) antibodies, and 204 (67.5%) patients were treated with a low dose of prednisolone (≤ 10 mg).

Validation of LDL cholesterol levels. Data on the distribution of genotypes and alleles in participants are presented in Table 2. The genotype frequency of each LDL cholesterol polymorphism agreed with that predicted by the Hardy-Weinberg equation in both patients with RA and controls (p > 0.05). We compared the LDL cholesterol levels according to each SNP. In controls carrying the unfavorable allele that was associated with high LDL cholesterol levels,

Table 1. Demographic features of patients with rheumatoid arthritis (RA) and controls. Data are presented as mean ± SD, median (interquartile range), or percentage as appropriate.

Characteristic	RA, n = 302	Controls, n = 1636	p
Age, yrs	53.5 ± 12.3	53.4 ± 8.2	0.999
Female, n (%)	237 (78.5)	1282 (78.4)	0.999
Current smoker, n (%)	33 (11.9)	201 (12.3)	0.776
Hypertension, n (%)	61 (20.2)	340 (20.8)	0.823
Diabetes, n (%)	27 (8.9)	179 (10.9)	0.300
Dyslipidemia, n (%)	58 (19.2)	131 (8.0)	< 0.001
Body mass index, kg/m²	22.8 ± 3.3	23.9 ± 3.2	< 0.001
Disease duration, yrs	6 (3–12)	NS	NS
DAS28	4.2 (2.9–5.4)	NS	NS
Rheumatoid factor+, n (%)	209 (69.2)	NS	NS
ACPA+, n (%)	226 (74.8)	NS	NS

DAS28: Disease Activity Score in 28 joints; ACPA: anticitrullinated protein antibody; NS: nonsignificant.

the difference in LDL cholesterol level between homozygote classes ranged from 5.8 to 17.4 mg/dl. In patients with RA, LDL cholesterol levels were increased in those carrying the minor allele, ranging from 14.1 to 31.4 mg/dl. However, there were too few subjects carrying the minor allele in rs693, and there was no significant trend toward LDL cholesterol levels between homozygote classes. The influence of the 2 SNP on LDL cholesterol levels remained significant after correction for age, sex, and medications (data not shown).

SNP and RA susceptibility. The frequency of unfavorable (minor) alleles linked to hyper-LDL cholesterolemia was significantly higher in patients with RA than in controls (p = 0.001 for rs688, p = 0.003 for rs4420638). In particular, findings for rs4420638 remained significant after the stratified analysis according to the presence of dyslipidemia (p < 0.001 for the dyslipidemic group, p = 0.001 for the nondyslipidemic group, data not shown). We further compared patients with RA to control subjects with diabetes mellitus, which is known to be more prevalent in those with dyslipidemia, to exclude the carryover effect. One hundred forty-one (51.6%) diabetic patients had dyslipidemia. Interestingly, there was no difference in LDL cholesterol levels between patients with RA and those with diabetes mellitus (131.2 ± 36.3 mg/dl in RA vs 134.8 ± 26.9 mg/dl in diabetes mellitus; p = 0.062), but the frequency of the minor allele was significantly higher in RA than in diabetes mellitus (rs688, p = 0.044; rs4420638, p = 0.002; Appendix 1). The influence of the 2 SNP on the risk of developing RA was still statistically significant after correction for age, sex, and the presence of dyslipidemia [OR 1.988 (95% CI 1.158–2.346) for rs688 and OR 1.327 (95% CI 1.241–3.648) for rs4420638, data not shown]. However, in a fully adjusted model, this significance was lost. The minor allele homozygote of rs4420638 increased the susceptibility for RA, regardless of the autoantibody status,

Table 2. Validation of low-density lipoprotein (LDL) cholesterol level according to single-nucleotide polymorphism (SNP)-associated LDL cholesterol in patients with RA and controls.

SNP	Allele	MAF	Major Allele Homozygotes		Heterozygotes		Minor Allele Homozygotes		p*
			No.	LDL Cholesterol Levels, mg/dl	No.	LDL Cholesterol Levels, mg/dl	No.	LDL Cholesterol Levels, mg/dl	
RA									
rs688 301	C/T	0.179	211	128.2 ± 34.9	76	136.5 ± 40.1	14	159.6 ± 38.8	0.013
rs693	C/T	0.048	282	130.9 ± 36.5	11	143.7 ± 31.1	9	145.0 ± 55.7	0.364
rs4420638	A/G	0.157	222	130.8 ± 35.3	67	132.5 ± 39.8	13	150.9 ± 46.9	0.032
Controls									
rs688	C/T	0.141	1204	112.0 ± 31.8	403	114.0 ± 31.8	29	129.1 ± 31.7	0.012
rs693	C/T	0.053	1469	112.7 ± 32.2	162	112.8 ± 30.9	5	119.2 ± 26.8	0.362
rs4420638	A/G	0.100	1326	107.7 ± 21.2	292	110.1 ± 29.2	18	113.5 ± 32.6	0.039

* For trends of 3 genotype groups after adjustment for age, sex, and presence of diabetes. MAF: minor allele frequency; RA: rheumatoid arthritis.

and the rs688 minor homozygotes increased the risk of developing RA, limited to seropositive patients (Figure 1A, 1B). Similarly, among current smokers, patients with the minor allele homozygote of rs688, but not that of rs4420638, exhibited a significant increase in the risk of RA (Figure 1C).

Genotype score and RA susceptibility. In line with a previous study[25], we constructed a genotype score for each patient by counting the number of minor alleles. This scoring system is based on the assumption that the combination of involved polymorphisms would be more relevant

to explain the phenotype of a certain disease than a single SNP, which reflects only a modest fraction of the variance in phenotype[29]. With zero, 1, or 2 minor alleles for each SNP, the possible genotype score ranged from 0 to 6. Indeed, the absolute differences in LDL cholesterol levels (57.9 mg/dl) were greater than those of each SNP (14.1 to 31.4 mg/dl). Because a small number (n = 24) of patients with RA had a score of 3 or more, these patients were regarded as a single group. As shown in Appendix 2, with a higher genotype score, plasma levels of LDL cholesterol were proportionally increased in both RA patients and controls, even after
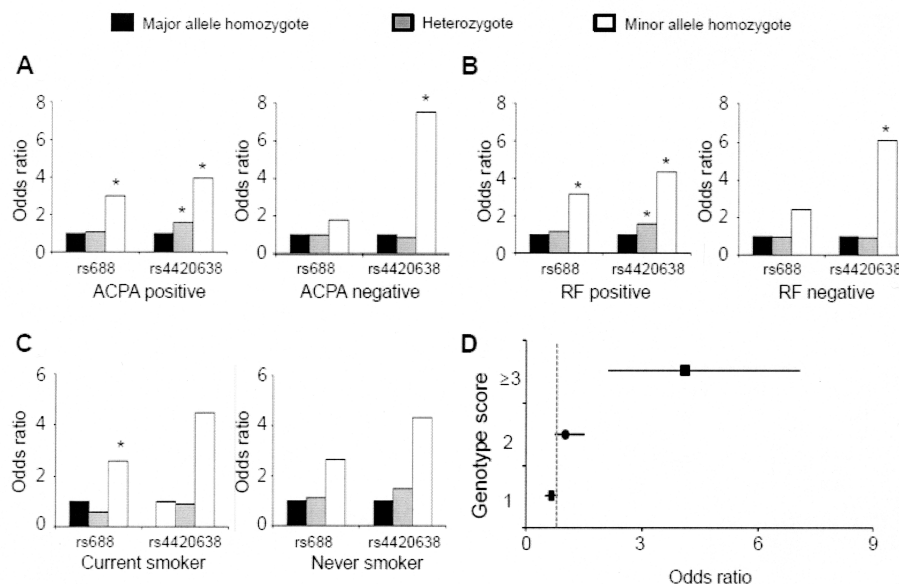

Figure 1. Low-density lipoprotein (LDL) cholesterol-associated polymorphisms and susceptibility to rheumatoid arthritis (RA). A-C. OR for developing RA. OR are calculated per single unfavorable allele, compared with major allele homozygotes. A and B. OR according to the presence or absence of anticitrullinated protein antibody (ACPA) or rheumatoid factor (RF). C. OR according to smoking status. D. RA susceptibility according to the genotype score. Symbols and lines represent OR and 95% CI, respectively. The logistic-regression model is adjusted for age, sex, and the presence of dyslipidemia. OR for RA susceptibility are calculated per single unfavorable allele as compared with genotype score = 0. *p < 0.05.

adjustment for age and sex. Moreover, subjects with a genotype score ≥ 3 had a higher risk for RA, by 4.12-fold, compared to healthy controls (Figure 1D).

Genotype score, RA activity, and RA severity. Next, we investigated whether each genotype influences RA activity and severity. RA patients carrying minor allele homozygotes of rs688 had higher levels of disease activity and those carrying rs4420638 showed a trend toward higher radiographic score (Appendix 3). However, the significance of the relationship between each SNP and RA disease activity/severity disappeared after adjustment for disease duration, presence of autoantibody, and therapeutic options (data not shown). Interestingly, when genotype score, rather than a single SNP, was introduced for the analysis, its association with disease activity and radiographic severity became statistically significant (Table 3). For example, the erosion score, narrowing score, and total SvdH score all increased proportionally as genotype score increased (Table 3). To ascertain the relationship between genotype score and radiographic severity, we performed additional exploratory analyses, considering other traditional risk factors that affect radiographic severity of RA (Appendix 4), and as described in other studies[30]. After adjustment for these confounders and medications, genotype score was still independently associated with radiographic severity (β coefficient = 0.162, p = 0.004; Figure 2A). Because genotype score correlated well with the LDL cholesterol level, we next investigated whether plasma LDL cholesterol concentrations reflect radiographic severity.

Genotype score and radiographic progression. Based on our finding that genotype score was involved in radiographic severity, we further investigated whether this score could serve as a predictor of radiographic progression. When the prevalence of cholesterol-raising SNP in RA patients with and those without radiographic progression was compared, patients carrying the minor allele of rs688 and rs4420638 were observed to have radiographic progression (Appendix 5). In agreement with previous reports[31,32], we observed that at baseline, platelet count, erythrocyte sedimentation rate (ESR), RF titer, and smoking status were significant indicators for predicting radiographic progression in our cohort (Appendix 6), which was defined as progression score ≥ 4. Using these variables, we constructed ROC curves to determine the relative contribution of genotype score to radiographic progression in comparison to other conventional risk factors. Surprisingly, the area under the ROC curve derived from genotype score was 0.704 (Figure 2C), which was greater than that from other risk factors, including ESR/C-reactive protein (CRP) at baseline, the presence of RF or ACPA, and current smoking (Figure 2C). Moreover, the effect of genotype score on radiographic progression remained significant when it was subjected to additional adjustment for age, presence of dyslipidemia, and treatment option [OR 2.175 (95% CI 1.448–3.049); Figure

Table 3. Comparison of disease activity, radiographic severity, autoantibody, and medications according to genotype score. The genotype score represents the number of unfavorable alleles (the allele associated with higher LDL cholesterol) at each of 3 single-nucleotide polymorphisms.

| | Genotype Score | | | | |
	0 (n = 157)	1 (n = 82)	2 (n = 39)	3 (n = 24)	p*
Disease activity					
DAS28	4.1 (2.8–5.2)	4.4 (3.1–5.2)	4.9 (2.9–6.8)	4.9 (2.9–7.1)	0.021
Tender joint counts	3 (1–5)	6 (1–7)	8 (1–13)	7 (1–13)	0.001
Swollen joint counts	2 (0–4)	4 (1–5)	5 (1–9)	4 (0–9)	0.017
ESR, mm/h	29 (13–38)	36 (15–47)	35 (14–55)	33 (12–60)	0.275
CRP, mg/dl	0.8 (0.1–0.9)	0.9 (0.1–1.4)	1.2 (0.3–1.6)	1.4 (0.5–1.6)	0.621
Radiographic severity					
Total SvdH score	34 (6–49)	37 (12–49)	37 (16–42)	62 (32–97)	0.005
Erosion score	18 (4–24)	19 (6–29)	19 (5–21)	33 (18–45)	0.004
Narrowing score	16 (2–24)	17 (4–23)	17 (8–20)	29 (14–49)	0.012
Disease phenotype					
Duration, yrs	9.8 (3–12)	8.6 (4–15)	7.4 (3–12)	9.4 (3–11)	0.479
RF-positive, n (%)	96 (61.1)	65 (79.3)	30 (76.9)	18 (75.0)	0.284
ACPA-positive, n (%)	110 (70.1)	66 (80.5)	31 (79.5)	19 (79.2)	0.635
Treatment					
Glucocorticoid	116 (73.9)	55 (67.1)	29 (74.4)	14 (58.3)	0.122
NSAID	117 (72.6)	70 (85.4)	27 (61.5)	20 (83.3)	0.906
Methotrexate	86 (54.8)	48 (58.5)	21 (53.8)	13 (54.2)	0.871
Anti-TNF-α	14 (8.9)	5 (6.1)	5 (12.8)	4 (16.7)	0.271

* For trends of 4 genotype scores and adjusted for age and sex. DAS28: Disease Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; SvdH: Sharp van der Heijde; RF: rheumatoid factor; ACPA: anticitrullinated protein antibody; NSAID: nonsteroidal antiinflammatory drugs; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; LDL: low-density lipoprotein.
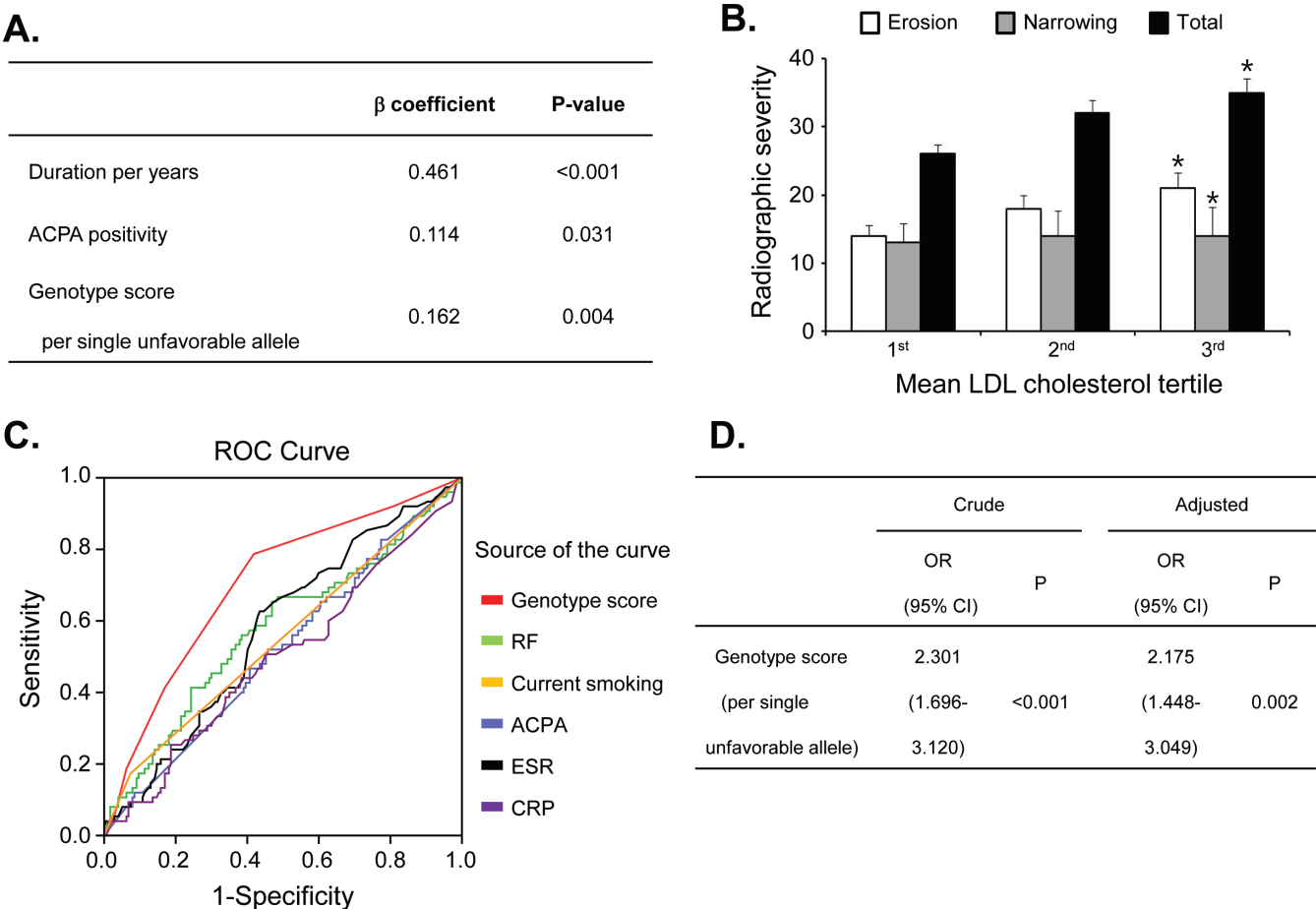
A.

	β coefficient	P-value
Duration per years	0.461	<0.001
ACPA positivity	0.114	0.031
Genotype score per single unfavorable allele	0.162	0.004

B.

C. ROC Curve

Source of the curve
- Genotype score
- RF
- Current smoking
- ACPA
- ESR
- CRP

D.

	Crude		Adjusted	
	OR (95% CI)	P	OR (95% CI)	P
Genotype score (per single unfavorable allele)	2.301 (1.696-3.120)	<0.001	2.175 (1.448-3.049)	0.002

Figure 2. Association of polymorphisms modulating LDL cholesterol levels with radiographic severity and progression in RA. A. Multivariable analysis of the association between genotype score and radiographic severity. Adjusted R^2 for the model is 24.9%. This model is adjusted for age, body mass index, sex, ESR, CRP, RF positivity, use of medications (glucocorticoid, methotrexate, hydroxychloroquine, anti-TNF-α, and statin), and dyslipidemia. B. Radiographic severity 2 years later according to plasma LDL cholesterol levels. Patients with high LDL cholesterol group (3rd tertile) had higher radiographic score than 1st tertile group. *p < 0.05. C. Receiver-operating characteristic (ROC) curves for radiographic progression during 2-year followup. D. Genotype score as a predictor for radiographic progression over 2 years. For adjusted OR, logistic regression was used after adjustment for age, smoking status, RF positivity, ACPA positivity, platelet levels, ESR levels, CRP levels, use of medications (glucocorticoid, methotrexate, hydroxychloroquine, anti-TNF-α, and statin), body mass index, and dyslipidemia. LDL: low-density lipoprotein; RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor; TNF: tumor necrosis factor; ACPA: anticitrullinated protein antibody.

2D]. Together, these observations suggest that genotype score might be one of the important indicators to predict radiographic progression of RA.

Genotype score and adipocytokine levels. Finally, we wondered how genotype score was related to severity and progression of RA. Evidence is emerging that adipo-cytokines with proinflammatory activity, produced mainly from adipose tissues, are increased in patients with RA, and their levels correlate with disease activity and radiographic severity[17,18]. Thus, we measured circulating adipocytokine levels in sera samples of 246 patients with RA and compared them with genotype score and radiographic severity. The results showed that patients with higher genotype score (≥ 3) had elevated adipocytokine levels compared to patients with genotype score = 0 after adjustment for age, sex, and BMI (adiponectin, p < 0.001;

leptin, p < 0.001; and IL-6, p = 0.041; Figure 3). Moreover, adiponectin and IL-6 levels correlated positively with radiographic scores (adiponectin, γ = 0.151, p = 0.033; IL-6, γ = 0.148, p = 0.036). However, leptin levels showed a trend for a positive correlation with the joint space narrowing score, although this was not statistically significant (γ = 0.141, p = 0.053). Collectively, these data suggest that the adipocytokine level varies depending on the genotype score, which might explain the destructive phenotype in RA patients with minor alleles.

DISCUSSION

Sometimes, 2 ostensibly unconnected conditions are closely related. Examples include Alzheimer's disease and dyslipidemia[33], baldness and coronary heart disease[34], and height and diabetes or cancer[35]. This interrelationship may be a
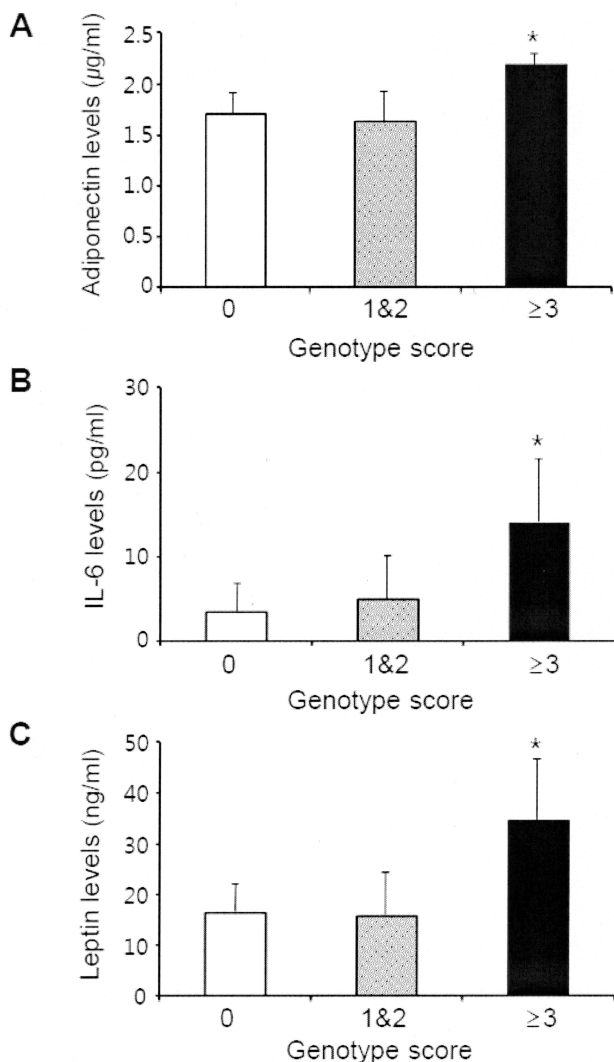
Figure 3. Correlation of genotype score with adipocytokine levels. Levels of adiponectin (A), interleukin (IL) 6 (B), and leptin (C) in the sera of patients with rheumatoid arthritis (n = 246) were determined by ELISA. Data show median ± SEM. *p < 0.05 versus genotype score 0.

genetic factor. The ε4 allele of the apolipoprotein E gene (APOE-ε4) has been confirmed as a genetic risk factor for late-onset Alzheimer's disease and dyslipidemia[36]. The high mobility group A gene associated with height frequently affects cancer, obesity, atherosclerosis, and diabetes[35]. In our study, we discovered that polymorphisms associated with LDL cholesterol (rs688 and rs4420638) previously thought to be unrelated are actually involved in susceptibility to RA. Moreover, they can be a good predictor for RA disease activity, severity, and progression as well as plasma cholesterol levels. To our knowledge, this is the first study to show the association of cholesterol-related polymorphisms with RA susceptibility, severity, and prognosis.

In our study, we created a genotype score from 3 validated SNP modulating LDL cholesterol, consistent with prospective studies of European ancestry[23]. Constructing a genotype score is known to be more effective in explaining some diseases or phenomena because each polymorphism acts independently and additively on the risk of disease, whereas a single-sequence variant is limited to explaining only a modest fraction of the variance (2% or less)[29]. Similar to studies conducted in diabetes, schizophrenia, bipolar disorder, and CVD[25,37,38], we found that the genotype score in RA displayed a stronger clinical relevance than a single SNP. In particular, the association of the genes with disease activity and severity became significant in analysis of the genotype score, although it was not evident with individual SNP, indicating that a combination of the involved SNP should be investigated to identify genetic mechanisms of the development and progression of RA.

Increasing evidence[9,10] indicates that genetic factors are related to the presence of dyslipidemia in RA. Yet these studies are limited in that they explained a high prevalence of dyslipidemia primarily in association with proinflammatory cytokine genes. Thus, it remains to be determined why patients with preclinical RA have dyslipidemia[7], and whether dyslipidemia plays a causal role in RA. We observed that the risk of RA and plasma LDL cholesterol levels increased proportionally as genotype score increased. This indicates that there should be a common genetic predisposition for the synchronicity of RA and dyslipidemia.

We also identified that genotype score was an independent risk factor for joint destruction. Importantly, it correlated well with radiographic progression over a period of up to 2 years, independent from the presence of ACPA, disease duration, and disease activity. How does the LDL cholesterol genotype score contribute to the radiographic severity of RA? Previous studies demonstrated that obese people are at increased risk of developing RA[39]. Also, 2 recent studies[40,41] reported that lipid-lowering therapy may be protective against development of RA, reducing disease activity and the number of swollen joints. An animal study demonstrated that hypercholesterolemia interferes with the bone marrow stromal cell-derived factor-1/CXCR-4 axis, resulting in lymphocytosis, thrombocytosis, and progenitor cell mobilization[42]. Further, hypercholesterolemia increases circulating inflammatory monocyte counts and renders these cells more prone to emigration into target lesions[13], indicating that dyslipidemia itself contributes to the inflammatory response. In our study, as the genotype score increased, measures of RA disease activity, including ESR/CRP, serum IL-6 level, and DAS28 score, all increased. This is in agreement with studies[43] showing that hyperlipidemic patients have more inflammation than nondyslipidemic patients. Moreover, mean LDL cholesterol levels, which we found increased with genotype score (Table 2), correlated positively with radiographic severity, suggesting that polymorphisms modulating LDL cholesterol

might contribute to rheumatoid inflammation, possibly by regulating plasma cholesterol levels.

Another possible explanation is that polymorphisms modulating LDL cholesterol may affect adipocytokine levels. Recently, adipocytokines have gained attention related to the pathogenesis of RA. It has been proven experimentally that leptin and adiponectin, as representative adipocytokines, were able to induce production of inflammatory mediators such as TNF-α and IL-6, promote T cell activation, and upregulate matrix metalloproteinase[44,45]. Concentrations of adipocytokines are increased in RA and correlate with disease activity, erosion, and joint space narrowing[46,47]. Our patients with a higher genotype score had elevated adipocytokine levels compared to those with a lower genotype score after adjustment for age, sex, and BMI. We also found that adiponectin and IL-6 levels were higher in patients with a higher radiographic score (data not shown). These findings, together with previous reports[45,46,47], imply that increased adipocytokines, according to genotype score, could contribute to joint destruction.

Our study has some limitations. First, we analyzed only LDL cholesterol levels. Because other lipid profiles (e.g., HDL cholesterol) may also be involved in inflammatory activity, they should be considered a part of the genotype score. Second, we analyzed relatively few SNP, because only 3 SNP were finally confirmed in the process of selecting polymorphisms modulating LDL cholesterol established well in Europeans and of filtering SNP for Korean population through GWAS. Further, among them, rs693 had a much lower frequency in Korean subjects (minor allele frequency was 0.053 in the general population and 0.048 in patients with RA) compared to Europeans (frequency 0.40–0.48). Similarly to the finding that HLA-DR3, known to be an RA susceptibility allele in those of European descent, was rare in an Asian RA cohort[48], SNP modulating LDL cholesterol levels seemed to show ethnic differences. Third, the 2-year followup period was too short

to reveal development of new cardiovascular events and so we could not include this issue as a subsidiary theme. Clarification of this important issue is required.

Our study provides 3 important assertions. First, patients with RA have a dyslipidemic genetic predisposition. Second, genetic predisposition to LDL cholesterol levels, estimated by a genotype score, is associated with an increased risk of RA, which may explain why RA patients are more prone to CVD. Finally, acting together, SNP modulating LDL cholesterol levels may have a substantial influence on the inflammatory process, promoting radiographic severity of RA. Our findings suggest that, in addition to conventional risk factors, the LDL cholesterol-associated genotype score may be a notable marker for prediction of radiographic progression in patients with RA.

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Appendix 1. Comparison of single-nucleotide polymorphism (SNP)-associated low-density lipoprotein (LDL) cholesterol levels in patients with rheumatoid arthritis (RA) and diabetes mellitus.

SNP	Allele	MAF	Major Allele Homozygotes No.	LDL Cholesterol Levels, mg/dl	Heterozygotes No.	LDL Cholesterol Levels, mg/dl	Minor Allele Homozygotes No.	LDL Cholesterol Levels, mg/dl	p*
RA									
rs688	C/T	0.179	211	128.2 ± 34.9	76	136.5 ± 40.1	14	159.6 ± 38.8	ND
rs693	C/T	0.048	282	130.9 ± 36.5	11	143.7 ± 31.1	9	145.0 ± 55.7	ND
rs4420638	A/G	0.157	222	130.8 ± 35.3	67	132.5 ± 39.8	13	150.9 ± 46.9	ND
Diabetes mellitus									
rs688	C/T	0.130	205	133.0 ± 31.7	65	137.5 ± 34.8	3	155.1 ± 35.2	0.044
rs693	C/T	0.042	250	134.7 ± 32.4	23	136.9 ± 30.7	0	NA	0.631
rs4420638	A/G	0.095	223	134.4 ± 31.6	48	136.6 ± 34.5	2	149.6 ± 43.8	0.002

* Comparison of minor allele frequency between RA and diabetes mellitus after adjustment for age, sex, and use of statin. MAF: minor allele frequency; ND: not done; NA: not applicable.
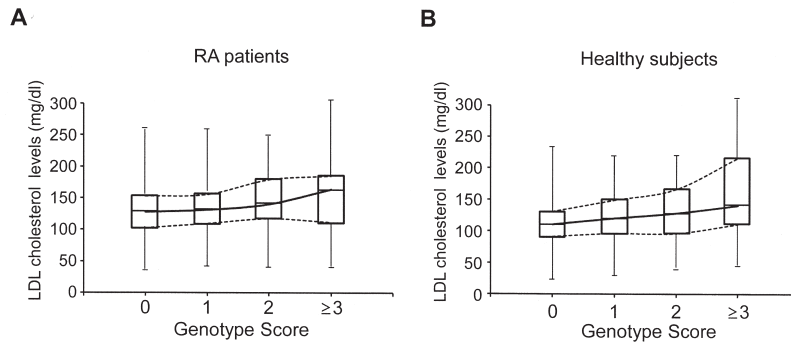
Appendix 2. Low-density lipoprotein (LDL) cholesterol genotype score and plasma LDL cholesterol levels in patients with rheumatoid arthritis (RA) and in healthy subjects. Solid lines indicate spline plot of LDL cholesterol levels and broken lines are interquartile ranges. Data were adjusted for age, sex, and presence of diabetes.

Appendix 3. Comparison of disease activity, radiographic severity, and disease phenotype according to each single-nucleotide polymorphism (SNP).

	rs688				rs4420638			
	Major Allele Homozygotes, n = 211	Heterozygotes, n = 76	Minor Allele Homozygotes, n = 14	p*	Major Allele Homozygotes, n = 222	Heterozygotes, n = 67	Minor Allele Homozygotes, n = 13	p*
Disease activity								
DAS28	4.1 (2.9–5.2)	4.3 (3.0–6.0)	4.3 (3.5–6.8)	0.103	4.2 (2.9–5.3)	4.2 (2.9–5.8)	4.2 (3.3–6.3)	0.799
Tender joint counts	3 (1–6)	4 (1–10)	6 (1–12)	0.019	3 (1–7)	4 (1–11)	4 (1–8)	0.226
Swollen joint counts	2 (0–4)	1 (0–6)	3 (1–8)	0.419	2 (0–5)	2 (0–7)	3 (1–7)	0.195
ESR, mm/h	23 (13–38)	29 (14–58)	51 (13–62)	0.005	24 (14–39)	27 (14–39)	14 (11–50)	0.728
CRP, mg/dl	0.2 (0.1–0.8)	0.4 (0.1–1.7)	0.5 (0.1–3.3)	0.008	0.3 (0.1–1.2)	0.2 (0.1–0.8)	0.5 (0.1–1.9)	0.267
Radiographic severity								
Total SvdH score	32 (7–48)	35 (15–59)	35 (10–35)	0.272	33 (7–49)	34 (13–60)	35 (33–36)	0.064
Erosion score	17 (5–24)	21 (7–30)	21 (4–24)	0.243	18 (5–25)	17 (5–31)	21 (19–23)	0.456
Narrowing score	14 (2–24)	14 (6–26)	14 (7–15)	0.428	14 (2–24)	14 (7–26)	14 (11–16)	0.439
Disease phenotype								
Duration, yrs	6 (3–12)	7 (3–13)	4 (2–12)	0.332	7 (3–13)	6 (3–11)	3 (1–9)	0.204
RF-positive, n (%)	143 (67.8)	56 (73.7)	10 (71.4)	0.772	151 (68.1)	49 (73.1)	9 (69.2)	0.423
ACPA-positive, n (%)	157 (74.4)	58 (76.3)	11 (78.6)	0.873	162 (73.0)	54 (80.6)	10 (76.9)	0.284

* For trends of each SNP and after adjustment for age and sex. DAS28: Disease Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; SvdH: Sharp van der Heijde; RF: rheumatoid factor; ACPA: anticitrullinated protein antibody.

Appendix 4. Association between radiographic severity score and risk factors.

	γ	p
Age	0.153	0.010
Sex	0.092	0.121
Disease duration	0.414	< 0.001
Body mass index	–0.076	0.208
Smoking status	0.036	0.589
Disease activity score-28 joints	0.043	0.475
Erythrocyte sedimentation rate	0.061	0.301
C-reactive protein	0.057	0.334
Rheumatoid factor	0.018	0.757
Anticitrullinated protein antibody	0.133	0.029

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Appendix 5. Comparison of frequency of single-nucleotide polymorphisms (SNP) and genotype score between radiographic progression group and nonprogression group.

SNP Associated with LDL Cholesterol	Radiographic Progression	No Radiographic Progression	p
rs688			
C vs T			< 0.001*
C/C (n = 174)	38 (21.8)	136 (78.2)	
C/T (n = 60)	24 (40.0)	36 (60.0)	
T/T (n = 12)	7 (58.3)	5 (41.7)	
rs4420638			
A vs G			< 0.001*
A/A (n = 182)	39 (21.4)	143 (78.6)	
A/G (n = 52)	28 (53.8)	24 (46.2)	
G/G (n = 12)	4 (33.3)	8 (66.7)	
Genotype score	0 (0–1)	1 (0–2)	< 0.001†

* Chi-square test and † Mann-Whitney test after adjustment for age and sex. LDL: low-density lipoprotein.

Appendix 6. Association between radiographic progression score and risk factors.

	γ	p
Age	0.122	0.047
Sex	0.014	0.820
Disease duration	0.003	0.965
Body mass index	–0.108	0.082
Smoking status	0.141	0.024
Disease activity score-28 joints	0.107	0.180
Erythrocyte sedimentation rate	0.121	0.049
C-reactive protein	0.033	0.398
Rheumatoid factor	0.127	0.022
Anticitrullinated protein antibody	0.030	0.635

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