The *APOM* polymorphism as a novel risk factor for dyslipidaemia in rheumatoid arthritis: a possible shared link between disease susceptibility and dyslipidaemia

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Abstract

Objective

A decrease in high-density lipoprotein (HDL) cholesterol during inflammation is common in many rheumatologic diseases, including rheumatoid arthritis (RA). Apolipoprotein M (apoM) is an apolipoprotein predominantly associated with HDL cholesterol. Recently, apoM polymorphisms have been related with RA susceptibility. We investigated the possible association between an APOM polymorphism and dyslipidaemia in Korean RA patients.

Methods

Two hundred and fifteen RA patients and 215 controls that provided complete genotyping were included. Genetic distribution, RA-associated phenotype, lipid profiles, and lipoproteins were evaluated.

Results

RA patients had increased frequencies of the APOM *C-1065A A allele compared to the controls. RA patients with A/A genotypes had lower levels of HDL cholesterol than those with C/C genotypes. After adjustment for confounding factors, the A/A genotype was a risk factor for low HDL cholesterolaemia (OR=1.070, p=0.001). Subgroup analyses according to disease activity showed that the association between APOM genotype and HDL cholesterol levels was still significant in all subgroups, indicating that this APOM polymorphism may increase the dyslipidaemia risk, independently of RA disease activity.*

Conclusion

These data support that the APOM *C-1065A polymorphism is associated with increased risk for developing RA and dyslipidaemia in RA patients. Reduced HDL cholesterol levels are independent of disease activity but are significantly influenced by APOM genotype. These findings suggest that a specific genetic factor for RA could be linked to dyslipidaemia and this could increase the risk of atherosclerosis in RA patients.*

Key words

rheumatoid arthritis, *APOM* polymorphism, dyslipidaemia

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Introduction

Cardiovascular disease (CVD) is a major cause of increased mortality in patients with rheumatoid arthritis (RA) (1). Although the precise mechanisms involved in increased CVD risk are various and remain elusive, one factor may be dyslipidaemia. Dyslipidaemia associated with RA is characterised by decreased total cholesterol levels and relatively more depressed high density lipoprotein (HDL) cholesterol levels (2). The fact that HDL cholesterol is decreased to a greater extent than the total cholesterol results in an increased atherogenic index, which is a leading predictor of cardiovascular risk. It has been suggested that the inflammatory state associated with RA may be closely involved in the pathogenesis of dyslipidaemia (3, 4). However, evidence suggests that dyslipidaemia is present years before arthritis develops, a phenomenon which cannot be explained by inflammation itself (5, 6).

Apolipoprotein M (apoM) is a recently discovered 25 kDa protein which belongs to the lipocalin protein superfamily (7). In animal studies, apoM has been shown to stimulate the formation of preβ-HDL, which is an acceptor of cellular cholesterol from peripheral cells (8). Two studies using genetically modified mice have reported that overexpression of apoM increases HDL cholesterol concentrations and has an atheroprotective role (9, 10). Additionally, human studies report a positive correlation between apoM and HDL cholesterol levels, suggesting an important role for apoM in human HDL metabolism (11, 12).

APOM genes are located within the major histocompatibility complex (MHC) class III region of chromosome 6p21.3 (7). Many genes in this region, including tumour necrosis factor-α (TNF-α), are related to immune and inflammatory responses. Recently, Hu *et al*. (13) have identified that apoM polymorphisms were associated with RA susceptibility. Several studies showed significant differences in plasma apoM levels between CVD patients, and *APOM* polymorphisms carry an increased risk of CVD in the Chinese population (14-16). This implicates that APOM plays a significant role in the susceptibility to RA and may contribute to the increased risk of dyslipidaemia, leading to excess CVD mortality in RA. In this study, we investigated the possible association of *APOM* polymorphism and dyslipidaemia in Korean RA patients.

Methods

Patients

Two hundred and fifteen RA patients were recruited from St. Vincent's Hospital and 215 age- and gender-matched healthy individuals were recruited from the Korean Genome Epidemiology Study. RA patients fulfilled the 1987 American College of Rheumatology criteria for the classification of RA (17). The following subjects were excluded: those with a history of CVD, uncontrolled arterial hypertension (>160/100 mmHg), diabetes, chronic renal failure, current or chronic infection, pregnancy, excessive alcohol use (>5 times per week), and malignancy. Three months of stable current treatment were necessary for inclusion. Dyslipidaemia was defined as total cholesterol ≥200 mg/dl, low-density lipoprotein (LDL) ≥130 mg/dl, HDL cholesterol <50 mg/dl for women and <40 mg/dl for men, triglyceride ≥150 mg/dl, or the use of lipid-lowering agents. The study protocol was approved by the Institutional Review Board of the Catholic Medical Center (XC09TIMI0070). All patients gave written informed consent to the study protocol.

Genotyping

We obtained genotyping data from 215 individuals from the Korean Association Resource project. Subjects had been genotyped using Affymetrix Genome-Wide Human SNP Arrays 5.0. Using the BRLMM algorithm, genotypes were called, and standard quality control procedures were adopted (18). Genomic DNA was extracted from whole blood by using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. ApoM C-1065A polymorphism analyses were performed by TaqMan analysis using the primer/probe sequence (ABI assay-on-demand C_7514748_10). Primers and probes were

designed by Applied Biosystems (Applied Biosystems, Foster, CA, USA). Polymerase chain reaction (PCR) was performed in a 10 μl reaction mixture containing 100 ng genomic DNA. The cycle conditions were as follows: 1 cycle at 95°C for 10 minutes and 50 cycles at 95°C for 15 sec and 60°C for 1 minute. PCR was carried out using the Rotor-Gene Thermal Cycler RG6000 (Corbett Research, Mortlake, NSW, Australia), and the products were read and analysed using Rotor-Gene 1.7.40 software (Corbett Research).

Clinical, laboratory, and radiographic assessment

All subjects underwent standard evaluation: age, sex, height, weight, and disease duration were ascertained by medical records. Patients with RA were assessed for the following clinical factors: a complete blood count, blood glucose, serum creatinine, erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibodies. Disease activity was evaluated with a Disease Activity Score 28-joint assessment (DAS28) (19). Disease activity status was defined as follows: DAS28 score <3.2, low disease activity; DAS28 score ≥5.1, high disease activity; and a score between 3.2 and 5.1 indicated moderate disease activity. Both hands and both feet radiographs were also taken in RA patients and then analysed by a board-certified physician, who was blinded to each patient's identity and clinical status. Radiographic severity was determined by measuring the erosion and narrowing score according to the Sharp method (20).

Measurement of plasma lipids and apoM levels

In fasting venous blood samples, we measured lipid parameters according to standard procedures at the Department of Clinical Chemistry, University Hospital St. Vincent. Plasma total cholesterol (TC), triglycerides, and lipoprotein (a) [Lp(a)] were assayed by enzymatic methods. HDL cholesterol was measured with a homogenous enzymatic colorimetric test. Plasma preβ-HDL concentration was measured by crossed immuno-electrophoresis essentially as previously described (21, 22). LDL cholesterol was calculated by the Friedewald formula. Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured by immunoturbidimetry. Plasma apoM concentrations were assayed using a commercial enzyme-linked immunosorbent assay Kit (USCN, Wuhan, China) according to the manufacturer's instructions. The concentration of apoM in the calibrator was determined using a standard of known apoM concentration. The range of the standard curve was 0.56–100 ng/ml.

Table I. Baseline characteristics of study participants.

Variables	Controls (n=215)	RA patients (n=215)	*p*-value
Age, yr	53.4 ± 5.8	53.3 ± 12.3	0.952
Female, n (%)	168 (78.6)	170 (79.1)	0.976
Hypertension, n (%)	32 (14.9)	50 (23.3)	<0.001
BMI, kg/m^2	24.5 ± 2.9	22.6 ± 3.2	<0.001
Glucose, mg/dl	83.3 ± 13.4	107.8 ± 41.8	<0.001
Creatinine, mg/dl	0.77 ± 0.09	0.73 ± 0.19	0.784
CRP, mg/dl	0.13 (0.06–0.23)	0.21 (0.08–0.87)	<0.001
Total cholesterol, mg/dl	189.4 ± 29.9	198.4 ± 34.2	<0.001
Triglyceride, mg/dl	131.1 ± 63.6	105.8 ± 61.5	<0.001
HDL cholesterol, mg/dl	50.3 ± 8.7	47.7 ± 15.5	<0.001
LDL cholesterol, mg/dl	104.2 ± 28.1	128.4 ± 31.6	<0.001
Disease duration, yr	NS	6.0 (3.0–12.0)	NS
Radiographic score	NS	22.0 (6.0–61.0)	NS
DAS28 score	NS	4.11 (2.93–5.24)	NS
RF†, n (%)	NS	146 (67.9)	NS
Anti-CCP Ab†, n (%)	NS	168 (78.1)	NS
Prednisolone, n (%)	NS	166 (77.2)	NS
Statin, n (%)	NS	19 (8.8)	NS
Hydroxychloroquine, n (%)	NS	137 (63.7)	NS
Methotrexate, n (%)	NS	121 (52.3)	NS
Anti-TNF-α, n (%)	NS	15 (7.0)	NS

Data are presented as mean±SD or median (IQR). *p*-values represent the comparison between RA patients and controls. NS: not stated. † RF and anti-CCP Ab positivity.

Statistical analysis

Variables with normal distribution were presented as mean±SD, and differences between the mean values were examined by ANOVA. Variables showing non-normal distribution were expressed as medians (interquartile range; IQR). Comparisons of the nonparametric data between groups were performed by Kruskal-Wallis test. For categorical data, the difference in prevalence was evaluated by a chi-square test or Fisher's exact test. Consistency of genotype frequencies with the Hardy-Weinberg equilibrium was examined using a chi-squared goodness-of-fit test. Correlations between the two variables were performed using the Spearman's rank correlation coefficient. ANCOVA and logistic regression analysis was used to adjust for confounders. Strength of associations between low HDL cholesterolaemia and genotypes of the *APOM* C-1065A polymorphism was estimated using odds ratios (ORs) and 95% confidence intervals (CIs), via multiple logistic regression. Estimates were adjusted for conventional factors (body mass index, presence of hypertension, smoking status, statin use, creatinine and fasting blood glucose) and further adjusted for RA-associated risk factors (disease duration, CRP, disease activity score 28-joints, rheumatoid factor positivity, anti-cyclic citrullinated antibody positivity, prednisolone, methotrexate, hydroxychloroquine, and anti-tumour necrosis factor inhibitors). The study had a 99.2% power to detect low HDL cholesterolaemia in the A/A genotype patients from the C/C genotype patients, with a two-sided significance level of 0.05. The results were considered significant if the two sided *p*-value was <0.05.

Table II. Genotype distributions and allele frequencies of *APOM* C-1065A in participants.

Group	Genotype			
	C/C	C/A	A/A	
RA	63 (29.3)	117 (54.4)	35 (16.3)	
Control	90 (41.9)	103 (47.4)	22 (10.2)	
Group	Allele		*p*-value	OR (95%CI)
	C	A		
RA	243 (56.5)	187 (43.5)	0.001	1.468 (1.171–1.842)
Control	283 (65.8)	147 (34.2)		

Values are number of subject (% frequencies). *p*-value represents comparison of A allele frequency between RA patients and control subjects.

Results

The APOM *C-1065A polymorphism and RA susceptibility*

The study group consisted of 430 participants: 215 patients with RA and 215 healthy controls. Dyslipidaemia was highly prevalent in 168 (79.2%) patients with RA. Baseline characteristics of RA patients and control subjects are presented in Table I. The genotype distributions of the *APOM* C-1065A polymorphism were in Hardy-Weinberg equilibrium in the RA patients (p=0.116) and in the control groups (p=0.169). The A allele frequency was statistically higher in patients with RA than in the control subjects (43.5% *versus* 34.4%, OR=1.468, p=0.001). The resulting genotypic and allelic frequencies are shown in Table II.

Association of the APOM *C-1065A polymorphism with RA-related phenotypes*

Table III shows the RA-related phenotypes between the genotypic distributions for the *APOM* C-1065A polymorphism. A allele carriers (A/C and A/A) had shorter disease durations and higher frequencies of anti-TNF-α therapy than those with C/C genotypes. However, no difference in disease activity and radiographic severity was observed between the two groups, which suggests that the *APOM* polymorphism is not a major determinant of RA disease activity and severity. Moreover, neither the prevalence of comorbid conditions, such as hypertension, nor other medications, including prednisolone, statin, hydroxychloroquine, and methotrexate differ across *APOM* genotypes.

Effect of the APOM *C-1065A polymorphism on lipid profiles*

Using univariate analysis, we found that patients with the A allele had significantly decreased HDL cholesterol and TC levels in comparison with those having C/C genotypes (Table IV). Although patients with A/C and A/A genotypes had somewhat decreased apoM, apoA-I, and apoB levels compared with those having the C/C genotype, no statistically significant difference of these apolipoprotein levels was observed among the genotypes (Table IV). Since apoM is known to induce preβ-HDL formation and apoM levels are affected by inflammation, we investigated the association between genotypes and preβ-HDL levels and between apoM levels and disease activity. The results showed that the A allele carriers tended to have lower levels of preβ-HDL, but this trend did not reach statistical significance.

Using multivariate analysis, genotype A/A was still associated with low HDL

Table III. *APOM* C-1065A genotype and clinical characteristics of RA patients.

Variables	Genotype				*p-value	**p-value
	C/C (n=63)	C/A (n=117)	A/A (n=35)	C/A+A/A (n=152)		
Age, yr	52.0 ± 12.2	53.4 ± 12.9	55.1 ± 10.1	53.7 ± 12.5	0.495	0.376
Female, n (%)	50 (79.4)	91 (77.8)	29 (82.9)	120 (78.9)	0.799	0.849
Hypertension, n (%)	11 (17.5)	28 (24.0)	11 (31.4)	39 (25.8)	0.281	0.187
BMI, kg/m^2	22.1 ± 3.4	22.8 ± 3.1	22.7 ± 3.2	22.8 ± 3.1	0.297	0.150
Glucose, mg/dl	99.2 ± 19.6	111.9 ± 51.1	108.7 ± 36.2	111.2 ± 47.9	0.222	0.022
Creatinine, mg/dl	0.70 ± 0.15	0.74 ± 0.21	0.71 ± 0.13	0.74 ± 0.20	0.321	0.207
CRP, mg/dl	0.21 (0.08–0.87)	0.23 (0.07–0.87)	0.19 (0.08–0.82)	0.24 (0.08–0.86)	0.906	0.743
ESR, mm/hr	23.0 (14.0–37.0)	27.0 (13.0–48.5)	20.0 (14.0–45.0)	26.0 (13.0–47.0)	0.841	0.537
Disease duration, yr	8.0 (6.0–17.0)	7.0 (4.0–12.0)	7.0 (3.0–17.0)	7.0 (4.0–12.0)	0.064	0.040
Radiographic score	26.5 (4.7–72.3)	25.0 (9.8–59.0)	23.0 (4.5–67.0)	25.0 (8.0–59.0)	0.888	0.916
DAS28 score	4.13 (2.73–5.23)	4.10 (2.96–5.15)	4.32 (3.11–5.55)	4.10 (2.99–5.21)	0.308	0.668
RF†, n (%)	42 (66.7)	80 (68.4)	24 (68.6)	104 (68.4)	0.969	0.802
Anti-CCP Ab†, n (%)	52 (82.5)	92 (78.6)	24 (68.6)	116 (79.5)	0.366	0.225
Prednisolone, n (%)	48 (76.2)	92 (78.6)	26 (74.3)	118 (78.1)	0.784	0.755
Statin, n (%)	6 (9.5)	10 (8.5)	3 (8.6)	13 (8.6)	0.977	0.830
Hydroxychloroquine, n (%)	39 (61.9)	78 (66.7)	20 (57.1)	98 (64.9)	0.506	0.677
Methotrexate, n (%)	32 (50.8)	71 (60.7)	18 (51.4)	89 (59.3)	0.289	0.251
Anti-TNF-α, n (%)	1 (1.6)	11 (9.4)	3 (8.6)	14 (9.3)	0.073	0.044

Data are presented as mean±SD or median (IQR). *p-values represent the comparison between C/C, C/A, and A/A genotypes; **p-values represent the comparison between C/C and C/A+A/A genotypes. †: RF and anti-CCP Ab positivity.

Table IV. *APOM* C-1065A genotype and lipid profiles of RA patients.

Variables	RA patients	Genotype				*p-value	**p-value
		C/C (n=63)	C/A (n=117)	A/A (n=35)	C/A+A/A (n=152)		
Total cholesterol, mg/dl	198.4 ± 34.2	206.4 ± 39.2	196.4 ± 31.9	189.3 ± 28.5	195.0 ± 31.1	0.039	0.025
Triglyceride, mg/dl	105.8 ± 61.5	101.4 ± 57.3	104.6 ± 64.2	119.7 ± 59.7	108.3 ± 63.4	0.342	0.455
HDL cholesterol, mg/dl	47.7 ± 15.5	52.8 ± 13.9	48.3 ± 14.4	46.1 ± 18.4	47.8 ± 15.3	0.001	0.010
Preβ-HDL, mg/dl	9.8 (5.7–14.4)	12.3 (5.6–15.1)	9.4 (5.4–14.9)	9.7 (6.5–13.9)	9.6 (5.7–14.4)	0.864	0.593
LDL cholesterol, mg/dl	128.4 ± 31.6	135.2 ± 32.7	128.0 ± 30.4	114.9 ± 27.3	125.3 ± 30.0	0.025	0.067
Lipoprotein (a), mg/dl	11.8 (5.9–20.7)	11.4 (5.1–33.0)	10.5 (5.9–20.4)	14.3 (8.6–20.6)	11.8 (6.1–20.4)	0.467	0.739
Apolipoprotein A-I, mg/dl	148.3 ± 22.6	155.8 ± 25.7	146.8 ± 22.5	147.0 ± 25.8	147.0 ± 21.7	0.197	0.064
Apolipoprotein B, mg/dl	87. 9 ± 22.2	89.6 ± 22.3	88.6 ± 23.2	85.6 ± 18.8	87.6 ± 22.5	0.710	0.526
Apolipoprotein M, mg/dl	7.6 (6.2–9.3)	7.7 (7.0–9.8)	8.0 (6.3–9.7)	7.0 (5.4–8.1)	7.6 (5.8–9.3)	0.185	0.309

Data are presented as mean±SD or median (IQR). *p-values represent the comparison between C/C, C/A, and A/A genotypes; **p-values represent the comparison between C/C and C/A+A/A genotypes.

cholesterolaemia as compared with the C/C genotype (OR=1.053, p<0.001) after adjusting for age and sex (Table V). As seen in Table I, RA patients were more hypertensive and had higher blood glucose levels than the controls. Therefore, we adjusted these factors in addition to conventional factors known to affect HDL levels, including body mass index (BMI), smoking status, statin use, and serum creatinine levels. Decreased HDL cholesterol levels remained significant even after adjusting for all these factors (OR=1.054, p<0.001). In addition, a link between the A/A genotype and decreased HDL levels was not changed by further adjustment for several clinical parameters associated with RA, including disease duration, activity, severity, and medication uses (OR=1.070, p=0.001) (Table V). However, the association of the A/A genotype with decreased total cholesterol and LDL cholesterol disappeared after adjusting for conventional factors and RA-related factors (supplementary Tables I and II), suggesting that the *APOM* genotype selectively affects the HDL cholesterol level but not total cholesterol or LDL cholesterol levels.

The APOM *C-1065A polymorphism affects HDL and apoM levels independent of disease activity*

Chronic inflammatory responses decrease total and HDL cholesterol levels in RA (4-6). Therefore, we finally investigated whether disease activity influences the association of the *APOM* C-1065A variant with fasting plasma HDL cholesterol levels in RA patients. To this end, RA patients were stratified by disease activity (DAS 28), and then the plasma HDL cholesterol levels were compared according to *APOM* genotype. As reported previously (3), HDL cholesterol levels were gradually decreased along with higher disease activity (low, moderate, and high disease activity; 54.14±9.84 mg/dl, 47.42±11.55 mg/dl, 46.84±10.18 mg/dl, respectively, p=0.019). In each subgroup of inactive, moderately active, and highly active patients, A/A genotype patients had significantly lower HDL cholesterol levels than C/C genotype (low, moderate, and high disease activity; 61.78±11.15 mg/dl versus 48.57±13.39 mg/dl [p=0.019], 57.13±7.22 mg/dl *versus* 45.88±10.53 mg/dl [p=0.037], 54.30±13.49 mg/dl *versus* 42.03±15.5 mg/dl [p=0.046], respectively) (Fig. 1A). These results indicate that the *APOM* polymorphism may increase the risk of dyslipidaemia independent of RA disease activity by further decreasing circulatory HDL cholesterol levels already decreased by chronic inflammatory responses.

Interestingly, plasma apoM levels also showed an increasing tendency according to disease activity (low, moderate, and high; 7.11 [5.70–8.52] mg/dl, 7.89 [5.95–9.70] mg/dl, 8.61 [6.41–9.82] mg/dl, respectively, p=0.072), and this association was more evident after correction for genotypes as potential confounders (p=0.045) (Fig. 1B). As a consequence, we stratified patients as low, moderate, and high disease activity patients and then compared plasma ApoM levels among the three groups. The results showed plasma apoM levels were lower in RA patients with A/A genotypes compared to those with C/C types (8.65 [6.70–9.45] mg/dl *versus* 6.44 [5.02–7.95] mg/dl in the DAS28<5.1 group [p=0.025], 9.31

Table V. Multivariate-adjusted HDL cholesterol levels across the *APOM* C-1065A genotypes in patients with RA.

Genotype	Odds ratio for low HDL-cholesterolaemia (95%CI)		
	Adjusted for age and sex	Further adjusted for conventional factors*	Further adjusted for RA-associated factors**
C/C (n=63)	1.000	1.000	1.000
C/A (n=117)	1.047 (1.021–1.073)	1.048 (1.020–1.076)	1.047 (1.014–1.080)
A/A (n=35)	1.053 (1.016–1.088)	1.054 (1.019–1.089)	1.070 (1.022–1.123)
p-value for trend	<0.001	<0.001	0.005

*Conventional factors: body mass index, presence of hypertension, smoking status, statin use, creatinine and fasting blood glucose. **RA-associated risk factors: disease duration, CRP, disease activity score 28-joints, rheumatoid factor positivity, anti-cyclic citrullinated antibody positivity, prednisolone, methotrexate, hydroxychloroquine, and anti-tumour necrosis factor inhibitors. Low HDL-cholesterolaemia was defined as HDL cholesterol <50 mg/dl for women and <40 mg/dl for men.

[5.70–9.65] *versus* 7.88 [7.00–8.75] in the DAS28>5.1 group [p=0.429]) (Fig. 1C), suggesting that circulating apoM levels cannot be sufficiently increased in RA patients with AA genotypes, resulting in relatively decreased levels of HDL cholesterol irrespective of inflammatory state of RA patients.

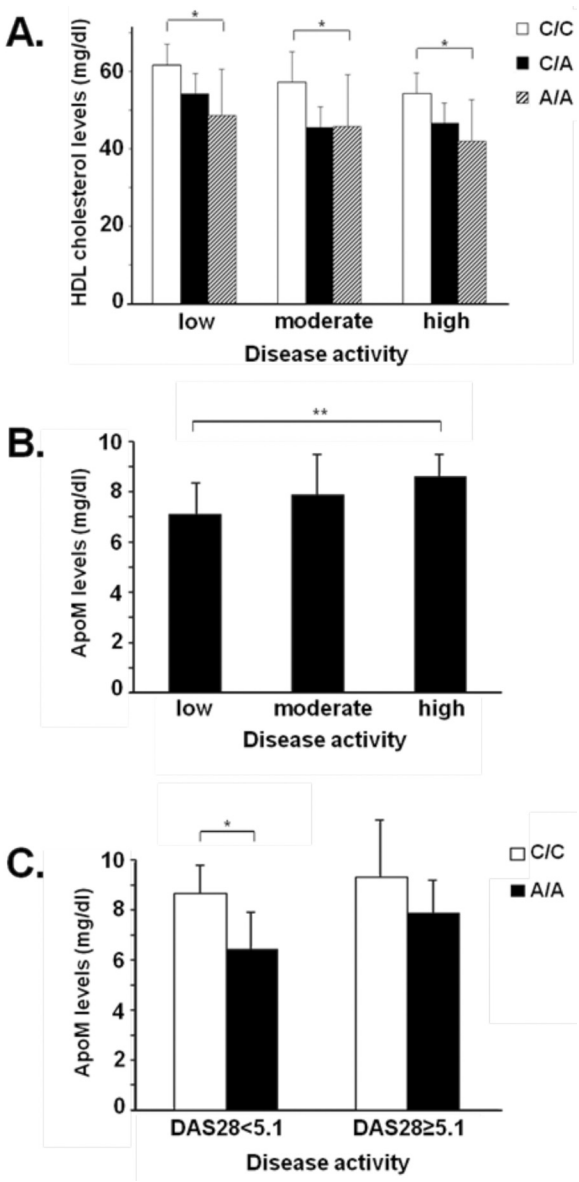

Fig. 1. The *APOM* C-1065A polymorphism affects HDL and apoM levels independent of disease activity.
A. Modifying effect of disease activity on the impact of the *APOM* C-1065A genotype on fasting serum HDL cholesterol levels in patients with RA.
B. Increasing tendencies of plasma apoM levels according to disease activity.
C. Decreased plasma apoM levels in patients with the A/A as compared to those with the C/C genotype in all subgroups. Error bars are SEM $^{*}p$-value and $^{**}p$<0.05. $^{*}p$-values represent the comparison between C/C and A/A genotypes. $^{**}p$-values after adjustment for *APOM* genotype by ANCOVA.

Discussion

Genetic polymorphisms associated with RA have been implicated in the pathogenesis of CVD (23-26). Gonzalez-Juanatey *et al*. have reported for the first time that MHC gene polymorphisms are linked to endothelial dysfunction in patients with RA (27). Since then, other studies have reported that *HLA-DRB1*- shared epitopes, particularly the HLA-DRB1*01/04 combination, which showed an increased risk of CVD (28-32). Recently, some kind of linkage disequilibrium among different genes in the MHC class II and class III regions have shown to increase the risk of CVD in RA (33). In the present study, we assessed the potential influence of a single SNP located in the promoter region of the apoM gene. We identified first that the *APOM* C-1065A polymorphism is associated with increased risk for developing RA and dyslipidaemia in RA patients. ApoM is a recently discovered plasma apolipoprotein predominantly associated with HDL. Given that HDL plays a critical role in the protection of CVD (34-36), our results provide additional evidences that the genetic mechanisms may lead to accelerated atherosclerosis in RA.

It is unclear how the *APOM* polymorphism contributes to the pathogenesis of RA. One possible explanation would be that the difference in alleles of apoM gene affects its expression, which may bring an influence on rheumatoid inflammation through HDL metabolism. It has been suggested that apoM controls the anti-inflammatory function of HDL (37, 38). Therefore, decreased apoM expression may negatively influence the physiologic anti-inflammatory balance, contributing to the uninhibited state of hyper-inflammation leading to chronic arthritis. Another hypothesis is that linkage disequilibrium among different genes may lead to the development of RA. *APOM* gene is located in MHC class III region of chromosome 6 (7), which is a high susceptibility region to RA (39). Moreover, the *APOM* gene is also closely located to pro-inflammatory genes that encode tumour necrosis factor alpha (TNF-α), lymphotoxin alpha (LTA) and beta (LTB) (40), which are known to be responsible for pro-inflammatory responses in RA, and to confer susceptibility to RA (41).

Previously, Hu *et al*. identified four different types of SNPs (rs805296, rs805297, rs1266078, and rs9404941) in the promoter region of *APOM* gene in the Korean population (13), but linkage disequilibrium was not observed among the SNPs. This suggests that the SNP rs805297 is independently associated with RA susceptibility. Although the SNP rs805296, a polymorphism near the rs805297 (*APOM* C-1065A), has been implicated in certain diseases such as type 1 diabetes mellitus and coronary artery disease, it has not yet been associated with RA (15, 16). Moreover, Hu *et al*. found that the SNP rs805297 only affected the transcription activity of *APOM* promoter in 239HEK cells (13). For these reasons, in this study we only determined the polymorphism of the SNP rs805297.

Some evidence has indicated that apoM is associated with pro-inflammatory cytokines as well as lipid metabolism. Xu *et al*. (42) demonstrated that platelet-activating factor significantly enhanced apoM mRNA levels and the

secretion of apoM in HepG2 cell culture. Although contradictory effects were shown *in vivo* and *in vitro*, other inflammatory factors including leptin, TNF-α, and interleukin-1β could up-regulate the expression of apoM mRNA in mice (42, 43). This evidence suggests that apoM may be involved in both inflammation and lipid metabolism. The present study shows that the genotype distributions and allele frequencies of the *APOM* C-1065A polymorphism found among RA patients were higher than those of control subjects, which is in line with a previous report (15). These data suggest that the *APOM* A/A genotype should be one of the genetic risk factors of RA and dyslipidaemia in Koreans, and provide evidence for a possible link between dyslipidaemia and RA susceptibility.

In epidemiological studies, plasma apoM levels were positively correlated with HDL cholesterol levels, and several *APOM* single nucleotide polymorphisms (SNPs) were associated with decreased apoM concentration (11, 40, 44, 45). Jiao *et al*. found that SNP T-778C was associated with total cholesterol level and G+1837T was related to elevated total- and LDL- cholesterol levels (16, 46). Moreover, T/T genotype patients had increased apoM levels, and these patients had a tendency towards higher cholesterol levels as compared to those with G/T and G/G genotypes. One cross-sectional study found an association between apoM levels and carotid intima-media thickness (IMT) or coronary computed tomography, but another study failed to find any relation between apoM levels and carotid IMT (12, 44). Although studies have been inconclusive on apoM as a reliable biomarker of cardiovascular disease, all these studies consistently reported that apoM was significantly associated with plasma HDL cholesterol levels.

In the present study, we found first that *APOM* C-1065A was associated with dyslipidaemia in RA, including increased total and LDL cholesterol but decreased HDL cholesterol levels. In particular, RA patients carrying the A allele had lower levels of HDL cholesterol after adjustment for various factors affecting HDL cholesterol concentration (Table V), which suggests that *APOM* genotypic differences among individual RA patients affect lipid profiles, particularly decreased HLD concentration, a strong risk factor for atherosclerosis.

In this study, the *APOM* C-1065A polymorphism was strongly associated with HDL cholesterol levels, but only modestly related to preβ-HDL concentration. Even though experimental studies have shown that *APOM* may regulate preβ-HDL levels, the findings from epidemiological studies have been inconsistent (8, 47). For example, Plomgaard *et al*. (47) found that plasma apoM levels were elevated in type 2 diabetes mellitus, but preβ-HDL levels were decreased in these patients. Given that the formation of preβ-HDL was also stimulated by phospholipid transfer protein and lecithin:cholesterol acyltransferase activities (48), it is also possible that the levels of preβ-HDL were affected by other enzymatic activities in addition to apoM.

It is well known that chronic inflammation adversely affects lipid profiles, increasing LDL cholesterol but decreasing HDL cholesterol levels (3, 49). A decrease in HDL cholesterol during inflammation is common in many rheumatologic diseases such as systemic lupus erythematosus, Behcet disease, gout, and RA (50-53). Park *et al*. suggested that lipid levels correlate with RA disease activity and that effective control of RA can reverse, at least partially, adverse lipid profiles (54). So, we further investigated whether disease activity influenced HDL cholesterol in RA patients with a certain *APOM* variant, C-1065A. In line with previous data, our results also showed a significant correlation between higher disease activity and lower levels of HDL cholesterol. Interestingly, A allele carriers consistently had lower levels of HDL cholesterol in each subgroup of inactive, moderately active, and severely active RA patients. These results indicate that this *APOM* polymorphism may be associated with dyslipidaemia, independent of RA disease activity. Furthermore, as stated in previous study that dyslipidaemia exists before RA develops, our results suggest that some genetic factors such as *APOM* polymorphism may be a fundamental cause of dyslipidaemia in RA patients and may explain why certain group of RA patients, but not all, still have dyslipidaemia after disease remission.

ApoM was speculated to play a role in anti-atherogenesis because it was identified as a component of HDL (43). Richter *et al*. (55) showed that patients with maturity onset diabetes of young type 3 (MODY3) had reduced plasma apoM levels compared to control diabetes patients. CVD is more common in MODY3 patients than in type 2 diabetes patients (56), suggesting that low apoM levels may increase CVD susceptibility in humans. In contrast, Josefin *et al*. (57) reported that apoM levels did not decrease in coronary heart disease, and several studies suggested that apoM levels can be increased in inflammatory conditions (42, 43, 58). In this study, we found that plasma apoM levels showed a lower tendency in RA patients with A/A genotypes than in those with C/C types, although they showed increasing tendency along with disease activity. Considering that apoM production was tightly linked to plasma HDL cholesterol levels, our results suggest that the *APOM* polymorphism affects circulating apoM levels, and insufficient production of apoM in A/A genotype individuals may lead to ineffective counter-regulation against pro-inflammatory drive to decrease HDL levels in a chronic inflammatory condition, increasing the risk of dyslipidaemia.

This study has some limitations. First, we did not test for the other relevant *APOM* polymorphisms nor considered other genes, including MHC class genes, which might be in linkage disequilibrium with the *APOM* C-1065A (rs805297). Further investigation is needed with larger and various ethnic groups to test this issue. Second, since plasma samples of the healthy controls were not available, we could not present the apoM levels in healthy controls in this study. Third, this study was cross-sectional nature and longitudinal study is needed in other cohort for testing the robustness of these results. Irrespective of these limitations, we provide evidences for the first time that the *APOM*

C-1065A polymorphism is associated with an increased risk of dyslipidaemia in Korean patients with RA. Reduced HDL cholesterol levels are significantly influenced by *APOM* genotype independently of disease activity. These results implicate that a certain genetic factor itself may contribute to developing both RA and dyslipidaemia, increasing the risk of atherosclerosis.

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