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The *RANTES* −403G > A promoter polymorphism in Korean men: association with serum RANTES concentration and coronary artery disease

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ABSTRACT

In the present study we investigated the association of the *RANTES* (regulated upon activation, normal T-cell expressed and secreted) −28C > G and −403G > A promoter polymorphisms with the concentration of serum RANTES and CAD (coronary artery disease) in Korean men. We included 553 male CAD patients with ($n = 176$) or without ($n = 377$) Type 2 diabetes, aged 40–65 years with previous myocardial infarction (~50%) or angiographically confirmed CAD (~50%), and 416 aged-matched healthy male controls. The main outcome measures were the OR (odds ratio) of CAD risk and the serum RANTES concentration evaluated by sandwich ELISA. Although the *RANTES* −28C > G genotype had no significant association with CAD risk, the presence of the minor allele of the *RANTES* −403G > A single nucleotide polymorphism was associated with a lower risk of CAD {OR 0.70 [95% CI (confidence interval) 0.54–0.92], $P = 0.011$} after adjusting for age, BMI (body mass index), cigarette smoking and alcohol consumption. Serum RANTES concentrations were significantly associated with the −403G > A genotype in controls (G/G: 44.7 ± 3.3 ng/ml, G/A: 36.5 ± 2.0 ng/ml, A/A: 28.7 ± 2.5 ng/ml; $P < 0.001$), non-diabetic CAD patients (G/G: 50.9 ± 3.0 ng/ml, G/A: 42.2 ± 2.6 ng/ml, A/A: 41.3 ± 4.4 ng/ml; $P < 0.05$) and diabetic CAD patients (G/G: 58.5 ± 3.5 ng/ml, G/A: 49.6 ± 4.1 ng/ml, A/A: 42.2 ± 4.3 ng/ml; $P < 0.05$); however, such associations were not observed in the subgroup of CAD patients taking lipid-lowering medication. Moreover, serum RANTES was positively correlated with C-reactive protein ($r = 0.289$, $P < 0.001$) and platelet counts ($r = 0.253$, $P < 0.001$). The results of the present study demonstrate that the *RANTES* −403A allele is associated with lower serum RANTES concentrations and consequently with reduced CAD risk.

Key words: −403G > A polymorphism, regulated upon activation, normal T-cell expressed and secreted (RANTES), coronary artery disease (CAD), myocardial infarction, single nucleotide polymorphism, Type 2 diabetes.

Abbreviations: ApoE, apolipoprotein E; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; CRP, C-reactive protein; HDL, high-density lipoprotein; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; LDL, low-density lipoprotein; LLD, lipid-lowering drug; MI, myocardial infarction; OR, odds ratio; RANTES, regulated upon activation, normal T-cell expressed and secreted; SNP, single nucleotide polymorphism.

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INTRODUCTION

Inflammation is emerging as a significant promoter of the initiation and progression of atherosclerotic lesion formation [1]. RANTES (regulated upon activation, normal T-cell expressed and secreted) is a CC chemokine, generated by circulatory lymphocytes and some types of tissue cell monocytes, that plays an important role in the inflammatory process [2]. RANTES has been implicated in two key initial stages of the atherosclerotic process: chemoattraction of leucocytes to the endothelial wall and induction of leucocyte transendothelial migration. This concept is supported by the protective effect seen following the blockage of RANTES receptors in ApoE (apolipoprotein E)-deficient mice [3], suggesting that RANTES levels affect the progress of atherogenesis through the inflammatory pathway.

RANTES is coded by the *CCL5* gene that has been localized to the region q11.2–q12 of chromosome 17. Two SNPs (single nucleotide polymorphisms) of $-28C > G$ and $-403G > A$ in the *RANTES* promoter region have been shown to have a possible association with *RANTES* gene expression [4–6]. Previously, Simeoni et al. [7] have reported that the A allele at the *RANTES* $-403G > A$ promoter polymorphism was associated with increased acute coronary syndrome, independent of conventional CAD (coronary artery disease) risk factors. However, a phenotype associated with polymorphic sites in the *RANTES* promoter has not definitely been determined, due to the complex nature of *RANTES* gene expression. Therefore in the present study our goal was to examine the association of *RANTES* $-28C > G$ and $-403G > A$ promoter polymorphisms with the serum RANTES concentration and CAD in Korean male participants in a Yonsei University Hospital Cardiovascular Genome Center case-control study. Moreover, we investigated the correlation of serum RANTES levels with platelet counts and acute-phase proteins such as hs-CRP (high sensitivity C-reactive protein).

MATERIALS AND METHODS

Study subjects

In the present study, we included 553 consecutive CAD patients and 416 healthy controls from an ethnically and geographically homogenous Korean population.

Male CAD patients with ($n = 176$) or without ($n = 377$) Type 2 diabetes, admitted to the Cardiovascular Center, Yonsei University Severance Hospital, Seoul, South Korea, were recruited. The inclusion criteria were: (i) aged 40–65 years, (ii) angiographic evidence with $\geqslant 50$ % occlusion of one or more major coronary artery or previous MI (myocardial infarction) confirmed according to the WHO (World Health Organization) criteria for symptoms, enzyme elevation or electrocardiographic changes, (iii) absence of non-atherogenic occlusion, such as osteal stenosis and spasm, and (iv) no orthopaedic limitations, no extreme weight loss or gain over the previous 6 months, or any diagnosis of liver disease, renal disease and thyroid or pituitary disease. The inclusion criteria for the healthy male control subjects ($n = 416$) included: aged 40–65 years, no history or diagnosis of atherosclerosis, vascular disease, diabetes mellitus, cancer (clinically or by anamnesis) or renal disease and no pathological ECG patterns. None of the healthy male subjects were taking medication. Written informed consent was obtained from all subjects. The study protocol complied with the Guidelines for Genome/Genetic Research issued by the Korean government and was approved by the Institute of Review Board of Yonsei University Severance Hospital, Seoul, South Korea.

Anthropometric parameters, blood pressure measurements and blood collection

Body weight and height were measured unclothed and without shoes in the morning. BMI (body mass index) was calculated as body weight in kilograms divided by height in metres square (kg/m^2). Blood pressure was determined from the left arm of seated patients with an automatic blood pressure monitor (TM-2654; A&D) after 20 min of rest. The average of three measurements was recorded for each subject.

Venous blood specimens were collected in EDTA-treated and plain tubes between 08.00 hours and 10.00 hours after an overnight fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory. EDTA-treated whole blood was immediately used for a complete blood cell count, and serum was withdrawn after centrifugation for 30 min at 1230 *g* at 4 °C and then aliquots were stored at −70 °C until analysis. Serum taken from plain tubes was used for analysing all metabolic variables (including RANTES levels) in the present study.

Genotyping of the *RANTES* $-28C > G$ and $-403G > A$ polymorphisms

Genomic DNA was prepared from peripheral blood samples using a Puregene® DNA purification kit (Gentra), following the manufacturer's protocol. *RANTES* SNPs $-28C > G$ (rs2280788) and $-403G > A$ (rs2107538) were genotyped using the TaqMan fluorogenic 5′ nuclease assay (Applied Biosystems). The final volume of PCR was 5 μl, which contained 2 ng of genomic DNA and 2.5 μl of TaqMan Universal PCR Master Mix, with 0.125 μl of 40× Assay Mix or 0.25 μl of 20× Assay Mix. Thermal cycling conditions were as follows: 50 °C for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95 °C for 10 min to activate the DNA polymerase, followed by 40 cycles

of 92 °C for 15 s and 60 °C for 1 min. All PCRs were performed using 384-well plates by a Dual 384-well GeneAmp PCR System 9700 (Applied Biosystems), and the end point fluorescent readings were performed on an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

Serum lipid profiles, glucose and insulin

Fasting serum concentrations of total cholesterol and triacylglycerols (triglycerides) were measured using commercially available kits on a Hitachi 7150 Autoanalyser. After using dextran sulfate magnesium to precipitate serum chylomicron, LDL (low-density lipoprotein) and VLDL (very LDL), the remaining HDL (high-density lipoprotein) cholesterol from the supernatant was measured by an enzymatic method. LDL cholesterol was indirectly estimated in subjects with serum triacylglycerol concentrations <4.52 mol/l (400 mg/ml) using the Friedewald formula. In subjects with serum triacylglycerol concentrations $\geqslant$4.52 mol/l, LDL cholesterol was measured directly using an enzymatic method on a Hitachi 7150 Autoanalyser. Fasting glucose was measured by the glucose oxidase method using a Beckman Glucose Analyser (Beckman Instruments). Insulin was measured using a commercially available RIA (Immuno-Nucleo Corporation).

Circulating levels of CRP and RANTES, and platelet count

Serum CRP concentrations were measured with an Express plus autoanalyser (Chiron Diagnostics) using an hs-CRP-Latex (II) X2 kit (Seiken Laboratories) that allowed detection of CRP concentrations as low as 0.001 mg/dl and as high as 32 mg/dl. The intra- and inter-assay coefficients of variation of CRP were 1.3 % and 1.5 % respectively. The platelet count was measured using an electric resistance method using an automatic Blood Cell Counter (LC-240A; Horiba). The intra- and inter-assay coefficients of variation of platelet counts were 1.7 % and 1.9 % respectively. The serum RANTES level was measured, in duplicate, using an ELISA (R&D Systems) according to the manufacturer's protocol. The resultant colour reaction was read using a Victor2 plate reader (PerkinElmer) at 450 nm and wavelength correction was set to 540 nm. The intra- and inter-assay coefficients of variation of RANTES were 2.9 % and 6.4 % respectively and the analytical sensitivity was 6.0 pg/ml.

Statistical analysis

Statistical analyses were performed with SPSS version 12.0 for Windows. HWE (Hardy–Weinberg equilibrium) and LD (linkage disequilibrium) were examined using the Executive SNP Analyzer 1.2A (http://snp.istech.info/snp/SNPAnalyzer.html). The association between CAD and genotype was calculated using the OR (odds ratio) [95 % CIs (confidence intervals)] of a χ^2 test and a logistic regression analysis with an adjustment for age, BMI, smoking and drinking status. An independent Student's t test with a general linear model followed by a Tukey's test was used to compare the differences between controls and CAD patients and among genotype group within controls and CAD patients. ANOVA followed by Tukey's test was also used to compare the differences among genotype groups in control subjects and CAD patients. Pearson correlation test was used to examine the relationship between RANTES concentrations and other biomarkers. Each variable was examined for normal distribution patterns. Significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are expressed as means $\pm$ S.E.M. A two-tailed value of $P < 0.05$ was considered statistically significant.

RESULTS

Clinical features of control subjects and CAD patients are shown in Table 1. Case patients were older ($P = 0.032$) and heavier ($P = 0.014$) than controls. Case patients had a lower prevalence of current smoking and alcohol drinking. Case patients had lower total and LDL cholesterol, probably because about two thirds of them ($n = 353$) had been treated with LLD (lipid-lowering drug) therapy. About one third of case patients had Type 2 diabetes, thus we subdivided CAD patients into two groups: those with or without Type 2 diabetes. CAD patients without Type 2 diabetes were treated with lipid-lowering (66.5 %), antihypertensive (94.1 %) or antiplatelet (96.0 %) drugs. Likewise, CAD patients with Type 2 diabetes were treated with LLDs (58.5 %), antihypertensive (97.7 %), antiplatelet (97.2 %) or hypoglycaemic (61.9 %) drugs. Case patients had higher levels of glucose, insulin, triacylglycerol, platelet counts, RANTES and CRP than controls before and after adjustment for age, BMI, cigarette smoking and alcohol drinking. The subgroup of CAD patients with diabetes showed higher levels of glucose, triacylglycerol, total cholesterol and RANTES than those without diabetes.

Distribution of *RANTES* promoter −28C > G and −403G > A gene polymorphisms

Genotype distributions were in HWE in the entire population as well as when patients and controls were separated. The relative −403G > A genotype and allele frequencies in all patients or in the subgroup of CAD patients with or without diabetes differed significantly

Table 1 Baseline characteristics of healthy controls and CAD patients with or without Type 2 diabetes

Values are means ± S.E.M. or percentages. §Values are log-transformed. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with controls; †$P < 0.05$ and ††$P < 0.001$ compared with CAD patients without Type 2 diabetes with adjustment for age, BMI, cigarette smoking and alcohol consumption status. BP, blood pressure.

		CAD patients		
	Controls ($n = 416$)	All ($n = 553$)	Without Type 2 diabetes ($n = 377$)	With Type 2 diabetes ($n = 176$)
Age (years)	53.8 ± 0.34	54.8 ± 0.29*	54.6 ± 0.35	55.1 ± 0.52*
BMI (kg/m^2)	24.6 ± 0.13	25.0 ± 0.11*	25.0 ± 0.13*	25.2 ± 0.24*
Cigarette smoker (%)	28.7	21.1**	19.7*	24.1
Alcohol drinker (%)	74.1	64.4**	65.8*	61.5**
Systolic BP (mmHg)	127.4 ± 0.82	126.5 ± 0.76	124.0 ± 0.86***	131.9 ± 1.40*††
Diastolic BP (mmHg)	81.9 ± 0.53	79.8 ± 0.42***	79.5 ± 0.48***	80.5 ± 0.81
Glucose (mg/dl)	93.6 ± 0.60	104.6 ± 1.51***	88.6 ± 0.58***	139.0 ± 3.33***††
Insulin (μ-units/ml)§	8.36 ± 0.18	10.4 ± 0.22***	10.3 ± 0.22***	10.5 ± 0.50***
Triacylglycerol (mg/dl)§	137.0 ± 3.51	147.9 ± 2.91***	142.4 ± 3.47*	159.9 ± 5.20***††
Total cholesterol (mg/dl)	196.5 ± 1.68	177.9 ± 1.71***	175.8 ± 1.98***	182.6 ± 3.25***†
LDL cholesterol (mg/dl)	118.1 ± 1.62	101.4 ± 1.53***	101.4 ± 1.79***	101.4 ± 2.92***
HDL cholesterol (mg/dl)	49.0 ± 0.63	44.6 ± 0.49***	44.3 ± 0.59***	45.3 ± 0.90*
Antidyslipidaemic therapy (%)	–	63.9	66.5	58.5
Antihypertensive therapy (%)	–	95.3	94.1	97.7
Antiplatelet therapy (%)	–	96.4	96.0	97.2
Hypoglycaemic therapy (%)	–	19.8	0	61.9††
Platelet count ($\times 10^3/\mu$l)§	237.6 ± 2.82	245.6 ± 2.58	246.8 ± 3.01*	243.1 ± 4.92
RANTES (ng/ml)§	37.7 ± 1.56	48.6 ± 1.50***	45.8 ± 1.85***	51.9 ± 2.42***†
CRP (mg/dl)§	1.64 ± 0.36	2.31 ± 0.20***	2.36 ± 0.26***	2.22 ± 0.33***

Table 2 Frequencies of the *RANTES* promoter −28C > G and −403G > A genotypes in all CAD patients, the subgroup of patients with or without Type 2 diabetes and in healthy controls

			CAD Patients								
	Controls ($n = 416$)		All ($n = 553$)		Without Type 2 diabetes ($n = 377$)		With Type 2 diabetes ($n = 176$)		*P* value compared with controls		
	n	%	*n*	%	*n*	%	*n*	%	All	Without	With
RANTES −28C/G											
CC	265	63.7	379	68.5	261	69.2	118	67.0			
CG	135	32.5	162	29.3	108	28.6	54	30.7	0.143	0.150	0.538
GG	16	3.8	12	2.2	8	2.1	4	2.3			
G allele frequency	167	20.1	186	16.8	124	16.4	62	17.6	0.074	0.069	0.376
RANTES −403G/A											
GG	135	32.5	229	41.4	154	40.8	75	42.6			
GA	211	50.7	242	43.8	170	45.1	72	40.9	0.017	0.048	0.049
AA	70	16.8	82	14.8	53	14.1	29	16.5			
A allele frequency	351	42.2	406	36.7	276	36.6	130	36.9	0.016	0.024	0.106

from those in controls (Table 2). However, the genotype and allele frequencies of −28C > G SNP failed to reach significance between controls and CAD patients.

The presence of the minor allele of the *RANTES* −403G > A SNP was associated with a lower risk of CAD [OR 0.68 (95% CI 0.52–0.89), $P = 0.004$]. The significance of the association remained after adjustments for age, BMI, cigarette smoking and alcohol consumption [OR 0.70 (95% CI 0.54–0.92), $P = 0.011$] (Table 3). Moreover, this association was independent of diabetes status (results not shown). With regard to the −28C > G polymorphism, there was no difference

Table 3 Unadjusted and adjusted OR for all CAD patients according to the *RANTES* − 28C > G and − 403G > A promoter genotype

The adjusted OR is tested by logistic regression with adjustment for age, BMI, cigarette smoking and alcohol consumption. ‡ indicates reference.

RANTES genotype		Unadjusted OR (95 % CI)	*P* value	Adjusted OR (95 % CI)	*P* value
− 28	C‡ compared with G	0.81 (0.64–1.02)	0.074	0.84 (0.66–1.06)	0.142
	CC + CG‡ compared with GG	0.56 (0.26–1.19)	0.128	0.57 (0.26–1.26)	0.167
	CC‡ compared with CG + GG	0.81 (0.62–1.05)	0.115	0.84 (0.64–1.11)	0.225
− 403	G‡ compared with A	0.80 (0.66–0.96)	0.014	0.82 (0.68–0.99)	0.039
	G/G + G/A‡ compared with A/A	0.86 (0.61–1.22)	0.397	0.91 (0.64–1.30)	0.592
	G/G‡ compared with G/A + A/A	0.68 (0.52–0.89)	0.004	0.70 (0.54–0.92)	0.011

in CAD risk both in the dominant or recessive model even after adjustment [CC + CG compared with GG: OR 0.57 (95 % CI 0.26–1.26), $P = 0.167$; CC compared with CG + GG: OR 0.84 (95 % CI 0.64–1.11), $P = 0.225$].

In the LD test, − 28C > G and − 403G > A had a positive LD; however, r^2 values were not considerably high ($r^2 = 0.347$). Also, the − 28C > G polymorphism failed to have any association with CAD risk, and the − 28C > G genotype-related phenotypes including serum RANTES levels were not significantly different in each of control or CAD patients, with or without LLD treatment (results not shown). Thus we did not perform further analysis on − 28C > G polymorphism.

Clinical characteristics and circulating levels of RANTES and CRP, and platelet count, associated with the − 403G > A polymorphism

There was a significant association between the serum RANTES concentration and the − 403G > A genotype in controls (G/G: 44.7 ± 3.3 ng/ml, G/A: 36.5 ± 2.0 ng/ml, A/A: 28.7 ± 2.5 ng/ml; $P < 0.001$), non-diabetic CAD patients (G/G: 50.9 ± 3.0 ng/ml, G/A: 42.2 ± 2.6 ng/ml, A/A: 41.3 ± 4.4 ng/ml; $P < 0.05$) and diabetic CAD patients (G/G: 58.5 ± 3.5 ng/ml, G/A: 49.6 ± 4.1 ng/ml, A/A: 42.2 ± 4.3 ng/ml; $P < 0.05$). Because of the role of LLD treatment on the modification of inflammatory markers [8], we subdivided further the CAD patients according to LLD treatment (Figure 1). Mean concentrations of RANTES in CAD patients treated with or without LLD were 49.0 ± 2.01 ng/ml and 48.1 ± 2.27 ng/ml respectively. However, the association between the serum RANTES level and the − 403G > A genotype was different according to LLD treatment. There was no difference in circulating levels of RANTES across the genotype in CAD patients with LLD treatment, whereas there was a significant association between the serum RANTES concentrations and the − 403G > A genotype in untreated CAD patients, with A/A subjects having values approx. 35 % lower than G/G subjects ($P < 0.05$) (Figure 1). The RANTES levels in patients carrying the − 403A allele were unaffected by LLD treatment; however, patients carrying the − 403G/G genotype in the LLD treatment group had approx. 15 % lower concentrations of RANTES than those in the non-LLD treatment group. This pattern was shown regardless of their diabetic status. There were no significant genotype-related differences among control subjects according to their − 403G > A SNP genotype with respect to age, BMI, cigarette smoking, alcohol consumption, serum glucose, insulin and lipid profiles (results not shown). Similarly, in CAD patients, there were no genotype-related differences for age, BMI, cigarette smoking, alcohol consumption, serum glucose, insulin, lipid profiles and pharmacological interventions (results not shown). No significant genotype associations with circulating levels of CRP and platelet count in control subjects and CAD patients were observed regardless of their LLD treatment status (results not shown).

Correlation between RANTES, CRP and platelet count

We found that serum RANTES levels were positively correlated with CRP concentration ($r = 0.289$, $P < 0.001$) and platelet count ($r = 0.253$, $P < 0.001$).

DISCUSSION

The major finding of the present study is that the frequency of the *RANTES* − 403A allele was significantly lower in patients with CAD regardless of the presence of Type 2 diabetes. The lower concentrations of serum RANTES in subjects carrying the AA genotype compared with those with the GG genotype could potentially explain the apparent CAD protection associated with the *RANTES* − 403A allele. In addition, a beneficial effect of LLD treatment on RANTES levels was observed in patients carrying the − 403G/G genotype. The main biological role of RANTES, a CC chemokine, relates to leucocyte chemoattraction. Although interactions between circulating leucocytes and endothelium are

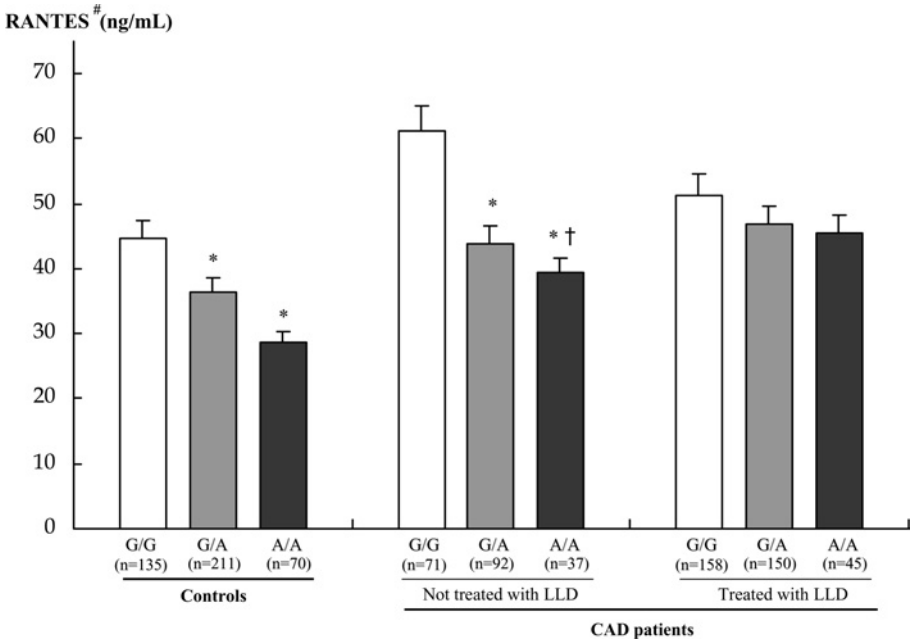

Figure 1 Relationship between the *RANTES* −403G > A genotype and serum RANTES concentration in healthy controls or CAD patients with or without LLD treatment

Values are means ± S.E.M. #RANTES concentrations were log-transformed. *$P < 0.05$ compared with G/G and †$P < 0.05$ compared with G/A, as determined by one-way ANOVA followed by Tukey's test.

beneficial in the host defence mechanism against infection, HIV and cancer, these interactions may also induce the initiation of arterial lesion formation [9,10]. This mechanism is supported by findings showing impaired T-cell and monocyte recruitment to inflammatory sites in mice deficient in the *RANTES* gene [11]. Furthermore, the RANTES antagonist Met-RANTES (an N-terminal-modified methionylated form of RANTES) was found to inhibit monocyte recruitment on carotid endothelium, neointima formation and macrophage accumulation after arterial injury, as well as atherosclerosis progression in uninjured arteries in ApoE-deficient mice [3,12,13]. RANTES antagonists that have been tested in heart transplantation [14] and HIV models [15] might also prove effective in preventing CAD.

Three SNPs in the *RANTES* gene (−403G > A and −28C > G in the promoter, and ln1.1T/C in the first intron) have been reported in different studies with regard to the extent of RANTES expression [4–6]. One study [4] showed that the ln1.1C allele strongly down-regulates the transcriptional activity of RANTES, whereas the −28G allele causes a modest up-regulation and the −403A allele has no obvious effect on RANTES expression. Another group [5] reported that the −403A allele resulted in an up to 8-fold increase in constitutive transcriptional activity after transient transfections of the human mast cell line HMC-1 and the T-cell line Jurkat with reporter vectors driven by either the mutant or wild-type *RANTES* promoter. However, both studies [4,5] did not determine the protein level of RANTES according to polymorphic sites in the *RANTES* gene. These conflicting results may come from the different experimental modelling, since RANTES production may be time- and cell-dependent even in the context of the same genetic background [5]. In addition, considering the complex system of chemokines and chemokine receptors, further studies determining the association of the *RANTES* gene with the RANTES receptor gene and other proximal genes are necessary in order to confirm such genotype effects and the functional significance of RANTES.

Clinical associations of the *RANTES* −403A allele have been reported with several inflammatory diseases [5,7,16–18]. The *RANTES* −403A allele has been associated with an increased susceptibility to atopy [5,16], asthma [16] and sarcoidosis [17] as well as atherosclerosis [7,18], suggesting *RANTES* is a candidate gene for predisposing to inflammatory diseases. Recently, Zhernakova et al. [19] reported that the −403A allele was associated with a lower serum level of RANTES, providing further protection from Type 1 diabetes. Also, Boger et al. [20] showed that individuals with the −403A allele, which is in almost complete LD with the ln1.1C allele, would be low-expressors of RANTES. These results corroborate the present observations of the association of the −403A allele with a low concentration of serum RANTES and consequently with a reduced CAD risk. However, the *RANTES* −28C > G promoter polymorphism had no association with CAD risk and no

genotype-related phenotype differences in the present study.

Overall, the lower concentration of serum RANTES observed in Korean men with the −403A allele was associated with a significantly lower CAD risk (30%) even after adjustment for age, BMI, cigarette smoking and alcohol consumption. However, the present findings are in contrast with a previous study in a White population, which reported an association of the *RANTES* −403A allele with an increased CAD risk [7]. This discrepancy could be attributed to ethnic differences; interestingly, this allele is at a much higher frequency in Koreans (approx. 0.42) compared with Caucasians (approx. 0.17), which may reflect some positive selection in favour of this allele involving differential susceptibility for the environmental changes [21]. Other differences between the present study and that of Simeoni et al. [7] relates to the population selection. Whereas the previous study investigated cases of White men and women with a mean age of 64 years undergoing coronary angiography because of chest pain or non-invasive tests consistent with MI, the population in the present study consisted of Korean men aged 40–65 years old. This selection was made based on the knowledge that CAD is greatly influenced by genetic factors at younger ages (below 65 years) [22]. Furthermore, differences existed between the clinical phenotypes (i.e. CAD with angiographic abnormalities and acute coronary syndrome compared with CAD, including ~50% myocardial infarct and ~50% coronary angiography defined atherosclerosis) in both studies.

A hyperglycaemic state has been suggested to increase the secretion of cytokines and, in turn, cytokines stimulate the expression of RANTES [23,24]. In the present study, CAD patients with Type 2 diabetes had higher concentrations of serum RANTES compared with those without diabetes. On the other hand, CAD patients carrying the −403G/G genotype in the LLD treatment group had an approx. 15% lower concentration of RANTES than those in the non-LLD treatment group regardless of the presence of Type 2 diabetes. This finding suggests a beneficial effect of LLD treatment on RANTES concentrations since the proportions of antihypertensive and antiplatelet therapies were not significantly different between LLD and non-LLD treatment groups. Indeed statins, the most common LLD treatment used by patients in the present study, is known to have both a lipid-lowering effect and anti-inflammatory properties [8]. Interestingly, patients carrying the *RANTES* −403A allele had no differences in serum *RANTES* concentrations between subjects taking or not taking LLDs. Therefore we suggest a pharmacogenetic effect associated with LLD treatment with the *RANTES* polymorphism, with G/G subjects getting additional benefit beyond their lipid-lowering effect.

RANTES stored in platelet secretory vesicles is released upon platelet activation and immobilized on the surface of inflamed endothelium [10,25]. In the present study, despite the positive relationship between platelet concentrations and RANTES levels, the lack of association between the *RANTES* −403G > A polymorphism and platelet levels indicate that the genotype effects on serum RANTES may not be related to platelet concentrations. RANTES has been suggested to activate the transcription of cytokines genes [11,26] which can activate hepatic synthesis of CRP. Although there was no significant association between the *RANTES* −403G > A polymorphism and circulating CRP levels, we observed a positive relationship between serum RANTES and pro-inflammatory CRP levels.

Several points should be considered when interpreting the present findings. First, current methods of measuring circulating RANTES levels in serum would not exclude the possibility of enhanced RANTES concentrations due to *ex vivo* platelet release during sample processing. Relevant to this limitation, the report that circulating RANTES levels does not correlate with local RANTES levels at a site of tissue damage has to be considered [27]. Secondly, our results share the limitations of cross-sectional observational studies by which we only evaluated association rather than prospective prediction. Thirdly, although the present results showed that the *RANTES* −403A allele is associated with CAD risk, the frequency of the subjects carrying the A/A homozygous genotype or G allele was not significantly different between control and CAD patients. However, different frequencies of each G/A genotype and G/G genotype between CAD patients and controls would contribute to the relationship of the *RANTES* −403G > A polymorphism and CAD risk. Finally, because gender is an important risk factor for CAD, in the present study we specifically focused on a representative group of Korean men aged 40–65 years. Therefore our results cannot be generalized to women or to other ethnic, age or geographical groups. Despite these limitations, our results show an interesting association between the *RANTES* −403A allele and reduced risk of CAD as well as a potential pharmacogenomic interaction between this allele and some of the pleiotropic effects of lipid-lowering medication.

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REFERENCES

1 Libby, P. (2002) Inflammation in atherosclerosis. Nature **420**, 868–874
2 Cocchi, F., Devico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C. and Lusso, P. (1995) Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. Science **270**, 1811–1815
3 Veillard, N. R., Kwak, B., Pelli, G. et al. (2004) Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. Circ. Res. **94**, 253–261
4 An, P., Nelson, G. W., Wang, L. et al. (2002) Modulating influence on HIV/AIDS by interacting RANTES gene variants. Proc. Natl. Acad. Sci. U.S.A. **99**, 10002–10007
5 Nickel, R. G., Casolaro, V., Wahn, U. et al. (2000) Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J. Immunol. **164**, 1612–1616
6 McDermott, D. H., Beecroft, M. J., Kleeberger, C. A. et al. (2000) Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. AIDS **14**, 2671–2678
7 Simeoni, E., Winkelmann, B. R., Hoffmann, M. M. et al. (2004) Association of RANTES G-403A gene polymorphism with increased risk of coronary arteriosclerosis. Eur. Heart J. **25**, 1438–1446
8 Waehre, T., Damas, J. K., Gullestad, L. et al. (2003) Hydroxymethylglutaryl coenzyme a reductase inhibitors down-regulate chemokines and chemokine receptors in patients with coronary artery disease. J. Am. Coll. Cardiol. **41**, 1460–1467
9 Hansson, G. K. (2001) Immune mechanisms in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. **21**, 1876–1890
10 Stemme, S., Faber, B., Holm, J., Wiklund, O., Witztum, J. L. and Hansson, G. K. (1995) T lymphocytes from human atherosclerotic plaques recognize oxidized low-density lipoprotein. Proc. Natl. Acad. Sci. U.S.A. **92**, 3893–3897
11 Makino, Y., Cook, D. N., Smithies, O. et al. (2002) Impaired T cell function in RANTES-deficient mice. Clin. Immunol. **102**, 302–309
12 Von Hundelshausen, P., Weber, K. S. C., Huo, Y. et al. (2005) RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. Circulation **103**, 1772–1777
13 Weyrich, A. S., Elstad, M. R., McEver, R. P. et al. (1996) Activated platelets signal chemokine synthesis by human monocytes. J. Clin. Invest. **97**, 1525–1534
14 Horuk, R., Clayberger, C., Krensky, A. M. et al. (2001) A non-peptide functional antagonist of the CCR1 chemokine receptor is effective in rat heart transplant rejection. J. Biol. Chem. **276**, 4199–4204
15 Strizki, J. M., Xu, S., Wagner, N. E., Wojcik, L. et al. (2001) SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **98**, 12718–12723
16 Fryer, A. A., Spiteri, M. A., Bianco, A. et al. (2000) The −403 G > A promoter polymorphism in the RANTES gene is associated with atopy and asthma. Genes Immun. **1**, 509–514
17 Takada, T., Suzuki, E., Ishida, T. et al. (2001) Polymorphism in RANTES chemokine promoter affects extent of sarcoidosis in Japanese. Tissue Antigens **58**, 293–298
18 Szalai, C., Duba, J., Prohaszka, Z. et al. (2001) Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD): coincidence of elevated Lp(a) and MCP-1 −2518 G/G genotype in CAD patients. Atherosclerosis **158**, 233–239
19 Zhernakova, A., Alizadeh, B. Z., Eerligh, P. et al. (2006) Genetic variants of RANTES are associated with serum RANTES level and protection for type 1 diabetes. Genes Immun. **7**, 544–549
20 Boger, C. A., Fischereder, M., Deinzer, M. et al. (2005) RANTES gene polymorphisms predict all-cause and cardiac mortality in Type 2 diabetes mellitus hemodialysis patients. Atherosclerosis **183**, 121–129
21 Sabeti, P. C., Schaffner, S. F., Fry, B. et al. (2006) Positive natural selection in the human lineage. Science **312**, 1614–1620
22 Marenberg, M. E., Risch, N., Berkman, L. F., Floderus, B. and de Faire, U. (1994) Genetic susceptibility to death from coronary heart disease in a study of twins. N. Eng. J. Med. **330**, 1041–1046
23 Satriano, J. A., Banas, B., Luckow, B., Nelson, P., Krensky, A. M. and Schlondorff, D. O. (1997) Regulation of RANTES and ICAM-1 expression in murine mesangial cells. J. Am. Soc. Nephrol. **8**, 596–603
24 Schwarz, M., Radeke, H. H., Resch, K. and Uciechowski, P. (1997) Lymphocyte-derived cytokines induce sequential expression of mono-human mesangial cells. Kidney Int. **52**, 1521–1531
25 Weyrich, A. S., Prescott, S. M. and Zimmermann, G. A. (2002) Platelets, endothelial cells, inflammatory chemokines, and restenosis. Circulation **106**, 1433–1435
26 Locati, M., Deuschle, U., Massardi, M. L. et al. (2002) Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. J. Immunol. **168**, 3557–3562
27 Gallo, R. C., Garzino-Demo, A. and DeVico, A. L. (1999) HIV infection and pathogenesis: what about chemokines? J. Clin. Immunol. **19**, 293–299

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