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Genetic diversity of RANTES gene promoter and susceptibility to coronary artery disease and restenosis after percutaneous coronary intervention

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ABSTRACT

Regulated on activation, normal T cell expressed and secreted (RANTES) gene promoter is a regulatory region and a site of notable genetic diversity. In order to explore a possible interaction between RANTES promoter genetic diversity and susceptibility to coronary artery disease (CAD) and in stent restenosis (ISR), we initially sequenced a locus extending from -516 to 40 covering the entire region of the RANTES promoter in 100 subjects randomly selected from our cohort. Four single nucleotide polymorphisms (SNPs) were identified: -403G/A, -256G/A, -109C/T and -28C/G. The frequency of the -109C/T and -256G/A variations was <0.01, and was considered to be of limited significance. The frequency of the -403G/A and -28C/G polymorphisms was evaluated in the entire sample, which consisted of 118 patients subjected to percutaneous coronary intervention (PCI) without ISR on angiographic re-evaluation (no IRS group), 74 CAD patients with ISR on angiographic re-evaluation (IRS group) and 146 controls without angiographic evidence of CAD (no CAD group). No association was established between the RANTES promoter genotype and ISR. A genotype-phenotype interaction was observed between the -403G/A polymorphism and CAD. The -403A homozygotes were significantly more common in the CAD group than in the controls. The severity of CAD among case subjects, expressed as the mean number of diseased vessels, was significantly higher among -403A homozygotes as compared to wild-type homozygotes and heterozygotes. In conclusion, the RANTES -403A allele was associated with the presence and severity of CAD independently of conventional cardiovascular risk factors. The RANTES promoter genotype did not influence susceptibility to ISR in patients subjected to PCI.

Introduction

Chemokines are small chemotactic cytokines with a pivotal role in leukocyte trafficking and activation [1]. Chemokine-induced pathways have been extensively implicated in the course of atherosclerosis [2,3].

Regulated on activation, normal T cell expressed and secreted (RANTES) is a member of the CC chemokine family. It is a strong chemoattractant for T-lymphocytes and macrophages [4,5]. Several studies have linked RANTES expression levels with the intensity of vascular inflammation and resulting lesion formation [6,7]. It has been demonstrated that the RANTES gene promoter is a regulatory region and a site of notable genetic diversity. Two RANTES promoter polymorphisms (-28 C/G, -403 G/A, rs2107538) have been identified and extensively investigated in case-control and functional studies [8–10]. Both have been associated with increased RANTES transcriptional activity, and thus with increased RANTES-mediated inflammatory response [11,12].

In the present study, we explored the possible impact of RANTES promoter genetic variability on susceptibility to coronary artery disease (CAD) and restenosis after percutaneous coronary intervention (PCI). In order to explore the hypothesized existence of a genotype-phenotype interaction not attributed to the two commonly studied polymorphisms (-28C/G and -403G/A), we applied direct sequencing in a large proportion of our study population.

Patients and methods

Study population

Two hundred CAD patients presenting with clinical signs of recurrent ischemia one month to two years after PCI and stent placement were recruited from two independent cardiology departments over a two-year period. Clinical signs of recurrent ischemia

Abbreviations: RANTES, regulated on activation, normal T cell expressed and secreted; CAD, coronary artery disease; PCI, percutaneous coronary intervention; ISR, in stent restenosis; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; BMS, bare metal stent; DES, drug eluting stent.

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Table 1
Demographic and clinical characteristics of patients with clinical signs of recurrent ischemia after percutaneous coronary intervention, and of the controls.

	All patients (n = 192)	Controls (n = 149)
Age	64 (0.7)	62 (0.9)
Male gender	171 (89.1)	106 (71.1)*
Hypertension	165 (85.9)	88 (59.1)*
Diabetes	91 (47.3)	25 (16.8)*
Dyslipidemia	190 (98.9)	100 (67.1)*
Smoking	114 (59.4)	59 (39.6)*
Positive family history	81 (42.2)	61 (40.9)
Number of diseased vessels:		
1	50 (26)	
2	44 (23)	
3	98 (51)	
Clinical presentation:		
Unstable disease	40 (20.1)	
Stable disease	152 (79.1)	

Values refer to the number of subjects (%) or the means (±SEM).
*p-values under the cut-off point of statistical significance.

were defined as: relapse of effort angina, occurrence of acute coronary syndrome (ACS), new evidence of myocardial ischemia revealed by stress electrocardiogram or SPECT myocardial perfusion imaging. All patients were angiographically re-evaluated and classified into the in stent restenosis (ISR) group or no in stent restenosis (no ISR) group based on the angiographic status of the intervened lesion. ISR was defined as ≥50% diameter reduction on follow-up angiography. The control group comprised 160 age-matched subjects without angiographic evidence of CAD (ns CAD group). Subjects selected as controls also had at least one conventional predisposing factor of CAD (besides age and gender). Nine patients and 11 controls were excluded from the study due to incomplete clinical data or failure of the genotyping process. A total of 192 CAD patients (median age 64; male : female ratio 8.1) were included in the genotype-phenotype association study along with 149 controls (median age 62; male : female ratio 2.5). All the subjects enrolled were Caucasian. Clinical and epidemiological characteristics are summarized in Tables 1 and 2.

Clinical definitions

Smoking was defined as a current or prior history of tobacco use. Diabetes was defined as a fasting blood glucose level >126 mg/dl, or treatment with dietary modification, oral hypoglycemic agents or insulin at the time of the study. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic pressure >90 mmHg in at least three distinct measurements, or in cases where such a diagnosis had been made in the past and the patient was being treated with medication or lifestyle modification. To define dyslipidemia in patients whose coronary status was unknown prior to the study, cut-off levels for the general population were applied. For patients with CAD established prior to the study, dyslipidemia was defined as treatment with lipid-lowering medication, dietary modification or lipid levels higher than those recommended by the Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice [13]. Parental history of myocardial infarction was considered to be positive family history. CAD was classified as 1, 2 or 3 vessel disease based on the number of major epicardial vessels with ≥70% lumen stenosis. Depending on the clinical presentation of CAD, patients were further classified according to whether they suffered from stable disease (asymptomatic patients or patients with stable angina) or unstable disease (unstable angina or myocardial infarction).

Informed consent was obtained from all the individuals participating in the study. Study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by a priori approval by the ethics committee of the University Hospital of Crete and Onassis Cardiac Surgery Centre.

DNA extraction and sequencing

Genomic DNA was extracted from blood samples using the standard phenol/chloroform and ethanol precipitation protocol. Direct sequencing of the promoter region of the RANTES gene was performed in 100 randomly selected samples. Polymerase chain reaction (PCR) was used to amplify the RANTES gene promoter region. A pair of primers framing a 556 bp fragment ranging from -516 to 40 in the gene sequence was applied. The PCR products were resolved on 2% agarose gel, excised and processed with the Qiaquick PCR purification kit (Qiagen Inc.) Sequences were analysed using ABI PRISM 310 Genetic Analyser (PE Applied Biosystems). The sequences were double-checked by the separate application of the forward and reverse primers.

PCR and RFLP analysis

Genotyping of the polymorphisms was originally performed using pairs of external primers framing the regions surrounding each polymorphic site of the RANTES gene. Allele assessment for the -28C/G and -408G/A polymorphisms was performed by subjecting the corresponding polymerase chain reaction products to restriction fragment length polymorphism (RFLP) analysis with, respectively, the MnlI (Fermentas, Canada) and MaeIII (Roche Applied Science Germany) restriction enzymes, as previously described [14].

Statistical analysis

Genotype distributions for each polymorphism were first compared to values predicted by the Hardy-Weinberg equilibrium (HWE) through χ^2 analysis. Haplotypes were calculated and linkage disequilibrium was measured using the classic statistic, disequilibrium coefficient.

The correlation of each of the studied polymorphisms to clinical parameters was first evaluated by χ^2 analysis with two degrees of freedom. The extent of the association of each genotype with the disease was initially estimated by Pearson's χ^2 analysis or the Fisher exact test when indicated (expected frequencies <5). Adjustment for conventional patient risk factors (age, gender, smoking status,

Table 2
Demographic and clinical characteristics of patients with clinical signs of recurrent ischemia after percutaneous coronary intervention.

	ISR group (n = 74)	no ISR group (n = 118)
Age mean (±SEM)	64 (1.2)	64 (0.9)
Male gender	68 (91.9)	103 (87.3)
Hypertension	68 (91.9)	97 (82.2)
Diabetes	38 (51.4)	53 (44.9)
Dyslipidemia	74 (100)	116 (98.3)
Smoking	50 (67.6)	64 (54.2)
Positive family history	30 (40.5)	51 (43.2)
Number of diseased vessels:		
1	15 (20.3)	35 (29.7)
2	15 (20.3)	29 (24.6)
3	44 (59.4)	54 (45.8)
Clinical presentation:		
Unstable disease	15 (20.3)	25 (21.2)
Stable disease	59 (79.7)	93 (78.8)
Type of stent:		
BMS	40(54)	50 (42.4)
DES	33(44.6)	61 (51.7)
BMS and DES	1 (1.4)	7 (5.9)

Values refer to the number of subjects (%) or the means (± SEM).
ISR: in stent restenosis; DES: Drug eluting stent; BMS: Bare metal stent.

diabetes mellitus, hypertension, dyslipidemia and family history) was performed by including these covariates in a logistic regression model. To provide separate odds ratios (ORs) for each genotype, the most common genotype was considered the reference group. Both adjusted and unadjusted ORs are reported in the presence of significant results or borderline significance. With the present sample size, the study had a power of 80% to detect a 1.4-fold increase in allele frequencies, assuming a 40% prevalence of the rare allele in the control group and a type I error probability of 0.05.

Numerical values are expressed as the mean ± SEM, and differences between the means were compared by the 2-tailed unpaired Student's t-test. In all cases, p-values<0.05 were considered statistically significant. Analyses were performed using SPSS v10 (SPSS Inc., Chicago, IL, USA).

Results

Sequencing analysis

Direct sequencing revealed four polymorphisms spanning the 556 bp fragment of the RANTES promoter region (Fig. 1): -403 G/A, -256 C/G, -109 C/T, -28 C/G. Allele frequencies in the sequenced samples were 0.25, 0.005, 0.005 and 0.015, respectively. Due to their extremely low frequency, the -256C/G and -109 C/T polymorphisms were not further genotyped in the study population.

Hardy-Weinberg Equilibrium and genetic Interaction within the RANTES gene

The -403A promoter allele was common in the case and control populations, with respective allele frequencies of 0.284 and 0.225, whereas the -28G promoter allele was rather infrequent. Genotyping yielded strong linkage disequilibrium between RANTES promoter SNPs (D' = 1). -28G always occurs on the haplotype containing 403A variant alleles. Therefore, genotyping resulted in 3 haplotypes and 5 of the 6 possible combined genotypes (Tables 3–5). In accordance with previous observations, the wild-type allele-containing haplotype ($C_{-28}G_{-403}$ or H1) was the most common in both patients and controls. Five of six permitted combined genotypes accounted for the entire sample. The genotype containing wild-type alleles in a homozygote state (28C/C 403G/G or G1) was the most common in all groups (Tables 4 and 5). Genotype frequencies for RANTES-403 complied with HWE in the CAD group as a whole and in the ISR and no ISR subgroups, but deviated from HWE in the controls ($\chi^2 = 9.4$, $p = 0.002$).

Genotype-phenotype interactions were evaluated for each genotype alone, and also for each of the 3 haplotypes and the 5 combined genotypes. The rarity of the -28C/G variation in the present sample did not allow for any genotype-phenotype association.

Table 3
Haplotype frequencies in patients and controls.

Haplotype		ISR	no ISR	Patients	Controls
1	$G_{403}C_{28}$	0.737	0.699	0.714	0.775
2	$A_{403}C_{28}$	0.25	0.288	0.273*	0.208*
3	$A_{403}G_{28}$	0.013	0.013	0.013	0.017
Total (n)		148	236	384	298

Total (n) count refers to the number of alleles.
*$p = 0.051$.
ISR: in stent restenosis.

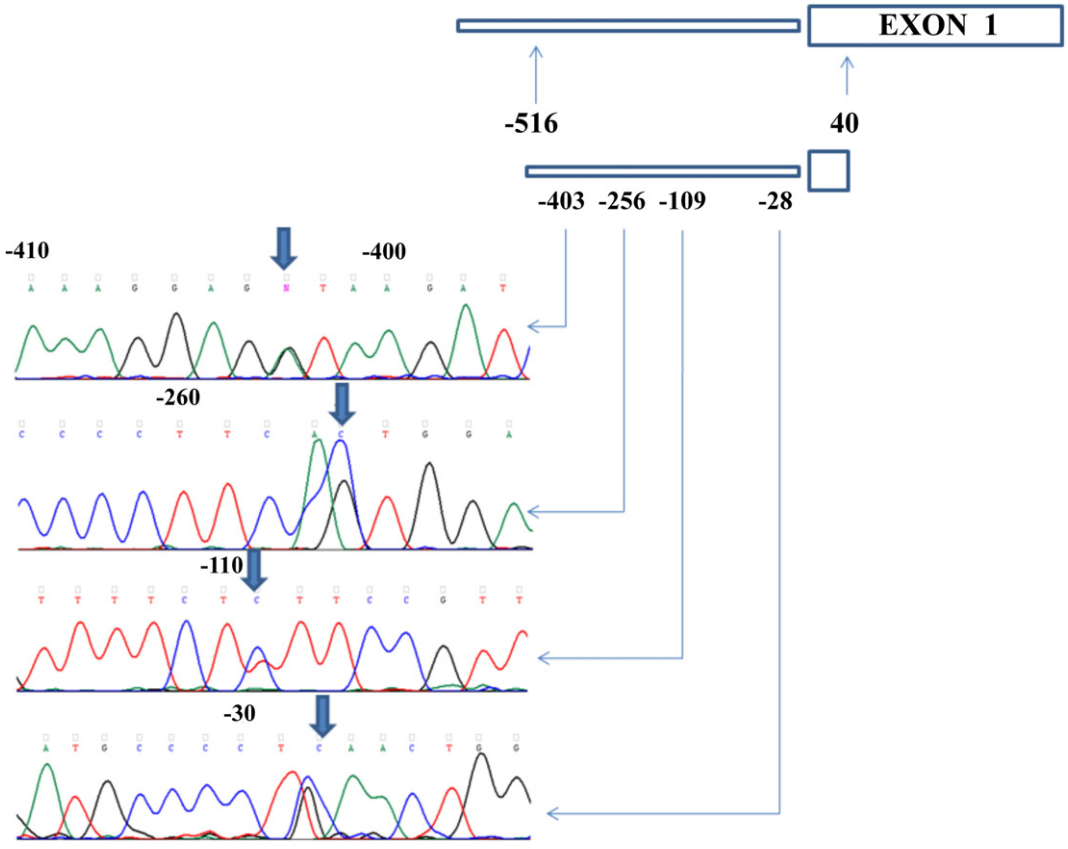

Fig. 1. The genomic structure of the RANTES promoter on chromosome 17q11.2 and representative chromatograms of the four polymorphisms observed in the study population. The polymorphic site is indicated by the arrow.

Table 4
Genotype frequencies in patients and controls.

Genotype	-403	-28	All patients n = 192	Controls n = 149	Odds ratio (95% Confidence Interval)	Adjusted odds ratio (95% Confidence Interval)	Adjusted p
1	GG	CC	96 (50)	83 (55.7)	1	1	-
2	GA	CC	79 (39.6)	60 (40.3)	1.14 (0.73-1.78)	1.08 (0.6-1.8)	0.80
3	AA	CC	12 (6.3)	1 (0.6)	10.4 (1.3-81)	12.6 (1.1-143)	0.041
4	GA	CG	4 (3.6)	5 (3.4)	0.69 (0.18-2.7)	0.27 (0.05-1.4)	0.13
5	AA	CG	1 (0.5)	0		-	-
6	AA	GG	0	0		-	-
3, 5, 6			13 (6.8)	1 (0.7)	11.2 (1.4-87.8)	12.8 (1.13-144)	0.039

All OR and p -values refer to a comparison with the most common genotype (-403GG-28CC). Adjusted OR values have been corrected for age, gender, presence of hypertension, diabetes mellitus, dyslipidemia, positive family history and smoking habits.
Values refer to the number of subjects (%).
ISR: in stent restenosis; OR: odds ratio; CI: confidence interval.

RANTES promoter polymophisms and in stent restenosis

Statistical analysis did not reveal any significant differences between genotype and haplotype frequencies in patients in the ISR and no ISR groups. A predominance of the -403A allele frequency was noted in the no ISR group; however it did not reach the cut-off point for statistical significance (Table 4).

RANTES promoter polymorphisms and coronary artery disease

A genotype-phenotype interaction between the -403G/A polymorphism and CAD was revealed by χ^2 analysis ($\chi^2 = 8.1$, $df = 2$, $p = 0.017$). Statistical significance was obtained for the -403A homozygotes using the wild-type homozygous genotype as the baseline risk. -403A homozygotes were significantly more common in the CAD group than in the controls (OR = 11.2, 95%CI: 1.4-87.8, $p = 0.004$). The latter observation retained statistical significance when the results were adjusted for age, gender and conventional CAD risk factors in a multiple regression analysis (adjusted OR = 12.8, 95%CI: 1.14-144, $p = 0.039$). Combined genotype analysis revealed a predominance of the AACC genotype (G3) in the patients compared to the controls (adjusted OR = 12.6, 95%CI: 1.1-143, p = 0.041), while the $A_{-403}C_{-28}$, haplotype (H2) was more common in the CAD group gene pool than in the controls, with borderline statistical significance ($p = 0.051$).

The severity of CAD among case subjects, expressed as the mean number of diseased vessels per patient, was significantly higher among -403A homozygotes as compared to wild type homozygotes and heterozygotes (2.9 vs. 2.2, $p = 0.02$) (Fig. 2).

No association was established between the RANTES gene promoter genotype and the clinical presentation of CAD.

Discussion

In the present study, no association was observed between the RANTES promoter genotype and ISR. However, a genotype-phenotype interaction was observed between the -403G/A polymorphism and CAD. -403A homozygotes were significantly more common in the CAD group than in the controls. Lastly, the severity of CAD among patients, expressed as the mean number of diseased vessels, was significantly higher among -403A homozygotes as compared to wild-type homozygotes and heterozygotes.

RANTES and coronary artery disease

Gene polymorphisms that modify the expression or bioactivity of chemokines and their cellular receptors may affect leukocyte trafficking and activation, and influence susceptibility to inflammatory diseases such as atherosclerosis.

RANTES has been implicated in the inflammatory pathways that promote atherosclerosis. It is expressed by T lymphocytes in advanced lesions and is highly produced in human transplant-associated accelerated atherosclerosis by macrophages, lymphocytes, myofibroblasts and endothelial cells [7]. Blocking RANTES-mediated signalling *in vivo* using the CC chemokine antagonist Met-RANTES has been shown to reduce the progression of atherosclerosis in animal models. This indicates that the blockade of chemokine receptor/ligand interactions is a potential novel therapeutic target for the deceleration of the progression of atherosclerosis [15].

The impact on CAD of the genetic polymorphisms that influence the CCR5/RANTES-mediated pathways has previously been assessed. Studies based on the screening of the 32-base-pair deletion in the CCR5 receptor gene and its association with CAD have reached conflicting conclusions. Szalai *et al* [16] reported that the CCR5 Δ32 genotype has an atheroprotective effect, as they found a higher frequency of CCR5-Δ32 homozygotes in controls than in CAD patients. Similarly, Gonzalez *et al* [17] demonstrated that non-carriers of the CCR5-Δ32 allele under the age of 55 years had a three-times increased risk of myocardial infarction. In a previous study of ours, the frequency of CCR5-Δ32 was evaluated in 210 angiographically-assessed CAD patients and 165 controls with negative coronary angiography. No differences were observed. However, the frequency of deletion in the population studied was relatively low, limiting the power of a negative

Table 5
Genotype frequencies in patients and controls.

Genotype	-403	-28	ISR n = 74	noISR n = 118	Odds ratio (95% Confidence Interval)	Adjusted odds ratio (95% Confidence Interval)	Adjusted p
1	GG	CC	40 (54)	56 (47.4)	1	1	-
2	GA	CC	30 (40.5)	49 (41.5)	0.86 (0.47-1.58)	1.08 (0.57-2)	0.8
3	AA	CC	2 (2.7)	10 (8.6)	0.28 (0.06-1.35)	0.26 (0.05-1.3)	0.1
4	GA	CG	1 (1.4)	3 (2.5)	0.46 (0.05-4.6)	0.45 (0.04-4.9)	0.5
5	AA	CG	1 (1.4)	0	-	-	-
6	AA	GG	0	0	-	-	-
3, 5, 6			3 (4.1)	10 (8.5)	0.42 (0.11-1.6)	0.4 (0.1-1.6)	0.2

All OR and p-values refer to a comparison with the most common genotype (-403GG-28CC). Adjusted OR values have been corrected for age, gender, presence of hypertension, diabetes mellitus, dyslipidemia, positive family history and smoking habits.
Values refer to the number of subjects (%).
ISR: in stent restenosis; OR: odds ratio; CI: confidence interval.

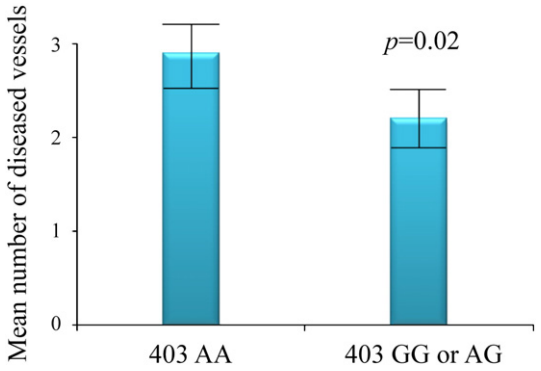

Fig. 2. Association of CAD severity with the RANTES -403 genotype. The mean number of diseased vessels is presented in subgroups defined by the -403 genotype: -403A homozygotes vs. -403 wild-type allele homozygotes and heterozygotes.

association [18]. It has been conclusively demonstrated that the RANTES promoter is an important regulatory region with genetic variability. The effect of polymorphisms of the RANTES promoter has been investigated in several cohorts. Szalai *et al* [16] found no association between RANTES polymorphisms -28G and -403A and CAD. In a study by Boger *et al* [19] of type 2 diabetics in end-stage renal disease, patients carrying the RANTES -403A or In1.1C allele of the intronic In1.1T/C polymorphism had a significantly higher "all cause" mortality risk, due mainly to cardiac events. Simeoni *et al* [20], in the largest cohort assessing the impact of RANTES promoter polymorphisms on susceptibility to CAD, concluded that the -403A allele is associated with CAD independently of conventional risk factors and C-reactive protein or fibrinogen as inflammatory biomarkers.

RANTES and in stent restenosis

There are no data available in the scientific literature regarding the impact of RANTES promoter genetic variability and susceptibility to ISR post PCI, and this is the first study to assess subject. In order to avoid omitting any genotype-phenotype association, we initially applied direct sequencing in a large proportion of our sample. Four polymorphisms spanning the promoter region were identified. However, the -109 C/T and -256 G/A polymorphisms had allele frequencies of <1%, and were unlikely to be associated with CAD or restenosis in the given sample size. We thus concluded that -403 G/A and, to a lesser extent, the -28 C/G polymorphism were more likely to result in clinically significant phenotype alterations.

Genotype-phenotype associations

We did not find a positive association between the RANTES promoter genotype and ISR independently of stent type (BMS or DES). However, the RANTES -403A allele was observed with significantly greater frequently in the CAD group. Statistical significance was established for the -403A homozygotes, which is to be expected assuming an additive effect of the -403A alleles. These findings are in accordance with previous observations in case-control studies, and are fully justified by the reported functional consequences of the RANTES -403A allele [19,20].

Functional studies have associated the -403A allele with increased transcriptional activity of the RANTES gene [11,12]. Increased RANTES expression at sites of endothelial dysfunction probably provokes the accumulation and migration of target cells and enhances inflammation, accelerating the formation of atherosclerotic lesions. It is therefore most likely that the biological mechanism underlying this genetic association is increased gene expression from the RANTES -403A promoter variant.

The most challenging finding in need of explanation is the departure of RANTES-403 genotypes from HWE proportions in the controls. Violation of HWE is commonly the result of genotyping errors. However, genotyping was repeated twice in the control group, and 30% of the samples were re-evaluated by direct sequencing. No deviation was observed between PCR-RFLP and sequencing results. Ethnic diversity was also ruled out as a possible cause of HWE disassociation, as all the study participants were Caucasian. This is not the first study to fail to produce results in accordance with HWE regarding the -403A/G polymorphism. Simeoni *et al* [20], in a control group consisting of 530 subjects, failed to produce genotype frequencies in HWE. Notably, in our study and in that of Simeoni *et al*, the control groups did not consist of randomly selected subjects from the general population, but rather of subjects without angiographic evidence of CAD. Consequently, we believe that this is a reproducible finding, not the result of genotyping error, and warrants further investigation in an unselected population.

Conclusions

In the present study, an association between the RANTES -403A allele in the homozygote state and CAD was found. This association was independent of conventional cardiovascular risk factors. These findings are in agreement with the functional consequences of the -403A polymorphism, and support a favourable effect of RANTES down-regulation regarding susceptibility to severe CAD. It is therefore conceivable that the blockade of RANTES receptor/ligand interaction could serve as a novel therapeutic target in the management of CAD. In fact, Veillard *et al* have already reported that blocking *in vivo* RANTES-mediated signalling using the CC chemokine antagonist Met-RANTES reduced the progression of atherosclerosis in a hypercholesterolemic mouse model [15]. We believe that our findings, from a different perspective, encourage further investigation of the favorable effects of RANTES antagonists in the management of CAD and other complications of atherosclerosis.

The current study did not establish a positive association between the RANTES promoter genotype and ISR. However, we believe that the possible implication of RANTES promoter genetic variability in ISR should be re-evaluated in a larger sample before a definite negative association is determined.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Luster AD. Chemokines - chemotactic cytokines that mediate inflammation. N Engl J Med 1998;338:436–45.
[2] Reape TJ, Groot PH. Chemokines and atherosclerosis. Atherosclerosis 1999;147:213–25.
[3] Apostolakis S, Papadakis EG, Krambovitis E, Spandidos DA. Chemokines in vascular pathology. Int J Mol Med 2006;17:691–701.
[4] Pattison JM, Nelson PJ, Huie P, Sibley RK, Krensky AM. RANTES chemokine expression in transplant-associated accelerated atherosclerosis. J Heart Lung Transplant 1996;15:1194–9.
[5] Song A, Nikolcheva T, Krensky AM. Transcriptional regulation of RANTES expression in T lymphocytes. Immunol Rev 2000;177:236–45.
[6] Wilcox JN, Nelken NA, Coughlin SR, Gordon D, Schall TJ. Local expression of inflammatory cytokines in human atherosclerotic plaques. J Arterioscler Thromb 1994;1(Suppl):S3–S10.
[7] Pattison JM, Nelson PJ, Huie P, Sibley RK, Krensky AM. RANTES chemokine expression in transplant-associated accelerated atherosclerosis. J Heart Lung Transplant 1996;15:1194–9.
[8] Fryer AA, Spiteri MA, Bianco A, Hepple M, Jones PW, Strange RC, et al. The - 403 G/A promoter polymorphism in the RANTES gene is associated with atopy and asthma. Genes Immun 2000;1:509–14.
[9] Nakajima K, Tanaka Y, Nomiyama T, Ogihara T, Ikeda F, Kanno R, et al. RANTES promoter genotype is associated with diabetic nephropathy in type 2 diabetic subjects. Diabetes Care 2003;26:892–8.
[10] Liu H, Chao D, Nakayama EE, Taguchi H, Goto M, Xin X, et al. Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. Proc Natl Acad Sci USA 1999;96:4581–5.

[11] Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, et al. Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. J Immunol 2000;164:1612–6.
[12] McDermott DH, Beecroft MJ, Kleeberger CA, Al-Sharif FM, Ollier WE, Zimmerman PA, et al. Chemokine RANTES promoter polymorphisms affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. Aids 2000;14:2671–8.
[13] Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice. European guidelines on cardiovascular disease prevention in clinical practice. Eur J Cardiovasc Prev Rehabil 2003;10:1–78.
[14] Amanatidou V, Sourvinos G, Apostolakis S, Neonaki P, Tsilimigaki A, Krambovitis E, et al. RANTES Promoter Gene Polymorphisms and Susceptibility to Severe Respiratory Syncytial Virus-Induced Bronchiolitis. Pediatr Infect Dis J 2007;27:38–42.
[15] Veillard NR, Kwak B, Pelli G, Mulhaupt F, James RW, Proudfoot AE, et al. Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. Circ Res 2004;94:253–61.
[16] Szalai C, Duba J, Prohaszka Z, Kalina A, Szabo T, Nagy B, et al. 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 2001;158:233–9.
[17] Gonzalez P, Alvarez R, Batalla A, Reguero JR, Alvarez V, Astudillo A, et al. Genetic variation at the chemokine receptors CCR5/CCR2 in myocardial infarction. Genes Immun 2001;2:191–5.
[18] Apostolakis S, Baritaki S, Kochiadakis GE, Igoumenidis NE, Panutsopulos D, Spandidos DA. Effects of polymorphisms in chemokine ligands and receptors on susceptibility to coronary artery disease. Thromb Res 2007;119:63–71.
[19] Boger CA, Fischereder M, Deinzer M, Aslanidis C, Schmitz G, Stubanus M, et al. RANTES gene polymorphisms predict all-cause and cardiac mortality in type 2 diabetes mellitus hemodialysis patients. Atherosclerosis 2005;183:121–9.
[20] Simeoni E, Winkelmannc BR, Hoffmannd MM, Fleurya S, Ruize J, Kappenbergera L, et al. Association of RANTES G-403A gene polymorphism with increased risk of coronary arteriosclerosis. Eur Heart J 2004;25:1438–46.