Increased risk for ischaemic events is related to combined RAS polymorphism

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Abstract

Objective—To determine whether the angiotensin converting enzyme (ACE) and the angiotensin II type 1 receptor (AT_1R A1166C) gene polymorphism interact to increase the risk of ischaemic events, and whether this can be explained by the progression of angiographically defined coronary atherosclerosis.

Design—Prospective defined substudy of the lipid lowering regression trial (REGRESS).

Setting—University hospital.

Patients—885 male patients with stable coronary artery disease.

Main outcome measures—Incidence of ischaemic events during a two year follow up; serial quantitative coronary arteriography (mean segment diameter and minimum obstruction diameter) at baseline and after two years.

Results—Patients who carried both the ACE-DD and AT_1R-CC genotype had significantly more ischaemic events during the two year follow up than those carrying other genotype combinations (p = 0.035, Mantel-Haenszel test for linear association). There was no association between the two genotypes and mean segment diameter or minimum obstruction diameter at baseline or after two years.

Conclusions—The suggestion that ACE-DD and AT_1R-CC genotypes interact to increase the risk of ischaemic events is confirmed. However, this increased risk was not accompanied by increased progression of angiographically defined coronary atherosclerosis.

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Polymorphism of the renin-angiotensin system (RAS) are reported to play a role in the development of various cardiovascular diseases. A polymorphism in the 16th intron of the angiotensin converting enzyme (ACE) gene, which is associated with higher ACE concentrations,[1][2] is reported to be a risk factor for myocardial infarction[3-5] and post-infarct cardiac dilation,[6] although another study has not found such an association.[7] An adenine/cytosine (A/C) base substitution at position 1166 in the angiotensin II type 1 receptor (AT_1R) has been associated with essential hypertension,[8] increased coronary artery vasoconstriction,[9] and cardiac hypertrophy.[10] Although both polymorphism seem to be associated with cardiovascular disease, only two studies have reported an interaction between them.[11][12]

It is therefore unclear at present whether an interaction between these two polymorphism is related to complications of atherosclerosis, or which underlying mechanism might be responsible. One could speculate that the mechanism involved is explained by the occurrence of a sudden thromboembolic processes, such as increased plaque instability, or conversely by the gradual progression of established coronary lesions.

We hypothesised that both polymorphism synergistically increase RAS activity, thereby increasing the risk of ischaemic events by augmented progression of coronary atherosclerosis. Therefore we genotyped male patients who were participating in the lipid lowering regression trial, REGRESS (regression growth evaluation statin study).[13] In this study, the analysis of genetic factors that might contribute to the progression of coronary artery disease was defined prospectively.

Methods

STUDY DESIGN

The genotype for ACE and AT_1R was determined in patients included in the REGRESS trial.[13] REGRESS is a double blind, placebo controlled multicentre (Netherlands) study to assess the effect of two years of treatment with the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor pravastatin on angiographically documented coronary atherosclerosis. The study involves 885 male patients with coronary artery disease with serum cholesterol ranging between 4–8 mmol/l and triglycerides < 4.0 mmol/l.

The REGRESS study was conducted under the auspices of the Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, Netherlands. Written informed consent was obtained from all patients.

LIPIDS AND LIPOPROTEINS

All lipid laboratory tests were carried out at the Lipid Reference Laboratory, as published previously.[13]

CLINICAL EVENTS

The following clinical events were analysed during the study and identified before unblinding: myocardial infarction (fatal or non-fatal); coronary heart disease death; non-scheduled

Table 1 Baseline characteristics according to the ACE and AT_1R genotypes

ACE DD genotype:	−	+	−	+	
AT_1R CC genotype:	−	−	+	+	p Value
Number of patients	522	180	47	25	
Age (years)	56 (8)	56 (8)	56 (8)	56 (8)	0.99
BMI (kg/m^2)	26.0 (2.7)	26.0 (2.6)	26.3 (2.5)	25.9 (3.3)	0.93
Ever smoked (%)	89	85	86	88	0.67
SBP (mm Hg)	135 (19)	136 (19)	136 (15)	134 (19)	0.89
DBP (mm Hg)	81 (10)	82 (10)	83 (9)	81 (8)	0.75
Total cholesterol (mmol/l)	6.0 (0.9)	6.1 (0.9)	6.0 (0.8)	6.1 (0.8)	0.66
LDL cholesterol (mmol/l)	4.3 (0.8)	4.3 (0.8)	4.2 (0.7)	4.4 (0.7)	0.75
HDL cholesterol (mmol/l)	0.93 (0.23)	0.91 (0.22)	0.91 (0.19)	0.94 (0.25)	0.85
Triglycerides (mmol/l)	1.79 (0.77)	1.88 (0.76)	1.76 (0.77)	1.70 (0.83)	0.50
Baseline MSD (mm)	2.72 (0.36)	2.75 (0.40)	2.75 (0.43)	2.71 (0.38)	0.86
Baseline MOD (mm)	1.75 (0.35)	1.76 (0.36)	1.83 (0.41)	1.68 (0.28)	0.39
Ejection fraction (%)	71 (12)	70 (12)	70 (14)	70 (14)	0.97
Extent of heart disease (%)					
1 vessel	42	43	51	24	
2 vessels	35	32	21	40	0.26
3 vessels	23	25	28	36	
β Blocking agent (%)	74	74	75	80	0.93
Calcium antagonist (%)	60	61	64	48	0.61
Nitrates (%)	56	52	57	56	0.80

Values are mean (SD) unless stated otherwise.
ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptor; BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; MOD, minimum obstruction diameter; MSD, mean segment diameter; SBP, systolic blood pressure.

Table 2 Change in lipids and lipoproteins in relation to the ACE and AT_1R genotypes for patients randomised to placebo or pravastatin treatment

ACE DD genotype:	−	+	−	+	
AT_1R CC genotype:	−	−	+	+	p Value
Placebo					
Number of patients	232	85	26	15	
Total cholesterol (mmol/l)	0.18 (0.72)	0.09 (0.82)	0.06 (0.56)	0.11 (1.01)	0.73
HDL cholesterol (mmol/l)	0.03 (0.15)	0.03 (0.14)	0.01 (0.13)	0.05 (0.26)	0.85
LDL cholesterol (mmol/l)	0.04 (0.67)	−0.01 (0.65)	−0.13 (0.48)	−0.07 (0.79)	0.56
Triglycerides (mmol/l)	0.21 (0.81)	0.12 (0.74)	0.40 (0.84)	0.53 (0.98)	0.20
Pravastatin					
Number of patients	252	82	20	9	
Total cholesterol (mmol/l)	−1.31 (0.86)	−1.46 (0.77)	−1.17 (1.11)	−0.87 (0.82)	0.14
HDL cholesterol (mmol/l)	0.11 (0.17)	0.09 (0.18)	0.11 (0.16)	−0.01 (0.08)	0.26
LDL cholesterol (mmol/l)	−1.36 (0.78)	−1.46 (0.64)	−1.28 (0.92)	−0.90 (0.94)	0.23
Triglycerides (mmol/l)	−0.13 (0.73)	−0.22 (0.67)	−0.02 (0.84)	−0.06 (0.61)	0.52

Values are mean change (SD). Change is defined as follow up measurement minus baseline measurement.

percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG); stroke and transient ischaemic attack; death from unknown cause.

QUANTITATIVE CORONARY ARTERIOGRAPHY

Serial coronary angiograms at baseline and after two years were analysed quantitatively using a cardiovascular measurement system (MEDIS Medical Imaging Systems, Nuenen, Netherlands). The quantitative coronary arteriographic procedures are described in detail elsewhere.[13] Primary end points were:

- Change in average minimum obstruction diameter per patient (this variable mainly reflects focal progression–regression of atherosclerosis).
- Change in average mean segment diameter per patient (this variable mainly reflects diffuse progression–regression of atherosclerosis).

Minimum obstruction diameter and mean segment diameter denote angiograpically defined coronary atherosclerosis. As previously,[13] we only analysed the changes in lesions and segments not influenced by angioplasty or bypass grafting.

GENOTYPING OF THE ACE AND AT$_1$r genes

Blood samples for DNA studies were obtained before randomisation. Thus DNA samples were available for prospectively defined purposes. Genomic DNA was extracted from white blood cells. The method used for genotyping of the ACE I/D polymorphism is a modification of that described by Rigat and colleagues.[14] To avoid mistyping heterozygotes, the DD genotype was confirmed by an additional polymerase chain reaction (PCR) with the same protocol but a separate sense primer specific for the insertion allele: 5'-TTT.GAG.ACG.GAG.TCT.CGC.TC.[15] The AT_1R polymorphism was identified by a mismatch PCR/restriction fragment length polymorphism strategy.[16] PCR products were separated by agarose gel electrophoresis.

STATISTICAL ANALYSIS

Patients were grouped according to ACE and AT_1R genotype and compared with each other for relevant baseline characteristics, change in lipid values, and changes in angiographic indices. Differences with respect to baseline variables were determined by analysis of variance and logistic regression as appropriate. The changes in lipid values and angiographic indices were assessed by a two way covariance analysis with randomised treatment (placebo or pravastatin) and the ACE and AT_1R genotypes and baseline values as covariates. An intention to treat analysis was judged unlikely to be of additional value. The occurrence of events was analysed using Cox regression. A probability p value of $p \leqslant 0.05$ was considered significant.

Results

BASELINE CHARACTERISTICS

Of the 885 patients from the REGRESS trial, genotyping was possible in 774; 394 of these received pravastatin and 380 placebo. Table 1 shows the baseline characteristics for patients not homozygous for the ACE-D and AT_1R-C allele (−/−), homozygous for only the ACE-D allele (+/−), homozygous for only the AT_1R-C allele (−/+), or homozygous for both the ACE-D and AT_1R-C allele (+/+). Neither the interaction between ACE-D and AT_1R-C alleles nor that between ACE-D and AT_1R-C alleles showed any significance.

CHANGE OF LIPIDS AND LIPOPROTEINS

The lipid lowering effect of pravastatin was similar in the genotype groups of both polymorphism (table 2).

CLINICAL EVENTS

There was no significant difference between genotypes with respect to the effect of lipid lowering treatment on the incidence of clinical events (Cox regression interaction test, p = 0.62). We therefore we pooled the two treatment groups to assess any differences between genotypes in the incidence of clinical events. Patients with the ACE-DD/AT_1R-CC
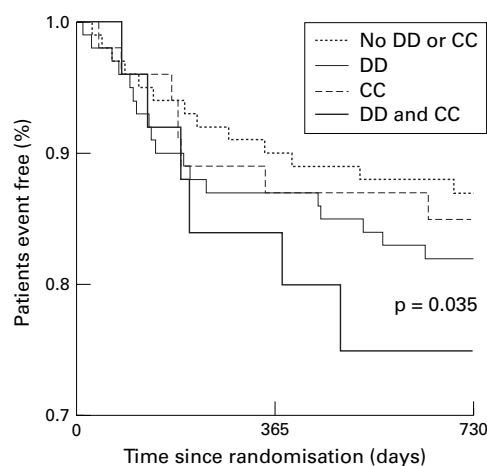
Figure 1   Kaplan-Meier curves for the event-free survival of patients according to ACE-DD and AT₁R-CC genotypes. The p value indicates the overall significance of the Mantel-Haenszel test for linear association between the curves.

genotype combination had significantly more ischaemic events during the two year follow up ($p = 0.035$ by the Mantel-Haenszel test for linear association (fig 1)). The relative risk of ischaemic events of each separate polymorphism was: ACE (DD *v* II/ID), 1.49 (95% confidence interval (CI) 1.01 to 2.20), $p = 0.047$; and AT₁R (CC *v* AA/AC), 1.42 (95% CI 0.81 to 2.48), $p = 0.22$.

ANGIOGRAPHIC RESULTS

Because of patient dropouts and revascularisations, follow up angiography was available in 587 patients only.[13] There was a mean (SD) of 6.6 (3.0) segments analysed in each patient. As shown in table 1, patients homozygous for both the ACE-D and the AT₁R-C allele showed no differences in angiographically defined coronary atherosclerosis (minimum obstruction diameter and mean segment diameter) at baseline compared with patients not homozygous for the ACE-D and AT₁R-C allele.

There was no significant difference between genotypes with respect to the effect of lipid lowering treatment on changes in minimum obstruction diameter and mean segment diameter during the trial ($p = 0.69$ and $p = 0.78$, respectively); thus the two treatment groups were pooled in order to assess the difference between genotypes with respect to changes in these variables.

The changes (follow up minus baseline) in mean segment diameter (MSD) and minimum obstruction diameter (MOD) after two years (mm; mean (SD)) were as follows ($p = 0.41$ for MSD and $p = 0.03$ for MOD):

- not homozygous for the ACE-D and AT₁R-C alleles (n = 394): MSD, 0.08 (0.20); MOD, 0.09 (0.23);
- homozygous for only the ACE-D allele (n = 135): MSD, 0.09 (0.16); MOD, 0.16 (0.44);
- homozygous for only the AT₁R-C allele (n = 43): MSD, 0.11 (0.19); MOD, 0.11 (0.19);
- homozygous for both the ACE-D and the AT₁R-C allele (n = 15): MSD, 0.01 (0.20); MOD, −0.05 (0.21).

Patients homozygous for the ACE-D and AT₁R-C allele showed the least progression of angiographically defined coronary atherosclerosis in comparison with the other genotype combinations.

Discussion

Our study shows that in male patients with established coronary artery disease the combination of ACE-DD and AT₁R-CC genotypes is a risk factor for recurrent ischaemic events. This increased risk did not result from increased progression of angiographically defined coronary atherosclerosis.

The pathophysiological mechanism involved in the relation between the ACE deletion genotype and the AT₁R-CC genotype on ischaemic events cannot be deduced from our study. However, patients carrying both the ACE-DD and the AT₁R-CC genotype showed the smallest increase in progression of coronary atherosclerosis. Therefore it seems justified to conclude that the increase in event number in this group is not caused by increased progression of coronary atherosclerosis.

ACE and angiotensin II are important constituents of advanced coronary atherosclerotic lesions.[17] Enhanced production of local angiotensin II by ACE stimulates adhesion molecule expression and increases oxidative stress, one of the triggering mechanisms of acute coronary syndromes.[18] In ruptured coronary plaques, enhanced ACE expression is found in macrophages accumulating around the attenuated fibrous cap.[19] Angiotensin II can induce proinflammatory cellular activity in human coronary atherosclerotic plaques and can raise concentrations of soluble intercellular adhesion molecule-1, suggesting a possible involvement of the RAS in inflammation and plaque instability.[20 21] It has been proposed that the higher tissue ACE activity found in patients with the ACE-DD genotype may increase plaque instability.[22 23] Although both polymorphism are associated with increased RAS activity,[1 2 9 24 25] no association was found with progression of coronary atherosclerosis in the present study. One could speculate that increased RAS activity leads to increased plaque instability (oxidative stress, proinflammation), and therefore this could be one of the reasons why patients with both a higher tissue ACE activity (DD patients) and an increased angiotensin II susceptibility (CC patients) have more recurrent ischaemic events. In line with this, it is of interest that the HOPE (heart outcomes prevention evaluation) trial confirms a potential plaque stabilising effect of RAS inhibition by showing that ramipril reduced morbidity and mortality in patients with severe vascular disease.[26]

Although patients with the ACE-DD and the AT₁R-CC genotype are more susceptible to ischaemic events, the "regression" of the minimum obstruction diameter in the two years of follow up is surprising. We cannot suggest a reason for this. However, some caution is needed in the interpretation of the findings, as the group of patients homozygous for both the ACE-D and the AT₁R-C allele was rather

small. Furthermore, only patients without an event were evaluated angiographically after two years, while patients with an event received no follow up. This means that selection of patients could have occurred. As the event rate was highest in patients with the ACE-DD and -CC genotypes, the drop out percentage was also the highest in that group in relation to angiographic follow up after two years. This drop out group could have biased our results on the progression/regression of atherosclerosis found in patients with the ACE-DD and AT$_1$R-CC genotypes after two years, leading to the surprising "regression" of MOD.

Others have previously attempted to link the ACE-DD and AT$_1$R-CC genotypes to the progression of coronary atherosclerosis.[5 27–29] Although there seems to be a positive association, negative studies have also been reported. Several factors may account for these conflicting data—for example, the retrospective nature of the analyses, the low prevalence of the AT$_1$R-CC genotype, and small sample size. In most reports, coronary angiography was defined at one time point, making it difficult to judge the progression of atherosclerosis over time. To our knowledge this present prospective study is the first to involve repeated computer assisted angiography in a well defined and substantial sample. Despite the limitations of the technique, it seems justified to conclude that the ACE-DD and AT$_1$R-CC genotypes, either separately or in combination, are not associated with progression of angiographically defined coronary atherosclerosis.

LIMITATIONS

A limitation of the present study is the lack of ACE activity data, which would have substantiated any relation between the DD genotype, ACE activity, and events. As the ACE deletion genotype is reported to be associated with higher ACE activity,[1 2] it may be assumed that this relation also existed in our patients.

It could be argued that we should have studied the progression/regression data on an intention to treat basis. Patients with cardiovascular death or myocardial infarction would then have been considered as having a total occlusion in one coronary segment. However, for our quantitative coronary arteriography, the coronary tree was divided into 13 segments according to the American Heart Association classification, excluding the posterolateral branches. Because angioplasty and coronary artery bypass procedures may influence progression considerably, lesions and segments modified by PTCA and CABG were excluded from the analysis. In the primary REGRESS study, 4209 coronary segments (6.6 (3.0) per patient) were measured quantitatively and included in the primary analysis. During the two year follow up of the study, only 28 of 885 patients suffered a myocardial infarct or other coronary heart disease event leading to death. It was considered that an intention to treat analysis which included 28 infarct related segments on top of the 4209 segments already evaluated would not to add substantially to the study, so it was not done.

CONCLUSIONS

The combination of the ACE-DD and the AT$_1$R-CC genotypes is a risk factor for recurrent ischaemic events in male subjects with established coronary artery disease. This increased risk is not related to an increased rate of progression of coronary lesions. As both polymorphism are associated with increased activity of the renin-angiotensin system but are not associated with increased progression of coronary atherosclerosis, increased plaque instability could be one of the reasons why these patients are at increased risk of recurrent ischaemic events.

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Commentary

This paper by van Geel and colleagues is a large prospective substudy within the REGRESS trial of lipid lowering treatment with pravastatin. Polymorphism of the angiotensin I converting enzyme (ACE) and the angiotensin II type 1 receptor (AT1 receptor) were examined in relation to the occurrence of ischaemic events in nearly 800 men. Although ACE DD genotype and AT1 receptor CC genotype did not influence response to lipid lowering treatment or plaque progression, men with both DD and CC genotypes showed a significantly greater risk of suffering an ischaemic event in the two year follow up period. This observation is suggestive of a functional interaction between the two genotypes. The authors speculate that increased activity of the renin angiotensin system could contribute to plaque instability through pro-oxidant and inflammatory mechanisms. Consideration of these data in the light of the recent HOPE study (confirming a reduction in ischaemic events in patients taking ACE inhibitor treatment) suggests that further investigation of the contribution of the renin-angiotensin system to ischaemic heart disease may be very rewarding.

G F BAXTER
Associate Editor

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