Increased risk for ischaemic events is related to combined RAS polymorphism

P P van Geel, Y M Pinto, A H Zwinderman, R H Henning, A J van Boven, J W Jukema, A V G Bruschke, J J P Kastelein, W H van Gilst, on behalf of the REGRESS Study Group

Abstract
Objective—To determine whether the angiotensin converting enzyme (ACE) and the angiotensin II type 1 receptor (AT1R 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 AT1R-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 AT1R-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.

Keywords: renin-angiotensin system; polymorphism; coronary atherosclerosis; ischaemic events

Department of Cardiology, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, Netherlands
P P van Geel
Y M Pinto
A J van Boven
W H van Gilst

Department of Clinical Pharmacology, University Hospital Groningen
R H Henning

Department of Medical Statistics, University of Leiden, Leiden, Netherlands
A H Zwinderman

Department of Cardiology, Leiden University Medical Centre
J W Jukema
A V G Bruschke

Department of Vascular Medicine, Academic Medical Centre, Amsterdam, Netherlands
J J P Kastelein

Correspondence to:
Dr van Geel
p.p.geel@med.rug.nl

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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, is reported to be a risk factor for myocardial infarction and post-infarct cardiac dilatation, although another study has not found such an association. An adenine/cytosine (A/C) base substitution at position 1166 in the angiotensin II type 1 receptor (AT1R) has been defined prospectively.

The suggestion that ACE-DD and AT1R-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. 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 AT1R was determined in patients included in the REGRESS trial. 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.

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

**Table 1** Baseline characteristics according to the ACE and AT1R genotypes

<table>
<thead>
<tr>
<th>ACE DD genotype:</th>
<th>−</th>
<th>+</th>
<th>+</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>522</td>
<td>180</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 (8)</td>
<td>56 (8)</td>
<td>56 (8)</td>
<td>56 (8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (2.7)</td>
<td>26.0 (2.6)</td>
<td>26.3 (2.5)</td>
<td>25.9 (3.3)</td>
</tr>
<tr>
<td>Ever smoked (%)</td>
<td>89</td>
<td>85</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>135 (19)</td>
<td>136 (19)</td>
<td>136 (15)</td>
<td>134 (19)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.0 (0.9)</td>
<td>6.1 (0.9)</td>
<td>6.0 (0.8)</td>
<td>6.1 (0.8)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.11 (0.17)</td>
<td>0.09 (0.18)</td>
<td>0.11 (0.16)</td>
<td>0.11 (0.16)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>0.93 (0.23)</td>
<td>0.91 (0.22)</td>
<td>0.91 (0.19)</td>
<td>0.94 (0.25)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.79 (0.78)</td>
<td>1.78 (0.76)</td>
<td>1.76 (0.77)</td>
<td>1.70 (0.83)</td>
</tr>
<tr>
<td>Extent of heart disease (%)</td>
<td>71 (12)</td>
<td>70 (12)</td>
<td>70 (14)</td>
<td>70 (14)</td>
</tr>
<tr>
<td>Number of patients</td>
<td>522</td>
<td>180</td>
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<td>25</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; AT1R, 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; Systolic blood pressure.

### Change in lipids and lipoproteins in relation to the ACE and AT1R genotypes for patients randomised to placebo or pravastatin treatment

<table>
<thead>
<tr>
<th>ACE DD genotype:</th>
<th>−</th>
<th>+</th>
<th>+</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>232</td>
<td>85</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>0.18 (0.72)</td>
<td>0.19 (0.82)</td>
<td>0.16 (0.56)</td>
<td>0.11 (1.01)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.03 (0.15)</td>
<td>0.03 (0.14)</td>
<td>0.01 (0.13)</td>
<td>0.05 (0.26)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>0.04 (0.07)</td>
<td>-0.01 (0.05)</td>
<td>-0.13 (0.48)</td>
<td>-0.07 (0.79)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.21 (0.81)</td>
<td>0.12 (0.74)</td>
<td>0.40 (0.84)</td>
<td>0.53 (0.98)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AT1R CC genotype:</th>
<th>−</th>
<th>+</th>
<th>+</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.13 (0.73)</td>
<td>-0.22 (0.67)</td>
<td>-0.02 (0.84)</td>
<td>-0.06 (0.61)</td>
</tr>
</tbody>
</table>

**Table 2** Change in lipids and lipoproteins in relation to the ACE and AT1R genotypes for patients randomised to placebo or pravastatin treatment

<table>
<thead>
<tr>
<th>Placebo</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td>0.01 (0.13)</td>
<td>0.05 (0.26)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
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<td>-0.01 (0.05)</td>
<td>-0.13 (0.48)</td>
<td>-0.07 (0.79)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.21 (0.81)</td>
<td>0.12 (0.74)</td>
<td>0.40 (0.84)</td>
<td>0.53 (0.98)</td>
</tr>
</tbody>
</table>

**Pravastatin**

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>252</th>
<th>82</th>
<th>20</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>-1.31 (0.86)</td>
<td>-1.46 (0.77)</td>
<td>-1.17 (1.11)</td>
<td>-0.87 (0.82)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.11 (0.17)</td>
<td>0.09 (0.18)</td>
<td>0.11 (0.16)</td>
<td>-0.01 (0.08)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>-1.36 (0.78)</td>
<td>-1.40 (0.64)</td>
<td>-1.28 (0.92)</td>
<td>-0.90 (0.94)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>-0.13 (0.73)</td>
<td>-0.22 (0.67)</td>
<td>-0.02 (0.84)</td>
<td>-0.06 (0.61)</td>
</tr>
</tbody>
</table>

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

### Results

**Statistical analysis**

Patients were grouped according to ACE and AT1R 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 AT1R 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 ≤ 0.05 was considered significant.

### 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 pooled the two treatment groups to assess any differences between genotypes in the incidence of clinical events. Patients with the ACE-DD/AT1R-CC genotypes and baseline values as covariates. An intention to treat analysis was judged unlikely to be of additional value.
Patients homozygous for the ACE-D and AT, R-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 AT, R-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 AT, R-CC genotype on ischaemic events cannot be deduced from our study. However, patients carrying both the ACE-DD and the AT, R-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. 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. In ruptured coronary plaques, enhanced ACE expression is found in macrophages accumulating around the attenuated fibrous cap. 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. It has been proposed that the higher tissue ACE activity found in patients with the ACE-DD genotype may increase plaque instability. Although both polymorphisms are associated with increased RAS activity, 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. 

Although patients with the ACE-DD and the AT, R-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 AT, R-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-R-CC genotypes after two years, leading to the surprising “regression” of MOD.

Others have previously attempted to link the ACE-DD and AT-R-CC genotypes to the progression of coronary atherosclerosis, 27–30 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-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-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 postero lateral 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-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.

We thank Hendrik Buikema PhD for his critical review and helpful suggestions. The REGRESS study was conducted under the auspices of the Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, Netherlands and supported by Bristol Myers Squibb Company, Princeton, New Jersey, USA. This study was supported by grant number 950-10-642 of the Netherlands Organisation for Scientific Research (NWO), Netherlands.

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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