Clinical research

Angiotensin converting enzyme insertion/deletion polymorphism and the risk of heart failure in hypertensive subjects

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\textbf{Aims} Cardiac angiotensin-I converting enzyme (ACE) activity is influenced by the ACE I/D polymorphism. Evidence suggests that the DD-genotype may be a risk factor for cardiac hypertrophy and heart failure, especially in hypertensive subjects. We assessed the relation between the ACE I/D polymorphism and the risk of incident heart failure in normotensive and hypertensive subjects.

\textbf{Methods and results} We investigated 4264 normotensive and 2174 hypertensive participants of the Rotterdam Study, a population based prospective cohort study. All subjects were available for follow-up from 1990 until 2000. Incidence rates (IR) of heart failure in normotensive subjects were the same over all genotype strata (10 per 1000 person-years). In hypertensive subjects, the IR increased with the number of D-alleles present (II: IR = 13, ID: IR = 18 and DD: IR = 20 per 1000 person-years). Hypertensive subjects carrying the II-genotype did not have an increased risk of heart failure compared to normotensive II subjects. However, hypertensive subjects carrying one or two copies of the D-allele did have a significantly increased risk of heart failure (ID: RR: 1.4 (1.1–1.9) and DD: RR: 1.5 (1.2–2.1)).

\textbf{Conclusion} Our findings suggest that the ACE I/D polymorphism may play a modifying role in the development of heart failure in hypertensive subjects.

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blood. Coronary artery disease and hypertension are among the most common risk factors. Regardless of the initial cause of cardiac stress, the heart will respond with a set of adaptive mechanisms in order to maintain its pumping function. Both clinical and experimental data suggest that activation of local renin angiotensin system (RAS) in the heart plays an important role in this compensatory mechanism to maintain adequate haemodynamic function. Recent studies have shown that cardiac expression of angiotensin-I-converting enzyme (ACE) and angiotensinogen is increased in experimental heart failure. In patients with chronic heart failure, cardiac expression of ACE was found to be increased up to threefold compared to the hearts of subjects without heart failure.

An insertion/deletion (I/D) polymorphism, characterised by the presence or absence of a 287-base pair alu repeat sequence in intron 16 of the ACE gene, has been reported to be responsible for about 50% of the interindividual variability in serum ACE levels. Both serum ACE levels and cardiac ACE activity were highest in subjects carrying two copies of the D-allele. The D-allele has been put forward as a risk factor for left ventricular remodelling in hypertensive subjects. Raymond et al. observed an increased frequency of the D-allele in patients with both ischaemic and idiopathic dilated cardiomyopathy.

The ACE I/D polymorphism has been associated with a set of adaptive mechanisms in order to maintain its pumping function. Both serum ACE and cardiac ACE activity were highest in subjects carrying two copies of the D-allele. The D-allele has been put forward as a risk factor for left ventricular remodelling in hypertensive subjects. Raymond et al. observed an increased frequency of the D-allele in patients with both ischaemic and idiopathic dilated cardiomyopathy.

We examined the role of the ACE I/D polymorphism in the development of heart failure in a population-based cohort study. Since several studies reported an effect of the D-allele on cardiac disease in hypertensive subjects only, we analysed normotensive and hypertensive subjects separately.

Methods

Study population

The study was conducted within the Rotterdam Study, a single-centre prospective follow-up study in which all residents, aged 55 years and over, of the Rotterdam suburb of Ommoord were invited to take part. The baseline examination of the Rotterdam Study was conducted between 1990 and 1993. The Medical Ethics Committee of Erasmus Medical Centre Rotterdam approved the study. Written informed consent was obtained from all participants. The design of the study has been described previously. 7983 participants were examined (response 78%). In 6869 subjects, the ACE I/D polymorphism was genotyped successfully (86%). In the remaining 1114 subjects, no genotypes were available. We excluded 211 subjects because no information on blood pressure levels was available.

At baseline, information concerning medical history, medication use and smoking behaviour was obtained with a computerised questionnaire. Blood pressure was measured twice, after a minimum of 5 min rest, in the sitting position at the right upper arm using a random zero sphygmomanometer. Participants were asked to abstain from smoking and drinking alcoholic or caffeine-containing beverages at least 2 h before blood pressure measurements were taken. The average of two measurements was used for analysis. Hypertension was defined as a diastolic blood pressure (DBP) of 100 mmHg or higher and/or a systolic blood pressure (SBP) of 160 mmHg or higher and/or use of anti-hypertensive medication indicated for treatment of hypertension (grade 2 and 3 of the 1999 WHO/ISH criteria and 2003 ESH/ESC criteria).

Heart failure assessment

Assessment of prevalent heart failure at the baseline examination in the Rotterdam Study has been described in detail earlier. We excluded subjects with prevalent heart failure from our study (n = 220). All participants of the Rotterdam Study were continuously monitored for the occurrence of heart failure during follow-up from 1990 until 2000, using automated linkage with files from general practitioners. All available medical data, such as hospital discharge letters and notes from general practitioners, were obtained from the medical records in case of possible heart failure. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area as described above.

The diagnosis of heart failure was classified as definite, probable, possible or unlikely. Definite heart failure was defined as a combination of heart failure diagnosed by a medical specialist and the presence of typical symptoms of heart failure, such as breathlessness at rest or during exertion, ankle oedema and pulmonary crepitations, confirmed by objective evidence of cardiac dysfunction (chest X-ray, echocardiography). This definition is in accordance with the criteria of the European Society of Cardiology. Probable heart failure was defined as heart failure diagnosed by a general practitioner, with at least two typical symptoms suggestive of heart failure, and at least 1 of the following: history of cardiovascular disease (e.g. myocardial infarction, hypertension), response to treatment for heart failure, or objective evidence of cardiac dysfunction, while symptoms could not be attributed to another underlying disease.

Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session. Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research physicians, the cardiologist’s judgment was considered decisive. The research physicians and the cardiologist based their decisions on the same data. Only definite and probable cases were included in the analyses.

After heart failure cases were diagnosed as definite or probable, the date of incident heart failure was defined as the day of the first occurrence of symptoms suggestive of heart failure or the date of the first prescription of a loop diuretic or an ACE-inhibitor.

Genotyping

The II, ID and DD genotypes were detected using the polymerase chain reaction technique (PCR) according to the method of Lindpaintner et al. with some modifications. In order to avoid misclassification of ID genotypes into DD genotypes, a second PCR was performed using an l-specific primer.

Statistical analysis

Overall general characteristics of normotensive and hypertensive subjects and those stratified by ACE genotype, were compared using univariate analysis of variance for continuous variables and \( \chi^2 \) statistics for dichotomous variables. Differences in median follow-up between the normotensive and hypertensive subjects separately.
sive group, and between genotypes within the normotensive and hypertensive group were tested using a Mann–Whitney non-parametric test for independent samples. Incidence rates (IR) were expressed as number of cases per 1000 person-years and presented with 95% confidence intervals (CI), based on the assumed Poisson distribution for the observed number of cases. We constructed a variable for the ACE-genotype groups (I–I; I–ID; DD) and performed a linear regression in order to test for trend of IR in the normotensive and hypertensive groups. Relative risks of incident heart failure were assessed using Cox proportional hazard regression analysis. In the first regression analysis we assessed the risk of heart failure for hypertensive subjects compared to normotensive subjects independent of the ACE genotype. For the second regression analysis we constructed an ordinal variable consisting of six categories, based on the ACE-genotype (II–ID–DD and hypertension (yes/no). Relative risks for each category were then assessed using normotensive II-subjects as the reference category. Proportionality of hazards was assessed and satisfied by means of a log-minus-log plot. All risk estimates are presented with 95% confidence intervals (CI). We adjusted for age and sex in all analyses. To assess the effect of the ACE I/D polymorphism independent of possible confounding or mediating factors, analyses were repeated adding body mass index (BMI), diabetes mellitus, smoking, myocardial infarction, total and HDL-cholesterol to the model. We tested for statistical interaction between the ACE I/D polymorphism and hypertension or blood pressure by adding different interaction terms to the regression model: hypertension (dichotomous) × ACE-genotype (categorical), systolic blood pressure (continuous) × ACE-genotype (categorical) and diastolic blood pressure (continuous) × ACE-genotype (categorical). All presented p-values are two-sided. We performed all analyses with SPSS version 11.0.

Results

A total of 6438 subjects were available for follow-up until 1st January, 2000. Baseline descriptives of the total study population are presented in Table 1. We included 4264 normotensive subjects and 2174 hypertensive subjects in our study. Both groups followed Hardy-Weinberg Equilibrium proportions for the ACE I/D polymorphism. Median follow-up was 7.2 (6.7;8.1) years for normotensive subjects and 7.0 (5.3;8.0) years for hypertensive subjects. In hypertensive subjects, median follow-up was significantly shorter for subjects carrying two copies of the D-allele than for subjects carrying two copies of the I-allele. Hypertensive subjects were significantly older and less often male than normotensive subjects. This difference was the same over all genotype strata. Within the normotensive group, DBP was significantly higher in subjects carrying the DD-genotype compared to subjects carrying the ID-genotype. Prevalence of diabetes mellitus and myocardial infarction, mean BMI and total cholesterol levels were significantly higher in hypertensive subjects compared to normotensive subjects. In the hypertensive group, BMI was significantly higher in subjects carrying the ID-genotype compared to subjects carrying the DD-genotype. HDL-cholesterol and percentage current smokers were significantly lower in hypertensive subjects than in normotensive subjects. This difference was the same over all genotype strata.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline descriptives of normotensive and hypertensive subjects: overall and stratified by ACE genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE genotype</td>
<td>Normotensive subjects</td>
</tr>
<tr>
<td></td>
<td>II</td>
</tr>
<tr>
<td>Number, n (%)</td>
<td>4264 (66.0)</td>
</tr>
<tr>
<td>Median follow-up (years)</td>
<td>7.2 (6.7;8.1)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.0 ± 8.9</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>42.9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130.7 ± 16.1</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70.9 ± 10.0</td>
</tr>
<tr>
<td>Diabtes mellitus (%)</td>
<td>7.1</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>11.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 3.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol)</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol)</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Smoking (current%)</td>
<td>24.7</td>
</tr>
</tbody>
</table>

All values are presented as percentage or mean ± standard deviation, except for follow-up, which is presented as median (interquartile range). *Significantly different from normotensive subjects, p < 0.05. **Significantly different from hypertensive DD-subjects, p < 0.05. ***Significantly different from normotensive ID-subjects, p < 0.05.
Table 2 shows number of cases, person-years and incidence rates (IR) of heart failure observed in normotensive and hypertensive subjects stratified by ACE genotype. During 44,883.1 person-years of follow-up 543 participants developed heart failure. In normotensive subjects, the IR of heart failure was about 10 per 1000 person-years, independent of genotype status. In hypertensive subjects, the IR of heart failure significantly increased with the number of D-alleles present ($p$ for trend = 0.04). In subjects carrying the II-genotype the IR of heart failure was 13 per 1000 person-years (95% CI: 9–17). In subjects carrying one or two copies of the D-allele the IR of heart failure increased up to 18 (15–21) and 20 (16–24) per 1000 person-years, respectively.

In Table 3, the relative risks (RR) of heart failure for hypertensive and normotensive subjects, overall and stratified by ACE genotype, are presented. Overall, hypertensive subjects had a significantly increased risk of 1.4 (1.2;1.7) of heart failure compared to normotensive subjects. In the normotensive group, the risk of heart failure did not differ between the different ACE genotype groups. Hypertensive subjects carrying two copies of the I-allele did not have an increased risk of heart failure compared to normotensive subjects carrying two copies of the I-allele (RR: 1.0 (0.7–1.4)) (model 2). However, hypertensive subjects carrying one or two copies of the D-allele had a significantly increased risk of heart failure compared to normotensive II subjects (ID: RR: 1.6 (1.2;2.2) and DD: RR: 1.6 (1.2;2.2)) (model 2). Additional analyses including also mild hypertensive subjects in the hypertensive group (cut-off value for diagnosis of hypertension: SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg) did not show a significant effect of the ACE-genotype on the risk of heart failure. Although the direction of the risk estimates was the same (RR: ID: 1.2 (0.8;1.8) and DD: 1.4 (0.8;2.5)) (results not shown in table).

The statistical interaction term between the D-allele of the ACE-genotype and hypertension was borderline significant ($p$ = 0.059). The interaction term with blood pressure as a continuous trait (either systolic or diastolic blood pressure) and the D-allele of the ACE-genotype did not reach statistical significance (SBP: $p$ = 0.36, DBP: $p$ = 0.80).

Table 3 Risk of heart failure in normotensive and hypertensive subjects: overall and stratified by ACE genotype

<table>
<thead>
<tr>
<th>Risk overall</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotension</td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>1.0 (ref)</td>
<td>1.6 (1.4;1.9)</td>
</tr>
<tr>
<td>Risk stratified by ACE genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.0 (ref)</td>
<td>1.1 (0.8–1.7)</td>
</tr>
<tr>
<td>ID</td>
<td>0.9 (0.6;1.2)</td>
<td>1.5 (1.1;2.0)**</td>
</tr>
<tr>
<td>DD</td>
<td>1.0 (0.7;1.4)</td>
<td>1.6 (1.2;2.2)**</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, BMI, smoking, diabetes mellitus, myocardial infarction, total and HDL-cholesterol.

* Significantly different from overall normotensive group, $p < 0.001$.
** Significantly different from II normotensive group, $p < 0.01$.
*** Significantly different from II normotensive group, $p < 0.05$.

Discussion

We observed an increased risk of heart failure in hypertensive subjects compared to normotensive subjects that was dependent on the presence of the D-allele of the ACE I/D polymorphism. Hypertensive subjects did not have a significantly increased risk of heart failure compared to normotensive subjects, unless they carried one or two copies of the D-allele. The incidence rate of heart failure in hypertensive subjects increased with the number of D-alleles present. As the incidence of heart failure marks the end of the follow-up period, this may also explain the shorter follow-up period observed in hypertensive DD subjects compared to II subjects.

Hypertension is the most common condition antedating heart failure in the general population. Especially in the elderly, heart failure is often preceded by long-
standing high blood pressure and LVH. However, the extent of cardiac remodelling does not always seem to correlate with the extent of cardiac stress. In fact, hypertension may lead to severe heart failure in one patient whereas hypertension may be without any perceivable effects on cardiac function in another patient. As a consequence, it has been hypothesised that genetic factors may modulate the manifestation or progression of cardiac remodelling.

The ACE I/D polymorphism is by far the most frequently studied candidate gene in the development of left ventricular hypertrophy and heart failure. Homozygosity for the D-allele has been associated with higher prevalence of LVH and increased heart weight in (untreated) hypertensive subjects. Raynolds et al. were the first to report an association between the ACE I/D polymorphism and heart failure. They observed an increased frequency of the DD-genotype in subjects with ischaemic and diluted cardiomyopathy. Since local formation of angiotensin II (ANG II) within the myocardium is thought to be involved in the cardiac remodelling process, elevated cardiac ANG II levels in subjects carrying the D-allele, may partly explain the association between the DD-genotype and various cardiac disorders. Although cardiac chymase is also an important ANG II generating enzyme in the heart, two studies observed that in failing hearts ACE enzyme gene expression was increased, whereas cardiac chymase enzyme gene expression was not. This may suggest that in failing hearts ANGII formation is more dependent on ACE than on cardiac chymase and therefore can potentially be modified by the ACE I/D polymorphism. Nevertheless, findings remain controversial and so far positive and negative results seem to outweigh each other.

Many of the conflicting findings on the ACE I/D polymorphism and cardiac disease are most likely due to small sample sizes and large heterogeneity of the populations that were studied. Another reason for the inconsistent findings may be that the ACE I/D polymorphism by itself does not have enough biological significance to exert an effect on cardiac tissue, especially since the RAS is normally under strict negative feedback inhibition. This has led to the hypothesis that an effect of the ACE I/D polymorphism on cardiac function may only become clinically relevant under specific conditions in which the cardiac growth machinery is already activated. In line with this hypothesis, Montgomery et al. observed increased left ventricular mass after rigorous exercise only in those participants who carried a copy of the D-allele. Another study observed increased adverse cardiac remodelling in subjects with the ACE ID- and DD-genotype after they had experienced a myocardial infarction.

We believe our findings provide additional evidence for a modifying effect of the ACE I/D polymorphism in the development of cardiac disease. In our study, the D-allele was associated with an increased risk of heart failure in hypertensive subjects only, which may suggest that the D-allele has an effect on the heart merely when local RAS is already activated because of increased haemodynamic load. Since asymptomatic cardiac remodelling usually precedes the development of clinically overt heart failure in hypertensive subjects, we believe our findings are in accordance with the observation that, particularly in subjects with hypertension, the D-allele of the ACE I/D polymorphism is associated with increased levels of various echocardiography measures of cardiac hypertrophy.

Our study is the largest population based study to date that assessed the role of the ACE I/D polymorphism in heart failure in a relatively homogenous population, as 98% of the participants in our study are Caucasians and they all live in the same area of Rotterdam. In contrast to case-control studies on heart failure that have been conducted so far, the prospective nature of our study makes our results less prone to survival bias. Still several issues still need to be addressed however. We observed a significantly increased risk of heart failure in moderate and severe hypertensive subjects only. Additional analyses, which also included mild hypertensive subjects, did not show a significantly increased risk of heart failure for ID and DD-carriers, although the risk estimates were in the same direction as those for moderate to severe hypertensive subjects. In addition, the interaction term including blood pressure as a continuous trait did not reach statistical significance. We believe this implies a 'threshold' effect of the ACE I/D polymorphism, as its detrimental effects on cardiac function only become present when the heart is already under severe cardiac stress due to substantially elevated blood pressure levels. Second, we did not account for lifestyle or dietary factors that may have influenced our genotype-phenotype relationship. Kuznetsova et al. recently observed that the relationship between left ventricular mass index and the ACE I/D polymorphism might be modulated by sodium intake. Finally, we were not able to discern the different aetiologies of heart failure (idiopathic, ischaemic or other) in our study. However, we think that the ACE I/D polymorphism may be more important as a modulator in the way the myocardium responds to cardiac stress ('remodelling') than in the events leading to cardiac stress. The ACE I/D polymorphism may increase the risk of heart failure through an effect on blood pressure or an increased risk of myocardial infarction; however, our results do not support this. On the contrary, our findings suggest that hypertension by itself is not a real strong predictor of heart failure, unless one or two copies of the D-allele are present. Furthermore, correction for baseline and incident MI in our analyses did not change the association between heart failure and the ACE I/D polymorphism. In addition, the prevalence of MI did not differ significantly between the genotype groups in hypertensive subjects.

In conclusion, our findings suggest that the ACE I/D polymorphism may play a modifying role in the development of heart failure in hypertensive subjects, regardless of the initial cause of cardiac damage. We believe these findings may provide an additional genetic clue as to why some hypertensive subjects develop cardiac hypertrophy resulting in heart failure and others do not.

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References