Circulating Activities of Angiotensin-Converting Enzyme, Its Homolog, Angiotensin-Converting Enzyme 2, and Neprilysin in a Family Study

Gillian I. Rice, Amy L. Jones, Peter J. Grant, Angela M. Carter, Anthony J. Turner, Nigel M. Hooper

Abstract—The renin–angiotensin system is a key regulator of blood pressure (BP), with inhibitors of angiotensin-converting enzyme (ACE) used clinically to treat hypertension and other cardiovascular conditions. ACE2 is a newly identified member of this system, which converts angiotensin II to angiotensin (1-7), and of which the occurrence in plasma has not been investigated. The aim of this study was to determine the heritability of circulating ACE, ACE2, and neprilysin (NEP), which may also be a regulator of BP, in a family study, and to determine covariates that contribute to the variation in plasma activity. ACE, ACE2, and NEP activities were measured in plasma from 534 subjects in the Leeds Family Study using selective fluorogenic substrates. Genetic factors accounted for 24.5%, 67%, and 22.7% of the phenotypic variation in circulating ACE, ACE2, and NEP, respectively. ACE insertion/deletion polymorphism and other measured covariates accounted for 23.8% of variance in circulating ACE. High-density lipoprotein cholesterol was a significant determinant of circulating ACE2. Measured covariates accounted for 17.3% of variation in circulating NEP. ACE and NEP were associated with systolic and diastolic BP in univariate analyses; however, only ACE was independently associated with systolic and diastolic BP after accounting for covariates and shared childhood household. (Hypertension. 2006;48:914-920.)

Key Words: angiotensin ■ blood pressure ■ genetics ■ metalloproteinases ■ population

The renin–angiotensin system (RAS) is a complex cascade of bioactive peptides and regulatory enzymes, which acts as a key regulator of blood pressure (BP) and fluid and electrolyte homeostasis.1 Angiotensin-converting enzyme (ACE) cleaves angiotensin I (Ang I) to form the biologically active peptide angiotensin II (Ang II). Ang II mediates a diverse range of biological effects including vasoconstriction, vascular smooth muscle cell proliferation, and hypertrophy of the heart vessel wall through its interaction with the Ang II type 1 receptor.2 Although ACE exists primarily as a transmembrane protein on the surface of endothelial and epithelial cells, a soluble form is present in plasma resulting from proteolytic shedding that can be readily measured.3 The Alu insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene is a strong predictor of plasma ACE levels.3,4

A recently identified homolog of ACE, ACE2, cleaves Ang II to form Ang (1-7),5,6 which, in turn, binds to a non-Ang II type I/Ang II type 2 receptor originally identified as the Mas oncogene7 and mediates functions that oppose the actions of Ang II.8 Thus, ACE2 may have a role to counterbalance the action of ACE in producing the vasoconstrictor Ang II, leading to the suggestion that ACE2 and ACE may be correlated.9 ACE2 displays 42% amino acid identity to ACE and, like ACE, is a type I integral membrane protein that can be proteolytically shed from the plasma membrane.10 As yet, ACE2 in plasma has not been measured, and its relationship to circulating ACE has not been reported.

Neprilysin (NEP) neutral endopeptidase cleaves a number of peptide components of the RAS, as well as the potent vasodilator atrial natriuretic factor, and studies suggest that NEP participates with ACE in BP regulation.11 On the basis of the role of NEP in the degradation of atrial natriuretic factor, vasopeptidase inhibitors, which simultaneously inhibit both NEP and ACE, have been developed, although their clinical use is still in question.12 Again, NEP is a transmembrane protein with a soluble counterpart present in plasma.13,14 However, the association of circulating NEP with ACE has not been determined.

Heritability describes the amount of phenotypic variation in a trait that can be attributed to additive genetic factors. Heritability analyses are, therefore, useful in indicating the extent to which interindividual differences in a given trait are a consequence of genetic factors. Heritability of plasma ACE has been studied in twin and family studies3,15,16 with estimates ranging from 15.8% to 65%. The heritability of plasma ACE2 and NEP has not been studied previously.

In the current study, we measured plasma ACE, ACE2, and NEP activities in the Leeds Family Study, a well-
characterized population of 89 healthy probands and their first-, second-, or third-degree relatives,\textsuperscript{17–19} and determined the heritability of the 3 enzymes to test the following hypotheses: (1) that circulating ACE, ACE2, and NEP are determined by genetic factors; (2) that circulating ACE, ACE2, and NEP are correlated; (3) that there is an inverse association between circulating ACE and ACE2; (4) that ACE, ACE2, and NEP contribute to variance in systolic BP (SBP) and diastolic BP (DBP); and (5) that phenotypic correlations among ACE, ACE2, NEP, SBP, and DBP can be explained, in part, by shared genetic regulation.

Methods

Study Population

The Leeds Family Study consisted of 537 subjects from 89 pedigrees from 133 households. The structure of the pedigrees and the clinical characteristics of the cohort have been published previously.\textsuperscript{17–19} Briefly, a list of potential subjects was generated at random from the community-based Family Health Authority Register in Leeds, and the potential subjects were contacted. Each subject (proband) was included in the study if healthy (defined as the absence of clinically overt vascular disease or diabetes mellitus) and if \( \geq 4 \) first-, second-, or third-degree relatives were able to take part. All of the probands were aged \( \geq 16 \) years, of white European origin, and gave informed consent according to a protocol approved by the Leeds Teaching Hospitals Trust Research Ethics Committee, in accordance with institutional guidelines and the Declaration of Helsinki. A total of 280 subjects were contacted, with 89 (32\%) having appropriate family size and health. Participating family members were interviewed to obtain information regarding their past medical history, smoking, and alcohol consumption patterns. The mean age of probands was 43.4 years, and the whole population (40.4\% male) had a mean age of 43.2 years. Of the family members (not probands), 7 subjects (1.3\%) had type II diabetes, and a further 2 subjects had fasting blood glucose \( \geq 7.0 \) mmol/L. Twenty-two subjects (4.1\%) had overt ischemic heart disease, 7 (1.3\%) had cerebrovascular disease (previous stroke or transient ischemic attack), and 44 subjects (8.2\%) were defined as being hypertensive. All of these subjects were included in the study. Three subjects did not have plasma samples available; therefore, 534 subjects were included in the present study. Fourteen subjects were taking ACE inhibitors and were excluded from all of the correlations and heritability analyses of ACE. SBP and DBP, the mean of 3 readings, were measured to the nearest 2 mm Hg with subjects in a sitting position.\textsuperscript{17–19} Blood was taken into lithium heparin at room temperature and centrifuged at 3000 \( \times \) g for 30 minutes. Aliquots of plasma were snap frozen in liquid nitrogen and stored at \(-40^\circ\text{C}\) until analyzed.

Assays

All of the assays were carried out in 50 mmol/L of Hepes/NaOH, 200 mmol/L of NaCl, 10 \( \mu \)mol/L of ZnCl\(_2\), and 1\% DMSO (pH 6.8) in the dark. ACE was assayed with 75 \( \mu \)mol/L of (7-methoxycoumarin-4-yl)acetyl (Mca)-Ala-Ser-Asp-Lys-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionic acid as substrate at 37\(^\circ\)C.\textsuperscript{20,21} ACE\(_{2}\) was assayed with 50 \( \mu \)mol/L of Mca-Ala-Pro-Lys-2,4-dinitrophenyl at room temperature.\textsuperscript{20} NEP was assayed with 200 \( \mu \)mol/L of succinyl-Ala-Ala-Ala-Ala-Phe-7-amido-4-methylcoumarin using a coupled assay with 50 \( \mu \)g/mL of leucine aminopeptidase at room temperature.\textsuperscript{20} Fluorescence was measured using a Biotek Synergy HT plate reader (\( \lambda_{\text{excitation}} \): 320 nm and \( \lambda_{\text{emission}} \): 405 nm for ACE and ACE\(_{2}\); \( \lambda_{\text{excitation}} \): 380 nm and \( \lambda_{\text{emission}} \): 460 nm for NEP). Enzyme activity (relative fluorescence units) in the plasma sample was converted to a concentration of enzyme by reference to a standard curve of recombinant enzyme assayed under the same conditions (further details of the assays can be found in the Supplementary Data available online at http://hyper.ahajournals.org). The ACE I/D polymorphism was genotyped as described previously.\textsuperscript{22}

Statistical Analysis

Activities of ACE, ACE2, and NEP were logarithmically transformed to remove skewness. Data are presented as geometric mean and 95\% CIs. For the genetic analysis, pedigree and phenotypic data were prepared using the Statistical Package for the Social Sciences for Windows version 11.0 (SPSS). The heritability of ACE, ACE2, and NEP was calculated using standard quantitative genetic variance components analysis\textsuperscript{23} using SOLAR v2.1.4 (Southwest Foundation for Biomedical Research) as described previously.\textsuperscript{17–19} Heritability is presented as the residual heritability (ie, variance after adjustment for covariates that could be attributed to the additive effect of genes). Shared childhood household effects were also considered in multivariate models.\textsuperscript{19} Only variables that were found to correlate significantly with ACE, ACE2, and NEP were entered into the appropriate multivariate analyses. Genetic and environmental correlations (\( \rho_{G} \) and \( \rho_{E} \)) between ACE and SBP and DBP were estimated as described previously.\textsuperscript{17}

Results

Heritability and Predictors of Plasma ACE

The mean (±95\% CI) plasma ACE in the study was 6.72 (6.50 to 6.96) nM, ranging from 0.23 to 16.74 nM. ACE was lower in subjects on ACE inhibitor therapy (1.74 [0.85 to 3.54] nM; \( n = 14 \)) than in those not taking ACE inhibitors (6.90 [6.71 to 7.10] nM; \( P = 0.02; n = 520 \)). ACE was lower in subjects taking aspirin who were not on ACE inhibitors (4.95 [3.16 to 7.78] nM; \( n = 18 \)) than in those who were not taking aspirin (10.61 [10.35 to 10.87] nM; \( P = 0.001; n = 503 \)) and lower in those taking aspirin and ACE inhibitors (1.11 [0.46 to 2.72] nM; \( n = 6 \)) than those on ACE inhibitors alone (2.72 [0.95 to 7.70] nM; \( P = 0.001; n = 7 \)). The ACE I/D genotype distribution was in Hardy–Weinberg equilibrium. ACE was lower in subjects possessing the ACE2 genotype (5.67 [5.26 to 6.12] nM; \( n = 121 \)) than in subjects with I/D genotype (6.72 [5.64 to 7.09] nM; \( P < 0.0005; n = 283 \)) or deletion/deletion (DD) genotype (7.64 [7.04 to 8.28] nM; \( P < 0.0005 \) for ACE2 versus DD and \( P = 0.025 \) for I/D versus DD; \( n = 129 \); Figure 1). ACE was lower in subjects with a history of myocardial infarction (MI), ischemic heart disease, or hypertension than in those without (data not shown), most likely because of ACE inhibitor or aspirin therapy. All of the subjects on ACE inhibitor therapy were removed from subsequent analyses of circulating ACE.

Figure 1. Scattergram of ACE I/D genotype against circulating ACE. Plasma ACE (nM) was measured as described in the Methods section. Lines show median, 2.5\%, and 97.5\% CIs.
TABLE 1. Correlations of Circulating ACE, ACE2, and NEP With Clinical Characteristics, Excluding Subjects on ACE Inhibitor Therapy

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>ACE</th>
<th>ACE2</th>
<th>NEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.111</td>
<td>0.245</td>
<td>0.267*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.071</td>
<td>−0.290</td>
<td>0.112</td>
</tr>
<tr>
<td>SBP</td>
<td>0.140*</td>
<td>0.197</td>
<td>0.216</td>
</tr>
<tr>
<td>DBP</td>
<td>0.116</td>
<td>0.170</td>
<td>0.197</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.040</td>
<td>0.294</td>
<td>0.137</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.161†</td>
<td>0.073</td>
<td>0.314†</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.173†</td>
<td>0.091</td>
<td>0.277*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.067</td>
<td>−0.381</td>
<td>0.039</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>0.112</td>
<td>−0.210</td>
<td>0.081</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td>0.121</td>
<td>0.073</td>
<td>0.314†</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.125</td>
<td>0.089</td>
<td>0.156</td>
</tr>
<tr>
<td>Homeostasis model assessment</td>
<td>0.119</td>
<td>0.100</td>
<td>0.170</td>
</tr>
<tr>
<td>ACE</td>
<td>−0.116</td>
<td>0.000</td>
<td>0.173</td>
</tr>
<tr>
<td>ACE2</td>
<td>−0.116</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>NEP</td>
<td>0.173</td>
<td>0.101</td>
<td></td>
</tr>
</tbody>
</table>

Excluding the individuals on ACE inhibitor therapy, plasma ACE was measured in 520 subjects. ACE2 was measurable in 40 subjects and NEP in 160 subjects. Data show Pearson’s partial correlation coefficients adjusted for age and sex.

*P<0.015 and †P=0.0075, data corrected for multiple comparisons.

Age- and sex-adjusted partial correlation coefficients for ACE are shown in Table 1. Circulating ACE correlated with SBP, cholesterol, and triglycerides (after correction for multiple comparisons). Quantitative genetic analyses in a model that excluded subjects on ACE inhibitor therapy indicated that genetic factors contributed to 24.5% of variance in ACE (Table 2). SBP accounted for 2.5% of variation in ACE and DBP accounted for 1.8%. The ACE I/D polymorphism accounted for only 8.8% of the total variance in circulating ACE. The best model for predicting circulating ACE was composed of ACE I/D polymorphism, age, sex, SBP, triglycerides, hypertensive therapy, and history of MI, which together accounted for 23.8% of the variance in ACE (ie, 76.2% of variance was not explained by the covariates studied).

Genetic factors were predicted to account for 17.8% of the residual variance. Inclusion of shared childhood household effects indicated that childhood household contributed to 16.2% of variance in circulating ACE, with genetic factors accounting for 8.7% of residual variance.

Heritability and Predictors of Plasma ACE

Circulating ACE was only detectable in 40 individuals (19 men and 21 women; mean: 33.02 pM; 95% CI: 22.08 to 49.36 pM; range: 3.27 to 463.63 pM). Age- and sex-adjusted partial correlation coefficients for ACE2 are shown in Table 1. Not adjusting for multiple comparisons, the only significant correlation of ACE2 was with high-density lipoprotein (HDL) cholesterol (P=0.026). There was no significant association of ACE2 with ACE inhibitor therapy or ACE I/D genotype (data not shown). Clinical characteristics were compared between subjects with and without detectable ACE2 as shown in Table 3. Subjects with detectable ACE2 were older, had a higher waist/hip ratio (WHR), SBP, DBP, fasting glucose, cholesterol, triglycerides, and NEP compared with subjects without detectable ACE2. In addition, 9 subjects had a history of diabetes or atherothrombosis, 5 had hypertension, and 12 women were postmenopausal. Of the 40 subjects, 22 had ≥1 other family member with detectable plasma ACE2; indeed, there was marked clustering of these individuals into 8 families having ≥2 members with detectable ACE2. There was no significant difference in circulating ACE2 between subjects taking aspirin (42.02 pM; 95% CI: 16.67 to 106.72 pM; n=8) and those not taking aspirin (31.35 pM; 95% CI: 20.01 to 48.69 pM; n=32). However, comparing subjects with and without measurable ACE2, a higher proportion of subjects with measurable ACE2 were taking aspirin than those without (20% versus 3.22%; P<0.0005). In quantitative genetic analyses (excluding subjects on ACE inhibitor therapy), 67.0% of variance in ACE2 was accounted for by genetic factors. HDL cholesterol, WHR, and fasting plasma glucose were found to be significant predictors of ACE2, accounting for 29.0% of the variance. Of residual variation, 32.6% was predicted to be genetic. Inclusion of shared childhood household effects indicated that childhood household contributed to 33.8% of variance in ACE2, with genetic factors accounting for 17.6% of residual variance.

Heritability and Predictors of Plasma NEP

Circulating NEP was detectable in 160 subjects with a mean value of 0.29 nM (95% CI: 0.23 to 0.36 nM), ranging from 3.07 pM to 10.40 nM. Age- and sex-adjusted partial correlation coefficients for NEP are shown in Table 1. Circulating NEP correlated with body mass index (BMI), cholesterol, triglycerides, and plasminogen activator inhibitor (PAI)-1 (after adjustment for multiple comparisons). NEP did not correlate with ACE or ACE2. There was no association of NEP with ACE inhibitor therapy, cardiovascular disease, diabetes, or hypertension (data not shown). There was no difference in NEP between subjects taking aspirin (0.23 nM; 95% CI: 0.09 to 0.55 nM; n=10) and those not taking aspirin (0.29 nM; 95% CI: 0.23 to 0.37 nM; n=150).

In quantitative genetic analysis, the unadjusted heritability estimate for NEP was 22.7%. Significant correlates of NEP (Table 1) were included in the analyses to determine the independent predictors of plasma NEP. The best model for...
TABLE 3. Comparison of Clinical Characteristics Between Subjects With and Without Detectable Circulating ACE2

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>No ACE2</th>
<th>ACE2</th>
<th>P (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42.60 (41.16 to 44.04)</td>
<td>51.04 (45.91 to 56.16)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.54 (25.14 to 25.95)</td>
<td>25.38 (24.07 to 26.75)</td>
<td>NS</td>
</tr>
<tr>
<td>WHR, cm</td>
<td>0.85 (0.84 to 0.86)</td>
<td>0.91 (0.87 to 0.94)</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120.8 (129.2 to 132.4)</td>
<td>141.3 (134.7 to 147.9)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>78.9 (77.5 to 79.9)</td>
<td>83.6 (80.5 to 86.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.06 (5.00 to 5.13)</td>
<td>5.15 (5.24 to 5.78)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.97 (4.88 to 5.07)</td>
<td>6.34 (3.26 to 6.05)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.24 (1.18 to 1.29)</td>
<td>1.91 (1.54 to 2.36)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.40 (1.36 to 1.44)</td>
<td>1.46 (1.34 to 1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mmol/L</td>
<td>2.65 (2.77 to 2.93)</td>
<td>3.02 (2.74 to 3.32)</td>
<td>NS</td>
</tr>
<tr>
<td>ACE, nM</td>
<td>6.80 (6.60 to 7.07)</td>
<td>5.47 (4.40 to 6.87)</td>
<td>NS</td>
</tr>
<tr>
<td>NEP, nM</td>
<td>0.25 (0.20 to 0.32)</td>
<td>0.67 (0.35 to 1.27)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Forty subjects had measurable circulating ACE2 activity, and in the remaining 494 subjects, ACE2 was undetectable. Data presented as mean or geometric mean and 95% CIs. NS indicates not significant.

Contribution of Plasma ACE, ACE2, and NEP to the Heritability of SBP and DBP

We have previously carried out quantitative genetic analyses for SBP in a healthy subset (n=504) of the current population and determined the association with age, sex, smoking, and BMI.17,19 In the present analysis, we have also included ACE, NEP, and additional significant correlates of SBP and DBP. ACE2 was undetectable. Data presented as mean or geometric mean and 95% CIs. NS indicates not significant.

Inclusion of significant covariates (ACE, age, sex, BMI, cholesterol, low-density lipoprotein cholesterol, PAI-1, homeostasis model assessment, aspirin therapy, and hypertensive therapy) accounted for 39.6% of variance in SBP. Childhood household contributed to 13.2% of variance in SBP, and there was no residual genetic contribution to variance after accounting for other covariates and household effects. We evaluated the age- and sex-adjusted genetic and environmental correlations between ACE and SBP and found only a significant environmental correlation (ρe=0.35; P=0.031). In a model including the subjects with detectable circulating NEP, NEP accounted for 5.1% of total variance. NEP was not a significant covariate in the final model.

In univariate analysis, circulating ACE accounted for 1.1% of total variance in DBP. Inclusion of ACE I/D genotype did not improve the model. In the final model, inclusion of significant covariates (ACE, age, sex, BMI, cholesterol, low-density lipoprotein cholesterol, PAI-1, homeostasis model assessment, aspirin therapy, and hypertensive therapy) accounted for 31.7% of variance in DBP. Childhood household accounted for an additional 12.2% of variance in DBP, with genetic factors accounting for 17.2% of residual variance. In a model including the subjects with detectable circulating NEP, NEP accounted for 4.9% of total variance in DBP. Plasma NEP was not a significant predictor for DBP in the final model. Exclusion of the 31 subjects on hypertensive medication did not substantially alter the models for SBP and DBP (data not shown).

Discussion

In the present study, we have measured circulating ACE2 for the first time and have investigated the heritability of circulating ACE, ACE2, and NEP and their relationship with SBP and DBP. The heritability estimate for ACE was 24.5%, which is consistent with previous studies in twins and families in which heritability estimates varied between 15.8% and
65%, 3, 15, 16 Previous studies have not reported consistent associations between environmental factors or clinical characteristics and circulating ACE. 24, 25 Correlations with BP and features of the insulin resistance syndrome might be expected, because ACE inhibitors are a successful therapy for the treatment of hypertension, heart disease, and diabetes. 25, 26 In support of this, BP, triglycerides, history of MI, and hypertensive therapy were significantly associated with plasma ACE, and together with the ACE I/D polymorphism, age and sex accounted for 23.8% of total variance in ACE activity, with the ACE I/D polymorphism accounting for 8.8% of this variance. Although it has been reported that the ACE I/D accounted for ≤ 43% of variation in plasma ACE in whites, 3 the majority of studies 15, 16, 27–29 indicate a contribution of the ACE I/D to variance of ACE in line with our findings.

Circulating ACE was found to be significantly lower in subjects taking aspirin than those who were not and lower in those taking aspirin and ACE inhibitors than in those taking only ACE inhibitors. Aspirin and ACE inhibitors are widely used in combination to treat a wide spectrum of cardiac disorders, 40 and there is much controversy as to whether these 2 agents interact negatively or positively. 31 Our observations suggest that aspirin directly lowers circulating ACE and augments the reduction in ACE activity seen with ACE inhibitors. Whether this observation is explained by the fact that aspirin has been reported to directly inhibit ACE activity in serum 42 remains to be determined. With the relatively small numbers involved, these observations have to be interpreted with caution, and further study is required to determine whether aspirin directly inhibits ACE activity and increases the clinical action of ACE inhibitors.

Circulating ACE2 was ≈ 100-fold lower than ACE and was detectable in only 40 subjects. This is probably not because of limited sensitivity of the assay, which could detect a 40-fold lower amount of ACE2 as compared with ACE. Lower circulating ACE2 could be because of less shedding of ACE2 than ACE from the plasma membrane. Recently, we have shown that the zinc metalloprotease ADAM17 is responsible for the regulated shedding of ACE2; however, there is little constitutive shedding of ACE2 in contrast to ACE. 10 Our data would indicate that there is no or very low circulating ACE2, at least in healthy individuals. It remains to be determined whether circulating ACE2 increases in certain disease states because of increased expression, increased shedding from the membrane, or a decrease in its clearance from the circulation.

There was evidence of clustering of circulating ACE2 in families, with 8 families having ≥ 2 members with detectable ACE2, and the estimated heritability was 67%. HDL cholesterol, WHR, and fasting plasma glucose were found to be significant predictors of ACE2, accounting for 29% of variance. However, these data need to be treated with caution because of the small number of subjects with measurable ACE2 activity. We found no evidence of a correlation between circulating ACE and ACE2, which is similar to findings in a range of primary and transformed cells in culture. 33 Subjects with detectable ACE2 were older than those without and had a higher prevalence of cardiovascular disease, diabetes, and hypertension, suggesting that ACE2 may also be upregulated in subjects with cardiovascular disease to counteract the adverse effects of Ang II.

Because NEP has previously been demonstrated to be regulated by glucose and fatty acids 34 and NEP is produced by adipocytes, 35 it is conceivable that circulating NEP might be correlated with other features of the metabolic syndrome. This is the first study to determine the heritability of circulating NEP, which was estimated to be 22.7%. We found that age, sex, cholesterol, and PAI-1 were significant determinants of NEP, together accounting for 17.3% of the variance. We did not find any evidence of an association of circulating NEP with either ACE or ACE2; however, circulating NEP was correlated with BMI, cholesterol, triglycerides, and PAI-1.

Figure 2. Scattergrams of SBP and DBP against circulating ACE, ACE2, and NEP. Plasma ACE, ACE2, and NEP were measured as described in the Methods section. Regression lines and $R^2$ values are shown on each plot.
suggesting that NEP may indeed be associated with the metabolic syndrome.

Vasopeptidase inhibitors, such as omapatrilat, which simultaneously inhibit ACE and NEP, produced greater decreases in SBP and DBP in patients with hypertension than ACE inhibition alone. Thus, NEP might be expected to correlate with BP. However, we found that ACE, but not NEP, was an independent determinant of SBP and DBP in this study. A significant shared childhood household effect was also observed for SBP and DBP. It is interesting to note that for SBP, inclusion of household effects indicated that there was no unexplained residual genetic contribution to variance in SBP. In contrast, genetic factors were predicted to contribute to 17.2% of residual variance in DBP, after accounting for covariates and shared childhood household (ie, 17.2% of 68.3% of variation not explained by covariates). In addition, we looked at the relative contribution of genetic and environmental components to the relationship between ACE and SBP; only a significant environmental correlation was identified. SBP was found to contribute to 2.5% of variation in ACE and DBP to 1.8%. Linkage analyses has suggested that a region close to the ACE gene is involved in BP regulation, but evidence for a specific genetic marker within the ACE gene itself is inconsistent. Our results of the present study suggest that the ACE gene only has a very minor role. Studies in ACE knockout mice with tissue-specific expression of ACE suggest that the RAS is able to compensate for small alterations in ACE expression, and only when ACE is almost entirely eliminated, is BP affected. Our study suggests that in a general population, the effect of the ACE gene is minor, supported by the fact that only 1% of variation in BP is because of ACE activity.

Perspectives

We have measured plasma ACE, ACE2, and NEP in a family study and shown that there is no correlation among circulating ACE, ACE2, or NEP. ACE was significantly lower in a variety of cardiac and metabolic disorders.

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Disclosures

None.

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