Correlates of ACE activity in macroalbuminuric type 2 diabetic patients treated with chronic ACE inhibition

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Abstract
The activity of the renin–angiotensin–aldosterone system (RAAS) plays an important role in the development and progression of diabetic nephropathy. However, the effect of angiotensin-converting enzyme (ACE) inhibition on the RAAS appears to be modulated by a number of factors including the I/D polymorphism of the ACE genotype. In this study, we attempted to find independent correlates of ACE activity in 121 macroalbuminuric type 2 diabetic Iranian patients under chronic ACE inhibition. Both univariate and multivariate analyses were used. The presence of the D allele was independently associated with significantly higher levels of ACE activity (with the II genotype as reference, P < 0.001, B = 27.3, 95% CI = 17.6–37.1), and this association was not eliminated by potentially confounding variables. In conclusion, the D allele is a significant independent correlate of ACE activity in macroalbuminuric type 2 diabetic Iranian patients under long-term ACE inhibition.

Keywords: ACE activity; diabetic nephropathy; macroalbuminuria; polymorphism; type 2 diabetes

Introduction
The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene, first identified by Rigat et al. [1], accounts for about half of the phenotypic variance in serum ACE levels. The DD genotype or D allele is associated with elevated circulating and tissue ACE activity compared to I allele. The I/D polymorphism may therefore influence the effect of ACE inhibitors on clinical outcomes in various disease states. However, evidence supporting an effect modification by the I/D polymorphism is still sparse [2].

Both systemic and renal renin–angiotensin systems are hyperactive in diabetic nephropathy [3]. Inhibition of the renin–angiotensin–aldosterone system (RAAS) exerts beneficial effects on renal outcomes in type 2 diabetic patients by delaying the progression of diabetic nephropathy [4,5], which is a major cause of morbidity and mortality especially in non-Caucasian populations [6]. Moreover, albuminuria has been identified as a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy [7,8]. It is therefore important to identify the determinants of ACE inhibitor effectiveness in decreasing ACE activity in patients with type 2 diabetic nephropathy. This new knowledge will help predict treatment outcomes and design appropriate therapeutic protocols on an individual basis.

The purpose of this study was to determine, for the first time, the potential determinants of ACE activity in macroalbuminuric type 2 diabetic patients under chronic ACE inhibition in a population of adult Iranian patients.

Subjects and methods
We evaluated, in a retrospective study, 121 adult type 2 diabetic patients with macroalbuminuria on chronic (≥2 years) ACE inhibitor treatment, who had been referred to our type 2 diabetes university clinic between September 2005 and December 2006. All referrals were for routine follow-up and there were no cases, such as proteinuria, that would render the cohort unrepresentative. All patients had reliable medical profiles from which relevant information was extracted when appropriate (see below). Exclusion criteria were non-Caucasian ethnicity, plasma creatinine >2 mg/dl, history of renal disease before the onset of diabetes, treatment with other antihypertensive medications, presence of any sign or symptom of inflammatory renal disease and non-compliance to therapy (e.g. due to side effects of drugs or irregular consumption of medications). Patients suspected of having any kidney disease other than diabetic nephropathy were referred to the nephrology clinic for further evaluation. All patients gave written informed consent and the local ethics committee at our centre approved the study protocol.

In addition to a thorough physical examination at referral, we examined the following variables: age, sex, body mass index (BMI; kg/m²) according to the Quetelet equation, diabetes duration and medication (oral agents, insulin or both), smoking habit, duration, type and dosage of ACE inhibitor treatment, history of coronary artery
ACE activity correlates in diabetic nephropathy under chronic ACE inhibition

Table 1. Characteristics of patients according to their ACE genotype

<table>
<thead>
<tr>
<th></th>
<th>DD group (n = 35)</th>
<th>ID group (n = 66)</th>
<th>II group (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median; range)</td>
<td>58 (49–79)</td>
<td>58.5 (45–61)</td>
<td>55.0 (50–65)</td>
<td>0.103</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>18:17</td>
<td>38:28</td>
<td>13:7</td>
<td>0.614</td>
</tr>
<tr>
<td>BMI (median; range)</td>
<td>26.4 (21.2–43.7)</td>
<td>26.6 (19.1–34.7)</td>
<td>23.5 (19.7–30.2)</td>
<td>0.094</td>
</tr>
<tr>
<td>Diabetes duration (years; mean ± SD)</td>
<td>12.1 ± 4.6</td>
<td>11.6 ± 4.8</td>
<td>11.5 ± 4.3</td>
<td>0.884</td>
</tr>
<tr>
<td>ACEI duration (years; mean ± SD)</td>
<td>6.3 ± 2.3</td>
<td>5.7 ± 2.0</td>
<td>5.8 ± 1.0</td>
<td>0.594</td>
</tr>
<tr>
<td>Captopril dosage (mg/day; mean ± SD)</td>
<td>37.5 ± 32.8</td>
<td>20.8 ± 12.4</td>
<td>16.7 ± 6.5</td>
<td>0.018*</td>
</tr>
<tr>
<td>Smoking</td>
<td>7 (20.0%)</td>
<td>11 (16.7%)</td>
<td>6 (30.0%)</td>
<td>0.424</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>32 (91.4%)</td>
<td>57 (86.4%)</td>
<td>18 (90.0%)</td>
<td>0.729</td>
</tr>
<tr>
<td>Proliferative diabetic retinopathy</td>
<td>13 (37.1%)</td>
<td>23 (34.8%)</td>
<td>4 (20.0%)</td>
<td>0.387</td>
</tr>
<tr>
<td>Insulin therapy</td>
<td>24 (68.6%)</td>
<td>48 (72.7%)</td>
<td>11 (55.0%)</td>
<td>0.326</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (60.0%)</td>
<td>36 (54.5%)</td>
<td>7 (35.0%)</td>
<td>0.187</td>
</tr>
<tr>
<td>FBS (median; range)</td>
<td>215.0 (129–393)</td>
<td>199.0 (124–365)</td>
<td>225.0 (135–330)</td>
<td>0.682</td>
</tr>
<tr>
<td>Chol (mean ± SD)</td>
<td>221.6 ± 49.3</td>
<td>218.2 ± 42.3</td>
<td>210.2 ± 48.2</td>
<td>0.669</td>
</tr>
<tr>
<td>TG (median; range)</td>
<td>185 (100–516)</td>
<td>162.5 (110–514)</td>
<td>140.0 (95–241)</td>
<td>0.078</td>
</tr>
<tr>
<td>LDL (mean ± SD)</td>
<td>125.0 ± 33.2</td>
<td>122.0 ± 27.9</td>
<td>108.8 ± 25.8</td>
<td>0.128</td>
</tr>
<tr>
<td>HDL (mean ± SD)</td>
<td>42.0 ± 11.5</td>
<td>43.6 ± 9.3</td>
<td>39.4 ± 9.0</td>
<td>0.240</td>
</tr>
<tr>
<td>Crt (mean ± SD)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.837</td>
</tr>
<tr>
<td>HbA1c (median; range)</td>
<td>8.1 (6.0–13.0)</td>
<td>8.2 (6.2–16.2)</td>
<td>10.6 (6.2–12.3)</td>
<td>0.148</td>
</tr>
<tr>
<td>24-h urine protein (median; range)</td>
<td>620.5 (320.5–1703.0)</td>
<td>715.4 (313.6–1890.0)</td>
<td>925.1 (19.7–30.2)</td>
<td>0.598</td>
</tr>
<tr>
<td>ACE activity (mean ± SD)</td>
<td>94.4 ± 19.6</td>
<td>64.4 ± 19.2</td>
<td>37.3 ± 6.6</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Non-normally distributed variables are presented as medians (range); *P < 0.05.

Results

Among 121 individuals included in the study, 69 (57%) were men and 52 (43%) were women, with a median age of 58.0 years (range: 45–81 years) and a median BMI of 25.8 (range: 19.1–43.7). Body weights in most patients were almost constant during the follow-up period. The duration of diabetes and ACE inhibitor treatment ranged from 1 to 13 years (11.7 ± 4.6 years) and 2 to 11 years (5.9 ± 2.0 years), respectively. The DD:ID:II ratio in the entire study population was 35:66:20. In univariate analyses (patients categorized according to their ACE genotypes), there were no significant differences between the three groups for age (P = 0.103), sex (P = 0.614), history of coronary artery disease (P = 0.729), smoking habit (P = 0.424), presence of proliferative diabetic retinopathy (P = 0.387), hypertension (P = 0.187), use of hypoglycaemic drugs (P = 0.326), diabetes duration (P = 0.884), duration of ACE inhibitor treatment (P = 0.594), FBS (P = 0.682), HbA1c (P = 0.184), TG (P = 0.078), Chol (P = 0.669), LDL (P = 0.128), HDL (P = 0.240), Crt (P = 0.837) or 24-h urinary albumin excretion (P = 0.598) (Table 1).
However, the presence of the D allele was associated with higher ACE activity. For example, the DD group had significantly greater ACE activity than the other two groups ($P < 0.001$) and ACE activity in the ID group was significantly higher than in the II group ($P < 0.001$). In addition to ACE activity, BMI was also different among the three groups ($P = 0.004$). BMI was significantly higher in the DD and ID groups than in the II group ($P = 0.003$ and $P = 0.016$, respectively). The dosage of ACE inhibitors was also different among the groups ($P = 0.018$). Patients in the DD group used significantly higher doses than those in the ID group ($P = 0.023$). In summary, the D allele was associated with higher ACE activities, while BMI and ACE inhibitor dosage appeared to act as potential confounders. Therefore, we proceeded to multivariate regression.

In multivariate regression analysis ($R^2 = 0.625$), we considered ACE activity as the dependent variable and ACE genotype, BMI, ACE inhibitor dosage (statistically significant variables), sex, age, diabetes duration and the duration of ACE inhibitor treatment (clinically significant variables) as covariates. The only independent correlate of ACE activity was the ACE genotype (with the II genotype as reference, $P < 0.001$, $B = 27.3$, 95% CI = 17.6–37.1). All other variables were non-significant (Table 2).

### Discussion

The results of this study show that the ACE genotype is an independent significant correlate of ACE activity in adult macroalbuminuric type 2 diabetic patients under chronic ACE inhibitor treatment. Specifically, the presence of the D allele was associated with higher ACE activity in these patients. In multivariate regression, the ACE genotype ($P < 0.001$) accounted for over half of the total variation in ACE activity. Age, sex, diabetes duration, ACE inhibitor treatment duration and ACE inhibitor dosage did not significantly confound the association with ACE. The immediate conclusion is that ACE activity after chronic ACE inhibition is higher in macroalbuminuric type 2 diabetic patients carrying D allele than in those lacking the D allele.

An effect of the I/D polymorphism on clinical outcomes in proteinuric patients under ACE inhibitor treatment has been shown repeatedly. However, results have not been completely consistent. Although the presence of the D allele appears to negatively affect the course of diabetic nephropathy [13], while renal outcomes are improved in macroalbuminuric type 2 diabetic individuals with the II genotype [14], a study with 36 Japanese proteinuric patients [15] and another with 83 macroalbuminuric type 2 diabetic Korean patients [16] showed that the effect of ACE inhibition on the severity of proteinuria is more prominent in the presence of the D allele. Caucasian DD carriers appear to show better responses to ACE inhibitors than do II carriers [2]. Finally, a study on 61 proteinuric Dutch patients given short-term ACE inhibition failed to show any effect of genotype on proteinuria. Thus, the area of genotype effects on outcome remains controversial and responses may vary according to ethnicity [17]. Consequently, our results obtained from a Caucasian population may not pertain to other populations.

We focused for the first time on macroalbuminuric type 2 diabetic patients given chronic ACE inhibition. The most similar previous study examined 60 insulin-dependent diabetic patients with diabetic nephropathy and showed a larger antiproteinuric effect during the 6-month follow-up period in patients with the II genotype [18]. The distinction between short-term and long-term ACE inhibition is important because the production of ACE is upregulated under chronic ACE inhibition [19–23]. The reason for this upregulation is unknown, but may be due, at least in part, to an increase in the synthesis and secretion of the enzyme by lung vascular endothelial cells [21]. This process has been shown to be generalized and is not limited to certain tissues [24]. The present findings suggest (but do not conclude) that this relationship is influenced by genotype. Chronic ACE inhibition is widely given for type 2 diabetics in Iran. Based on the results of our study, this protocol may not provide an ideal treatment, at least for obese DD patients. Better alternatives for this group may include ultra-high doses of ACE inhibitors and angiotensin II receptor blockers [17].

In a previous study, we showed that genotype is related to hypertension in type 2 diabetic patients [10]. Our macroalbuminuric type 2 diabetic patients under chronic ACE inhibition in the present study were obviously not representative of the general type 2 diabetic population. However, it is interesting that the prevalence of hypertension in this study was (though not significantly) greatest in DD, lower in ID and lowest in II patients. A possible explanation is that the association between the ACE genotype and hypertension in type 2 diabetes is so strong that it was not completely cancelled out in the non-representative subgroup examined in the present study.

There were a number of limitations to our study. First, the retrospective nature of the study, the lack of data on ACE activity and the severity of proteinuria at the time of ACE inhibitor initiation made it impossible to evaluate the efficacy of chronic ACE inhibition on lowering ACE activity and diminishing the severity of proteinuria using a before/after treatment statistical methodology. Specifically, DD patients may have had a better response to treatment but nevertheless a worse outcome. As a second limitation, the presence of diabetic nephropathy and the absence of other kidney diseases were not conclusively shown from renal biopsies. Although we attempted to include only patients with pure diabetic nephropathy, it is possible though unlikely that active inflammation interfered with the outcomes. Appropriate laboratory measures of active inflammation such as C-reactive protein (CRP) should be considered in future studies. Despite these limitations, our findings indicate that
DD macroalbuminuric patients under chronic ACE inhibition have higher ACE activities (and probably more severe nephropathy) than their otherwise matched ID or II counterparts.

Conflict of interest statement. None declared.

References


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