The Angiotensin-Converting Enzyme Genotype and Microalbuminuria in Autosomal Dominant Polycystic Kidney Disease

MARJAN A. VAN DIJK,* DORIEN J.M. PETERS,‡ MARTIJN H. BREUNING,† and PETER C. CHANG*

*Department of Nephrology and †Clinical Genetic Centre, Leiden University Medical Centre, and ‡Department of Human Genetics, Sylvius Laboratories, Leiden University, The Netherlands.

Abstract. Autosomal dominant polycystic kidney disease (ADPKD) has a variable clinical course. Clinical parameters associated with a worse prognosis are hypertension and proteinuria or microalbuminuria (MA). Because chronic stimulation of the renin-angiotensin system is likely to be present in ADPKD patients, the effect of the angiotensin-converting enzyme insertion/deletion (ACE I/D) genotype on the variability of these clinical parameters was examined in untreated ADPKD patients. Proteinuria and MA were determined in 24-h urine collections. BP measurements were performed with an ambulatory monitor, over 24 h. With analysis of covariance, the ACE genotype was found to be significantly associated with MA, corrected for age, gender, GFR, mean arterial pressure, body surface area, and urinary Na⁺ excretion (P < 0.05). The patients homozygous for the deletion (DD) had the highest rate of MA (P < 0.05) compared to the patients homozygous for the insertion (II). There was no relationship between the ACE genotype and BP or renal function. A significant positive correlation was found between MA and mean arterial pressure (r = 0.31, P < 0.05), whereas a significant negative correlation was found between MA and renal function (r = −0.28, P < 0.05). In conclusion, in ADPKD patients, MA is partly determined by the ACE I/D polymorphism. Because MA is associated with an enhanced progression toward renal failure, the ACE genotype could help in identifying patients at risk for a worse prognosis.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common heritable renal disease, with an estimated incidence of 1:1000. This disease is genetically heterogeneous; in approximately 85% of the cases, the disease is caused by a mutation localized on chromosome 16 (PKD1) and in 15% by a mutation localized on chromosome 4 (PKD2) (1–3), while a few families have been identified in which the disease is caused by a mutation in an unmapped locus (4). This genetic heterogeneity causes differences in clinical appearance, i.e., the disease tends to run a milder course with a longer patient survival, slower progression toward end-stage renal failure (ESRF), and a lower prevalence of hypertension in PKD2 (5). However, within the two identified forms of the disease, there is a remarkable variability in clinical features. For instance, the age at which ESRF occurs varies from early 20s to 90s or not at all (6). Proteinuria and microalbuminuria (MA) also occur with a highly variable severity and are associated with a more progressive course of the disease (7,8). The most common and easily treatable complication, hypertension, is likewise related to progression of the disease (9–12). However, the influence of these factors on progression cannot wholly account for the variability in the clinical course.

It has been assumed that different mutations in the PKD1 and PKD2 gene are responsible for the differences in clinical course of this disease. However, several reports describe wide differences in complications and progression of the disease among affected members of the same family, having the same mutation of the PKD gene (13,14). A logical explanation for the clinical variability of the disease, apart from environmental factors, therefore, seems to be the presence of genetic modifying factors. Earlier studies have shown an enhanced function of the renin-angiotensin system (RAS) in ADPKD patients (15–17). This, together with the finding that hypertension of the unaffected parent is related to a less favorable course of ADPKD in the patient (18), makes it likely that genetic polymorphisms of the RAS genes are candidate factors for modifying the course of this disease.

One of the described polymorphisms of the RAS is the angiotensin-converting enzyme insertion/deletion (ACE I/D) polymorphism. This polymorphism accounts for half of the variability of serum ACE levels in humans (19). Individuals homozygous for the deletion (DD) have an approximately twofold higher serum ACE level than individuals who are homozygous for the insertion (II). Nevertheless, in the majority of studies, the expected relationship between ACE genotype and hypertension was not found, despite the well-established function of ACE in BP regulation (20,21). However, in...
ACE Genotype and Microalbuminuria in ADPKD

ADPKD patients with an already stimulated RAS, this may be different. Although the impact of the ACE genotype on BP is lacking, the effects may be present at the tissue level. Several studies (22,23) suggest that ACE is likely to be the rate-limiting step in the local production of angiotensin II (AngII). The associations found between the DD genotype and an increased risk for various cardiac diseases, the progression of renal diseases, and the antiproteinuric response to ACE inhibitors (23–26) may therefore be explained by the influence of the ACE genotype on the AngII that is generated at specific sites, such as in the heart or kidneys.

We hypothesized that the ACE genotype is a determinant of the clinical course in ADPKD. In a cross-sectional study in untreated ADPKD patients, we investigated the impact of the ACE genotype on clinical features such as renal function, BP, proteinuria, and MA.

Materials and Methods

Patients

Seventy-three untreated ADPKD patients were recruited. None of the patients used antihypertensive medication. Dietary intake of sodium was normal, as was demonstrated by the urinary excretion of sodium, 150 ± 6.3 mmol/24 h and potassium, 80 ± 2.8 mmol/24 h (mean ± SEM, untreated and without dietary prescriptions or restrictions). All gave informed consent for our protocol, which was approved by our Medical Ethics Review Committee.

Measurements

Patients were investigated twice, with 2 to 4 wk in between. On these occasions, an intravenous cannula was inserted and blood samples were taken for biochemical, hematologic, and hormonal tests, including serum ACE levels, plasma renin activity, and aldosterone. To establish serum ACE activity, a colorimetric method, using p-hydroxybenzoyl-glycyl-L-histidyl-L-leucine (ACEcolor, Fujirebio, Inc., Tokyo, Japan), was used. Plasma renin activity and aldosterone were measured by RIA.

GFR and effective renal plasma flow were measured after an overnight fast, by infusion of inulin and para-aminohippurate. A loading dose was given in 10 min, followed by 3 h of continuous infusion. During the infusion, patients stayed in the resting position and maintained hydration by oral water intake. After 1.5 h, three 30-min timed urine collections were obtained, with blood samples before and at the end of each collection period, for determination of inulin and para-aminohippurate concentrations.

Before attending our ward, patients collected urine for 24 h for the determination of Na⁺, K⁺, creatinine, proteinuria, and MA. MA was determined using an immunoturbidimetric method (27). A 24-h BP profile was recorded at the second visit, using an ambulatory BP monitor (Spacelab 90207). Hypertension was defined as a mean diastolic BP during daytime (10 a.m. to 11 p.m.) ≥90 mmHg.

ACE I/D Genotyping

DNA was isolated from peripheral blood leukocytes, using standard techniques (28). The ACE gene I/D polymorphism was detected by performing the PCR as described by Rigat (29). The PCR product is a 190-bp fragment in the absence of the insertion and a 490-bp fragment in the presence of the insertion. To prevent mistyping of ACE heterozygotes, we used a specific primer for the insertion (30) whenever a DD genotype was found. In case of an I-allele, a fragment of 408 bp was present; in case of a true DD homozygote, no such band was present. In both cases, the PCR product was visualized after electrophoresis in 2% agarose gels.

Statistical Analyses

One-way ANOVA was performed on all clinical parameters and patient characteristics. Further analysis of covariance (ANCOVA) was accomplished on BP, renal function parameters, and MA, correcting for different covariates. Multiple comparison tests were performed according to Bonferroni to detect differences between the ACE genotypes. All data were normally distributed, except MA, which was therefore analyzed after log transformation. Correlation coefficients were calculated according to Pearson. All values are reported as means ± SEM. Statistical significance was defined as P < 0.05.

Results

None of the patients had proteinuria >500 mg/24 h; therefore, proteinuria was further analyzed as MA. MA was expressed as urinary albumin/creatinine ratio, to avoid bias by incomplete 24-h urine collections (31). The results of the one-way ANOVA on clinical parameters and patient characteristics of the ADPKD patients are shown in Table 1. There was a significant relationship between the ACE genotype and serum ACE levels (sACE) and between the ACE genotype and MA. Bonferroni multiple comparison test showed that for sACE this significance was due to differences between the DD and the II genotype (P < 0.05) and between the DD and II genotype (P < 0.05). For MA, significant differences were found between the DD and II genotype (P < 0.05) and between the DD and ID genotype (P < 0.05).

ANCOVA was performed to further explore the relationship between MA and the ACE genotype, with age, gender, GFR, MAP, body surface area (BSA), and urinary Na⁺ excretion as covariates. This relationship still appeared to be significant (P < 0.05). With Bonferroni multiple comparison test, this effect was due to a significant difference between the DD group and the II group (P < 0.05). This relationship is shown in Figure 1, with the DD genotype associated with the highest rate of MA, and the II genotype with the lowest. In this analysis, the explained variability was 34%. BP parameters, e.g., systolic BP and diastolic BP, were analyzed likewise with ANCOVA, correcting for age, BSA, and GFR. No significant differences between the genotypes could be established. After further analysis of renal function parameters (e.g., creatinine clearance and GFR) correcting for age, BSA, and MAP, no significant relation with the ACE genotype was found.

In addition, we looked at the relationship between MA and the clinical parameters. Significant positive correlations were found between MA and age (r = 0.25, P < 0.05) and MAP (r = 0.31, P < 0.05), and significant negative correlations were found with GFR (r = −0.28, P < 0.05) and BSA (r = −0.34, P < 0.01).

Distribution of the ACE I/D polymorphism in our ADPKD population was in Hardy-Weinberg equilibrium, with a distribution of 0.27/0.51/0.23 for the DD/ID/II genotypes, respectively. The allele distribution for the D and I allele was 0.52/
Table 1. Clinical parameters, patient characteristics, and the ACE genotype in ADPKD patientsa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DD (n = 19)</th>
<th>ID (n = 38)</th>
<th>II (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>10/9</td>
<td>16/22</td>
<td>5/11</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39 ± 2</td>
<td>39 ± 1</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.97 ± 0.05</td>
<td>1.94 ± 0.04</td>
<td>1.80 ± 0.06</td>
</tr>
<tr>
<td>CCr (ml/min)</td>
<td>104 ± 6</td>
<td>105 ± 5</td>
<td>106 ± 6</td>
</tr>
<tr>
<td>MA/Cr (mg/mmol)</td>
<td>4.31 ± 1.16</td>
<td>2.70 ± 0.56</td>
<td>1.85 ± 0.38b</td>
</tr>
<tr>
<td>Renin (µg/L per h)</td>
<td>1.74 ± 0.23</td>
<td>1.87 ± 0.22</td>
<td>2.34 ± 0.47</td>
</tr>
<tr>
<td>Aldosterone (mmol/L)</td>
<td>0.46 ± 0.05</td>
<td>0.44 ± 0.03</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>sACE (nmol/min per ml)</td>
<td>41.8 ± 3.8</td>
<td>39.1 ± 2.0</td>
<td>31.0 ± 2.8c</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137 ± 3</td>
<td>132 ± 2</td>
<td>132 ± 3</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>91 ± 2</td>
<td>86 ± 2</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>113 ± 5</td>
<td>111 ± 4</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>ERPF (ml/min)</td>
<td>466 ± 32</td>
<td>454 ± 23</td>
<td>430 ± 27</td>
</tr>
<tr>
<td>FF (%)</td>
<td>25.4 ± 1.1</td>
<td>25.7 ± 0.7</td>
<td>26.2 ± 1.2</td>
</tr>
</tbody>
</table>

a ACE, angiotensin-converting enzyme; ADPKD, autosomal dominant polycystic kidney disease; BSA, body surface area; CCr, creatinine clearance; MA/Cr, ratio urinary excretion albumin/creatinine per 24 h; sACE, serum angiotensin-converting enzyme; SBP, systolic blood pressure; DBP, diastolic blood pressure; ERPF, effective renal plasma flow; FF, filtration fraction.

b One-way ANOVA P < 0.05. Post hoc Bonferroni: DD versus II: P < 0.05 and ID versus ID: P < 0.05.

c One-way ANOVA P < 0.05. Post hoc Bonferroni: DD versus II: P < 0.05 and ID versus II: P < 0.05.

Discussion

Proteinuria and MA are not prominent features of ADPKD. However, their presence in individual patients is important because they are associated with a worse renal prognosis in ADPKD patients (7,8) and an increased cardiovascular morbidity (33). So, the presence of established MA helps in identifying ADPKD patients who are at risk for an adverse clinical course of the disease. Factors of known influence on MA are BP, renal function, renal volume, and filtration fraction (FF) (8). In this study, we confirmed the correlation between MA and MAP and renal function. We were not able to confirm the correlation between MA and FF, and renal volume was not measured in this study. A significant relationship between MA and the ACE genotype could be established in our patient group. Although this relationship was demonstrated earlier in patients with non-insulin-dependent diabetes mellitus and hypertensive nephropathy (25,34,35), it is the first time that the ACE genotype is associated with MA in an ADPKD population.

Although BP was higher, albeit not statistically significant, in the DD group both diastolic and systolic BP could not be related to the ACE genotype. However, given the positive correlation between MA and BP, the higher BP in the DD group could be responsible for the higher rate of MA in that group. When we corrected for MAP in our analysis, the relationship between MA and the ACE genotype remained significant. Therefore, apart from BP, MA is influenced by the ACE genotype. Together with MAP, age, GFR, BSA, gender, and urinary Na+ excretion, the ACE genotype explains 34% of the variability of MA in our patient group.

Most of our patients were normotensive (44 patients, 60%) and had normal renal function (7% had GFR ≥90 ml/min). The FF is known to decrease as renal function deteriorates (36). In our study, only small interindividual differences in both GFR and FF were present. This could explain why in these patients the expected relationship between FF and MA was not found. The same holds true for proteinuria. Because proteinuria is associated with high BP and diminished renal function (37,38), the absence of proteinuria is likely to be due to the normal renal function and relatively normal BP of our patients. The relationship between MA and the ACE polymorphism makes it likely that proteinuria is also influenced by the ACE genotype.

In this study, the significant relationship between the ACE genotype and sACE was confirmed. A relationship between BP and the ACE genotype was not found in this study. Several other investigators reported a discrepancy between sACE levels and BP in other populations such as healthy control subjects or essential hypertensive patients (21,39,40). Recently, two large studies were presented in which the ACE DD genotype was associated with higher BP in males (41,42). This ambiguous relationship is probably due to the fact that BP is regu-
lated by multiple systems and the serum ACE levels are not rate-limiting for the conversion of AngI into AngII in the plasma (43). The local, i.e., tissue, level of AngII, however, seems to be partly regulated by the ACE genotype (22,44). The finding in this study that the ACE genotype is one of the determinants of the amount of MA indicates that it is likely to be related to tissue damage, and it therefore is possible that the ACE genotype could affect the course of renal function loss in ADPKD patients.

Determination of progression of renal disease was not the aim of this study. Moreover, the determination of progression in ADPKD patients in relation to the ACE genotype would need a relatively long observation period. Besides, during that period many factors may interfere, like the occurrence of hypertension, hematuria, and infections for which specific treatment would become necessary. A short-term, retrospective, small Japanese study was not able to show a relationship between ACE genotype and the 1/creatinine slopes. MA was not evaluated in their population (45). A somewhat larger study of 184 patients revealed that the cumulative renal survival was 7 yr shorter in patients with the DD genotype compared to patients with the II genotype (46).

The finding that ACE genotype determines MA may have therapeutic implications. It has already been established that MA is an indicator of a worse prognosis, whereas treatment with ACE-inhibitors and angiotensin type I receptor antagonists is capable of reducing MA. Therefore, in ADPKD patients with the DD ACE genotype, early treatment with these compounds might be important in reducing progression toward ESRF.

Acknowledgments

This study was supported by Grant 28-1911 of the Dutch Foundation for Preventive Medicine. We thank Jasper Saris and Lia Spruit for their assistance in determination of the ACE ID genotype and for performing linkage analyses. We thank the collaborators of our statistical department, in particular Wendy Post, for help with the statistical analyses. Finally, we thank Prof. Van Es for careful revision of the manuscript.

References


This article can be accessed in its entirety on the Internet at http://www.lww.com/JASN along with related UpToDate topics.