Original Article

The ACE insertion/deletion polymorphism has no influence on progression of renal function loss in autosomal dominant polycystic kidney disease

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Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) shows a variable clinical course that is not fully explained by the genetic heterogeneity of this disease. We looked for a possible genetic modifier, the ACE I/D polymorphism, and its influence on progression towards end-stage renal failure (ESRF).

Methods. Forty-nine ADPKD patients who reached ESRF <40 years, and 21 PKD1 patients who reached ESRF >60 years or were not on dialysis at 60 years of age were recruited. Clinical data were provided by questionnaires. Blood was collected for the determination of the ACE insertion/deletion (I/D) polymorphism genotype. The ACE genotype was also determined in a general, control PKD1 group (\(n=59\)).

Results. Patients who reached ESRF <40 years had significantly more early onset hypertension than patients reaching ESRF >60 years (80% vs 21%; \(P<0.001\)). The ACE genotype distribution showed no differences between the groups of the rapid progressors (DD 20%, ID 56%, II 24%), the slow progressors (DD 29%, ID 52%, II 19%) and the general PKD1 control population (DD 31%, ID 47%, II 22%).

Conclusion. There is no relationship between progression towards ESRD and the ACE I/D polymorphism in ADPKD patients.

Keywords: ACE insertion/deletion polymorphism; autosomal dominant polycystic kidney disease; progression

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited renal disorder. Its clinical course is highly variable. End-stage renal failure (ESRF) in ADPKD can be reached as early as childhood or not at all [1]. Part of this variation can be attributed to genetic heterogeneity [2]. In the majority of cases (85%) the PKD1 gene, located on chromosome 16, is mutated. However, when the disease is caused by a mutation in the PKD2 gene, located on chromosome 4, the disease generally runs a milder course: the mean age at which ESRF is reached is 54 years in PKD1 patients and 73 years in PKD2 patients [3]. Nevertheless, the clinical variability cannot be explained fully by these two different genes. Considerable interfamilial variability between either PKD1 or PKD2 families is frequently observed. Different mutations in the PKD gene may have different effects on renal function. A clear genotype–phenotype relationship though, has not been found so far. A possible genotype–phenotype relationship certainly cannot explain the diversity in clinical course that can be seen between members of the same family, known to carry the same mutation [4,5]. Environmental and other genetic modifying factors can to a large extend be held responsible for this intrafamilial variability. Factors such as hypertension, haematuria, urinary tract infections in men, or more than four pregnancies are known to have a negative influence on progression [6]. The influence of genetic modifiers on progression has not been clarified yet.

The renin–angiotensin system (RAS) activity has been implicated in the pathogenesis of hypertension [7,8]. In view of the effect of hypertension on progression towards ESRF, the genetic polymorphisms of the RAS have recently received great interest, in particular the ACE insertion/deletion (I/D) polymorphism [9]. Although this polymorphism is located in an intron of the ACE gene (chromosome 17), it is associated with a 50% variability in serum ACE levels. Individuals homozygous for the deletion (DD) have the highest ACE levels, individuals homozygous for the insertion (II) the lowest. Several reports have been published trying to relate this polymorphism with hypertension [7,10]. Such an association was demonstrated in two recent reports in a population of young men [11,12].
The ACE I/D polymorphism was also shown to have prognostic value in various cardiovascular disorders [13,14]. Enhanced progression in renal disease could also be linked to DD genotype, as shown for IgA nephropathy and focal segmental glomerulosclerosis [15,16]. Therefore the ACE I/D genotype may also explain some of the variation in progression seen in ADPKD patients.

We performed a case-control study, comparing the differences in frequencies of the ACE I/D genotype between PKD1 patients who reached ESRF above the age of 60 years of age and ADPKD patients who reached ESRF under 40 years of age. These groups of rapid and slow progressors were also compared with a control, general PKD1 population.

Subjects and methods

Patients

After the protocol was approved by the Medical Ethics Review Committee of the LUMC, nephrologists of 47 (small) dialysis centres in our country were asked for their co-operation. We received a positive answer from 37 centres, and with their consent the nephrologists of the participating centres. Patients with the diagnosis of PKD who became dialysis dependent under 40 years of age and patients who became dialysis dependent above 60 years of age were identified to the nephrologist of the participating centres. The participating centres were applied with questionnaires and material necessary for blood sampling. The forms contained questions about age at ESRF, the existence of hypertension before ESRF (defined as diastolic blood pressure >95 mmHg and systolic blood pressure >165 mmHg, or treatment with antihypertensive medication), haematuria, urinary-tract infections, kidney stones, cerebrovascular accidents, pregnancies, nephrectomies, important other complications before reaching ESRF, and family history. Blood was collected in heparinized tubes and sent to our hospital for DNA isolation and determination of the ACE genotype.

Collected data

Blood samples were received from 49 patients who had reached ESRF <40 years (rapid progressors), and from 21 patients who had reached ESRF >60 years (slow progressors). We were unable to retrieve data from five patients (three rapid progressors and two slow progressors), whereas DNA isolation was impossible in three rapid progressors. In both groups, only two patients were from the same family. Patients were diagnosed as having ADPKD by the presence of cysts in both kidneys and a positive family history for ADPKD. In all patients with a negative family history for ADPKD, ultrasound investigation showed enlarged kidneys with numerous cysts throughout the kidneys and liver.

We presumed that the group of rapid progressors consisted of PKD1 patients. Because PKD2 patients are known to be slow progressors, we needed to ensure that all patients in the group of slow progressors were PKD1 patients. We were able to link all the slow progressors to the PKD1 gene.

To collect a general PKD1 control population, we randomly took samples from our collection of genetically identified PKD1 families and determined the ACE genotype.

Families who had members included in our study population were excluded.

ACE I/D genotyping

DNA was isolated from peripheral blood leukocytes, using standard techniques [17]. The ACE gene I/D polymorphism was detected by performing the polymerase chain reaction (PCR) as described by Rigat et al. [18]. 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 [19], 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 products were visualized after electrophoresis in 2% agarose gels.

Statistical analysis

Chi square tests according to Pearson were performed to compare the frequency of ACE genotype between the groups. A P value <0.05 was considered significant.

Results

Clinical characteristics of rapid and slow progressors

Although more women were present, the gender distribution was not significantly different between the two groups (Table 1). In the rapid progressors hypertension was present in 80% before the age of 40, when all had reached ESRF, whereas in the slow progressors only 21% had hypertension before they reached the same age (Table 1). This difference was significant (P<0.001).

Four patients with rapid progression had major complications that might have had a deleterious effect on the disease progression. Two patients were on non-steroidal anti-inflammatory drugs, one because of gout and the other because of Bechterew’s disease. Another patient had undergone a nephrectomy at the age of 6 for unknown reasons. In one patient pyostasis occurred for which the kidney was removed, followed by hypertensive periods after which dialysis became necessary. Exclusion of these patients from our data set had no influence on the results. Other complications were nephrolithiasis in one patient and an intracranial haemorrhage before the age of 40 years in the >60 group. Percentages are shown in parentheses.

| Table 1. Clinical characteristics of ADPKD patients who reached ESRF <40 years and PKD1 patients who reached ESRF >60 years |
|------------------------------------------|----------------|----------------|
|                                          | ESRF <40 years | ESRF >60 years |
| Number                                   | 46             | 19             |
| Male/female                              | 25/21          | 7/14           |
| Hypertension*                            | 37 (80)        | 4 (21)         |
| **P value**                              | **<0.001**     |                |

*Hypertension before reaching ESRF in the <40 group and hypertension before the age of 40 years in the >60 group. Percentages are shown in parentheses.
orrhage due to a ruptured cerebral aneurysm at the age of 16 in another patient.

**Distribution of the ACE genotype**

In the randomly collected 59 control PKD1 patients, the ACE genotype distribution was 31% for the DD genotype, 47% for the ID genotype, and 22% for the II genotype (Table 2). This was in Hardy–Weinberg equilibrium and did not differ from the distributions in other populations. No significant differences in the ACE-genotype distribution between the groups of rapid and slow progressors could be established. ACE genotype frequencies were DD 20%, ID 56%, II 24% in the rapid progressors and DD 29%, ID 52%, II 19% in the slow progressors. These distributions were comparable with the general PKD1 population (Table 2).

An effect of the ACE genotype on hypertension was not found (data not shown).

**Discussion**

We found no influence of the ACE polymorphism on the progression towards ESRF in ADPKD patients. This is in agreement with a recently published study of a large American ADPKD population (Fick et al., abstract ASN 1998), but contradict the first study published on this subject [20]. Babooal et al. [20] found a difference of 7 years in renal survival between the DD and the II genotype. Patients with the DD genotype had a relative risk of 17 for reaching ESRF under 40 years of age. However, these outcomes were based on a small group of patients who reached renal failure <40 years [20]. Recently, a negative effect of the DD genotype of the ACE polymorphism on progression towards ESRF was also reported by Perez-Oller et al. [21]. In this study, 48 ADPKD patients reached ESRF at a mean age of 48 ± 7 years. Only when the authors compared the patients who reached ESRF <50 years (n = 25) with the total study population of 151 patients, the DD genotype was significantly more frequently present in the group of patients who reached ESRF <50 years. However, the total group of patients who reached ESRF had an abnormal distribution of the ACE genotype (with only 26% heterozygotes), and the cut-off point for rapid progression was 50 years, which is 2 years higher than the mean age at which their patients reached ESRF. To perform a reliable statistical analysis, we took 40 years as cut-off point to define rapid progressors, because ESRF is generally reached in 5th decade [22]. Furthermore, Perez-Oller et al. were unable to demonstrate a difference in renal survival time between patients with the DD and the II genotypes [21]. In our study we found that the distribution of the ACE genotype in ADPKD patients who reached ESRF <40 years did not differ from PKD1 patients reaching ESRF >60 years and equalled the distribution in the control PKD1 group. This was determined in a group of rapid progressors four times, respectively twice larger than in the studies mentioned above. No effect of the ACE genotype on hypertension was found, as previously reported [20,21]. Therefore, evidence is lacking for a role of the ACE polymorphism on progression in autosomal dominant polycystic kidney disease.

In our study the majority of rapid progressors was hypertensive before reaching renal failure. This was significantly different compared to the slow progressors, in whom only 21% were identified as hypertensive before the age of 40 years. Unfortunately, the age at onset of hypertension and the cause of hypertension could not be verified. Therefore, it remains unclear whether early onset hypertension (arbitrarily defined as hypertension <40 years) hastened progression in the rapid progressors, or whether hypertension was due to deteriorating renal function. Therefore, early onset hypertension could be simply a consequence of a more severe disease. Nevertheless, various studies have reported a negative effect of hypertension on progression of ADPKD [6,23–25]. In ADPKD patients hypertension frequently occurs before the decline of renal function and can start as early as childhood [26,27]. Based on this study it may be suggested that the early onset hypertension could have contributed to the enhanced progression towards ESRF in the group of early progressors. However, studies have provided no clear evidence for a beneficial effect of antihypertensive treatment on the decline of renal function in ADPKD patients [28,29].

Although the rate of microalbuminuria was linked to the ACE genotype [30], there is no indication that the ACE genotype influences progression in this disease. Other genetic polymorphisms of the renin–angiotensin system, such as the M235T angiotensinogen polymorphism and the A1166C angiotensin II type I receptor polymorphism, do not seem to interfere with the disease’s severity either [20]. In our study, hypertension was presumably associated with progression. Hypertension in ADPKD is probably RAS related, but does not seem to be influenced by the ACE polymorphism. Therefore other determinants than the genetic modifying effect of the ACE polymorphism are needed to explain variability in clinical course. Recently, the ‘two hit’ model has been proposed as the basis of cyst growth [31]. The occurrence of a somatic mutation in the previously normal PKD allele could be a rate-limiting step in cystogenesis. This could well

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<tr>
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<th>ESRF &lt;40 years</th>
<th>ESRF &gt;60 years</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Number</td>
<td>46</td>
<td>21</td>
<td>59</td>
</tr>
<tr>
<td>DD</td>
<td>9 (20)</td>
<td>6 (29)</td>
<td>18 (31)</td>
</tr>
<tr>
<td>ID</td>
<td>26 (56)</td>
<td>11 (52)</td>
<td>28 (47)</td>
</tr>
<tr>
<td>II</td>
<td>11 (24)</td>
<td>4 (19)</td>
<td>13 (22)</td>
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Percentages are shown in parentheses.
serve as an explanation for intrafamilial variability. Consequently, to find the source of clinical variation in ADPKD, factors influencing the mutation rate of the PKD gene need to be identified.

In conclusion, we were unable to establish a relationship between the ACE I/D genotype and progression in a PKD1 population. The ACE genotype also had no effect on hypertension.

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References


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