Angiotensin-converting enzyme gene polymorphism in autosomal dominant polycystic kidney disease

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Abstract

Background and aim: Autosomal dominant polycystic kidney disease shows considerable variability in clinical features, including differences in severity of hypertension, rate of decline of renal function and variability in rate of cystogenesis, which are not fully explained by the genetic heterogeneity of this disease. Many different modifier variables have been proposed to explain this variability. This study aims to look at the role played by polymorphism of the ACE gene as a possible modifier in the clinical course and rapidity of progression. Material and Methods: Thirty seven patients diagnosed as ADPKD were recruited to the study. 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 population (n = 40). The data was analyzed using the SPSS software. ACE genotype polymorphism frequencies were compared across groups using the one-way ANOVA tests. λ² cross tabulation statistics was used to test for difference between frequency data. Results: The ACE genotype distribution showed no differences between the study (II 29.7%, ID 43.2%, DD 27.1%) and the control (II 35%, ID 45%, DD 20%) populations. Although patients on hemodialysis had a significantly higher Blood Pressure levels (p = 0.004) when compared to non-dialysis patients, no significant differences were demonstrated between genotypes of the study population. No difference was also demonstrated between the genotypes for rate of decline in renal function. Conclusion: No relationship between the ACE I/D polymorphism in ADPKD patients and severity of hypertension or progression towards ESRD was demonstrated.

Key words: genotype of ACE gene, end stage renal disease (ESRD), chronic kidney disease (CKD), hemodialysis

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most commonly inherited conditions in humans affecting 1 in 1000 persons. The disease is caused by a mutation in either the PKD 1 or the PKD 2 gene, with PKD 1 mutations accounting for over 80% of patients. The cardinal clinical manifestation is the presence of renal cysts, the progressive enlargement of which may lead to chronic renal failure and the eventual need for renal replacement therapy(RRT) accounting for 5-10% of patients on RRT. Around 50% of the ADPKD patients require RRT by the age of 60 years, however there is considerable variation in the age and severity of presentation. Moreover, hypertension is the most frequent trait of patients with ADPKD, probably because of increased renin secretion caused by ischemia secondary to cyst compression. The extreme variability in renal function, presentation of liver cysts and hypertension and rate of progression to ESRD have made it difficult to predict prognosis in individual patients. The factors contributing to the variability remain largely unknown. To this purpose we evaluated the patterns of ACE gene polymorphism and the influence of ACE gene polymorphism on rate of progression of CKD in ADPKD patients of South Indian origin.
Methods

The study protocol was approved by the Institutional Ethics Committee of Narayana Medical College and Hospital, Nellore, Andhra Pradesh. Patients were diagnosed as ADPKD based on the ultra sonographic findings as outlined in the Unified criteria by Pei et al for individuals with unknown PKD genotype. Patients who were satisfying these criteria were included in the study. A pre-prepared questionnaire including questions regarding age at diagnosis, family history, basic complaints and lab parameters was used to collect and accumulate data. In those patients for whom data was available from the point of diagnosis, these were also included in the study. Hypertension was defined as BP > 140/90 mmHg recorded on more than 2 occasions or the use of anti-hypertensive medication for control of blood pressure. End Stage Renal Disease was defined as the requirement of Renal Replacement Therapy in the form of Hemodialysis during the study period. Two ml of blood was collected in EDTA tubes and ACE genotype analysis carried out in molecular biology lab of Advanced Research Centre in Narayana Medical College and DNA extraction and ACE genotype determination was done as mentioned below.

Angiotensin Converting Enzyme Insertion/Deletion Polymorphism (Genotypes) Analysis

Genomic DNA was extracted from peripheral blood using spin column genomic DNA extraction kit (Axxygen Biosciences USA) and ACE intron 16 gene was amplified by Polymerase chain reaction (MG series Thermocycler USA). For amplification, a flanking primer pair 5' - CTGGAGACCCTCCCATCTTCTTCT-3' and 5' - GATGTGCGCA TCACATTC GTCAAGGTAC-3' (synthesized by Bioserve Biotechnology) was used. PCR amplification was performed with a 50μl reaction mixture containing 40pmol of each primer, 200μmol/L of each dNTP, 1.5mmol/L MgCl₂, 1U of thermo stable DNA polymerase (DYNAZYME II Espoo, Finland) and 20mMol of Tris-HCL (pH 8.8 at 25°C). PCR cycling conditions were carried out with an initial denaturation step of 5 minutes at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 1 minute and extension at 72°C for 2 minutes, followed by final extension for 5 minutes at 72°C before the storage of sample at 4°C. PCR products were separated by Agarose gel electrophoresis (GENI Bangalore, India) (Fig.1). DNA fragments were stained with ethidium bromide and visualized under UV light (Gel documentation system, Biorad USA). The PCR fragments consist of three genotypes, a 490bp band (II), a 190bp band (DD), and both 490 and 190 bp band (ID).

To increase the DD genotyping, PCR amplifications were also performed with insertion specific primer pair PCR cycling conditions were carried out with an initial denaturation step of 1 minutes at 95°C, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 67°C for 45 seconds and extension at 72°C for 2 minutes. The PCR product shows a 335 bp band for I allele and no band for DD genotype. 5'-TGCGACTCTCCGCTGCGGTAC-3' and 5'-TGCGACTCTCCGCTGCGGTAC-3' for each sample which had the DD genotype to avoid mistyping of ID heterozygotes as D homozygotes.

Statistical Analysis : The data was analyzed using the SPSS software Version-19. ACE genotype polymorphism frequencies were compared across groups using the one-way ANOVA tests. χ2 cross tabulation statistics was used to test for difference between frequency data. A p value of <0.05 was considered significant.

Results

The ACE genotype distribution in the randomly selected control population was II 35% (N=14), ID 45% (N=18) and DD 20% (N=8). This was within the Hardy-Weinberg equilibrium. In the study population characteristics of the ACE genotype distribution was II 29.7% (N=11), ID 43.2% (N=16) and DD 27.1% (N=10). This observed distribution also conformed to
Table-1: Variables analyzed for each Genotype of ACE I/D Polymorphism

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( yrs)</td>
<td>12.40</td>
<td>13.86</td>
<td>7.47</td>
<td>.245</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>27.09</td>
<td>23.31</td>
<td>20.20</td>
<td>.525</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.50</td>
<td>2.15</td>
<td>2.81</td>
<td>.463</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>37.90</td>
<td>50.12</td>
<td>30.20</td>
<td>.190</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>103.85</td>
<td>101.75</td>
<td>108.49</td>
<td>.144</td>
</tr>
</tbody>
</table>

Table - 2 : Subgroup Analysis of ESRD patients

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( yrs)</td>
<td>52.00±7.8</td>
<td>35.75±7.5</td>
<td>52.8±7.8</td>
<td>0.705</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>41.75±17.9</td>
<td>33.0±18.6</td>
<td>22.20±8.4</td>
<td>0.157</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>3.42±0.7</td>
<td>4.40±0.9</td>
<td>3.52±1.5</td>
<td>0.057</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>20.25±7.9</td>
<td>14.75±2.2</td>
<td>23.40±10.1</td>
<td>0.079</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>109.87±9.8</td>
<td>111.85±9.4</td>
<td>107.14±4.7</td>
<td>0.236</td>
</tr>
</tbody>
</table>

the Hardy-Weinberg equilibrium. Out of 37 patients, 10 were females. The mean age was 44.9±12.19 yrs. Twenty two of the 37 (59.5%) study patients were hypertensives with a mean arterial pressure (MAP) of 104.19±8.5mmHg. The mean duration of follow up was 23.59±13.6 months. Thirteen (35.1%) developed ESRD and were on hemodialysis.

Baseline serum creatinine, GFR, age and gender were found to be similar across the three polymorphisms. The percentage of patients with hypertension for each polymorphism was 31.8% for II, 31.8% for ID, and 36.4% for DD were also similar (P = 0.17). (Table- 2). There was no significant differences in the percentage of patients with ESRD for each polymorphism 30.8% for II, 30.8% for ID, and 38.5% for DD. (p = 0.428)

**Discussion**

One of the most striking features of ADPKD is the wide variability in presentation of the disease and variability in decline of renal function⁹. It is in this respect that studies involving genetic modifiers of clinical course assume importance, in that they provide new insights into the pathogenesis of ADPKD and also possible pathways for medical interventions. The description by Rigat et al⁸ of a polymorphism in the ACE gene and the association of the D allele with greater plasma levels of ACE and therefore an increased production of Angiotensin II has been followed up by several studies suggesting a relationship between this polymorphism and essential hypertension¹⁰⁻¹¹. Type 2 Diabetes Mellitus¹², IgA Nephropathy¹³ and a number of other diseases in which the Renin Angiotensin Aldosterone System (RAAS) may have a pathogenic role¹⁴⁻²¹. However, some reports have not found an association²²; thus, this relationship still remains to be elucidated. A role for RAAS in cystogenesis in ADPKD was elucidated by Norman et al²³ when they demonstrated that Angiotensin
II was a growth factor in renal cell systems. This hypothesis was further enhanced by Torres et al\textsuperscript{24} who demonstrated that experimentally induced renal cystogenesis was enhanced by conditions that activated RAAS and diminished by suppression of RAAS. Hypertension is the most important potentially treatable variable associated with a faster progression to ESRD in patients with ADPKD\textsuperscript{25}. In addition, it has an important role in the survival of these patients by producing cardiovascular damage. Several studies have shown a relationship between increased RAAS activity and hypertension in patients with ADPKD. All these features have led to an increased interest in the relationship between the ACE gene polymorphism and ADPKD.

The present study aimed to investigate the role of the ACE gene polymorphism on the progression of CKD in ADPKD and also its role in the prevalence of hypertension in the 37 patients that were recruited. The study could not demonstrate a role for the DD polymorphism on the rate of decline in renal function. Also while the study demonstrated a significantly higher Mean Arterial Pressure among those patients who already reached ESRD, a higher prevalence of the DD genotype among these patients was not evident.

The first study published on this subject by Baboolal et al\textsuperscript{26}, found a difference of 7 years in renal survival between the II and the DD genotype. This was followed by another positive study by Perez-Oller et al\textsuperscript{37}, which also demonstrated a negative effect of the DD genotype of the ACE gene on progression towards ESRD. This study showed that the DD genotype was significantly more frequent in the 48 ADPKD patients who reached ESRD before 50yrs when compared to the total study population of 151 patients. This finding was in direct contradiction to that reported by Dijk et al\textsuperscript{24} who found no such correlation between the DD and II genotypes in patients who reached ESRD early (defined in the study as onset <40yrs) and those who progressed at a more conventional rate. The issue has since then been investigated multiple times with only 2 other groups demonstrating a positive correlation between DD genotype and a worse outcome\textsuperscript{29,30}. But multiple studies with larger study populations have concluded that ACE gene polymorphism has no influence on renal outcomes in ADPKD\textsuperscript{31-37}. Four of these studies included patients of a predominantly Oriental population and the remaining study groups were largely of European descent. Many of these were included in a meta-analysis which also reached the conclusion that ACE gene has no influence on renal or patient outcomes in ADPKD\textsuperscript{38}. To date no studies have been conducted on a population of Indian origin.

In this study, all the patients who progressed to ESRD, were hypertensive and the difference in MAP between patients on dialysis and those still in various stages of CKD was statistically significant. Unfortunately the age at onset of hypertension and the exact cause for hypertension could not always be determined. Therefore, it remains unclear whether early onset hypertension hastened the progression of CKD or hypertension was really due to deteriorating renal function. Therefore early onset of hypertension could simply be an indicator of more severe renal disease. In ADPKD patients hypertension occurs before the decline of renal function and can frequently start as early as childhood\textsuperscript{39}. However studies have provided no clear evidence for a beneficial effect of anti-hypertensive treatment on the decline of renal function in ADPKD\textsuperscript{40}. In the current study hypertension was presumably associated with progression. Thus hypertension in ADPKD is probably RAAS mediated but does not seem to be related to the ACE gene polymorphism.

Therefore other mechanisms than the genetic modifying effect of ACE gene polymorphism are needed to explain the variability in clinical course. The role of other modifier genes has been explored by Tazon-Vega et al\textsuperscript{41} but except for PKD 2 which had a mild modifying effect on the expression of PKD1 gene, all other
proposed candidate genes were found to be insignificant. Recently the two-hit model has been proposed as the basis of cyst growth. The occurrence of a somatic mutation in the previously normal PKD allele could be the rate limiting step in cystogenesis. This could serve as an explanation for intrafamilial variability. Therefore to find the source of variability in ADPKD, factors influencing the mutation rate of the PKD gene need to be identified.

**Limitations**: One of the limitations of the study is the small sample size which limits the strength of the statistical analysis. Another limitation of this study is the fact that the ADPKD type was not defined in the study population. While PKD1 type accounts for 85% of the mutations worldwide, no data exist specifically for the Indian population and PKD2 mutations have a modifying role on PKD1 expression. In the present work we did not attempt to adjust age, gender, diet and other potential confounding variables which are well described to influence ADPKD progression.

**Conclusion**

The ID polymorphism of the ACE gene was the most frequently documented genotype. Based on the evidence accumulated in this study, ACE gene polymorphisms do not seem to have an influence on the prevalence or severity of hypertension in ADPKD patients or on the rate of progression of CKD in ADPKD patients.

**References**


among North Indian end stage renal disease patients. BMC Nephrology 2006; 7:15.


19. Prasad N, O'Kane KP, Johnstone HA, Wheeldon NM, McMahon AD, Webb DJ, MacDonald TM. The relationship between blood pressure and left ventricular mass in essential hypertension is observed only in the presence of the angiotensin converting enzyme deletion allele. Q J Med 1994;87:659-62.


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