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Susceptibility and progression of end stage renal disease are not associated with angiotensin II type 1 receptor gene polymorphism

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Abstract

Context: The role of the angiotensin II type 1 receptor (AT1R) gene polymorphism, A1166C, has been shown to be associated with end stage renal disease (ESRD) and its progression. There is also some evidence that HLA class II alleles are associated with ESRD independent of other factors. Objective: To examine the association between AT1R gene polymorphism in the susceptibility and progression to ESRD in patients with chronic renal failure and to investigate if the AT1R genotypes and HLA-DR alleles predict the time to ESRD. Materials and methods: Genotyping was performed in 50 ESRD patients and 44 control subjects for the AT1R A1166C gene polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). ESRD patients were examined for HLA-DRB1 alleles according to a reverse hybridization line probe assay. Results: Allele and genotype frequencies of the AT1R polymorphism did not differ significantly between ESRD patients and controls. Furthermore, there was no association between the AT1R gene polymorphism or HLA-DRB1 alleles with the risk of developing ESRD. However, the AT1R genotype did not contribute to the genetic susceptibility to ESRD and is not associated with progression of chronic kidney failure to ESRD.

Introduction

End stage renal disease (ESRD) is a complex phenotype, which results from the presence of underlying kidney disease of different etiologies (1). It has been suggested that genetic susceptibility, independent of the etiologic factors of kidney disease, alters the risk of the development of end-stage renal failure (2). However, the genes influencing the development and rate of progression to ESRD have yet to be identified.

The role of the renin–angiotensin system (RAS) in the pathophysiologic changes that lead to progression of renal disease has been stressed (3). RAS plays a central role in the regulation of blood pressure, electrolyte and volume homeostasis, with its actions exerted primarily by angiotensin II (Ang II) (4). Ang II effects are predominately mediated by the angiotensin II type 1 receptor (AT1R), a G-protein coupled receptor, which is particularly present in the vascular smooth muscle and the kidney (5).

Ang II concentrations within the kidney are 1000-fold higher than the circulating blood, indicating the presence of an intrarenal RAS (6). Ang II plays a critical role in the regulation of the glomerular filtration rate and the development of glomerulosclerosis by increasing glomerular capillary pressure caused by constriction of efferent arterioles (7). Importantly, pharmacological targeting of the RAS with Ang II receptor blockers or angiotensin converting enzyme (ACE) inhibitors preserves kidney function better than non-RAS blood pressure lowering regimens (8–10). Furthermore, agonistic autoantibody-triggered stimulation of AT1R induces severe vascular pathology in allogeneic renal transplantation and higher risk of graft failure independently of classical immunological risk factors (11–13).

Besides the hemodynamic effects of Ang II in the kidney, Ang II has been shown to have direct actions on mesangial cells which exclusively express AT1R, including cell proliferation, apoptosis, inflammation and angiogenesis, and these actions may play a crucial role in Ang II-mediated glomerular injury (4,14,15). In addition, Ang II was found to be injurious to the kidney through up-regulation of adhesion molecules and cytokines in particular transforming growth factor beta (8,16,17). Ang II acts as a potent proinflammatory modulator that augments and perpetuates the immune response (18).

Several studies have shown a relationship between genetic variants of the RAS genes and renal disease as well as the rate of progression of renal damage (19,20). In this context, the AT1R gene, located on chromosome 3q21.25 spanning over 55 kb, presents several polymorphic sites, with important participation in the susceptibility to disease (21,22). One biallelic AT1R polymorphism, A1166C, due to a substitution of cytosine for adenine at the position 1166 in the
DNA was amplified by PCR using 5'-GCCAGCACCCTACTA CCAATGGGCG-3' (sense) and 5'-CAGGACAAAGCA GGCTAGGGAGA-3' (antisense) primers spanning AT1R 3'-untranslated region containing A1166C site (20). PCR reactions were carried out in a 25-μl reaction mixture containing 12.5 μl of 2× PCR master mix (Thermo Fisher Scientific) composed of 0.05 units/μl Taq DNA polymerase in reaction buffer, 4 mM MgCl₂, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP, 0.1 μM of forward and reverse primers, and 1 ng of template DNA.

The PCR amplification cycles were performed as follows: initial denaturation at 95°C for 6 min followed by 30 cycles of denaturation at 94°C, annealing at 55°C and elongation at 72°C, 1 min each (Perkin Elmer 9600).

RFLP

PCR products were then digested by BsuRI restriction enzyme (Fermentas, Germany), according to the manufacturer's instructions. BsuRI (including 2 μl of 10× Buffer, 1 μl of BsuRI enzyme, 10 μl of PCR product, 18 μl of nuclease free water) for digestion of the amplification products of AT1R was incubated for 16 h at 37°C. In the presence of cytosine (C allele), there is a restriction site for the enzyme, resulting in a 231-bp fragment and a 24-bp fragment. Undigested 255-bp fragment indicates the presence of the A allele. DNA fragments were separated by electrophoresis in 3% agarose gel stained with ethidium bromide.

HLA

All patients in the study were awaiting renal transplantation in Kasr Al Ainy hospital, Cairo University. HLA DRB1 alleles were performed according to a reverse hybridization line probe assay (InnoLiPa HLA typing kits, Innogenetics, Belgium). DNA amplification by polymerase chain reaction followed by hybridization with a panel of biotinylated sequence specific oligonucleotide probes as supplied in the HLA DRB1*Kit INNO-LiPA (Innogenetics). The alleles were allocated based on the hybridization pattern for various probes using the interpretation software provided with the kit.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (±SD), number of cases and percentages. Comparison of quantitative variables between the study groups was done using Student t test for independent samples. For comparing categorical data, χ² test was performed. Exact test was used instead when the expected frequency is <5. Cox proportional hazards regression analysis was used to examine the genetic variables associated with the progression to ESRD. HLA DR alleles with frequency <5 were not included. A probability value (p Value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL) version 15 for Microsoft Windows.

Results

AT1R A1166C genotypes and susceptibility to ESRD

The characteristics of ESRD patients are presented in Table 1. Genotype distributions and allele frequencies of AT1R A1166C in ESRD patients and controls are shown in Table 2. The frequencies of AA, AC and CC genotypes showed no significant difference in the susceptibility to ESRD (p = 0.823). C homozygotes were rare in the population studied, so further genotypic comparison was performed between AA and non-AA (AC + CC) genotypes. Patients, homozygous
Angiotensin II type 1 receptor gene polymorphism

Table 1. Clinical and laboratory data of ESRD patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ESRD patients, N = 50</th>
<th>Controls, N = 44</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.9 ± 14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>32/18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>4 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney</td>
<td>5 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>17 (34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (years)</td>
<td>6.2 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV positive patients</td>
<td>12 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>7.2 ± 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predialysis urea (mg/dl)</td>
<td>132.6 ± 51.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD or number (%) as applicable.

Table 2. Distribution of AT1R A/C alleles and genotypes in ESRD patients and controls.

<table>
<thead>
<tr>
<th>AT1R A1166C polymorphism</th>
<th>ESRD patients, N = 50</th>
<th>Controls, N = 44</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, n (%)</td>
<td>37 (74)</td>
<td>30 (68.2)</td>
<td>0.823</td>
</tr>
<tr>
<td>AC, n (%)</td>
<td>12 (24)</td>
<td>13 (29.5)</td>
<td></td>
</tr>
<tr>
<td>CC, n (%)</td>
<td>1 (2)</td>
<td>1 (2.3)</td>
<td></td>
</tr>
<tr>
<td>A, n (%)</td>
<td>86 (86)</td>
<td>73 (83)</td>
<td>0.708</td>
</tr>
<tr>
<td>C, n (%)</td>
<td>14 (14)</td>
<td>15 (17)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Distribution of AT1R genotypes in ESRD patients with and without diabetic nephropathy.

<table>
<thead>
<tr>
<th>AT1R A1166C polymorphism</th>
<th>Diabetic ESRD, N = 13</th>
<th>Non-diabetic ESRD, N = 37</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA, n (%)</td>
<td>9 (69.2)</td>
<td>28 (75.7)</td>
<td>0.719</td>
</tr>
<tr>
<td>AC + CC, n (%)</td>
<td>4 (30.8)</td>
<td>9 (24.3)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Factors studied for association with time to ESRD by Cox proportional hazards regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1R gene polymorphism*</td>
<td>1.254</td>
<td>0.658–2.389</td>
<td>0.491</td>
</tr>
<tr>
<td>HLA-DRB1 alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DRB B1*01</td>
<td>1.994</td>
<td>0.765–5.198</td>
<td>0.158</td>
</tr>
<tr>
<td>HLA-DRB B1*02</td>
<td>1.289</td>
<td>0.619–2.685</td>
<td>0.497</td>
</tr>
<tr>
<td>HLA-DRB B1*03</td>
<td>1.103</td>
<td>0.601–2.023</td>
<td>0.752</td>
</tr>
<tr>
<td>HLA-DRB B1*04</td>
<td>1.102</td>
<td>0.599–2.026</td>
<td>0.755</td>
</tr>
<tr>
<td>HLA-DRB B1*07</td>
<td>0.728</td>
<td>0.341–1.556</td>
<td>0.412</td>
</tr>
<tr>
<td>HLA-DRB B1*11</td>
<td>1.002</td>
<td>0.504–1.992</td>
<td>0.997</td>
</tr>
<tr>
<td>HLA-DRB B1*13</td>
<td>0.713</td>
<td>0.393–1.294</td>
<td>0.265</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>0.997</td>
<td>0.974–1.021</td>
<td>0.835</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>1.053</td>
<td>0.590–1.879</td>
<td>0.862</td>
</tr>
</tbody>
</table>

*AT1R AA genotype is the reference group.

AT1R A1166C genotypes and risk for progression of renal disease to ESRD

Genetic polymorphism of the AT1R gene was analyzed for its association with the rate of progression to end stage disease and was found not to be influenced by the polymorphism in our patient group. The time to end stage renal disease was 6.3 ± 2.4 years for AA genotype and 5.9 ± 2.1 years for AC/CC genotypes (p = 0.672).

Table 4 shows the variables studied for association with time to the occurrence of ESRD by Cox regression analysis. These variables were the AT1R CC/AC genotypes, HLA-DRB1 alleles, age at diagnosis and gender. Although AT1R CC and AC genotypes were associated with time to ESRD (hazard ratio = 1.254, 95% CI: 0.658–2.389), statistical significance was not reached. Similarly, HLA-DRB1 alleles were not associated with time to the occurrence of ESRD. HLA-DRB1*01, 02, 03 and 04 alleles seemed more likely to experience a shorter time to ESRD that was not statistically significant.

Discussion

The prevalence of chronic kidney disease (CKD) is on the rise in all ethnic groups (10). While the prevalence of CKD is high, the incidence of ESRD differs substantially (30).

In this study, the distribution of AT1R A1166C genotypes and C allele frequency was similar in Egyptian ESRD patients and controls, in accordance with the findings in the Japanese population (25) and Chinese population (26), and supported by a recent meta analysis in overall populations, Caucasians and Asians (27). This is in contrast with reports of increased frequency of the C allele and the homozygous CC genotype of the AT1R polymorphism in adult and pediatric dialysis patients (1,24,31).

The differences in the incidence of ESRD in ethnic groups have been explained to a large extent by different prevalences of diabetes and by differences in the rate of progression from early chronic kidney disease stages to ESRD (30). Some renal diseases as diabetic nephropathy cluster within families, and this is consistent with a genetic component of the development or progression of these diseases. Diabetes is a leading cause of progression from CKD to ESRD and is becoming more frequent in the general population making a large contribution to the rising incidence of ESRD (10,32).
observed that the genotypes of AT1R A1166C polymorphism were comparable between patients with and without diabetic nephropathy. The A1166C allele was previously found to be associated with an increased risk of microalbuminuria (33) and a faster progression of nephropathy (34) in patients with type II diabetes, findings that were in contrast with reports from other studies showing no association between the polymorphism and diabetic renal disease (35). Studies focusing on specific non-diabetic renal diseases, such as focal segmental glomerulosclerosis, IgA nephropathy and adult autosomal-dominant polycystic kidney disease, failed to detect an association between the polymorphism and progression to ESRD (36–38).

The rate of progression of renal disease to end stage failure in our patient group was not influenced by the AT1R genotypes. The time to the onset of ESRD was similar for patients carrying the C allele and for the homozygous AA genotype. In contrast, one study showed that patients carrying the C allele showed more rapid deterioration of renal function than those with the AA genotype, and the time from diagnosis to the onset of ESRD was significantly shorter for patients carrying the C allele (20). It was thus suggested that the CC/AC genotype might serve as a predictor of early ESRD.

Importantly, the risk of renal disease progression is multifactorial, including many genetic and environmental factors and their interactions. Variation at the HLA-DRB1 genetic locus has been studied in relation to ESRD. In some studies, certain HLA-DRB1 alleles appear to be associated with the risk of development of ESRD including, HLA-DRB1*03 (39), HLA-DRB1*04 (40) and HLA-DRB1*11 (28), while in others no correlation was observed (41). However, studies focusing on HLA alleles and the rate of progression are limited. In the present study, HLA-DRB1 alleles were not associated with time to occurrence of ESRD. Due to the low frequencies of the alleles, further examination of the potential effect requires analysis of larger datasets. Furthermore, the rate of progression seems to be affected by factors as race, socioeconomic status and predialytic care which includes blood pressure control, control of proteinuria and glycemic control (30,42).

It was proposed that the mechanism by which the AT1R A/C polymorphism affects the development of renal disease and its progression to ESRD is related to genetic variability in the sensitivity of target tissues to Ang II whose actions are mediated by the AT1R receptor (1,43). However, the polymorphism is a non-functional mutation located in the 3'-untranslated region of the gene, thus it was postulated that it may be in linkage disequilibrium with a functional mutation that alters Ang II responsiveness (44) or in the AT1R or closely linked gene possibly located in regulatory regions, involved in the development and progression of renal damage (1). Furthermore, it was suggested that patients carrying the genetic set ACE DD, angiotensinogen TT and AT1R AC/CC polymorphism in combination may be at increased risk for vascular and renal damage (45).

One study revealed that renal transplant recipients with the C allele of the AT1R are inclined to have the angiotensin II type I receptor agonistic auto-antibodies. Renal transplant patients with these antibodies had a less favorable prognosis, greater graft loss and poor long-term function (46).

Significantly, the pre-transplant prevalence of AT1R-antibodies positive individuals was 47% in one study and 17% in another (12,47). Important questions are raised why AT1R antibodies develop in patients with ESRD and what the role of AT1R genetic predisposition is.

It has been challenging to uncover variants elucidating the genetically determined variability of kidney function. This is largely because of the multifactorial nature of CKD, the different mechanisms involved in progressive CKD stages, and the challenges in explaining the role of low-frequency variants (30). It is noteworthy to mention that our study has certain limitations such as the small sample size and lack of functional analysis of the polymorphism. The combinational effect of individual genes in susceptibility and progression of renal failure needs to be more emphasized.

**Conclusions**

In conclusion, the A1166C polymorphism in the angiotensin II type 1 receptor gene does not contribute to the genetic susceptibility or the rate of progression of ESRD in Egyptian patients.

**Declaration of interest**

The authors report no declarations of interest.

**References**


