The DD genotype of the angiotensin-converting enzyme gene occurs in very low frequency in Australian Aboriginals

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Abstract

Background. The DD genotype of the angiotensin-converting enzyme (ACE) gene appears to be an independent risk factor for myocardial infarction, left ventricular hypertrophy and an increased incidence and rate of progression of renal disease. The high incidence of renal disease and end-stage renal failure in the Australian Aboriginal population has prompted investigation of ACE genotypes in these people.

Methods. ACE genotypes were determined in four groups: (i) normal Australian Caucasian blood donors (n = 100), (ii) Caucasian renal transplant recipients (n = 173), (iii) normal Australian Aboriginals from a single tribe (n = 184), and (iv) Australian Aboriginals included in the renal-transplant programme (n = 94).

Findings. The D allele frequency in the normal Australian Caucasian (54.5%) and renal transplant groups (57.2%) was similar. However, the D allele frequency in the normal Australian Aboriginal (3%) and Aboriginal renal patient group (14.4%) was significantly lower than both Caucasian groups.

Interpretation. The D allele of the ACE gene has little or no influence on the renal disease of Australian Aboriginals.

Key words: ACE genotype; Australian Aboriginals; renal failure

Introduction

The high incidence of renal disease and end-stage renal failure in Australian Aboriginals is of great social, economic, and medical concern.

The Australian continent has been colonized for approximately 50 000 years [1]. The original small colonizing population(s), of ultimate Asian origin [2], were geographically isolated and evolved into the independent Australoid race which has only been influenced by the Caucasian gene pool in the last 200 years.

In Caucasians, the insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin converting enzyme (ACE) gene has attracted considerable attention as the DD genotype appears to be an independent risk factor for renal disease [3–8] as well as myocardial infarction and left ventricular hypertrophy [9]. It would seem that while susceptibility to acquire renal parenchymal disease may not be affected by the ACE genotype, the presence of the DD genotype may potentiate renal, diabetic, and vascular components. Evidence with respect to the progression of renal disease is, however, more convincing. An increased rate of progression to renal failure is reported in patients with insulin [10] and non-insulin-dependent diabetes mellitus [11], IgA nephropathy [5] and polycystic kidney disease [8] who have the DD genotype. DD homozygotes have elevated serum and tissue ACE concentrations [12,13] increased vascular conversion of angiotensin [14] and increased pressor response to angiotensin II [15]. For these reasons, angiotensin-converting enzyme inhibitor (ACEI) drugs have become standard therapy to slow progression of renal failure and are deemed most efficacious in those patients with the DD genotype [16]. Because of the high incidence of renal failure in Australian Aboriginals, ACEI drugs may be of prophylactic value in the early stages of their renal disease. This study was therefore performed to determine the incidence of DD genotype in an Australian Aboriginal population whose renal disease is frequently manifest by metabolic abnormalities in which diabetes, hyperlipidaemia and vascular complications predominate and in which relatively rapid decline in renal function frequently occurs.

Subjects and methods

Blood was sampled with appropriate consent from four groups.

(i) Australian Caucasian normal samples (n = 100) were collected from random blood donors.
(ii) Renal patient samples \( n = 173 \) were received by the Tissue Typing Laboratory from end-stage renal failure patients in the renal transplant programme at the time of commencing dialysis. All of these patients proceeded to transplantation. These samples came from predominantly Caucasian patients (one Vietnamese patient was included).

(iii) Yuendumu Australian Aboriginal normal samples \( n = 184 \) were collected from random volunteers. These people belong to the Wailbri tribe of Australian Aboriginals which still occupies traditional tribal lands in the region of Yuendumu in the Central Desert of the Australian Northern Territory. This group has minimal evidence of Caucasian admixture as judged by HLA class I and class II haplotypes \((4/368 = 1.1\% \text{ were of Caucasian origin})\).

(iv) Australian Aboriginal renal patient samples \( n = 94 \) were received by the Tissue Typing Laboratory from end-stage renal failure patients in the renal transplant programme at the beginning of their dialysis treatment. Only samples from patients of known Australian Aboriginal extraction were selected. Not all of these patients received kidney transplants, largely attributable to the difficulty in matching kidneys from an almost exclusively Caucasian cadaver kidney pool. The degree of Caucasian admixture as judged by HLA class II haplotypes was greater than in the Yuendumu group \((12/188 \text{ haplotypes} = 6.4\% \text{ were of Caucasian origin})\). This group was expected to be more diverse in terms of Caucasian admixture having been drawn from a broad geographical area (South Australia and Northern Territory) and urban and non-urban regions.

**DNA extraction**

Genomic DNA was isolated from buffy coats or Epstein–Barr virus-transformed B lymphocyte cell lines by modification of the salt precipitation method [17]. The DNA was dissolved in TE buffer \((10 \text{ mM Tris HCl, 0.1 mM EDTA, pH 8.0})\) at an approximate concentration of \(75–150 \text{ ng/μl}\).

**Oligonucleotide primers**

Oligonucleotide primers were synthesized by DNA Express (Colorado State University, USA).

**ACE genotyping by PCR amplification**

The intron 16 deletion/insertion polymorphism in the ACE gene was typed by PCR using primers ACE-1 \(5' - \text{CTGGAGACCACCTCCATCCCTTTCT} <^3'>\) and ACE-2 \(5' - \text{GATGGCCATCACATCGTCAGAT} <^3'>\) which span the insertion/deletion site [18].

PCR reactions were prepared in a 20-μl volume consisting of PCR buffer \((10 \text{ mM Tris.HCl pH 8.8, 1.5 mM MgCl}_2, 50 \text{ mM KCl, 0.1% Triton X-100})\), 200 μM each dNTP, 0.3 μM each primer, 0.4U Dynazyme (Finnzymes OY, Finland), and approximately 0.2 μg of DNA. PCR was performed in a Gene-Amp PCR System 9600 (Perkin Elmer, USA). The reactions were pre-denatured at 96°C for 2 min, followed by 35 cycles consisting of denaturation at 95°C for 20 s, annealing at 57°C for 40 s, and extension at 72°C for 20 s with a final 3-min extension at 72°C. PCR products were subjected to 2% agarose gel electrophoresis, stained with ethidium bromide, and photographed under ultraviolet light. Genotype interpretation was based on the length of the PCR products, approximately 200 and 450 base pairs for the deletion (D) and insertion (I) alleles respectively.

**Statistics**

Calculation of allele frequencies, exact tests for Hardy–Weinberg equilibrium and population differentiation were performed with Arlequin software for population genetic data analysis [19].

**Results**

The ACE I/D polymorphism genotype and allele distributions observed in the Australian Caucasian, renal patients, Yuendumu Australian Aboriginal, and Australian Aboriginal renal patients are listed in Table 1.

The genotype distribution in all four sample groups did not differ significantly from expected Hardy–Weinberg proportions. Therefore there was no observed preponderance of the DD genotype in any sample group. This indicates no association of this genotype with either renal patient group and no systematic mistyping of ID genotypes as DD genotypes which has previously been suggested as a shortcoming of this typing method [20]. The conformance to Hardy–Weinberg proportions means that the sample groups can adequately be described and compared in terms of allele frequencies.

A D allele frequency of 54.5% was observed in the Australian Caucasian normal sample group. This allele frequency was slightly elevated in the renal patient

### Table 1. ACE insertion (I) and deletion (D) polymorphism genotype distribution and allele frequencies in four sample groups

<table>
<thead>
<tr>
<th>Sample group</th>
<th>n</th>
<th>DD genotypes (observed)</th>
<th>ID</th>
<th>II</th>
<th>ACE allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australian Caucasian</td>
<td>100</td>
<td>28.0%</td>
<td>53.0%</td>
<td>19.0%</td>
<td>0.545</td>
</tr>
<tr>
<td>Renal patients</td>
<td>173</td>
<td>34.7%</td>
<td>45.1%</td>
<td>20.2%</td>
<td>0.572</td>
</tr>
<tr>
<td>Yuendumu Australian Aboriginal</td>
<td>184</td>
<td>0.5%</td>
<td>4.9%</td>
<td>94.6%</td>
<td>0.0030†</td>
</tr>
<tr>
<td>Australian Aboriginal renal patients</td>
<td>94</td>
<td>2.1%</td>
<td>24.4%</td>
<td>73.4%</td>
<td>0.144†</td>
</tr>
</tbody>
</table>

The genotype distribution in each sample group did not differ significantly from the expected Hardy–Weinberg distribution.

†Allele frequency significantly less \(P < 0.0001\) than each of the Australian Caucasian and ‘Other’ renal patients sample groups.

‡Allele frequency significantly less \(P < 0.0001\) than each of the other three sample groups.
group (D = 57.2%), but this difference was not significant (P = 0.42).

The D allele frequency was comparatively low in Yuendumu Australian Aboriginal population sample (D = 3.0%). This frequency was significantly increased in the Australian Aboriginal renal patient group (D = 14.4%, P < 0.0001), but was still significantly less than the frequency observed in either the Caucasian normal or renal patient groups (P < 0.0001).

Discussion

Population differences in genetic polymorphic systems are to be expected. From the data available from varied ethnic groups (Table 2), the frequencies of the ACE D allele observed in this study in the Caucasian normal and renal transplant groups are comparable to those previously reported in European Caucasians and renal transplant [21]. The frequency observed in several black populations is similar to that observed in Caucasians, whereas those observed in several Asian, Amerindian, and Polynesian populations (both ultimately of Asian ancestry) are less. By comparison the frequency observed in this study in the Yuendumu Australian Aboriginal population (also of ultimate Asian ancestry) (D = 3%) is very low. Indeed the D allele frequency (D = 14%) in the Australian Aboriginal renal patient group is also very low given the diverse source of study material from different tribes of urban and non-urban regions and almost certain Caucasian admixture. Whether these observations have implications on the understanding of the evolution of the renin–angiotensin–aldosterone system in a nomadic hunter-gatherer population living in an extremely dry climate can only be a matter of conjecture. Certainly, stochastic factors enhanced by small breeding population sizes, limited gene pool, and geographical isolation probably played a major role in this population’s loss of genetic polymorphism. Limited genetic polymorphism in this Australian Aboriginal population has also been observed in previous studies in such systems such as HLA [29] platelet antigens [30], and the haemochromatosis-associated HFE gene (Lester S, unpublished data).

While tribal differences in genetic polymorphism are known [25], the very low frequency of the D-allele in the Yuendumu Australian Aboriginal population (D = 3%) and the Australian Aboriginal renal patient group (D = 14%) suggests that a low frequency of the ACE D allele is a common feature of Australian Aboriginal populations, and that the increased frequency in the renal patient group (14%) is due to Caucasian admixture rather than any intrinsic involvement with renal disease. This assumption is supported by the observation that the sixfold increase in Caucasian admixture as judged by HLA class II haplotypes (from 1.1 to 6.4%) is almost identical to the proportional increase in the frequency of the D allele in the patient group.

Therefore the ACE D allele is not a major genetic factor in the renal disease of Australian Aboriginals, a disease characterized by a high incidence and a rapid rate of progression. However, other polymorphisms in this and other genes should be investigated.

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Table 2. ACE insertion (I) and deletion (D) polymorphism allele frequencies reported in different populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Reference</th>
<th>ACE allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>Caucasian</td>
<td>[22]</td>
<td>0.54</td>
</tr>
<tr>
<td>Nigerian black</td>
<td>[23]</td>
<td>0.54</td>
</tr>
<tr>
<td>Jamaican black</td>
<td>[23]</td>
<td>0.59</td>
</tr>
<tr>
<td>US black</td>
<td>[23]</td>
<td>0.63</td>
</tr>
<tr>
<td>Japanese</td>
<td>[24]</td>
<td>0.36</td>
</tr>
<tr>
<td>Taiwanese</td>
<td>[25]</td>
<td>0.36</td>
</tr>
<tr>
<td>Korean</td>
<td>[26]</td>
<td>0.35</td>
</tr>
<tr>
<td>Pima Indian</td>
<td>[27]</td>
<td>0.29</td>
</tr>
<tr>
<td>Yanomami Indian</td>
<td>[28]</td>
<td>0.15</td>
</tr>
<tr>
<td>Samoan Polynesian</td>
<td>[28]</td>
<td>0.09</td>
</tr>
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References


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