Lack of Association Between the Insertion/Deletion Polymorphism of the Angiotensin-Converting Enzyme Gene and Idiopathic Dilated Cardiomyopathy

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Objectives. We sought to investigate the role of polymorphisms of the gene for angiotensin-converting enzyme in the development and progression of idiopathic dilated cardiomyopathy.

Background. Cardiovascular renin-angiotensin systems may be involved in cardiac remodeling and fibrosis. The absence (deletion [D]) of a 287-base pair marker in the angiotensin-converting enzyme gene (intron 16) has been associated with increased serum angiotensin-converting enzyme levels. The DD genotype may be a risk factor for the development of end-stage heart failure due to cardiomyopathy. We therefore examined the relation of the angiotensin-converting enzyme genotype to idiopathic dilated cardiomyopathy and to markers of disease severity.

Methods. We studied 364 control subjects and 99 consecutive patients with idiopathic dilated cardiomyopathy. When the incidence of the DD genotype in our control group was assumed to be similar to that previously reported (27%), this study had a power of 0.9 to detect a different incidence in the patient group, if the true incidence in patients was 42%. Deoxyribonucleic acid (DNA) was isolated from blood samples, and angiotensin-converting enzyme genotype was determined by specific polymerase chain reaction and separation of amplified fragments by agarose gel electrophoresis. We also compared genotype distribution with that in previously reported European control subjects. Functional status, clinical course over a mean ± SD of 28 ± 33 months and outcome were documented. Cardiac morphology and function and evidence of rhythm disturbance were noninvasively determined.

Results. Angiotensin-converting enzyme genotype distribution and allele frequencies were similar in patients and control subjects to within 10% (with 95% confidence) and were also similar between patients and European control subjects. No markers of disease severity or progression other than duration of symptoms before diagnosis and the number of ventricular ectopic beats/h were significantly associated with the presence of the DD alleles.

Conclusions. We find no evidence to support an association between angiotensin-converting enzyme genotype and either the diagnosis of idiopathic dilated cardiomyopathy itself or progression of the disease.

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Systemic or local cardiovascular renin-angiotensin systems may be involved in cardiac remodeling and fibrosis (1–3). In formal segregation analysis studies, the absence (deletion [D]) rather than the presence (insertion [I]) of a 287-base pair marker in the angiotensin-converting enzyme gene (intron 16) has been shown (4) to be associated with raised serum levels of the enzyme responsible for the conversion of angiotensin I to angiotensin II. Furthermore, the deletion polymorphism has been reported to be a risk factor for the development of myocardial infarction (5,6). Against this background, Raynolds et al. (7) reported that possession of the angiotensin-converting enzyme DD genotype was a risk factor for the development of end-stage heart failure due to cardiomyopathy. However, their conclusion has been questioned. The allele frequencies were similar in both the case and control group in their study. Their control group data were not in Hardy-Weinberg equilibrium, as there were fewer patients with the DD and II genotypes than either expected (8) or previously reported (5,9). The use of a small control group composed of young potential organ donors and genetic heterogeneity among both the patient and control groups were additional limitations of their study. The involvement of the angiotensin-converting enzyme genotype in the genesis of idiopathic dilated cardiomyopathy as opposed to its progression was not directly addressed. We therefore determined the angiotensin-converting enzyme genotype in 99 consecutive patients with well-characterized idiopathic dilated cardiomyopathy referred for management of heart failure and in 364 control subjects. The relations among genotype, the diagnosis of idiopathic...
dilated cardiomyopathy itself and markers of disease severity and clinical progression of the disease were examined.

Methods

This study was performed with hospital ethical committee approval. No patients were subjected to invasive investigation unless the procedure was clinically indicated. All gave written informed consent.

Patients. The diagnosis of idiopathic dilated cardiomyopathy was based on the criteria recommended by the World Health Organization and the National Heart, Lung, and Blood Institute (10). All patients had left ventricular dilation (end-diastolic diameter >2.7 cm/m²) (11) and impaired systolic contraction (left ventricular ejection fraction <40% or fractional shortening <25%). Patients with >50% obstruction of one or more coronary arteries, active myocarditis (12), specific primary or secondary heart muscle disease, sustained systemic arterial hypertension, isolated right ventricular dilation and valvular or pericardial disease were excluded. Twenty-seven patients with a history of chronic alcohol consumption (>8 U/day for male patients and >6 U/day for female patients) were included.

We studied 99 consecutive unrelated white patients with idiopathic dilated cardiomyopathy (mean age ± SD 41 ± 14 years [range 12 to 73]; 79 male) who presented to St. Georges Hospital between January 1989 and March 1994. Functional assessment at presentation demonstrated that 42 were in New York Heart Association class I, 17 in class II, 25 in class III and 15 in class IV when first seen. They were assessed by 12-lead electrocardiography, chest radiography, two-dimensional transthoracic Doppler echocardiography, 24-h ambulatory electrocardiographic (ECG) monitoring, radionucleotide ventriculography and maximal symptom-limited exercise testing. M-mode guided short-axis views at the level of the papillary muscles were used to assess left ventricular cavity dimensions. Cardiac catheterization with selective coronary angiography was performed in 81 patients. The remaining 18 patients were all <40 years old with no risk factors for ischemic heart disease and no evidence of ischemia on exercise ECG testing. Endomyocardial biopsy in the 56 patients who consented to the procedure was normal in 23 and showed fibrosis in 33. The clinical and demographic characteristics of those consenting to biopsy and those who declined were similar. The left ventricular ejection fraction at diagnosis was 25 ± 11% and left ventricular end-diastolic volume 68 ± 11 mm³. Duration of symptoms was defined empirically as the time from first reported symptoms to time of first confirmation of diagnosis of idiopathic dilated cardiomyopathy recorded in the case notes. Patients were followed up for a mean of 28 ± 33 months. During this period 35 patients showed clinical deterioration, including 22 who required heart transplantation. In addition, four patients had a sudden cardiac death (13).

Control groups. The study control group consisted of 364 white men (mean age 54 ± 3 years [range 49 to 60]) drawn from a local general practice group. Subjects with symptoms suggestive of coronary artery disease or with a known diagnosis of ischemic heart disease were excluded. We also compared our patients with the previously reported European control sample from the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) study (5).

Statistics. We assumed that the frequency of the DD genotype in our control subjects would be similar to that previously reported (27%) (5). Given 99 patients and 364 control subjects, this gave the study a power of 0.9 to detect a different incidence in the patient group of 42%. Differences in the distribution of the DD, DI and II genotypes were assessed by contingency table/chi-square analysis. Numeric data between different groups were compared by using unpaired two-tailed t tests, and outcomes were compared by a one-tailed analysis of variance test. All p values were expressed with continuity corrections. A p value < 0.05 was considered statistically significant.

Results

We compared angiotensin-converting enzyme genotype/allele frequencies in our patient, our control and previously reported healthy European control (5) groups (Table 1). Allele frequencies in the control and patient groups were consistent with Hardy-Weinberg equilibrium. The genotype distribution in the control group fit well with that previously reported (p = 0.1 [5]; p > 0.1 [9]) and was similar to that in the patient group (p = 0.75). The DD genotype frequency was the same (within 10%, with 95% confidence) between groups; the difference in DD genotype frequency between the patient and control groups was 0.005 (31/99 vs. 112/364; 95% confidence interval ± 0.103). The same held true when the patient group was compared with European control subjects (p = 0.75). The allele frequencies in the patient group were also not significantly different from those in the control groups (p = 0.5). The difference in D allele...
Table 2. Characteristics of Patients With Dilated Cardiomyopathy According to Genotype for Angiotensin Deletion (D) or Insertion (I) Polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>DD</th>
<th>ID</th>
<th>II</th>
<th>ID + II</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43 ± 13</td>
<td>36 ± 14</td>
<td>47 ± 17</td>
<td>40 ± 15</td>
<td>0.32</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>41</td>
<td>11</td>
<td>52</td>
<td>0.08</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>9</td>
<td>7</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>17</td>
<td>33</td>
<td>9</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>III + IV</td>
<td>14</td>
<td>17</td>
<td>9</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Duration (mo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>30 ± 29</td>
<td>29 ± 34</td>
<td>26 ± 37</td>
<td>28 ± 35</td>
<td>0.71</td>
</tr>
<tr>
<td>Symptoms before diagnosis</td>
<td>50 ± 52</td>
<td>29 ± 34</td>
<td>26 ± 40</td>
<td>27 ± 51</td>
<td>0.16</td>
</tr>
<tr>
<td>Ejection fraction (n = 58) (%)</td>
<td>22 ± 8</td>
<td>28 ± 12</td>
<td>22 ± 11</td>
<td>26 ± 12</td>
<td>0.93</td>
</tr>
<tr>
<td>Left ventricular end-diastolic dimension (n = 77) (mm)</td>
<td>72 ± 12</td>
<td>65 ± 9</td>
<td>72 ± 12</td>
<td>67 ± 10</td>
<td>0.96</td>
</tr>
<tr>
<td>Left ventricular fractional shortening (n = 77) (%)</td>
<td>14 ± 6</td>
<td>15 ± 7</td>
<td>14 ± 7</td>
<td>14 ± 7</td>
<td>0.91</td>
</tr>
<tr>
<td>Pulmonary artery wedge pressure (n = 45) (mm Hg)</td>
<td>20 ± 11</td>
<td>18 ± 7</td>
<td>28 ± 7</td>
<td>20 ± 8</td>
<td>0.095</td>
</tr>
<tr>
<td>Ventricular ecotopic beats (n = 48) (beats/h)</td>
<td>333 ± 334</td>
<td>143 ± 294</td>
<td>81 ± 134</td>
<td>128 ± 264</td>
<td>0.06</td>
</tr>
<tr>
<td>Nonsustained ventricular tachycardia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with</td>
<td>11</td>
<td>12</td>
<td>5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Patients without</td>
<td>7</td>
<td>18</td>
<td>6</td>
<td>24</td>
<td>0.66</td>
</tr>
<tr>
<td>Fibrosis on endomyocardial biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with</td>
<td>14</td>
<td>13</td>
<td>6</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Patients without</td>
<td>7</td>
<td>13</td>
<td>3</td>
<td>16</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± SD or number of patients. NYHA class = New York Heart Association functional class; other abbreviations as in Table 1.

frequency was negligible (0.027 ± 0.078), being within 10% with 95% confidence.

In the patient group, the relation between angiotensin-converting enzyme genotype and clinical markers of disease severity and progression were examined (Tables 2 and 3). Patients with the DD genotype (n = 31) were compared with patients homozygous for the I allele (n = 18) and with the total group of “non-DD” (ID and II; n = 68) patients. The age and gender characteristics of these groups were similar. Only the duration of symptoms before diagnosis and the number of ventricular ecotopic beats/h were significantly associated with the presence of the DD alleles, with patients homozygous for the D allele having the longer duration of symptoms and the greater number of ventricular ecotopic beats/h. In particular, the presence of fibrosis at endomyocardial biopsy was not associated with the DD genotype, and the incidence of fibrosis was similar in patients with different genotypes. Genotype distribution and allele frequency were similar in patients who had progressive heart failure or required orthotopic heart transplantation when values were compared with those in the clinically stable patients (p = 0.5), the European control group (p = 0.5) or our control group (p > 0.5).

Discussion

Systemic and paracrine renin-angiotensin systems. Physiologically and pathophysio logically modulated expressions (15-18) of renin-angiotensin system components and messenger ribonucleic acids have been identified in cardiovascular tissues including the heart (19-21). Cardiac renin-angiotensin systems may thus have an autocrine/paracrine function that may influence cardiac architecture, growth and function and through which the action of angiotensin-converting enzyme inhibitors may be partly mediated (15,22-24).

Potential role of renin-angiotensin systems in cardiomyopathy. Many findings suggest a potential role for this system in cardiomyopathic processes. Components such as angiotensin II activity may damage myocytes, causing increased membrane permeability and microscopic scarring (25). Cultured rat fibroblasts have functional angiotensin II receptors (26), and angiotensin II increases collagen synthesis (27). Rat cardiac collagen deposition may be regulated by renin-angiotensin system activity (1,28,29) and prevented by nonhypotensive doses of angiotensin-converting enzyme inhibitors (2,3) or angiotensin-converting enzyme inhibitor treatment after experimental myocardial infarction (30,31).

Cardiac function and structure may also be influenced. Angiotensin II impairs myocyte relaxation (32,33), and angiotensin-converting enzyme inhibition improves diastolic function in animals (34) and the hypertensive human heart (35).
Angiotensin-converting enzyme genotype and dilated cardiomyopathy. Recently, a polymorphism of the gene for angiotensin-converting enzyme has been discovered, consisting of the presence (insertion allele or “I”) or absence (deletion allele or “D”) of a 250-base pair fragment. This polymorphism is strongly associated with circulating (and possibly tissue) levels of the enzyme, with those of a DD genotype having highest levels (9). If renin-angiotensin system activity is associated with myocardial pathogenicity, then patients with the highest levels might be expected to suffer most.

Given these findings, an association between angiotensin-converting enzyme genotype and dilated cardiomyopathy would seem feasible. Raynolds et al. (7) compared 112 white patients with end-stage dilated cardiomyopathy, of whom 93 required heart transplantation, with 79 younger white control subjects and found that the frequency of the DD genotype was 36% in patients and 24% in control subjects (p < 0.01). However, an associated excess of the II genotype counters the concept that DD genotype exerts a pathophysiologic action through associated raised levels of angiotensin-converting enzyme activity. Further, they did not examine the association of angiotensin-converting enzyme gene polymorphism with the diagnosis of dilated cardiomyopathy itself, or with disease severity or progression of disease within a cohort. Our study has addressed these issues.

The DD genotype is only strongly associated with myocardial infarction in an otherwise low risk group in which only 35% of cases might be ascribed to its possession (5). Distortion of allele distribution in our control group due to the presence of, or mortality from, ischemic heart disease can therefore be only minimal. Similarly, idiopathic dilated cardiomyopathy is uncommon, and undiagnosed control cases are unlikely to introduce significant bias.

Our results demonstrate no association between angiotensin-converting enzyme genotype or allele frequencies and a diagnosis as such of idiopathic dilated cardiomyopathy or biopsy-proved myocardial fibrosis among patients. The D allele is slightly (but not statistically significantly, p = 0.5) more common among patients than among control subjects. However, like the study by Raynolds et al. (7), our study would have lacked the power to detect a small increase in relative risk associated with the presence of the D allele.

We also examined the association of polymorphism with progression of disease by comparing clinical variables in patients with the DD genotype (n = 31) with those in patients in the II (n = 18) and non-DD groups (II and ID: n = 68) (Table 2). The association between DD genotype and increased time to diagnosis suggests, if anything, that patients with a DD genotype had slower progression of disease, because clinical grading of symptoms at presentation and all measures of ventricular contractility were similar irrespective of patient genotype. However, “time to diagnosis” (first recalled symptoms to first recorded diagnosis) is an empiric measure subject to various social, personal, professional and pathologic influences.

The frequency of the DD genotype and D allele was similar in the 35 patients with progressive disease (including the 4 patients who died suddenly) and in the 60 patients with a stable course. However, the risk of end-stage idiopathic dilated cardiomyopathy previously attributed to having two D alleles is small (odds ratio 1.43), and these numbers may be insufficient to rule out such a weak association of the angiotensin-converting enzyme genotype with progression to end-stage heart failure.

Our results do accord with some features of the study of Raynolds et al. (7). The frequency of the D allele in their patient and control groups (M. Raynolds, personal communication, February 1994) and in our patient group was remarkably similar (D frequency 0.56, 0.57, 0.57, respectively.) Our failure to demonstrate any association between the gene polymorphism and the diagnosis of idiopathic dilated cardiomyopathy as such may be due to several factors. There are theoretical difficulties in population studies such as these in determining what makes an association (or lack of it) significant, and there are pitfalls in applying the investigational standards derived for Mendelian traits to studies of more complex phenotypes or diseases (36). Differences in patient or control characteristics may also have played a role: The two groups of patients studied were different, the group of Raynolds et al. (7) comprising those with end-stage disease of undefined etiology and characteristics on the one hand, and our well characterized unrelated probands with various levels of disease severity on the other. The control groups also differed. Ours was drawn from a local general practice population and was slightly older than our patient group (mean ± SD 54 ± 3 vs. 41 ± 14 years). Theirs largely comprised potential heart donors and the subjects were younger (mean ± SEM 33 ± 1.8 vs. 44.8 ± 1.5 years). The subjects in neither group had a proved normal heart. Ejection fractions as low as 30% were accepted as normal in their small (n = 79) control group, which itself did not obey the Hardy-Weinberg equilibrium. The incidence of the II genotype (12.7%) was far lower than that previously reported (8) in control groups (19.5% [5] and 17.5% [9]). The frequency of the DD genotype (24%) was also lower than expected (8) or previously reported (36.25% [9] and 27% for ECTIM control subjects [5]). It is this unusual distribution of alleles among control subjects that accounts for much of the statistically significant excess of the DD genotype in their patient group over that of their control subjects. In contrast, our control group was much larger (n = 364), its data obeyed the Hardy-Weinberg equilibrium, and the genotype and allele frequencies were similar to those seen in a large (n = 733) European control sample (5).

Raynolds et al. (37) correctly state that the issue under discussion is whether this genetic variant of the angiotensin-converting enzyme gene predisposes a person to the development of cardiomyopathy. Using similar numbers of patients, we found no evidence that it does. Neither do our data suggest a major role for the angiotensin-converting enzyme DD genotype in the progression of idiopathic dilated cardiomyopathy. A large long-term follow-up study is required if such associations of this angiotensin-converting enzyme gene polymorphism are to be investigated further.
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References