Rapid Communication

Excess of DD homozygotes in haemodialysed patients with type II diabetes

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Abstract The role of the insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene in the genesis of diabetic nephropathy has been controversial. It has recently been proposed that progression occurs more rapidly in individuals with diabetic and non-diabetic renal disease who are homozygous for the D allele. We studied 658 patients with type II diabetes, 347 without diabetic nephropathy and 311 with various stages of diabetic nephropathy, and determined the I/D polymorphism of the ACE gene. Patients at the extremes of renal risk, i.e. normotensive patients without antihypertensive treatment and without nephropathy (n = 144), vs patients on dialysis (n = 61), differed with respect to genotype (DD 36.8% vs 57.4%; P = 0.007) and allele frequencies (D 0.59 vs 0.76; P < 0.001). In contrast, patients with and without presumed nephropathy as assessed by albuminuria did not differ with respect to DD genotype. In conclusion, this study, which was limited by sample size, patients with the highest renal risk more frequently had the DD genotype. This would be compatible with a greater risk of (or rate of) progression to end-stage renal failure.

Key words: ACE gene polymorphism; diabetic nephropathy; genetics; haemodialysis; type II diabetes

Introduction

The role of the angiotensin-converting enzyme (ACE) gene polymorphism in development of diabetic nephropathy [1–5] and renal disease in general [6–9] has remained controversial. It has been proposed that patients with type I diabetes who are homozygous for the I allele are less likely to develop nephropathy [1], but this has not been uniformly confirmed [2,3], particularly in type II diabetes [3].

We have recently expanded the original [3] cohort of patients with type II diabetes. Using this larger, but still limited, patient sample we reassessed the relation of the ACE gene polymorphism to albuminuria and to renal function.

Subjects and methods

We examined 358 patients with type II diabetes of at least 10 years duration. Patients were recruited from four German diabetes clinics, one Polish diabetes clinic and four German dialysis centres. Diabetic nephropathy was defined by the minimum criterion of albumin excretion ≥ 30 mg/24 h. As controls we examined 256 healthy blood donors from the Heidelberg region.

The ACE gene polymorphism was determined using PCR amplification as described previously [3]. To avoid preferential amplification of the D allele we added 5% DMSO to the reaction mix [10]. The alleles were separated on agarose gels.

Statistical analysis

All statistical calculations were carried out using the statistical package SPSS for windows 6.0 (SPSS Inc.).

Results

Clinical characteristics of albuminuric and non-albuminuric patients, as well as of normotensive, normoalbuminuric and dialysis patients, are given in Table 1.

As shown in Table 2, patients without and with nephropathy did not differ with respect to genotype distribution and allele frequency. In contrast, at the extremes of renal risk, i.e. normotensive patients without antihypertensive medication and without

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Table 1. Clinical data of diabetic patients with type II diabetes with and without diabetic nephropathy

<table>
<thead>
<tr>
<th></th>
<th>Without DN (n = 347)</th>
<th>With DN (n = 311)</th>
<th>P</th>
<th>Patients without antihypertensive treatment and without nephropathy (n = 144)</th>
<th>Patients on dialysis (n = 61)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M)</td>
<td>189/158</td>
<td>158/153</td>
<td>NS</td>
<td>63/81</td>
<td>34/27</td>
<td>0.116</td>
</tr>
</tbody>
</table>
| Age (years)
 1          | 63 ± 9               | 65 ± 9            | <0.05   | 61 ± 9                                                                          | 69 ± 7                       | <0.001|
| Duration of diabetes (years)
 1          | 17 ± 6               | 19 ± 7            | <0.0005 | 17 ± 5                                                                          | 21 ± 9                       | 0.0005|
| BMI (kg/m²)          | 28.8 ± 4.3           | 28.7 ± 4.9        | NS      | 28.1 ± 4.0                                                                      | 26.1 ± 3.9                   | 0.0041|
| Hba1c (%)            | 8.8 ± 1.7            | 8.7 ± 1.8         | NS      | 8.7 ± 1.8                                                                      | 6.8 ± 1.5                    | 0.0005|
| Hypertensives (%)     | 57.1                 | 74.3              | <0.00001 | 0                                                                                | 83.6                         | <0.00001|
| Diabetic retinopathy (%) | 35.3               | 64.7              | <0.000001 | 29.7                                                                            | 90.2                         | <0.00001|
| Background            | 75.7                 | 51.3              | <0.0001 | 69.7                                                                            | 50.0                         | 0.08  |
| Proliferative (%)     | 24.3                 | 48.7              |         | 30.3                                                                            | 50.0                         | 0.00001|
| Patients treated with ACE inhibitors (%) | 28.1                   | 42.9              | <0.0005 | 0                                                                                | 27.5                         | <0.00001|

1Data given as mean ± SD.
2% of patients with diabetic retinopathy.

Table 2. Genotype distribution and allele frequencies of the I/D polymorphism in patients with type II diabetes

<table>
<thead>
<tr>
<th></th>
<th>Without nephropathy (n = 347)</th>
<th>With nephropathy (n = 311)</th>
<th>Patients without antihypertensive and without nephropathy (n = 144)</th>
<th>Patients on dialysis (n = 61)</th>
<th>Controls (n = 256)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype distribution in per cent (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>37.7 (131)</td>
<td>38.9 (121)</td>
<td>36.8 (53)</td>
<td>57.4 (35)</td>
<td>32.4 (83)</td>
</tr>
<tr>
<td>ID</td>
<td>44.4 (154)</td>
<td>41.5 (129)</td>
<td>45.1 (65)</td>
<td>37.7 (23)</td>
<td>46.5 (119)</td>
</tr>
<tr>
<td>II</td>
<td>17.9 (62)</td>
<td>19.6 (61)</td>
<td>18.1 (26)</td>
<td>4.9 (3)</td>
<td>21.1 (54)</td>
</tr>
<tr>
<td>P vs controls</td>
<td>0.346</td>
<td>0.273</td>
<td>0.612</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.60</td>
<td>0.60</td>
<td>0.59</td>
<td>0.76</td>
<td>0.56</td>
</tr>
<tr>
<td>I</td>
<td>0.40</td>
<td>0.40</td>
<td>0.41</td>
<td>0.24</td>
<td>0.4</td>
</tr>
<tr>
<td>P vs controls</td>
<td>0.137</td>
<td>0.177</td>
<td>0.309</td>
<td>&lt;0.00005</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.931</td>
<td></td>
<td></td>
<td>&lt;0.00001</td>
<td></td>
</tr>
</tbody>
</table>

nephropathy, differed from patients on dialysis both with respect to genotype distribution and allele frequencies, i.e. a higher proportion of patients with dialysis were homozygous for the D allele and the frequency of the D allele was significantly higher. Genotype distribution of all groups, except the group of patients with nephropathy (n = 311), were in Hardy–Weinberg equilibrium.

Discussion

The negative findings in the comparison between albuminuric and non-albuminuric patients with type II diabetes are in line with previous observations suggesting that in patients with type II diabetes the risk of developing significant albuminuria is not related to polymorphism in the ACE gene [3]. The strength of this conclusion may be limited by several considerations. It has been suggested that in type II diabetes albuminuria is not a specific indicator of nephropathy [11]. More recent autopsy [12] and biopsy studies [13,14] argue against contamination of this patient group with a high proportion of non-diabetic renal disease. Undoubtedly, however, renal morphology is more heterogeneous in type II than in type I diabetes [14,15]. It is not known whether albuminuric patient with type II diabetes and non-specific lesions carry the same renal risk as microalbuminuric patients with type I diabetes.

A striking observation, however, was the fact that patients with type II diabetes on dialysis had a large excess of the D allele. The strength of this observation is limited: (i) by the small sample size; (ii) by the fact that it is the result of subsample testing; and (iii) by the fact that longitudinal data are not available. Nevertheless the magnitude of the difference is so striking as to warrant preliminary reporting.

Several explanations as to the pathomechanisms involved are conceivable. It has been proposed that individuals who are homozygous for the D allele progress more rapidly to end-stage renal failure; this was reported in non-diabetic renal disease [7,8], as well as in diabetic renal disease [4,5]. The observation of accumulation of the DD genotype in the cohort of dialysed patients would be consistent with the notion
that these individuals progress more rapidly to end-stage renal failure (so that accumulation outweighs loss secondary to the known increased cardiac risk [16]). In other words, it is assumed that under non-steady state conditions the cohort of patients with end-stage renal failure is enriched with rapid progressors. Alternatively, in albuminuric patients with type II diabetes the DD genotype may be related to a higher absolute risk to progress to end-stage renal failure.

Yoshida et al. [4] reported a higher frequency of DD homozygotes in Japanese patients with type II diabetes who showed evidence of progression. In the dialysed cohort, however, no excess of DD homozygotes was noted. This is not necessarily in conflict with the present data, since cardiac mortality is higher in DD homozygotes. The net impact of this factor may have been different in Japanese and European patients.

Recently, concerns have been raised concerning testing multiple genetic loci in cohorts and the implication of the Bonferroni problem [17]. We are aware of this problem and therefore these preliminary data require confirmation.

References


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