Renal function after solid organ transplantation
Broekroelofs, Jan

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Chapter 6

Risk factors for long-term renal survival after renal transplantation: a role for angiotensin-converting enzyme (insertion/deletion) polymorphism?

Broekroelofs J, Stegeman CA, Navis GJ, Tegzess AM, De Zeeuw D, De Jong PE
Abstract

Chronic progressive renal function loss is a main cause of long-term graft loss after initially successful renal transplantation. Transplanted kidneys share some risk factors for renal function loss, such as hypertension or proteinuria, with diseased native kidneys. Recently, it has been shown that renal function loss is influenced by the angiotensin-converting enzyme (ACE)(insertion/detection [I/D]) genotype in renal disease in diseased native kidneys. This study examines whether donor or recipient ACE (I/D) genotype is a risk factor for graft loss after renal transplantation.

To avoid bias by acute events, graft survival was studied, with patients dying with a functioning graft censored, starting at 12 months after transplantation in a cohort of 367 patients transplanted between 1987 and 1994 with at least two years of follow up. Mean follow up was 58 months. ACE (I/D) genotype was determined by PCR on stored donor and recipient lymphocytes.

Neither donor nor recipient ACE (I/D) genotype was associated with graft survival. However, Cox proportional hazards analysis identified recipient, but not donor, ACE (I/D) genotype D-allele to be independently associated with a shorter time to graft loss in subgroups of patients at high risk for graft loss defined by a creatinine clearance < 50 ml/min (n=108, p=0.017) or proteinuria ≥ 0.5 g/24h at 12 months (n=97, p=0.0051) after transplantation.

In conclusion, recipient ACE (I/D) genotype was associated with time to graft loss in a specific high risk subgroup of our population. This suggests that the effect of ACE (I/D) genotype on graft survival only becomes apparent when other risk factors are simultaneously present.
Introduction

Chronic renal function loss occurs in a substantial number of patients with an initially successful renal transplantation and is a main cause of graft loss during long-term follow up. Multiple risk factors have been identified, suggesting that the pathogenesis of this progressive renal function loss is multifactorial. Some of these factors, such as HLA-mismatching and previous rejection episodes are specific for renal transplants, whereas others, such as high blood pressure and proteinuria are risk factors common to both native and transplanted kidneys. In chronic renal disease in native kidneys, the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene has recently been identified as a risk factor for progressive renal function loss and decreased renal survival. The association of the D-allele with an increased risk for renal failure in a spectrum of renal disorders of different origin suggests that the D-allele is a renal risk factor regardless of the primary cause of renal damage.

In the present study, therefore, the primary objective was to identify whether the ACE (I/D) genotype contributes to graft loss after renal transplantation. The study of this polymorphism in renal transplantation provides the unique opportunity to investigate whether the recipient or donor ACE (I/D) genotype is associated with renal risk. This might allow us to distinguish between the influence of tissue and systemic ACE genotype and thus could provide a clue to mechanism of action of ACE activity in renal disease progression. To avoid bias induced by acute pathology, such as technical failure and therapy-resistant episodes of acute rejection, the study involved a cohort of patients with a functioning graft 12 months after transplantation. To account for the multifactorial nature of graft loss after renal transplantation, the influence of recipient or donor ACE (I/D) genotype on graft survival was assessed both by univariate and multivariate survival analysis including other identified risk factors for graft loss.

Patients and methods

Patients

We retrospectively studied data from all patients transplanted with a cadaveric renal graft between April 1987 and December 1994 who had at least 12 months of follow up with a functioning graft. Patients were included if stored lymphocytes of both donor and recipient were available for ACE I/D genotype determination.

Immunosuppressive treatment consisted of 3 mg/kg cyclosporin A intravenously for 72 hours, followed by oral cyclosporin A (10 mg/kg) in 2 divided doses, with dose adjustments to obtain trough levels of 200 to 250 ng/ml for the first 3 months and 150 to 200 ng/ml...
thereafter, and 20 mg/day prednisolone, tapered over 8 weeks to a maintenance dose of 10 mg/day. Patients with anti-HLA antibodies (maximum panel reactivity > 60%) received induction with murine monoclonal anti-CD3 antibody (OKT3, 5mg/day) for 12 days followed by cyclosporin A started at day 10, azathioprine (1.5 mg/kg/day) and prednisolone (1 mg/kg/day initially and tapered to 10 mg/day after 8 weeks). Acute rejection episodes diagnosed on clinical findings or by renal biopsy were treated with maximal two courses 1000 mg of methylprednisolone intravenously for 3 consecutive days. Steroid-resistant or acute vascular rejection was treated with rabbit anti-thymocyte globulin (4 mg/kg on alternating days for 10 days). ACE (I/D) genotype was determined by PCR on stored lymphocytes from donor and recipient11. Mistyping was checked by intron specific primers as described by Shanmugan et al12. Serum and urinary creatinine concentrations and 24-hour urinary protein excretion were determined by standard laboratory techniques.

Data analysis

Survival curves until graft failure were calculated by the Kaplan-Meier method. Graft failure was defined as the need to restart renal replacement therapy or death of the patient due to renal failure. Patients dying with a functioning graft were censored at the moment of death. Thus, renal survival is analysed as pure graft survival. Data are given as means with standard deviation (SD). Differences in categorical variables between groups were tested with Chi-square test. Differences in continuous variables between two groups were tested with the Student-t test or the non-parametric Wilcoxon rank sum test, where appropriate. Differences in continuous variables between more than two groups were tested by ANOVA or the non parametric Kruskall Wallis test. Post tests were performed with the Bonferroni method. Differences in survival between groups were tested by logrank test. Cox proportional hazards analysis with time to graft failure as the dependent variable was performed; independent variables tested were recipient and donor ACE I/D genotype, age, gender, number of HLA class I and II mismatches, ischaemia times, number of acute rejection episodes, and creatinine clearance, blood pressure, the use of antihypertensive medication and proteinuria at 12 months after transplantation. Hazard ratios with 95% confidence interval calculated from the exponential in the regression model, including all covariates with a p-value < 0.1, are reported as relative risks. All p-values are two-tailed.

Results

Four hundred and ninety-seven patients received a cadaveric renal transplant at our centre from April 1987 to December 1994. In 62 patients, either donor or recipient material for
ACE (I/D) genotype determination was unavailable. Of the remaining 435 patients, 68 (15.6%) lost their graft within 12 months after transplantation due to technical failure (21 patients, 4.8%), death (15 patients, 3.4%), therapy resistant rejection (25 patients, 5.7%), or other causes (7 patients, 2.0%). Genotype distribution in patients with graft failure within 12 months after transplantation was similar to that in patients with a functioning graft at 12 months (II: 21, ID: 30, and DD: 17 for recipient ACE (I/D) genotype and II: 18, ID: 30, and DD: 20 for donor ACE (I/D) genotype, respectively).

The characteristics of the 367 patients with a functioning graft at 12 months after transplantation are shown in Table 1, grouped according to recipient ACE (I/D) genotype.

<table>
<thead>
<tr>
<th>Recipient ACE (I/D) genotype</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>91</td>
<td>187</td>
<td>89</td>
</tr>
<tr>
<td>Male/female</td>
<td>56/35</td>
<td>102/85</td>
<td>55/34</td>
</tr>
<tr>
<td>Recipient age (year)*</td>
<td>44 ± 12</td>
<td>45 ± 13</td>
<td>43 ± 13</td>
</tr>
<tr>
<td>Donor age (year)*</td>
<td>36 ± 16</td>
<td>37 ± 15</td>
<td>38 ± 17</td>
</tr>
<tr>
<td>Total number of HLA mismatches*</td>
<td>1.2 ± 0.9</td>
<td>1.2 ± 0.9</td>
<td>1.3 ± 1.0</td>
</tr>
<tr>
<td>Number of HLA DR mismatches*</td>
<td>0.3 ± 0.6</td>
<td>0.2 ± 0.4</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Acute rejection episodes in first year (%)</td>
<td>36</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>Serum creatinine level (mmol/l)*</td>
<td>170 ± 85</td>
<td>158 ± 59</td>
<td>171 ± 87</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)*</td>
<td>61 ± 22</td>
<td>61 ± 21</td>
<td>65 ± 25</td>
</tr>
<tr>
<td>Proteinuria ≥ 0.5 g/24h (%)*</td>
<td>31</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)*</td>
<td>112 ± 13</td>
<td>113 ± 12</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>Antihypertensive medication (%)*</td>
<td>70</td>
<td>68</td>
<td>74</td>
</tr>
<tr>
<td>Follow up (months)*</td>
<td>59 ± 24</td>
<td>58 ± 24</td>
<td>55 ± 25</td>
</tr>
</tbody>
</table>

* Results are given as mean ± standard deviation

ACE (I/D) genotype determination was unavailable. Of the remaining 435 patients, 68 (15.6%) lost their graft within 12 months after transplantation due to technical failure (21 patients, 4.8%), death (15 patients, 3.4%), therapy resistant rejection (25 patients, 5.7%), or other causes (7 patients, 2.0%). Genotype distribution in patients with graft failure within 12 months after transplantation was similar to that in patients with a functioning graft at 12 months (II: 21, ID: 30, and DD: 17 for recipient ACE (I/D) genotype and II: 18, ID: 30, and DD: 20 for donor ACE (I/D) genotype, respectively).

The characteristics of the 367 patients with a functioning graft at 12 months after transplantation are shown in Table 1, grouped according to recipient ACE (I/D) genotype. The ACE (I/D) genotype distribution is in accordance with the Hardy-Weinberg equilibrium. No statistically significant differences in baseline characteristics were found between the three recipient ACE (I/D) genotype groups. At 12 months after transplantation, approximately 70% of the recipients used antihypertensive medication without significant differences between the three recipient ACE (I/D) genotype groups. Less than 10% of the
Figure 1. Death-censored graft survival curves for patients with a functioning graft at 12 months after renal transplantation (n=367) grouped according to recipient ACE (I/D) genotype. The number of patients for each recipient ACE (I/D) genotype still at risk during follow up is indicated at the bottom of the figure. Graft survival is not statistically significantly different between the 3 groups (log-rank test).

Figure 2. Death-censored graft survival curves for patients with a functioning graft at 12 months after renal transplantation (n=367) grouped according to donor ACE (I/D) genotype. The number of patients for each donor ACE (I/D) genotype still at risk during follow up is indicated at the bottom of the figure. Graft survival is not statistically significantly different between the 3 groups (log-rank test).
patients in all groups were treated with ACE inhibitors. Also, the number of antihypertensive agents used was not different between the different groups. Grouped according to donor ACE (I/D) genotype (109, 163 and 95 with II, ID and DD genotype, respectively) no differences in any of the above parameters were found either (data not shown).

Graft survival was not significantly different between the three recipient ACE (I/D) genotype groups (figure 1); during follow up 6, 14 and 7 patients lost their graft in the recipient II, ID and DD genotype group, respectively. Patient survival was not different between these groups; 8, 23, and 11 patients died with a functioning graft in the recipient II, ID and DD genotype group, respectively. Grouped according to donor ACE (I/D) genotype no significant difference in graft survival was found between these groups either (figure 2); 7, 9 and 11 patients lost their graft during follow up in the donor II, ID and DD genotype group, respectively. Patient survival also was not different between these three groups; 11, 17 and 14 died with a functioning graft in the donor II, ID and DD genotype groups, respectively. To identify risk factors for graft loss, univariate analysis of graft survival was performed by log-rank test. Creatinine clearance below 50 ml/min at 12 months (p<0.0001; relative risk (RR) 4.82; 95% confidence interval (CI), 2.21 to 10.52), proteinuria ≥ 0.5 g/24h at 12 months (p<0.0001; RR 8.86; 95% CI, 3.74 to 20.96), and an acute rejection episode in the first year after transplantation (p=0.046; RR 2.12; 95% CI, 1.00 to 4.52) were identified as variables associated with graft loss. The presence of ≥ 1 class I HLA mismatches (p=0.074; RR 2.74; 95% CI, 0.89 to 8.85) just failed to reach statistical significance.

To assess the relative importance and interaction of the different risk factors for graft loss, Cox proportional hazards analysis was performed. Creatinine clearance (p<0.0001) and a proteinuria of ≥ 0.5 g/24h (p<0.0001) at 12 months, recipient age (p=0.013), ≥ HLA class I mismatches (p=0.011), and recipient ACE (I/D) genotype (p=0.053) were identified as covariates with a p-value < 0.10. The model including these covariates had a r² value of 0.33.

A lower creatinine clearance at 12 months was significantly associated with an increased risk for graft loss during long-term follow up, as was a lower recipient age. Proteinuria ≥ 0.5 g/24h at 12 months and the presence of ≥ 1 class I HLA mismatches were also significantly associated with an increased risk for graft loss. The presence of one or two D alleles in the recipient ACE (I/D) genotype is associated with time to graft loss at borderline statistical significance (table 2, pag. 86).

To identify a possible interaction between different risk factors, subgroups of patients with a poor renal prognosis, i.e. those with creatinine clearance < 50 ml/min at 12 months after transplantation (n=108; 29% of all patients) or with proteinuria ≥ 0.5 g/24h (n=97; 26% of all patients), were analysed separately.

In the patients with a creatinine clearance < 50 ml/min at 12 months after transplantation 17 of 27 graft losses (63%) occurred. In these patients acute rejection episodes during the first year (54 of 108 (50%) vs 94 of 259 (36%), respectively; p=0.033) and proteinuria
≥ 0.5 g/24h at 12 months (40 of 108 (37%) vs 57 of 259 (22%), respectively; p=0.004) were more frequently found compared to patients with a creatinine clearance ≥ 50 ml/min at 12 months (n=259). Also, donor age was significantly higher in this group (45 ± 25.5 vs 34 ± 15.2 year; p<0.0001). Otherwise the characteristics at transplantation and 12 months after transplantation were similar. In particular, no significant differences in blood pressure or the

<table>
<thead>
<tr>
<th>Variable (unit)</th>
<th>Relative risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min)*</td>
<td>0.93 (0.91 to 0.95)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Proteinuria ≥ 0.5 g/24h*</td>
<td>7.48 (2.93 to 19.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>≥ 1 HLA class I mismatch</td>
<td>5.47 (1.47 to 20.3)</td>
<td>0.011</td>
</tr>
<tr>
<td>Recipient age (year)</td>
<td>0.96 (0.93 to 0.99)</td>
<td>0.013</td>
</tr>
<tr>
<td>Recipient ACE (I/D) genotype (per D allele)</td>
<td>1.73 (0.99 to 3.01)</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Given are results from 367 patients with functioning grafts at 12 months with time until graft failure as dependent variable including all covariates with a p-value < 0.10.

At 12 months after transplantation

Figure 3. Death-censored graft survival curves for patients with a creatinine < 50 ml/min at 12 months after transplantation (n=108) grouped according to recipient ACE (I/D) genotype. The number of patients still at risk during follow up is indicated at the bottom of the figure. Graft survival is not statistically significantly different between the 3 groups (log-rank test).
use of antihypertensives agents at 12 months after transplantation were found. *Figure 3* shows graft survival according to recipient ACE I/D genotype in the group with a creatinine clearance < 50 ml/min. Log-rank did not identify significant differences in graft survival between patients with different recipient or donor (data not shown) ACE (I/D) genotype in this subgroup. Cox proportional hazards analysis identified creatinine clearance, proteinuria ≥ 0.5 g/24h at 12 months, the presence of ≥ 1 HLA class I mismatch, recipient age, and recipient ACE (I/D) genotype as variables independently associated with time to graft loss (*table 3*).

In the patients with proteinuria ≥ 0.5 g/24h at 12 months 20 of 27 graft losses (74%) occurred. These patients had an increased frequency of acute rejection episodes during the first year (53 of 97 (55%) vs 95 of 270 (35%), respectively; p=0.0004), a lower creatinine clearance at 12 months (56 ± 26 vs 64 ± 20 ml/min, respectively; p=0.0086), and higher donor age at time of transplantation (40 ± 17 vs 36 ± 16 year, respectively; p=0.039) compared to the patients with proteinuria < 0.5 g/24h at 12 months (n=270). No significant differences in blood pressure or the use of antihypertensive agents at 12 months after transplantation were found. *Figure 4 (pag. 88)* shows graft survival according to recipient ACE (I/D) genotype in this subgroup. By log-rank test, no significant differences in graft survival between the 3 recipient ACE (I/D) genotype groups were found. In this subgroup, Cox proportional hazards analysis identified creatinine clearance at 12 months, recipient ACE (I/D) genotype, and recipient age as variables independently associated with time to graft loss (*table 4, pag. 88*).

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**Table 3** Relative risk with 95% confidence interval (CI) for renal graft loss calculated from the Cox proportional hazards analysis model.

<table>
<thead>
<tr>
<th>Variable (unit)</th>
<th>Relative risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min)*</td>
<td>0.89 (0.85 to 0.94)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Proteinuria ≥ 0.5 g/24h*</td>
<td>34.7 (3.97 to 303)</td>
<td>0.0013</td>
</tr>
<tr>
<td>≥ 1 HLA class I mismatch</td>
<td>9.74 (1.70 to 55.7)</td>
<td>0.011</td>
</tr>
<tr>
<td>Recipient age (year)</td>
<td>0.94 (0.90 to 0.99)</td>
<td>0.012</td>
</tr>
<tr>
<td>Recipient ACE (I/D) genotype (per D allele)</td>
<td>2.54 (1.18 to 5.47)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

* Given are the results from a subgroup of 108 patients with a creatinine clearance < 50 ml/min at 12 months with time until graft failure as dependent variable including all covariates with a p-value < 0.10 (r² of the model including these variables: 0.48).

* At 12 months after transplantation
Figure 4. Death-censored graft survival curves for patients with proteinuria > 0.5 g/24h at 12 months after transplantation (n=97) grouped according to recipient ACE (I/D) genotype. The number of patients at risk during follow up is indicated at the bottom of the figure. Graft survival is not statistically significantly different between the 3 groups (log-rank test).

Table 4 Relative risk with 95% confidence interval (CI) for renal graft loss calculated from the Cox proportional hazards analysis model*.

<table>
<thead>
<tr>
<th>Variable (unit)</th>
<th>Relative risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance (ml/min)*</td>
<td>0.91 (0.88 to 0.94)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>≥ 1 HLA class I mismatch</td>
<td>-</td>
<td>not significant</td>
</tr>
<tr>
<td>Recipient age (year)</td>
<td>0.96 (0.93 to 0.99)</td>
<td>0.0084</td>
</tr>
<tr>
<td>Recipient ACE (I/D) genotype (per D allele)</td>
<td>3.02 (1.39 to 6.54)</td>
<td>0.0051</td>
</tr>
</tbody>
</table>

* Given are the results from 97 patients with proteinuria ≥ 0.5 g/24h at 12 months with time until graft failure as dependent variable including all covariates with a p-value < 0.10* (r² of the model including these variables: 0.49).

* At 12 months after transplantation
Discussion

The presence of one or two D-alleles in the recipient, but not donor, ACE (I/D) genotype was found to be associated with a shorter time to graft loss in subgroups at high risk for graft loss from a retrospective cohort of 367 patients with a functioning graft 12 months after cadaveric renal transplantation. However, the relation between ACE (I/D) genotype and graft survival was not found by univariate analysis and could only be demonstrated after controlling for other risk factors for graft loss by multivariate analysis. For the whole cohort of 367 patients, this relation was only of borderline statistical significance.

Multiple risk factors have previously been identified for long-term renal graft loss. The inability to demonstrate an association between graft survival and either recipient or donor ACE (I/D) genotype for the whole population studied could mean that such an association is absent, or that its influence is obscured by selection bias or by interaction with other risk factors. Because our study was performed retrospectively, it may have been subject to selection bias, for instance due to effects of ACE (I/D) genotype on patient or kidney survival during the first year after transplantation. Genotype distribution in the 68 patients with graft loss or death within one year after transplantation was not different from the patients included in the study (i.e., those with a functioning graft at 12 months). Furthermore, in the group of patients studied, the distribution of ACE (I/D) genotypes was in accordance with the Hardy-Weinberg equilibrium and the D-allele frequency was similar to that in the normal population in the Netherlands. Although this does not exclude selection with certainty, it renders such selection effects less likely. A recently published cohort study in 269 renal transplant recipients also failed to show an association between recipient or donor ACE (I/D) genotype and graft survival. In contrast to our study, the latter study analysed graft survival from the moment of transplantation, and follow up was restricted to 30 months after transplantation. The same authors recently reported a large case-control study in which no differences were found in the recipient and donor ACE (I/D) genotype distribution in patients with graft survival less than 3 years compared to patients with graft survival of at least 3 years. The mean graft survival in the patients with graft survival less than 3 years, however, was only 5.2 months. The differences in time frame and, therefore, patient selection should be taken into account when comparing the results of these two analyses.

Progressive renal function loss after renal transplantation is a multifactorial process. To assess the relative importance of other risk factors for graft loss and the possible interaction of these risk factors with recipient or donor ACE (I/D) genotype, we applied different approaches. First, Cox proportional hazards analysis was used to account for the influence of other risk factors. This analysis showed the recipient, but not donor, ACE (I/D) genotype to be an independent determinant of graft loss in the whole population, albeit of borderline
statistical significance, with a relative risk of 1.73 per recipient D-allele present. The other risk factors identified in this analysis, a low creatinine clearance, a greater urinary protein loss, more HLA class I mismatches and a lower recipient age, are in accordance with previous findings, with the sole exception of recipient blood pressure. In this small study, blood pressure was not identified as an independent risk factor associated with graft survival, which is in contrast with larger studies. This indicates that the risk factor profile in our population is largely in line with reports from literature. This suggests that in renal transplant recipients, recipient ACE (I/D) genotype may influence time to graft loss, but that its effect is not prominent, or only operative in the presence of other risk factors. Another explanation could be that our study group was of inadequate size and the number of events too small to detect differences in graft survival associated with ACE (I/D) genotype alone.

In addition, we analysed the effect of ACE (I/D) genotype in two subgroups of our patient cohort with an increased renal risk identified by a low creatinine clearance (< 50 ml/min) or proteinuria (≥ 0.5 g/24h) at 12 months after transplantation, respectively. Interestingly, in these two subgroups, the presence of the recipient D-allele was associated with time to graft loss. This more clearcut effect of the D-allele in these subpopulations compared with the overall population might reflect the greater statistical power in subpopulations with a greater proportion of events. On the other hand, it could also implicate that the D-allele exerts an effect on graft survival only when other risk factors are simultaneously present. Current evidence on the nature of the renal risk associated with the D-allele, as also apparent from a large meta-analysis that addressed cardiovascular risk, indicates that the D-allele acts as a course modifying gene rather than as a disease inducing gene. Our data are consistent with this view because we did not find a difference in long-term graft survival, as demonstrated by the convergence of the survival curves after 5 years for the three recipient ACE (I/D) genotype groups, but did find a difference in time to graft loss in those destined to lose their graft. Thus, presence of a D-allele does not appear to enhance renal risk in itself, but once a sequence of events leading to progressive renal function loss is initiated by whatever cause, its course is more rapid in presence of the D-allele.

We did not find any association between graft loss and donor ACE (I/D) genotype. A comparison between the risk associated with donor versus recipient ACE (I/D) genotype could provide a clue as to the mechanism of risk modulation by ACE (I/D) genotype. The D-allele is associated with increased levels of circulating as well as tissue ACE and, possibly, although not uniformly, with enhanced conversion of angiotensin I in angiotensin II. It should be noted that it is still unknown whether the D-allele is just a marker or a mediator of increased renal risk. Nevertheless, the above findings, taken together with the key role of angiotensin II in the pathophysiology of progressive renal function loss, fuelled the hypothesis that increased circulating or tissue ACE activity is a mediator of the increased renal risk associated with the D-allele. An alternative hypothesis could be that
graft-infiltrating mononuclear cells, which *in vitroc* have been found to express ACE activity influenced by ACE (I/D) genotype polymorphism, are a major source of renal tissue ACE activity after renal transplantation\textsuperscript{21,22}. The present results, however, do not support a role for donor derived renal tissue ACE as a mechanism of risk modulation by ACE (I/D) genotype in renal transplant recipients. Finally, like many studies on the influence of genetic polymorphisms like ACE (I/D) genotype, our study was a retrospective one. Clearly, to allow for definite conclusions, large prospective studies are mandatory.

In conclusion, in patients with a high risk for graft loss, we found an association between the recipient, but not donor, ACE gene D-allele and time to graft loss. This suggests that an adverse effect of the D-allele on renal prognosis is present, but only becomes manifest when other risk factors for graft loss are simultaneously present.

**Acknowledgement**

References


