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Risk Factors for Long-Term Renal Survival after Renal Transplantation: A Role for Angiotensin-Converting Enzyme (Insertion/Deletion) Polymorphism?

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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/deletion [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 mo after transplantation in a cohort of 367 patients transplanted between 1987 and 1994 with at least 2 yr of follow-up. Mean follow-up was 58 mo. 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/24 h at 12 mo (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 the study population. This suggests that the effect of ACE (I/D) genotype on graft survival only becomes apparent when other risk factors are simultaneously present.

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 (1,2). Some of these factors, such as HLA-mismatching and previous rejection episodes, are specific for renal transplants, whereas others, such as high BP 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 (3–7). 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 (8).

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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 the mechanism of action of ACE activity in renal disease progression (9,10). 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 mo 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.

Materials 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 mo 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 h, followed by oral cyclosporin A (10 mg/kg) in two divided doses, with dose adjustments to obtain trough levels of

200 to 250 ng/ml for the first 3 mo and 150 to 200 ng/ml thereafter, and 20 mg/d prednisolone, tapered over 8 wk to a maintenance dose of 10 mg/d. Patients with anti-HLA antibodies (maximum panel reactivity >60%) received induction with murine monoclonal anti-CD3 antibody (OKT3, 5 mg/d) for 12 d followed by cyclosporin A started at day 10, azathioprine (1.5 mg/kg per d), and prednisolone (1 mg/kg per d initially and tapered to 10 mg/d after 8 wk). Acute rejection episodes diagnosed on clinical findings or by renal biopsy were treated with maximal two courses of 1000 mg of methylprednisolone intravenously for 3 consecutive days. Steroid-resistant or acute vascular rejection was treated with rabbit antithymocyte globulin (4 mg/kg on alternating days for 10 d). ACE (I/D) genotype was determined by PCR on stored lymphocytes from donor and recipient (11). Mistyping was checked by intron-specific primers as described by Shanmugam *et al.* (12). Serum and urinary creatinine concentrations and 24-h urinary protein excretion were determined by standard laboratory techniques.

Statistical Analyses

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 analyzed as pure graft survival. Data are given as means with SD. Differences in categorical variables between groups were tested with the χ^2 test. Differences in continuous variables between two groups were tested with the *t* test or the nonparametric Wilcoxon rank sum test, when appropriate. Differences in continuous variables between more than two groups were tested by ANOVA or the nonparametric Kruskal–Wallis test. Post tests were performed with the Bonferroni method. Differences in survival between groups were tested by the log-rank 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, ischemia times, number of acute rejection episodes, and creatinine clearance, BP, the use of anthypertensive medication, and proteinuria at 12 mo 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 center 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 mo 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 (seven patients, 2.0%). Genotype distribution in patients with graft failure within 12 mo after transplantation was similar to that in patients with a functioning graft at 12 mo (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 mo 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 (13). No statistically significant differences in baseline characteristics were found between the three recipient ACE (I/D) genotype groups. At 12 mo 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 patients in all groups were treated with ACE inhibitors. Also, the number of antihypertensive agents used was not different between the 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

Table 1. Baseline characteristics at the moment of cadaveric transplantation and renal function and BP in 367 patients with functioning grafts at 12 mo grouped according to recipient ACE (I/D) genotype[a]

Recipient ACE (I/D) genotype	II	ID	DD
n	91	187	89
Male/Female	56/35	102/85	55/34
Recipient age (yr)	44 ± 12	45 ± 13	43 ± 13
Donor age (yr)	36 ± 16	37 ± 15	38 ± 17
Total no. of HLA mismatches	1.2 ± 0.9	1.2 ± 0.9	1.3 ± 1.0
No. of HLA class II mismatches	0.3 ± 0.6	0.2 ± 0.4	0.1 ± 0.3
Acute rejection episodes in first year (%)	36	41	44
Serum creatinine level (μmol/L)[b]	170 ± 85	158 ± 59	171 ± 87
Creatinine clearance (ml/min)[b]	61 ± 22	61 ± 21	65 ± 25
Proteinuria ≥0.5 g/24 h (%)[b]	31	24	28
Mean arterial BP (mmHg)[b]	112 ± 13	113 ± 12	113 ± 13
Antihypertensive medication (%)[b]	70	68	74
Follow-up (mo)	59 ± 24	58 ± 24	55 ± 25

[a] Results are given as mean ± SD. ACE, angiotensin-converting enzyme; I/D, insertion/deletion.
[b] At 12 mo after transplantation.

three recipient ACE (I/D) genotype groups (Figure 1): During follow-up, six, 14, and seven patients lost their graft in the recipient II, ID, and DD genotype groups, respectively. Patient survival also was not different between these groups: Eight, 23, and 11 patients died with a functioning graft in the recipient II, ID, and DD genotype groups, respectively. Grouped according to donor ACE (I/D) genotype, no significant difference in graft survival was found between these groups either (Figure 2): Seven, nine, and 11 patients lost their graft during follow-up in the donor II, ID, and DD genotype groups, 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 <50 ml/min at 12 mo ($P < 0.0001$; relative risk [RR] 4.82; 95% confidence interval [CI], 2.21 to 10.52), proteinuria ≥0.5 g/24 h at 12 mo ($P < 0.0001$; 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 mismatch ($P = 0.074$; 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/24 h ($P < 0.0001$) at 12 mo, recipient age ($P = 0.013$), ≥1 HLA class I mismatch ($P = 0.011$), 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 an r^2 value of 0.33. A lower creatinine clearance at 12 mo 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/24 h at 12 mo and the presence of ≥1 class I HLA mismatch 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).

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 mo after transplantation ($n = 108$; 29% of all patients) or with proteinuria ≥0.5 g/24 h ($n = 97$; 26% of all patients), were analyzed separately.

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

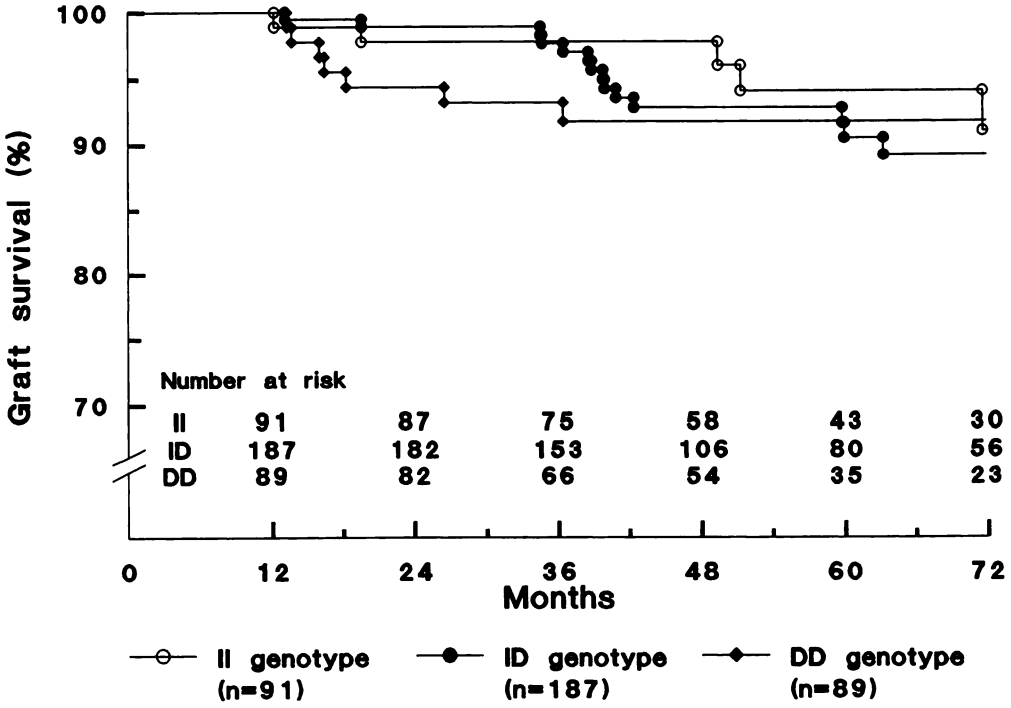

Figure 1. Death-censored graft survival curves for patients with a functioning graft at 12 mo after renal transplantation ($n = 367$) grouped according to recipient angiotensin-converting enzyme (ACE) (insertion/deletion [I/D]) genotype. The number of patients for each recipient ACE (I/D) genotype still at risk during follow-up is indicated at the bottom of the figure. Graft survival is not significantly different between the three groups (log-rank test).

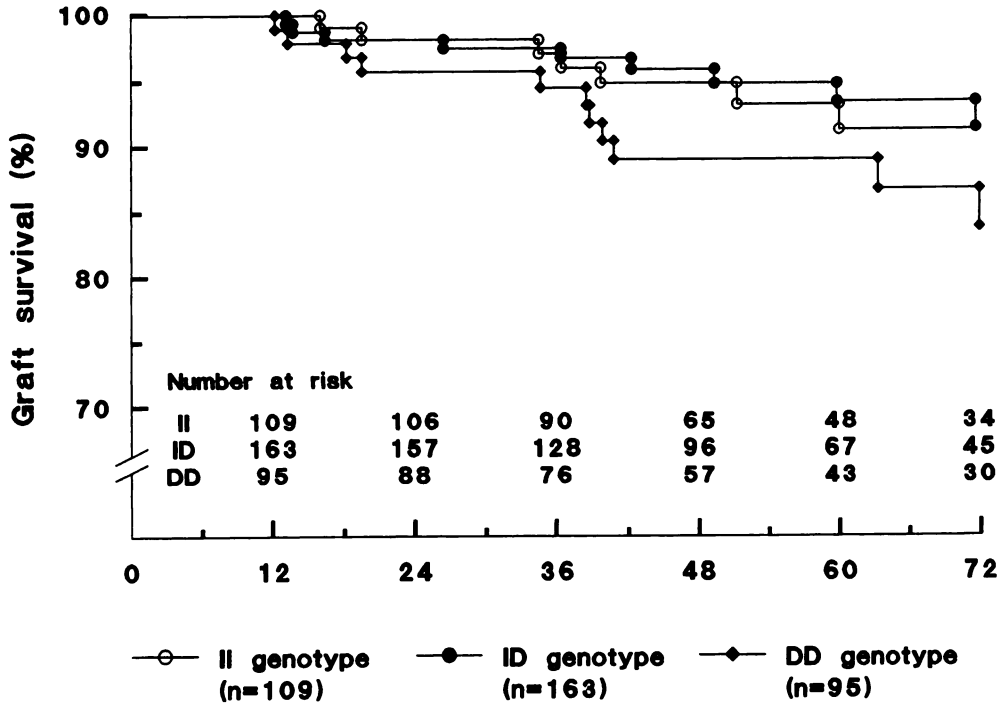

Figure 2. Death-censored graft survival curves for patients with a functioning graft at 12 mo after renal transplantation (n = 367) grouped according to donor ACE (I/D) genotype. The number of patients for each donor ACE (I/D) genotype still at risk during follow-up is indicated at the bottom of the figure. Graft survival is not significantly different between the three groups (log-rank test).

Table 2. Relative risk with 95% confidence interval (CI) for renal graft loss calculated from the Cox proportional hazards analysis model[a]

Variable	Relative Risk (95% CI)	P Value
Creatinine clearance (ml/min)[b]	0.93 (0.91 to 0.95)	<0.0001
Proteinuria ≥0.5 g/24 h[b]	7.48 (2.93 to 19.1)	<0.0001
≥1 HLA class I mismatch	5.47 (1.47 to 20.3)	0.011
Recipient age (yr)	0.96 (0.93 to 0.99)	0.013
Recipient ACE (I/D) genotype (per D-allele)	1.73 (0.99 to 3.01)	0.053

[a] Given are results from 367 patients with functioning grafts at 12 mo with time until graft failure as dependent variable including all covariates with a P value <0.10.

[b] At 12 mo after transplantation.

agents at 12 mo 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/24 h at 12 mo, 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/24 h at 12 mo, 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%] *versus* 95 of 270 [35%], respectively; P = 0.0004), a lower creatinine clearance at 12 mo (56 ± 26 *versus* 64 ± 20 ml/min, respectively; P = 0.0086), and higher donor age at time of transplantation (40 ± 17 *versus* 36 ± 16 yr, respectively; P = 0.039) compared to the patients with proteinuria <0.5 g/24 h at 12 mo (n = 270). No significant differences in BP or the use of antihypertensive agents at 12 mo after transplantation were found. Figure 4 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 three recipient ACE (I/D) genotype groups were found. In this subgroup, Cox proportional hazards analysis identified creatinine clearance at 12 mo, recipient ACE (I/D) genotype, and recipient age as variables independently associated with time to graft loss (Table 4).

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 mo 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.

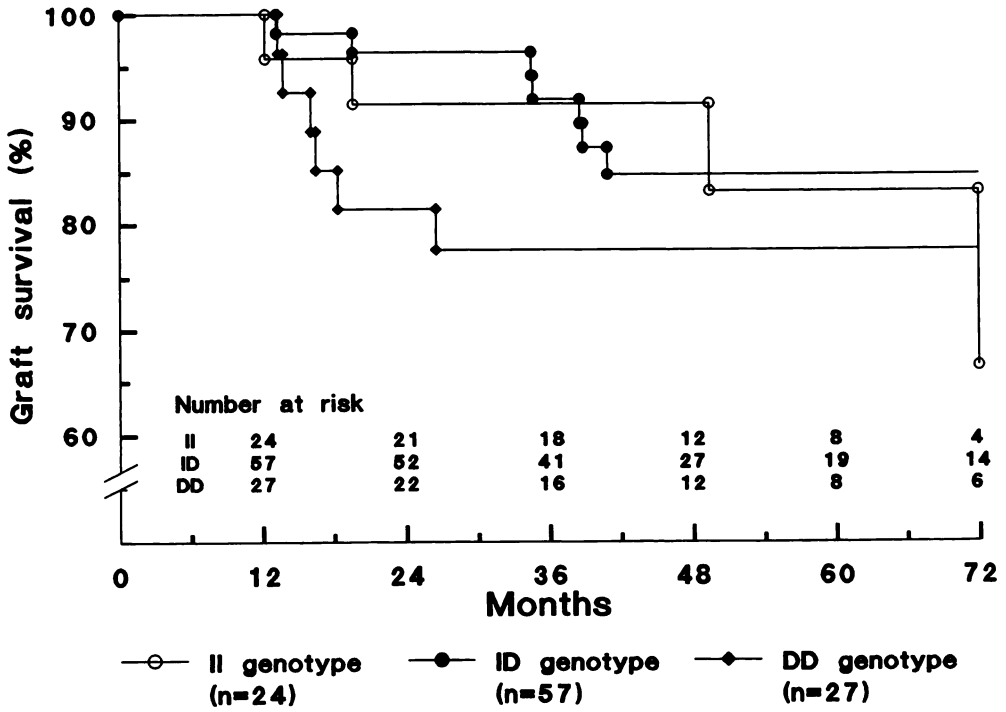

Figure 3. Death-censored graft survival curves for patients with a creatinine <50 ml/min at 12 mo after transplantation (n = 108) grouped according to recipient ACE (I/D) genotype. The number of patients still at risk during follow-up is indicated at the bottom of the figure. Graft survival is not significantly different between the three groups (log-rank test).

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

Variable	Relative Risk (95% CI)	*P* Value
Creatinine clearance (ml/min)[b]	0.89 (0.85 to 0.94)	<0.0001
Proteinuria ≥0.5 g/24 h[b]	34.7 (3.97 to 303)	0.0013
≥1 HLA class I mismatch	9.74 (1.70 to 55.7)	0.011
Recipient age (yr)	0.94 (0.90 to 0.99)	0.012
Recipient ACE (I/D) genotype (per D-allele)	2.54 (1.18 to 5.47)	0.017

[a] Given are results from a subgroup of 108 patients with a creatinine clearance <50 ml/min at 12 mo with time until graft failure as dependent variable including all covariates with a *P* value <0.10 (r^2 of the model including these variables: 0.48).
[b] At 12 mo after transplantation.

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 1 yr after transplantation was not different from the patients included in the study (*i.e.*, those with a functioning graft at 12 mo). 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 (14). 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 (15). In contrast to our study, the latter study analyzed graft survival from the moment of transplantation, and follow-up was restricted to 30 mo 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 yr compared to patients with graft survival of at least 3 yr (16). The mean graft survival in the patients with graft survival less than 3 yr, however, was only 5.2 mo. 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

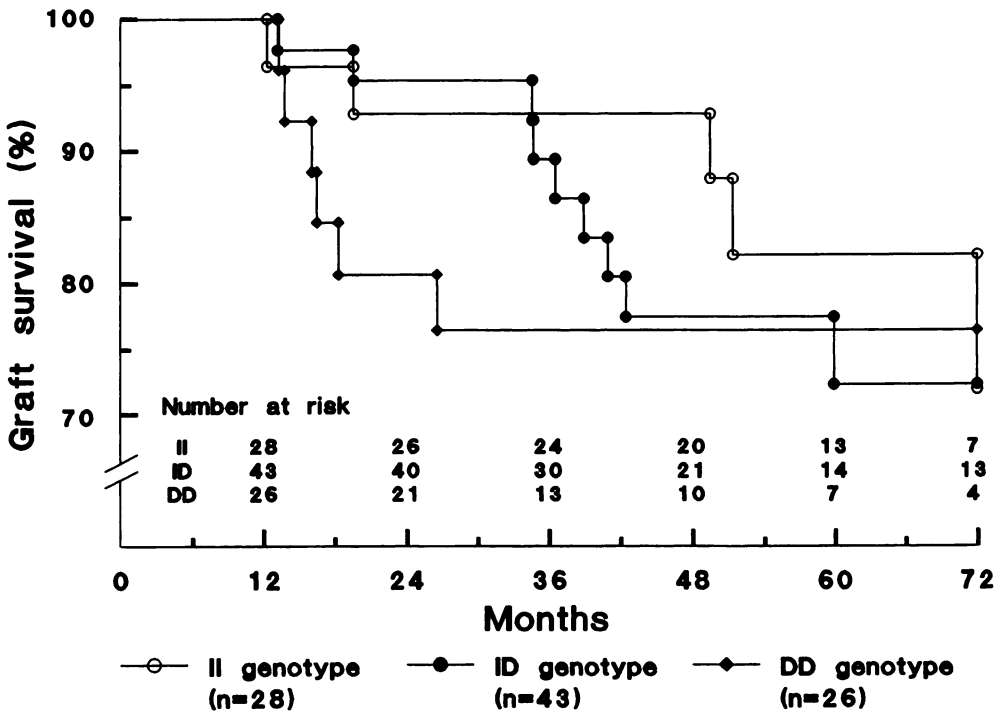

Figure 4. Death-censored graft survival curves for patients with proteinuria ≥0.5 g/24 h at 12 mo after transplantation (n = 97) grouped according to recipient ACE (I/D) genotype. The number of patients at risk during follow-up is indicated at the bottom of the figure. Graft survival is not significantly different between the three 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[a]

Variable	Relative Risk (95% CI)	P Value
Creatinine clearance (ml/min)[b]	0.91 (0.88 to 0.94)	<0.0001
≥1 HLA class I mismatch		NS
Recipient age (yr)	0.96 (0.93 to 0.99)	0.0084
Recipient ACE (I/D) genotype (per D-allele)	3.02 (1.39 to 6.54)	0.0051

[a] Given are results from 97 patients with proteinuria ≥0.5 g/24 h at 12 mo with time until graft failure as dependent variable including all covariates with a P value <0.10 (r^2 of the model including these variables: 0.49).

[b] At 12 mo after transplantation.

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 BP. In this small study, BP was not identified as an independent risk factor associated with graft survival, which is in contrast to larger studies (17). This indicates that the risk factor profile in our population is largely in line with reports from the literature (1,2). 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 analyzed 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/24 h) at 12 mo after transplantation, respectively. Interestingly, in these two subgroups, the presence of the recipient D-allele was associated with time to graft loss. This more clear-cut 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 indicate 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 (18), indicates that the D-allele acts as a course-modifying gene rather than as a disease-inducing gene (8). 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 yr 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, the 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 to angiotensin II (19,20). 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, fueled 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 vitro* 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 (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 such as 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.

References

1. Hostetter TH, Cohen JJ, Harrington JT, Madias NE, Zusman CJ: Chronic transplant rejection. *Kidney Int* 46: 266–279, 1994
2. Pirsch JD, Ploeg RJ, Gange S, D'Alessandro AM, Knechtle SJ, Sollinger HW, Belzer FO: Determinants of graft survival after renal transplantation. *Transplantation* 61: 1581–1586, 1996
3. van Essen GG, Rensma PL, de Zeeuw D, Sluiter WJ, Scheffer H, Apperloo AJ, de Jong PE: Association between angiotensin-converting enzyme gene polymorphism and failure of renoprotective therapy. *Lancet* 347: 94–95, 1996
4. McLaughlin KJM, Harden PN, Ueda S, Boulton-Jones JM, Connell JMC, Jardine AG: The role of genetic polymorphism of angiotensin converting enzyme in the progression of renal disease. *Hypertension* 28: 912–915, 1996
5. Parving HH, Jacobsen P, Tarnow L, Rossing PP, Lecerf L, Poirier O, Cambien F: Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin converting enzyme: Observational follow-up study. *Br Med J* 313: 591–594, 1996
6. Yoshida H, Kuriyama S, Atsumi Y, Tomonari H, Mitarai T, Hamaguchi A, Kubo H, Kawaguchi Y, Kon V, Matsuoka K, Ichikawa I, Sakai O: Role of the deletion polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J Clin Invest* 96: 2162–2169, 1995
7. Harden PN, Geddes C, Rowe PA, McIlroy JH, Boulton-Jones M, Rodger RSC, Junor BJR, Briggs JD, Connell JMC, Jardine AG: Polymorphism in angiotensin-converting enzyme gene and progression of IgA nephropathy. *Lancet* 345: 1540–1542, 1995
8. Navis GJ, de Jong PE, de Zeeuw D: Insertion/deletion polymorphism of the angiotensin converting enzyme gene: A clue to heterogeneity of renal prognosis and renal response to therapy? *Nephrol Dial Transplant* 12: 1097–1100, 1997
9. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An Insertion/Deletion polymorphism in the angiotensin-I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86: 1343–1346, 1990
10. Danser JAH, Schalekamp MADH, Bax WA, van den Brink AM, Saxena PR, Riegger GA, Schunkert H: Angiotensin-converting-enzyme in the human heart: Effect of the insertion/deletion polymorphism. *Circulation* 92: 1387–1388, 1995
11. Rigat B, Hubert C, Corvol P, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin-converting enzyme gene (DCP1). *Nucleic Acids Res* 20: 1433, 1992
12. Shanmugam V, Sell KW, Saha BK: Mistyping ACE heterozygotes. *PCR Methods Appl* 3: 120–121, 1993
13. Elston RC, Forthofer R: Testing for Hardy-Weinberg equilibrium in small samples. *Biometrics* 33: 536–542, 1977
14. Schmidt S, van Hooft IMS, Grobbee DE, Ganten D, Ritz E: Polymorphism of the angiotensin I converting enzyme gene and its predisposition to high blood pressure. *Hypertension* 21: 455–460, 1993
15. Beige J, Scherer S, Weber A, Engeli S, Offermann G, Opelz G, Distler A, Sharma AM: Angiotensin-converting enzyme genotype and renal allograft survival. *J Am Soc Nephrol* 8: 1319–1323, 1997
16. Beige J, Offermann G, Distler A, Sharma AM: Angiotensin-converting enzyme insertion/deletion genotype and long term renal allograft survival. *Nephrol Dial Transplant* 13: 735–738, 1998
17. Opelz G, Wujciak T, Ritz E: Association of chronic kidney graft failure with recipient blood pressure. *Kidney Int* 53: 217–222, 1998
18. Staessen JA, Wang JG, Ginocchio G, Saavedra AP, Soubrier F, Vlietinck R, Fagard R: The insertion/deletion polymorphism of the angiotensin converting enzyme gene and cardiovascular risk: A meta-analysis. *J Hypertens* 15: S86, 1997
19. Buikema H, Pinto YM, Rooks G, Grandjean JG, Schunkert H, van Gilst WH: The deletion polymorphism of the angiotensin-converting enzyme gene is related to phenotypic differences in human arteries. *Eur Heart J* 17: 787–794, 1996
20. Kitamura H, Moriyama T, Izumi M, Yokoyama K, Ueda N, Kamada T, Imai E: Angiotensin I-converting enzyme insertion/deletion polymorphism: Potential significance in nephrology. *Kidney Int Suppl* 55: S101–S103, 1996
21. Friedland J, Setton C, Silverstein E: Induction of angiotensin-converting enzyme in human circulating monocytes in culture. *Biochem Biophys Res Commun* 83: 843–849, 1978
22. Costerousse O, Allegrini J, Lopez M, Alhenc Gelas F: Angiotensin I-converting enzyme in human circulating mononuclear cells: Genetic polymorphism of expression in T-lymphocytes. *Biochem J* 290: 33–40, 1993