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ACE I/D polymorphism is associated with mortality in a cohort study of patients starting with dialysis

FEMKE VAN DER SMAN-DE BEER, CLAUDIA VERHAGEN, SASKIA M. ROMBACH, PETER BOORSMA, JEANNETTE G. VAN MANEN, JOHANNA C. KOREVAAR, RUUD VAN DEN BOGAARD, ELISABETH W. BOESCHOTEN, RAYMOND T. KREDIET, GERJAN J. NAVIS, JAN P. VANDENBROUCKE, and FRIEDO W. DEKKER, FOR THE NECOSAD STUDY GROUP

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; Hans Mak Institute, Naarden, The Netherlands; Departments of Clinical Epidemiology and Biostatistics, Clinical Genetics, and Nephrology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and Division of Nephrology, Department of Medicine, University Hospital Groningen, Groningen, The Netherlands

ACE I/D polymorphism is associated with mortality in a cohort study of patients starting with dialysis.

Background. In dialysis patients, only a few follow-up studies have addressed the relationship between the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene and mortality, but the available data are contradictory.

Methods. A cohort of 453 consecutive patients starting dialysis between January 1999 and January 2002 and participating in a Dutch multicenter prospective study was examined. Patients who died within 3 months after the start of dialysis were excluded. Patients were followed until date of death or censoring in November 2003.

Results. The ACE II, ID, and DD genotype frequencies were 24.3% ($N = 110$), 50.1% ($N = 227$), and 25.6% ($N = 116$). Besides a slightly higher number of Caucasians in the DD group, all other patient characteristics of the 3 ACE groups were similar at the start of dialysis. After adjustment for age, comorbidity, and ethnic background, patients with the ID and DD genotype showed an increased hazard ratio (HR) for all-cause mortality of 1.55 (95% CI 1.00–2.42) and 2.30 (95% CI 1.41–3.75), compared to patients with the II genotype. Slightly lower HRs were found for cardiovascular mortality. All groups of primary kidney disease showed a 2- to 3-fold increased adjusted HR for DD.

Conclusion. The DD genotype identifies dialysis patients at an increased risk for mortality.

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in dialysis patients [1, 2]. In the United States about 50% of deaths among dialysis patients is caused by CVD [2], whereas in Europe cardiac death accounts for 36% of deaths [1]. Compared to the general population, cardiovascular mortality is 10 to 20 times higher in dialysis patients [3].

Angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin-aldosterone system (RAAS). The ACE gene contains a diallelic polymorphism in intron 16. This insertion/deletion (I/D) polymorphism accounts for about 50% of the variation in plasma and tissue ACE levels [4–6]. Compared to subjects with the II genotype, DD homozygotes have the highest plasma and tissue ACE levels, whereas ID heterozygotes have intermediate levels [4–6].

In 1992, Cambien et al [7] found that the DD genotype is associated with myocardial infarction. Since then numerous studies have investigated the possible role of I/D polymorphism as cardiovascular risk factor in various study populations, but consensus of opinion was not reached. Three meta-analyses showed a positive association between the DD genotype and CVD [8–10]. However, these findings were contradicted by a meta-analysis of Agerholm-Larsen et al [11], who performed sensitivity analyses on small and large studies separately.

In patients with renal failure, most studies have focused on the impact of I/D polymorphism on the progression of renal disease. A review published in 1999 [12], but also more recent prospective follow-up studies [13, 14], showed that the DD genotype is associated with increased progression of renal failure towards end-stage renal disease (ESRD). The effect of I/D polymorphism on the occurrence of CVD in renal disease is less pronounced. A positive association of the DD genotype with left ventricular hypertrophy [15], fatal and nonfatal CVD [16], and cerebrovascular disease [17] has been reported in ESRD patients, whereas others did not find such an effect [18–20].

Key words: end-stage renal disease, dialysis, angiotensin-converting enzyme, polymorphism, mortality.

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In dialysis patients, there are only a few follow-up studies available that have investigated the relationship between I/D polymorphism and mortality [16, 18, 20–22]. The results of these studies were contradictory, probably because most of these study populations consisted of patients who had been on dialysis treatment for a long time before the study was started. Furthermore, the 2 follow-up studies reporting a positive association between DD genotype and mortality were performed in diabetic dialysis patients [20] or in hemodialysis (HD) patients only [22]. These studies were both conducted in Japan, a country with a rather low D-allele frequency (about 38%) [20, 22] compared to Caucasian populations [23].

At the moment it is unclear whether the DD genotype is a risk factor for mortality in dialysis patients. Therefore, we have investigated the relationship between I/D polymorphism and all-cause and cardiovascular mortality in Dutch dialysis patients who were followed from the start of dialysis onwards (i.e., incident patients). Due to the large number of dialysis patients in our follow-up study, we were also able to study the ACE-related mortality risk in patients with various primary kidney diseases either treated with HD or peritoneal dialysis (PD).

METHODS

Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) patients

NECOSAD is a multicenter, prospective follow-up study in which incident ESRD patients are included consecutively at the time of initiation of dialysis. Eligibility criteria were: 18 years and older and no previous renal replacement therapy. All patients gave informed consent before inclusion. All local medical ethics committees gave their approval to the study.

For the current analyses, data were used from patients who were included between January 1999 and January 2002 in dialysis centers that gave permission for DNA analyses (19 out of 38 participating centers). Patients who died within 3 months after the start of dialysis were excluded from the current analyses because other than dialysis-related clinical factors might have contributed to their rapid decease. Patients were followed until date of death or date of censoring. Surviving patients were considered to have their survival times censored at the date of leaving the study as a result of a renal transplant, a transfer to a nonparticipating dialysis center, withdrawal from the study, or at the end of the follow-up period (November 2003).

Comparison group

Data of 472 Dutch healthy adult control subjects from the Leiden Trombophilia Study (LETS) were used for comparison of ACE genotype and allele distribution with the NECOSAD patients. The LETS is a large population-based case-control study on risk factors for venous thrombosis [24, 25]. Control subjects were friends or partners of patients with venous thrombosis from the Leiden, Amsterdam, and Rotterdam area. Mean age of control subjects was 45 years (range 15–72 years), and 57% was female.

Demographic and clinical data of NECOSAD patients

The following data were collected between 4 weeks prior and 2 weeks after the start of chronic dialysis treatment: age, gender, ethnic background, primary kidney disease, systolic and diastolic blood pressure, comorbidity, body mass index (BMI), and modality. A blood sample and 24-hour urine sample were obtained simultaneously. Serum albumin, plasma creatinine, and plasma urea levels were determined. Urea and creatinine were also analyzed in the urine sample. Renal function at 3 months after start of chronic dialysis treatment was expressed as glomerular filtration rate (GFR), calculated as the mean of creatinine and urea clearances, adjusted for body surface area (mL/min/1.73m^2).

Primary kidney disease and causes of death were classified according to the codes of the European Renal Association-European Dialysis and Transplantation Association [1]. The following codes were designated as cardiovascular mortality: 0 (cause of death uncertain/not determined), 11 (myocardial ischemia and infarction), 12 (hyperkalemia), 14 (other causes of cardiac failure), 15 (cardiac arrest, cause unknown), 16 (hypertensive cardiac failure), 17 (hypokalemia), 18 (fluid overload), 22 (cerebrovascular accident), 26 (hemorrhage from ruptured vascular aneurysm), and 29 (mesenteric infarction). Comorbidity was defined according to the risk criteria of Khan et al [26]. The Khan index is a combination of age and comorbidity leading to three risk groups: low, medium, and high.

DNA isolation and ACE I/D polymorphism genotyping

Genomic DNA was isolated from peripheral blood with the Puregene® DNA isolation kit (Gentra, Minneapolis, MN, USA). To identify carriers of the I/D polymorphism in intron 16 of the ACE gene, a two-step polymerase chain reaction (PCR) was performed with the following primers [27]: GIIS: 5′-CTC AAG CAC GCC CCT CAC AGG ACT G-3′, GAS: 5′- GAT GTG GCC ATC ACA TTC GTC AGA T-3′ and FYM: 5′-ATC ACG AGG TCA GGA GAT CGA GAC-3′. Primers GIIS and GAS were used in the first PCR step, yielding a PCR product of 274 bp for DD homozygotes, PCR products of 274 and 561 bp for ID heterozygotes, and a PCR product of 561 bp for II homozygotes. Because the D-allele in heterozygous samples is preferentially amplified, mistyping between DD and ID is possible. Therefore, a second

PCR step (using GIIS and FYM primers) that recognizes an insertion specific sequence, was performed on each sample. This reaction yields a PCR product (376 bp) only in the presence of an I-allele.

The PCR conditions for both PCR steps were similar. All reactions were performed with 8 pmol of each primer in a final volume of 25 μL containing 10 mmol/L Tris-HCl (pH 9.0), 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.01% (w/v) gelatin, 0.1% Triton X-100, 0.5 units *Taq* polymerase (SuperTaq; HT Biotechnology, Ltd., Cambridge, UK), 1 mol/L betaïne, 2% DMSO, 1.25 μL of each dNTPs (Roche Diagnostics, Basel, Switzerland), and 50 to 100 ng genomic DNA. Amplification was performed for 35 cycles of 35 sec at 94°C, 40 sec at 62°C, and 45 sec at 72°C, with an initial denaturation period of 3 minutes. After the last cycle a final extension period of 7 minutes at 72°C was used.

Statistical analysis

Hardy-Weinberg equilibrium was calculated using the gene-counting method and differences were assessed by chi-square test. Chi-square test was applied to compare genotype and allele frequencies between NECOSAD patients and the comparison group.

In patients, differences between ACE groups were tested with the chi-square test for dichotomous and categorical variables, and analysis of variance for continuous variables. The survival curve of each ACE group was determined with the Kaplan-Meier method. The log rank test was used to determine differences between survival curves. Hazard ratios (HRs) were calculated by Cox proportional-hazard regression analysis from the 3-month visit onward. Patients with the II genotype were used as reference group. HRs were adjusted for age at the start of dialysis, comorbidity, and ethnic background.

To evaluate whether the association between ACE genotype and mortality might be the same or different according to dialysis modality, Cox regression analysis was performed using a model with 5 dummy variables as covariates: PD ID, PD DD, HD II, HD ID, and HD DD. The sixth dummy variable, PD II, was not included in the Cox regression model because this group was used as reference group. HRs were adjusted for age at the start of dialysis, comorbidity, ethnic background, and residual GFR (measured at 3 months after the start of dialysis). A difference in effect was evaluated by calculating whether the differences in HR departed from additivity [28].

All statistical analyses were performed with SPSS statistical software (version 11; SPSS, Chicago, IL, USA).

RESULTS

Between January 1999 and January 2002, 532 patients were included in NECOSAD centers that gave permission for DNA analyses. Seventy-nine out of 532 patients (15%) were not genotyped due to various reasons. In 9 out of these 79 patients either the DNA isolation or the ACE genotyping failed. Because full blood sampling for DNA analysis in NECOSAD started in March 2000, we missed 70 patients that had left the study before blood could be drawn: 28 patients had died, 20 left the study for a kidney transplantation, 14 refused further participation in the study, in 1 patient kidney function recovered, and 7 patients were missed for other reasons. The 79 patients who were not genotyped showed similar baseline characteristics as the 453 genotyped patients but had a significantly lower BMI (23.8 ± 4.6 vs. 25.5 ± 4.8 kg/m^2, $P = 0.004$; data not shown). Further statistical analyses were performed on the 453 genotyped patients.

Table 1. ACE genotype and allele distribution

	NECOSAD					
	All patients ($N = 453$)		Caucasians only ($N = 415$)		Comparison group ($N = 472$)	
ACE	*N*	%	*N*	%	*N*	%
II	110	24.3	96	23.1	112	23.7
ID	227	50.1	208	50.1	235	49.8
DD	116	25.6	111	26.7	125	26.5
	$P = 0.95$[a]		$P = 0.98$[b]			
I-allele	447	49.3	400	48.2	459	48.6
D-allele	459	50.7	430	51.8	485	51.4
	$P = 0.76$[a]		$P = 0.86$[b]			

[a] All NECOSAD patients versus the comparison group as determined with the chi-square test.
[b] Caucasian NECOSAD patients versus the comparison group as determined with the chi-square test.

Table 1 shows the ACE genotype and allele frequencies of all NECOSAD patients, Caucasian patients only, and the comparison group of healthy volunteers. I/D polymorphism distribution was within Hardy-Weinberg equilibrium in all groups. The ACE genotype and allele distribution of all NECOSAD patients and of Caucasian patients only was not different from the comparison group.

As shown in Table 2, patient characteristics of the 3 ACE groups were highly similar at the start of dialysis, except for a slightly higher number of Caucasians in the DD group.

The mean follow-up duration of the 453 patients was 2.3 ± 1.2 years, with a maximum of 4.6 years. A total of 154 of the 453 patients died during follow-up. A total of 90 patients received a renal transplant and were censored at the time of transplantation. Three patients left the study because of recovery of kidney function and were censored at that date. Twenty-five patients refused further participation, 2 patients were transferred to a nonparticipating dialysis center, and 8 patients left the study for other reasons. The survival times of those 35 patients were censored at the date of leaving the study. The survival times of the 171 patients who had not left the study at the end

Table 2. Baseline demographics of dialysis patients grouped according to ACE genotype

	ACE genotype		
	II ($N = 110$)	ID ($N = 227$)	DD ($N = 116$)
Age *years*	59 ± 14	59 ± 15	59 ± 14
Gender% *male*	64	58	63
Modality% *HD*	66	67	72
Ethnic background[a]% *Caucasian*	87	92	96
Primary kidney disease%			
Diabetes mellitus	15	21	10
Glomerulonephritis	16	10	12
Renal vascular disease	17	17	16
Other	52	52	61
Comorbidity[b]%			
Low	40	34	43
Medium	36	36	34
High	24	30	23
Systolic blood pressure *mm Hg*	147 ± 26	151 ± 25	148 ± 23
Diastolic blood pressure *mm Hg*	82 ± 12	83 ± 13	84 ± 13
Antihypertensive medication[c]% *yes*	84	88	86
Albumin *g/L*	35.3 ± 6.8	35.0 ± 6.1	35.7 ± 5.7
BMI kg/m^2	25.2 ± 4.5	25.8 ± 4.9	25.3 ± 4.8
Creatinine *μmol/L*	769 ± 300	743 ± 292	732 ± 253
Urea *mmol/L*	34.8 ± 12.8	32.1 ± 10.7	35.0 ± 11.3
rGFR $mL/min/1.73m^2$	3.5 ± 2.4	3.9 ± 2.7	4.6 ± 4.9
Renal Kt/V_{urea} per week	3.0 ± 1.3	2.9 ± 0.9	3.1 ± 1.0

BMI, body mass index; rGFR, residual glomerular filtration rate. All variables are measured at the start of dialysis, except rGFR and renal Kt/V_{urea}, which have been measured 3 months after the start of dialysis. Mean values ± SD are given for continuous variables.

[a] $P = 0.01$, determined with the chi-square test.

[b] Khan index.

[c] Includes the use of β-blockers, α1-blockers, nonselective β- and α-blockers, central α2-agonists, diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists, and vasodilators.

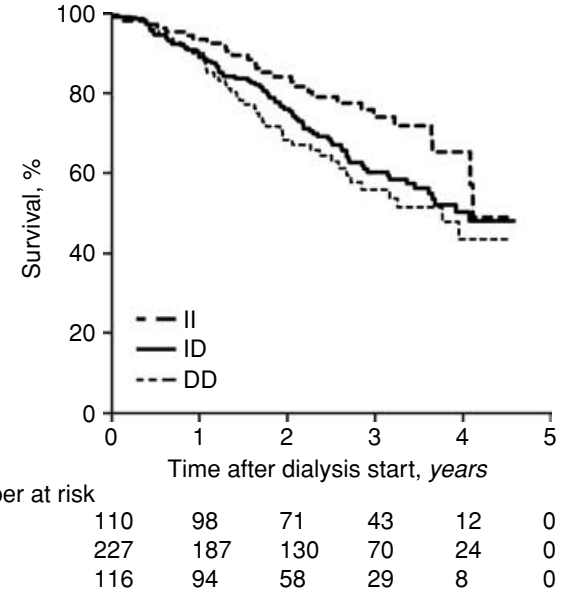
Table 3. Association between ACE genotype and all-cause and cardiovascular mortality

		Crude		Adjusted[a]	
ACE	*N*	HR	95% CI	HR	95% CI
		All-cause mortality			
II[b]	110	1.00		1.00	
ID	227	1.51	0.99–2.33	1.55	1.00–2.42
DD	116	1.81	1.13–2.91	2.30	1.41–3.75
		Cardiovascular mortality			
II[b]	110	1.00		1.00	
ID	227	1.30	0.67–2.54	1.33	0.67–2.67
DD	116	1.59	0.76–3.33	2.06	0.96–4.43

HR, hazard ratio; 95%CI, 95% confidence interval.

[a] Adjusted for age at the start of dialysis, comorbidity (Khan index), and ethnic background.

[b] Reference group.



Number at risk

II	110	98	71	43	12	0
ID	227	187	130	70	24	0
DD	116	94	58	29	8	0

Fig. 1. Kaplan-Meier survival curve.

of the follow-up period were censored at November 1, 2003. The 2-year survival was 76%. Fifty-nine out of 154 deaths (38%) were cardiovascular. Other causes of death were infections ($N = 16$), liver diseases ($N = 3$), social ($N = 27$), miscellaneous ($N = 25$), accident ($N = 1$), and other identified causes of death ($N = 3$). In 20 of the 154 deceased patients the cause of death was unknown.

Kaplan-Meier curves for all-cause mortality for each ACE genotype group are shown in Figure 1. There was a significant difference in crude survival between ACE genotypes ($P = 0.04$, log rank test). The 1-year mortality of II homozygotes, ID heterozygotes, and DD homozygotes was 7%, 11%, and 10%, respectively. The 2-year mortality was as follows: II subjects 16%, ID subjects 24%, and DD subjects 32%.

HRs for all-cause and cardiovascular mortality were calculated with Cox regression analyses. Table 3 shows the crude and adjusted HRs of each ACE genotype group. After adjustment for age, comorbidity, and ethnic background, the HRs for all-cause mortality for ID and DD were 1.55 (95% CI 1.00–2.42) and 2.30 (95% CI 1.41–3.75), compared to II homozygotes. Regarding cardiovascular mortality, patients with the ID and DD genotype showed adjusted HRs of 1.33 (95% CI 0.67–2.67) and 2.06 (95% CI 0.96–4.43). Because the I/D genotype distribution is known to vary across races [23], we have also determined HRs for the 415 Caucasians patients separately. Adjusted HRs of Caucasian patients were similar to those of the whole study population. Compared to II subjects, all-cause mortality risks for ID and DD subjects were 1.49 (95% CI 0.95–2.34) and 2.15 (95% CI 1.31–3.55). For cardiovascular mortality, ID and DD subjects had adjusted HRs of 1.32 (95% CI 0.64–2.72) and 1.91 (95% CI 0.86–4.23).

To study whether the association between ACE genotype and all-cause mortality differed between patients with various primary kidney diseases, HRs were calculated for each group of primary kidney disease

Table 4. Association between ACE genotype and all-cause mortality, stratified by primary kidney disease

		Crude		Adjusted[a]	
ACE	*N*	HR	95% CI	HR	95% CI
		Renal vascular disease ($N = 76$)			
II[b]	19	1.00		1.00	
ID	38	1.46	0.64–3.35	2.04	0.83–4.98
DD	19	1.89	0.76–4.72	3.03	1.12–8.21
		Diabetes mellitus ($N = 75$)			
II[b]	16	1.00		1.00	
ID	47	1.28	0.58–2.87	1.82	0.73–4.53
DD	12	1.83	0.63–5.33	1.99	0.58–6.82
		Other ($N = 302$)			
II[b]	75	1.00		1.00	
ID	142	1.67	0.87–3.19	1.44	0.73–2.84
DD	85	2.21	1.12–4.36	2.60	1.30–5.19

HR, hazard ratio; 95%CI, 95% confidence interval.
[a]Adjusted for age at the start of dialysis, comorbidity (Khan index), and ethnic background.
[b]Reference group.

Table 5. Effect of ACE genotype and modality on all-cause mortality

			Crude		Adjusted[a]	
ACE	Modality	*N*	HR	95% CI	HR	95% CI
II[b]	PD[b]	37	1.00		1.00	
ID	PD	74	2.82	0.96–8.24	2.58	0.85–7.84
DD	PD	32	2.36	0.67–8.38	3.09	0.86–11.12
II	HD	73	3.52	1.22–10.15	2.04	0.68–6.12
ID	HD	153	4.49	1.63–12.36	2.09	0.73–5.98
DD	HD	84	5.55	1.98–15.54	3.17	1.07–9.33

HR, hazard ratio; 95%CI, 95% confidence interval.
[a]Adjusted for age at start dialysis, comorbidity (Khan index), ethnic background, and residual GFR (measured three months after the start of dialysis).
[b]Patients with the II genotype on PD are the reference group.

separately (Table 4). All groups of primary kidney disease showed a 2- to 3-fold increased adjusted HR for DD subjects compared to II subjects with the same primary kidney disease.

Three hundred and ten out of 453 patients had HD as starting modality, whereas 143 patients started with PD. To study whether the association between ACE genotype and all-cause mortality was different for HD and PD patients, Cox regression analysis was performed using PD patients with the II genotype as reference group (Table 5). Among PD patients, DD subjects had an adjusted HR of 3.09, which means that the presence of the DD genotype increased the HR from 1.00 to 3.09. The HR difference, 2.09, is the effect of DD genotype in PD patients. Among HD patients, the adjusted HR increased from 2.04 in II subjects to 3.17 in DD subjects, resulting in an HR difference of 1.13. Thus, the HR difference among HD patients was smaller than among PD patients, which is predominantly due to the higher mortality of HD patients with the II genotype. However, the confidence intervals around the estimates were large.

DISCUSSION

In this prospective study of patients who were followed from the start of dialysis onward, we found a significantly increased all-cause mortality risk for ID and DD carriers. The DD genotype also tended to be associated with increased cardiovascular mortality. The positive association between mortality and the DD genotype was observed in all groups of primary kidney disease.

The limited amount of available data concerning the relationship between I/D genotype and mortality in dialysis patients have been contradictory. Two follow-up studies found a positive association between the D-allele and mortality [20, 22]. Three follow-up studies [16, 18, 21] that did not find an association were based on follow-up data of patients who were already on dialysis treatment for a varying amount of time. In these studies with so-called prevalent patients, selection bias might have occurred since a cohort of prevalent dialysis patients is characterized by an over-representation of patients who already survived relatively long on dialysis treatment. These long-term survivors might not be equally susceptible anymore to mortality risk factors compared to ESRD patients who had already died before they could be included in the study. As a consequence, the effect of the DD genotype in prevalent cohorts might be underestimated. To circumvent selection bias, our study was performed in so-called incident patients (i.e., ESRD patients who were followed from the start of dialysis onward).

One follow-up study among incident dialysis patients found a significant HR of 2.5 for DD subjects compared to the other ACE genotypes [20]. Although this study was performed in Japanese dialysis patients with diabetic nephropathy, the HR for all-cause mortality was in accordance with the findings in our overall study population and in our patients with diabetes mellitus. In addition, we observed an increased mortality risk for patients with renal vascular disease and other causes of primary renal failure, indicating that the DD genotype is a risk factor for mortality irrespective of the underlying kidney disease.

We were interested in the effect of ACE genotype on mortality in patients who had reached a stage of kidney disease requiring dialysis treatment. For this reason we have studied ACE-related mortality in ESRD patients who were followed from the start of dialysis onward. The possibility exists that some patients with the DD genotype have died from CVD before they could initiate dialysis treatment. In that case, we would expect to find an under-representation of the DD genotype in our dialysis population. To explore this possibility, we have compared the ACE genotype distribution of our dialysis population with a comparison group of healthy adults. We found no differences in genotype distribution between our dialysis patients and the comparison group.

Because the NECOSAD study has included HD and PD patients, we wondered whether the association between ACE genotype and mortality was different for HD and PD patients. We showed that the HR difference between II and DD subjects was larger among PD patients than among HD patients. This slight difference was predominantly due to a higher mortality of HD patients with the II genotype. Given the large confidence intervals around the estimates, these findings are uncertain. Furthermore, although we have adjusted the analyses for known confounders, some residual confounding might still be present. Thus, our findings should at present have no consequences for the choice of modality in DD subjects.

In our study, ACE genotype data were available for the majority of our included patients (85%). Although the 79 untested patients had a lower BMI than the 453 genotyped patients, there were no differences in important prognostic factors. Therefore, it does not seem very likely that serious distortion of our results has occurred.

In an observational follow-up study like NECOSAD, it is necessary to adjust for confounding factors like age and comorbidity because these confounders may distort the association between the risk factor of interest and the outcome. However, controlling for confounders is in principle unnecessary when studying the relationship between a genetic risk factor and outcome [29]. This is because in the population at large the polymorphism is not linked to age or any other possible confounder. However, age and age-related comorbidity could be confounders in our study if some ESRD patients with the DD genotype have already died before they could start with dialysis. In that case, dialysis patients with the DD genotype would be younger than those with the other ACE genotypes. In our study we did not observe a difference in age and comorbidity between the genotype groups. Although adjustment was probably unnecessary, adjustment for age and comorbidity further increased the HRs. This effect of adjustment must be the result of some association with age and comorbidity within the NECOSAD data that is not present in the population at large [29].

Population stratification (PS) is another form of confounding that may occur in genetic association studies. This "confounding by ethnicity" is commonly being referred to as one of the main reasons for the lack of replication across population-based studies [30]. Bias due to PS may occur when a distinct population comprises subgroups with different genetic background. For ACE I/D polymorphism it is known that there are ethnic differences in genotype distribution [23]. Today, there is growing evidence that the magnitude of bias caused by PS is small unless extreme differences exist in genotype frequencies across the ethnicities [30–32]. In our study, the genotype distribution of the total dialysis population was in Hardy-Weinberg equilibrium, indicating that the population's genotype and allele frequencies have remained unchanged over successive generations. Therefore, it seems unlikely that PS is a major problem in our study population. Furthermore, genotype frequencies of the total dialysis population and Caucasian patients were similar. Nevertheless, we have adjusted the HRs for ethnic background because we observed a slightly increased number of Caucasians in the DD group. In addition, restriction of the survival analyses to Caucasian patients only showed HRs similar to the ones of the whole study population.

In our study, cardiovascular HRs were slightly lower than all-cause HRs. This suggests that the DD genotype is not specifically related to cardiovascular death. In fact, at the moment, the mechanisms by which the D-allele could lead to an increased risk for CVD and cardiovascular mortality are unknown. Recently, several studies have provided evidence for a role of the I/D genotype in the progression and pathogenesis of noncardiovascular diseases, such as prostate cancer [33] and sepsis [34]. Finally, it should be noted that the I/D polymorphism is located within an intron. Therefore, the effect on mortality might be caused by another (functional) variant that is in linkage disequilibrium with the I/D polymorphism, and located within or near the ACE gene.

CONCLUSION

We have shown that the DD genotype is associated with an increased mortality risk in ESRD patients new on dialysis. This positive association was observed in all groups of primary kidney disease. Further research is necessary to determine the mechanisms by which the DD genotype leads to increased mortality.

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Reprint requests to Dr. F. van der Sman-de Beer, Leiden University Medical Center, Dept. of Clinical Epidemiology, C9-P, PO Box 9600, 2300 RC Leiden, The Netherlands.
E-mail: sman@lumc.nl

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