ACE I/D Polymorphism, Plasma ACE Levels, and Long-term Kidney Outcomes or All-Cause Death in Patients With Type 1 Diabetes

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PATHOPHYSIOLOGY/COMPLICATIONS

OBJECTIVE

The deletion (D) allele of the *ACE* insertion/deletion (I/D) polymorphism is a risk factor for diabetic kidney disease. We assessed its contribution to long-term kidney outcomes and all-cause death in patients with long-standing type 1 diabetes.

RESEARCH DESIGN AND METHODS

A total of 1,155 participants from three French and Belgian cohorts were monitored for a median duration of 14 (interquartile range 13) years. The primary outcome was the occurrence of end-stage kidney disease (ESKD) or a 40% drop in the estimated glomerular filtration rate (eGFR). Secondary outcomes were the individual components of the primary outcome, rapid decline in eGFR (steeper than –3 mL/min/1.73 m^2 per year), incident albuminuria, all-cause death, and a composite ESKD or all-cause death. Hazard ratios (HRs) for XD versus II genotype and for baseline plasma ACE levels were computed by Cox analysis. Genotype performance in stratifying the primary outcome was tested.

RESULTS

Genotype distribution was 954 XD and 201 II. The primary outcome occurred in 20% of XD and 13% of II carriers: adjusted HR 2.07 (95% CI 1.32–3.40; $P = 0.001$). Significant associations were also observed for rapid decline in eGFR, incident albuminuria, ESKD, all-cause death, and ESKD or all-cause death. Baseline plasma ACE levels were higher in XD carriers and significantly associated with an increased risk of the primary outcome. The *ACE* genotype enhanced net reclassification improvement (0.154, 95% CI 0.007–0.279; $P = 0.04$) and integrated discrimination improvement (0.012, 95%CI 0.001–0.021; $P = 0.02$) for primary outcome stratification.

CONCLUSIONS

The D-allele of the *ACE* I/D polymorphism was associated with an increased risk of major kidney events and all-cause death in patients with long-standing type 1 diabetes.

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The renal prognosis of patients with type 1 diabetes is primarily determined by diabetes duration and glycemic control (1,2), although other clinical (3), biological

(4,5), and genetic factors also play an important role (6,7). Candidate gene and genome-wide studies have shown that allelic variations in many biological systems contribute to the genetic risk of diabetic kidney disease (DKD) in people with type 1 diabetes (6–10). Early studies from our and other groups have shown that the Alu insertion/deletion (I/D) polymorphism (rs1799752) in intron 16 of the angiotensin 1 converting enzyme gene (*ACE*) was associated with diabetic nephropathy (11,12). The rationale for this association is strong. The variant is frequent and the deletion (D) allele has been shown to be associated with increased intracellular and circulating levels of ACE with a dominant or codominant effect, both in the general population (13,14) and in people with type 1 diabetes (11). The D-allele was associated with increased risk of diabetic nephropathy, with a dominant effect in type 1 diabetes (11,15) as well as in type 2 diabetes (16). Several levels of evidence support a causal link between the *ACE* I/D variant, circulating ACE levels, and DKD in type 1 diabetes, including cross-sectional (15,17) and prospective studies (18,19), clinical investigations (20), experimental manipulation of *ACE* (21), and randomized clinical trials with ACE inhibitors stratified by *ACE* I/D genotype (22–24).

However, data on the long-term impact of the *ACE* I/D polymorphism on kidney outcomes and all-cause death in type 1 diabetes are lacking. In the present investigation, we assessed associations between the *ACE* I/D polymorphism and the incidence of major kidney outcomes, including end-stage kidney disease (ESKD), and with all-cause death over a two-decade follow-up in three cohorts of patients with long-standing type 1 diabetes. Associations of baseline plasma ACE levels with genotypes and outcomes were also examined.

RESEARCH DESIGN AND METHODS

Study Participants

This is the latest follow-up of three French and Belgian cohorts of people with long-standing type 1 diabetes (duration of diabetes 23 ± 11 years at baseline) designed to study the genetic components of DKD: Génétique de la Néphropathie Diabétique (GENEDIAB) (15), Genesis France-Belgium (GENESIS) (25), and Survival Genetic Nephropathy (SURGENE) (18) studies. Previous interim reports were published (4,5,8,9,26–29). The GENEDIAB study enrolled patients with type 1 diabetes diagnosed for at least 5 years, with a proliferative or severe nonproliferative retinal disease requiring laser treatment (15). GENESIS was a family study in which index case subjects were people who had type 1 diabetes for at least 5 years and any stage of diabetic retinopathy (25). SURGENE was a single-center, prospective, follow-up study of all volunteers with type 1 diabetes attending the diabetes clinic at the CHU d'Angers, France, whatever their baseline retinal or renal condition (18). Characteristics of participants at baseline by cohort membership are shown in Supplementary Table 1. The Angers University Hospital (Angers, France) Ethics Committee approved the study protocol, and all participants gave written informed consent.

Participants were monitored until death or the latest clinical visit up to 31 May 2019. Clinical and biological data were obtained from hospital case records or by contacting the family physician of the participants. Vital status was cross-checked by contacting the civil registry of the birth place of participants. Data from 1,155 of the 1,347 participants enrolled in one of the three cohorts were analyzed. The earliest baseline set of data were considered for participants enrolled in more than one cohort.

In the present investigation, we excluded patients with a history of kidney replacement therapy (hemodialysis, peritoneal dialysis, or kidney transplantation, $n = 82$) or without estimated glomerular filtration rate (eGFR) data ($n = 1$) at baseline, those without kidney or mortality follow-up data ($n = 96$), and those for whom *ACE* I/D genotyping was not available ($n = 13$). The clinical profile of excluded participants is summarized in the Supplementary Material. We checked that *ACE* genotype distribution was not significantly different in excluded participants and in those who remained in the analysis. The number of patients evaluated for the primary outcome and for each secondary outcome is shown in the Supplementary Fig. 1.

Clinical Outcomes

The primary outcome was defined as the occurrence during follow-up of ESKD or a 40% drop in the eGFR, whichever occurred first (30). ESKD was defined as the requirement of hemodialysis or kidney transplantation, or eGFR <15 mL/min/1.73 m^2 at the end of the follow-up. Secondary outcomes were the individual components of the primary outcome, plus an eGFR slope steeper than -3 mL/min/1.73 m^2 per year (rapid decline in eGFR), the incidence of micro- or macroalbuminuria in the subset of participants with normoalbuminuria at baseline, all-cause death, and the combined outcome ESKD or all-cause death.

Laboratory and Clinical Procedures

ACE I/D genotypes were determined as previously described (15). Plasma ACE was measured centrally at baseline in a subset of 379 of the 1,155 participants using an immunoradiometric method quantifying ACE independently of its enzymatic activity (11,31). The following biological variables were measured centrally at baseline and locally during follow-up using standardized methods: HbA_{1c} by high-performance liquid chromatography, total cholesterol and triglycerides by colorimetric methods, urinary albumin concentration by nephelometry, and serum creatinine by a derivation of the Jaffé method with adjustment to the enzymatic method when it was introduced in routine practice. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration study equations for serum creatinine (32). Systolic and diastolic blood pressures (SBP, DBP) were measured with automatic devices by trained nurses or physicians as described earlier (15,18,25). Mean arterial pressure (MAP) was computed as DBP + 1/3(SBP − DBP).

Statistical Analysis

Categorical variables are expressed as the number of participants with the corresponding percentage. Continuous variables are expressed as mean ± SD or as median and interquartile range (IQR) for those with skewed distribution. Characteristics of participants at baseline were compared using χ^2, Fisher exact, ANOVA, or Wilcoxon tests.

Cox proportional hazards regression models were fitted to estimate associations of *ACE* genotype or baseline plasma

ACE with the outcomes. Hazard ratios (HRs) with associated 95% CIs were computed in these analyses for the XD (DD or ID) versus II genotype or for tertiles of baseline plasma ACE. Regression models were adjusted for relevant confounding variables, including cohort membership, sex, age, duration of diabetes, MAP, HbA_{1c}, eGFR, and use of ACE inhibitors and antihypertensive drugs at baseline (model 1). Use of antihypertensive drugs was expressed as the number of drug classes used by each participant. An extended regression model was also used as sensitivity analyses for all-cause death and ESKD or all-cause death outcomes (model 2). It included the covariates of model 1 plus BMI, tobacco smoking, history of cardiovascular disease (myocardial infarction or stroke), and use of lipid-lowering drugs at baseline. We also tested, as sensitivity analyses, the association between the *ACE* genotype and the risk of the primary outcome in each individual cohort. Interactions between the *ACE* genotype and cohort membership or the use of ACE inhibitors were assessed by including multiplicative interaction terms in the regression model. Of note, the use of angiotensin receptor blockers was infrequent in our cohorts at baseline ($n = 24$), and thus, we did not consider this class of drugs independently in the analysis. Because death during follow-up could compete with the occurrence of the kidney outcomes, we estimated the subdistribution HR (SHR) for the risk of the primary outcome and the risk of ESKD by *ACE* genotype, with all-cause death considered as a competing risk (33).

The Harrell C statistic, integrated discrimination improvement (IDI), and continuous net reclassification improvement (NRI) indices were computed to evaluate prognostic value of the *ACE* genotype on top of adjustment model 1 in the discrimination and classification of the primary outcome as assessed by survival methodology (34). A stepwise regression analysis with backward selection was used to assess the covariates contributing to the interindividual variance of baseline plasma ACE levels. Statistics were performed using SAS 9.4 and JMP Pro 14SW software (SAS Institute, Cary, NC) and Stata 13 software (StataCorp, College Station, TX). Two-sided P values of <0.05 were considered significant.

RESULTS

Primary Outcome

Genotype distribution in the study population was 400 DD (35%), 554 ID (48%), and 201 II (17%) (Hardy-Weinberg equilibrium $\chi^2 = 0.15$, $P = 0.70$). Characteristics of participants at baseline by *ACE* genotype (XD vs. II) are summarized in Table 1. The number of case subjects, incidence rates, and relative risks of outcomes by genotype are reported in Table 2. The primary outcome occurred in 195 of 1,039 participants (19%) for whom data were available, over a median follow-up of 14 (IQR 13) years, and corresponding to 13,614 person-years and an incidence rate of 14.3 (95% CI 12.4–16.5) per 1,000 person-years. Baseline characteristics of participants who presented or not the primary outcome during follow-up are reported in Supplementary Table 2. Detailed antihypertensive and lipid-lowering drugs at baseline by *ACE* genotype and by primary outcome during follow-up are reported in Supplementary Table 3.

The Kaplan-Meier outcome-free curve for the primary outcome during follow-up by *ACE* I/D genotype is shown in Fig. 1. The primary outcome occurred more frequently in XD than in II genotype carriers (20% vs. 13%): HR 1.64 (95% CI 1.09–2.58, $P = 0.01$). This association was stronger after adjustment for confounders (Table 2): adjusted HR 2.07 (95% CI 1.32–3.40, $P = 0.001$). The association remained significant when we considered all-cause death as competing risk further to adjusting for model 1: SHR 1.96 (95% CI 1.21–3.17, $P = 0.006$). No significant interaction between *ACE* genotype and use of ACE inhibitors was observed in these analyses ($P = 0.12$ for interaction). In sensitivity analysis, we looked for the association of the *ACE* I/D variant with the primary outcome in individual cohorts (Supplementary Table 4). The XD genotype association with increased risk for the primary outcome remained significant in the GENESIS and GENEDIAB cohorts but not in the SURGENE cohort, although no significant interaction was observed between cohort membership and *ACE* genotype in their association with the outcome. The performance of *ACE* I/D for predicting kidney prognosis in the whole study population was assessed. When added to model 1, *ACE* genotype improved IDI (0.012, 95% CI 0.001–0.021, $P = 0.02$) and NRI (0.154, 95% CI 0.007–0.279, $P = 0.04$), but not the Harrell C statistic index (change –0.0004, 95% CI –0.0006 to 0.005, $P = 0.89$) for risk stratification of the primary outcome (Supplementary Table 5).

Secondary Outcomes: Kidney Events

Incident ESKD occurred in 107 of 1,039 participants (10.3%) during follow-up. When adjusted for confounders, the relative risk of ESKD was significantly higher in XD than in II carriers: adjusted HR 2.06 (95% CI 1.16–3.94, $P = 0.01$) (Table 2). The association was impacted when we considered all-cause death as competing risk: SHR 1.90 (95% CI 0.96–3.81, $P = 0.07$). Rapid decline in eGFR occurred during follow-up in 180 of 984 participants (18.3%) and incident albuminuria in 175 of the 596 participants (29.4%) who were normoalbuminuric at baseline. The relative risk of both outcomes was significantly higher in XD than in II carriers (Table 2). The Kaplan-Meier outcome-free curve for the incidence of albuminuria during follow-up by *ACE* I/D genotype is shown in Supplementary Figure 2. No significant interaction between *ACE* genotype and cohort membership or use of ACE inhibitors was observed on the associations with incident ESKD, rapid decline in eGFR, or incident albuminuria. No association between *ACE* I/D genotype and the 40% drop in eGFR outcome was observed.

All-Cause Death

Among the 1,144 participants monitored for survival, 289 (25.3%) died during a median duration of follow-up of 17 (IQR 11 years), corresponding to 18,135 person-years. The incidence rate of all-cause death was 15.9 (95% CI 14.2–17.9) per 1,000 person-years. The relative risk of all-cause death was significantly higher in XD than in II carriers when adjusted for confounding covariates (model 1): adjusted HR 1.39 (95% CI 1.01–1.96, $P = 0.04$) (Table 2). A significant association of the XD genotype with the combined outcome ESKD or all-cause death was also observed: adjusted HR 1.58 (95% CI 1.16–2.18, $P = 0.003$). No significant interactions were observed on these associations between

Table 1—Characteristics of participants at baseline by *ACE* I/D genotype

		ACE genotype		
	All	XD	II	*P*
n (%)	1,155 (100)	954 (83)	201 (17)	
Cohort membership, *n* (%)				0.66
SURGENE	336 (29)	280 (29)	56 (28)	
GENEDIAB	337 (29)	273 (29)	64 (32)	
GENESIS	482 (42)	401 (42)	81 (40)	
Sex (women), *n* (%)	520 (45)	432 (45)	88 (44)	0.69
Age, years	40 ± 13	40 ± 13	41 ± 14	0.23
Age at diabetes diagnosis, years*	15 (14)	15 (14)	14 (13)	0.95
Diabetes duration, years	23 ± 11	23 ± 11	24 ± 11	0.37
BMI, kg/m^2	23.8 ± 3.4	23.9 ± 3.5	23.5 ± 3.2	0.12
Blood pressure				
Systolic, mmHg	132 ± 18	132 ± 18	131 ± 18	0.61
Diastolic, mmHg	76 ± 11	76 ± 11	75 ± 11	0.30
MAP, mmHg	94 ± 12	95 ± 12	94 ± 12	0.39
HbA_{1c}, %	8.8 ± 1.8	8.8 ± 1.9	8.8 ± 1.7	0.73
HbA_{1c}, mmol/mol	73 ± 20	73 ± 20	72 ± 19	0.73
Total cholesterol, mmol/L†	5.59 ± 1.41	5.58 ± 1.35	5.64 ± 1.66	0.70
Triglycerides, mmol/L*†	1.03 (0.64)	1.03 (0.67)	0.91 (0.60)	0.19
Serum creatinine, μmol/L	93 ± 54	92 ± 50	96 ± 68	0.32
eGFR, mL/min/1.73 m^2	89 ± 28	89 ± 28	88 ± 28	0.77
Urinary albumin concentration, mg/L*	14 (86)	14 (109)	11 (37)	0.12
Urinary albumin concentration stages, *n* (%)				0.11
Normoalbuminuria (<30 mg/L)	650 (56)	526 (55)	124 (62)	
Microalbuminuria (30–300 mg/L)	229 (20)	189 (20)	40 (20)	
Macroalbuminuria (>300 mg/L)	276 (24)	239 (25)	37 (18)	
Use of ACE inhibitors, *n* (%)	353 (31)	297 (31)	56 (28)	0.40
Use of any antihypertensive drug, *n* (%)	462 (40)	392 (41)	70 (35)	0.11
No. of classes of antihypertensive drugs	0.68 ± 0.99	0.69 ± 1.00	0.59 ± 0.98	0.19
Use of lipid-lowering drugs, *n* (%)	50 (4.3)	41 (4.3)	9 (4.5)	0.90
Tobacco smoking, *n* (%)‡	363 (31)	302 (32)	61 (31)	0.72
History of myocardial infarction, *n* (%)	47 (4.1)	42 (4.4)	5 (2.5)	0.24
History of stroke, *n* (%)	24 (2.1)	21 (2.2)	3 (1.5)	0.78
Plasma ACE levels, ng/mL§	464 ± 179	487 ± 183	358 ± 114	<0.0001

Quantitative data are expressed as mean ± SD or *median (IQR). The XD genotype represents the combined data of ID and DD genotype carriers of rs1799752. Statistics are Fisher exact, ANOVA, or Wilcoxon tests. $P < 0.05$ was significant. †Total cholesterol and triglycerides were measured at baseline only in GENEDIAB and SURGENE participants: 553 XD and 120 II carriers. ‡Current or former smokers. §Plasma ACE levels at baseline were measured in 310 XD and 69 II carriers.

ACE genotype and cohort membership or the use of ACE inhibitors. The use of an extended regression model in sensitivity analyses (model 2) had only a minimal impact on these results, with adjusted HRs of 1.38 (95% CI 1.00–1.95, $P = 0.046$) for all-cause death and 1.51 (95% CI 1.11–2.09, $P = 0.008$) for ESKD or all-cause death.

Baseline Plasma ACE and Outcomes During Follow-up

Baseline plasma ACE was 464 ± 179 ng/mL in the subset of participants for whom data were available. It was lower in II, intermediate in ID, and higher in DD carriers at 358 ± 114, 444 ± 162, and 550 ± 194 ng/mL, respectively ($P < 0.0001$). The *ACE* I/D polymorphism accounted for 15% of the interindividual variance of plasma ACE ($P < 0.0001$) in a stepwise regression analysis with backward selection. The use of ACE inhibitors was positively associated, and age at baseline was inversely associated with plasma ACE levels and accounted for 13% ($P < 0.0001$) and 2% ($P = 0.0009$), respectively, of its interindividual variance. Other covariates, such as cohort membership, sex, duration of diabetes, mean arterial pressure, HbA_{1c}, eGFR, urinary albumin concentration, and use of antihypertensive drugs at baseline did not have a significant independent impact on plasma ACE (data not shown). Primary and secondary outcomes by tertiles of baseline plasma ACE are reported in Table 3. The Kaplan-Meier outcome-free curve for the primary outcome during follow-up by tertiles of baseline plasma ACE is shown in Fig. 1. Plasma ACE was higher in individuals who presented the primary outcome during follow-up than in those who did not: 514 ± 179 vs. 450 ± 177 ng/mL ($P = 0.004$). The cumulative incidence of the primary outcome during follow-up was 19%, 36%, and 38% ($P =$

Table 2—Primary and secondary outcomes during follow-up by *ACE* I/D genotype

	Outcomes		Incidence rate	Crude model		Adjusted model 1	
	No, *n* (%)	Yes, *n* (%)	(95% CI)	HR (95% CI)	*P*	HR (95% CI)	*P*
Primary outcome							
II genotype	161 (87)	24 (13)	10.3 (6.9–15.3)	1		1	
XD genotype	683 (80)	171 (20)	15.2 (13.1–17.6)	1.64 (1.09–2.58)	0.01	2.07 (1.32–3.40)	0.001
ESKD							
II genotype	167 (90.3)	18 (9.7)	7.6 (4.8–12.1)	1		1	
XD genotype	765 (89.6)	89 (10.4)	7.8 (6.3–9.6)	1.15 (0.71–1.97)	0.58	2.06 (1.16–3.94)	0.01
40% drop in eGFR							
II genotype	152 (89)	18 (11)	8.3 (5.2–13.2)	1		1	
XD genotype	681 (84)	133 (16)	12.1 (10.2–14.3)	1.55 (0.97–2.63)	0.07	1.40 (0.87–3.82)	0.17
Rapid decline in eGFR							
II genotype	148 (87)	22 (13)	9.9 (6.5–15.1)	1		1	
XD genotype	656 (81)	158 (19)	14.1 (12.1–16.5)	1.56 (1.03–2.51)	0.03	1.56 (1.01–2.51)	0.04
Albuminuria							
II genotype	91 (81)	22 (19)	16.1 (10.3–21.9)	1		1	
XD genotype	330 (68)	153 (32)	21.4 (18.3–24.5)	1.40 (0.92–2.26)	0.12	1.60 (1.03–2.63)	0.04
All-cause death							
II genotype	153 (77)	46 (23)	15.1 (11.3–20.2)	1		1	
XD genotype	704 (74)	243 (26)	16.1 (14.2–18.3)	1.10 (0.81–1.53)	0.54	1.39 (1.01–1.96)	0.04
ESKD or all-cause death							
II genotype	141 (71)	58 (29)	19.7 (15.2–25.2)	1		1	
XD genotype	656 (69)	291 (31)	20.0 (17.9–22.5)	1.07 (0.81–1.43)	0.63	1.58 (1.16–2.18)	0.003

Primary outcome: ESRD or a 40% drop in eGFR during follow-up. Data are expressed as *n* (%) of participants. Incidence rates are expressed per 1,000 person-years. HRs (95% CIs) for the XD vs. the II genotype of rs1799752 computed by Cox regression analysis, adjusted for cohort membership (crude model), plus sex, age, duration of diabetes, MAP, HbA_{1c}, eGFR, and use of ACE inhibitors and antihypertensive drugs at baseline (adjusted model 1). The XD genotype represents the combined data of ID and DD genotypes. Genotypes were in Hardy-Weinberg equilibrium in the whole study population and in all outcome-related subsets of participants. $P < 0.05$ was significant.

0.008) for the first, second, and third tertiles of baseline plasma ACE, respectively. High plasma ACE levels remained significantly associated with the primary outcome after adjustment for confounders (HR 1.94, 95% CI 1.03–3.78, $P = 0.04$) for the third versus first tertile of plasma ACE distribution. The higher tertiles of plasma ACE distribution were also significantly associated with rapid decline in eGFR, a 40% drop in eGFR, the incidence of albuminuria, and the combined outcome ESKD or all-cause death (Table 3). Association with the combined outcome ESKD or all-cause death remained significant in sensitivity analyses with an extended regression model (model 2), with HRs of 1.59 (95% CI 1.06–2.39, $P = 0.02$) and 1.84 (95% CI 1.25–2.373, $P = 0.002$) for the third and second tertiles, respectively, versus the first tertile of plasma ACE distribution.

CONCLUSIONS

In the present investigation in people with long-standing type 1 diabetes, the *ACE* I/D polymorphism was found to be associated with major kidney outcomes over an ~15-year follow-up, and remarkably, with all-cause death over a slightly longer follow-up. These associations were independent of other DKD risk factors such as glycemic control and duration of diabetes at baseline. Carriers of the D-allele showed an increased risk of incidence of the primary outcome (ESKD or a 40% drop in eGFR) during follow-up. The D-allele was also significantly associated in secondary analyses with an increased risk of ESKD, a hard and highly relevant outcome, of incident albuminuria, and of a rapid decline in eGFR, a less severe outcome based on the slope of the eGFR variation throughout the study. The D-allele was also associated with an increased risk of all-cause death and of the combined outcome ESRD or all-cause death.

In the subset of participants for whom plasma ACE levels were measured at baseline, consistent associations were observed between plasma ACE levels and the primary outcomes and between the *ACE* I/D polymorphism and plasma ACE levels. These associations suggest a pattern of Mendelian randomization, which could not be properly tested in the present investigation conducted with a single genetic variant. However, our findings are consistent with a cause-to-effect relationship between *ACE* allelic variations, ACE expression, and kidney outcomes, including the risk for ESKD or all-cause death. Moreover, our data confirm and expand results from clinical (20) and experimental (21) investigations and clinical trials (22–24) supporting the causality of the association between the *ACE* I/D II genotype and protection against DKD in patients with type 1 diabetes.

We found no interaction between the *ACE* I/D genotype and the use of ACE inhibitors on the associations with any of the outcomes, while we and others reported the renal response to ACE inhibitors to be more beneficial in II than in ID or DD genotype carriers (22–24). The current study was observational, and prescription of ACE inhibitors or other antihypertensive drugs reflected principally the severity of DKD. However, after adjustment for the use of ACE inhibitors, the impact of *ACE* genotypes on kidney outcomes and mortality remained significant. Thus, the contribution of *ACE* I/D genotypes to the risk of outcomes seems to be

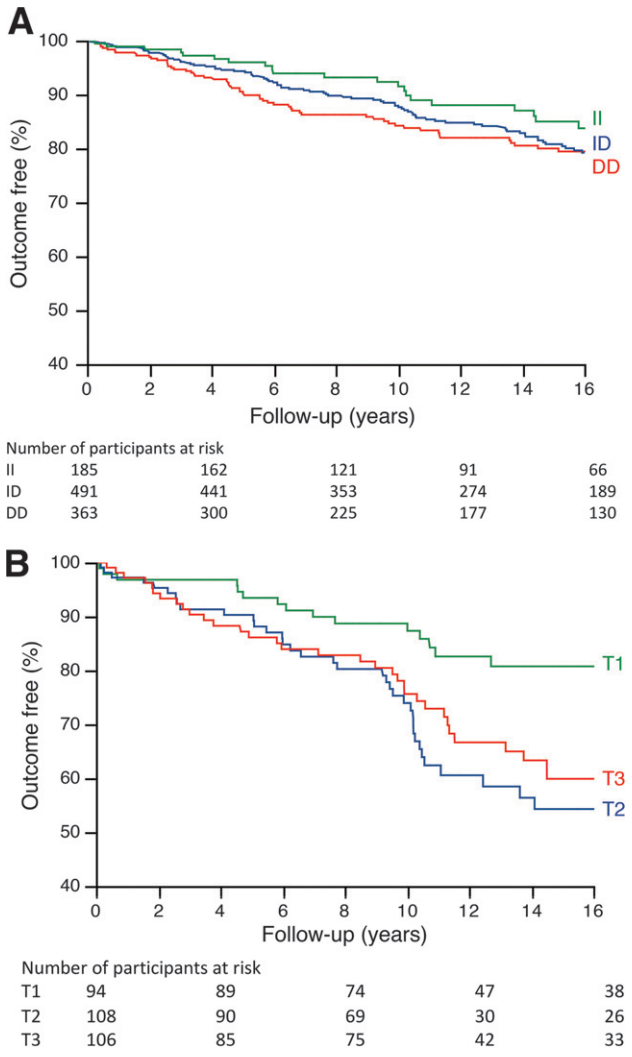

Figure 1—Kaplan-Meier outcome-free curves for the primary outcome during follow-up by *ACE* I/D genotype (*A*) or by tertiles of plasma ACE levels at baseline (*B*).

independent, at least in part, from pharmacological inhibition of ACE activity after the onset of the renal disease. In this regard, experimental and clinical studies have shown that ACE levels can have an effect on kidney development (35,36), thereby conditioning renal prognosis on the long-term.

Despite the significant association of the *ACE* I/D polymorphism with kidney outcomes, the performance of the variant for predicting kidney prognosis on the top of other relevant risk factors was modest. It was significant in NRI and IDI tests but not in the Harrell C statistic, which is less sensitive to changes of small magnitude (34). One possible explanation is that the I/D polymorphism does not account for all of the interindividual genetic variance of ACE levels (37) and thus reflects only partially the contribution of genetically determined ACE activity to renal prognosis. Moreover, the diabetes state per se is associated with increased plasma ACE levels, thereby minimizing the proportion of plasma ACE variance attributable to the I/D polymorphism (15). Also, allelic variations in many other genes contribute to kidney outcomes in type 1 diabetes (6–10). Finally, no association was observed in genome-wide association studies (7), suggesting that the variant might only be a minor contributor to the genetics of DKD in people with type 1 diabetes.

The major strengths of our study were its relatively large sample of people with long-standing type 1 diabetes (39-year duration on average at the end of follow-up), with a good retention over a 20-year follow-up, and its binational and multicentered design. Also, participants at baseline were in their 40s, an age at which premature mortality is unlikely to affect representativeness at baseline in the case of a frequent gene variant distribution. Indeed, Hardy-Weinberg equilibrium of genotypes was verified for all outcome-related subsets of participants. Clinical events in relation with diabetes, including premature death, could be observed in a sizeable proportion of participants during the lengthy follow-up. Premature death in type 1 diabetes is primarily related to kidney disease (3,38). Impaired kidney function may aggravate cardiovascular risk factors such as hypertension, oxidative stress, insulin resistance, dyslipidemia, inflammation, and arterial calcification (39,40). Thus, the association of the D-allele with all-cause death could be accounted for, at least in part, by its deleterious effects on the kidney. On the other hand, results of competitive risk analyses suggest that premature mortality might have been a competitive risk to the development of ESKD in our cohorts.

There are limitations of our study to acknowledge. Firstly, we investigated one single gene variant in one candidate gene. The impact of the *ACE* I/D variant on circulating ACE levels was reported 30 years ago (13), and its additive effect to that of diabetes status some years later (15). However, the I/D polymorphism is in linkage disequilibrium with other *ACE* variants that have a similar impact on circulating ACE levels (37), and it is still unclear which functional variant or variants are responsible for the genetic effect on ACE levels and on the risk of clinical outcomes. Secondly, only an internal replication of results was possible in this investigation. Thirdly, the effects of lifestyle modification and drug changes during follow-up could not be considered in the analyses due to study design. Finally, we studied three cohorts consisting predominantly of people of European descent, and our conclusion may not apply to people from other ethnic backgrounds.

In conclusion, this is the first report of the effect of a common gene variant on the long-term kidney prognosis and all-cause death of patients with type 1 diabetes. The associations between *ACE* genotype, plasma ACE level, and clinical outcomes strongly suggest a cause-to-effect relationship. Studies in larger cohorts with more extensive genotyping of the *ACE* locus are required to further

Table 3—Primary and secondary outcomes during follow-up by tertiles of plasma ACE levels at baseline

	Outcomes		Incidence rate	Crude model		Adjusted model 1	
	No, *n* (%)	Yes, *n* (%)	(95% CI)	HR (95% CI)	*P*	HR (95% CI)	*P*
Primary outcome							
1st tertile	76 (81)	18 (19)	14.7 (9.4–23.0)	1		1	
2nd tertile	69 (64)	39 (36)	33.6 (24.4–46.2)	2.51 (1.46–4.51)	0.008	1.99 (1.08–3.81)	0.03
3rd tertile	66 (62)	40 (38)	31.5 (23.1–42.9)	2.23 (1.32–4.10)	0.003	1.94 (1.03–3.78)	0.04
ESKD							
1st tertile	83 (88)	11 (12)	9.1 (5.1–16.0)	1		1	
2nd tertile	85 (79)	23 (21)	19.2 (12.6–29.1)	2.34 (1.63–5.00)	0.02	1.34 (0.62–3.06)	0.46
3rd tertile	81 (76)	25 (24)	18.8 (12.7–27.8)	2.29 (1.15–4.87)	0.02	1.51 (0.67–3.53)	0.32
40% drop in eGFR							
1st tertile	76 (85)	13 (15)	11.1 (6.6–18.7)	1		1	
2nd tertile	71 (70)	31 (30)	26.7 (18.7–38.2)	2.92 (1.56–5.79)	0.0007	4.06 (2.04–8.54)	<0.0001
3rd tertile	72 (71)	30 (29)	22.5 (15.7–32.1)	2.10 (1.11–4.20)	0.02	2.37 (1.13–5.21)	0.02
Rapid decline in eGFR							
1st tertile	81 (91)	8 (9)	7.0 (3.7–13.5)	1		1	
2nd tertile	71 (70)	31 (30)	25.8 (18.0–36.8)	4.17 (2.01–9.75)	<0.0001	5.66 (2.57–13.92)	<0.0001
3rd tertile	77 (75)	25 (25)	17.7 (12.0–26.3)	3.07 (1.44–7.29)	0.003	4.62 (1.97–11.92)	0.0003
Albuminuria							
1st tertile	29 (78)	8 (22)	14.8 (4.6–24.9)	1		1	
2nd tertile	23 (74)	8 (26)	20.2 (6.3–34.1)	1.39 (0.51–3.79)	0.51	4.53 (1.19–19.45)	0.03
3rd tertile	25 (66)	13 (34)	26.6 (12.3–40.8)	1.85 (0.78–4.68)	0.17	4.42 (1.20–19.12)	0.02
All-cause death							
1st tertile	71 (59)	49 (41)	25.6 (19.5–33.8)	1		1	
2nd tertile	73 (55)	59 (45)	29.9 (23.0–38.8)	1.23 (0.84–1.81)	0.28	1.40 (0.94–2.10)	0.10
3rd tertile	67 (54)	56 (46)	29.0 (22.4–36.7)	1.17 (0.78–1.73)	0.43	1.43 (0.94–2.19)	0.09
ESKD or all-cause death							
1st tertile	67 (56)	53 (44)	28.9 (22.2–37.6)	1		1	
2nd tertile	60 (46)	72 (54)	40.0 (31.6–50.8)	1.48 (1.04–2.12)	0.03	1.54 (1.06–2.25)	0.02
3rd tertile	55 (45)	68 (55)	38.6 (30.5–48.8)	1.42 (0.99–2.04)	0.06	1.55 (1.04–2.32)	0.03

Primary outcome: ESKD or a 40% drop in eGFR during follow-up. Data are expressed as *n* (%) of participants. Incidence rates expressed per 1,000 person-years. HRs with 95% CIs for the 2nd and 3rd tertiles vs. the 1st tertile of baseline plasma ACE levels computed by Cox regression analysis, adjusted for cohort membership (crude model), plus sex, age, duration of diabetes, MAP, HbA_{1c}, eGFR, and use of ACE inhibitors and antihypertensive drugs at baseline (adjusted model 1). $P < 0.05$ was significant.

assess the impact of *ACE* allelic variations on the variance of ACE expression and the incidence of clinical outcomes. Moreover, the interactions between *ACE* variants, circulating ACE, and the use of ACE inhibitors or angiotensin receptor blockers on the risk of clinical outcomes deserve additional investigations.

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