ACE Gene Polymorphism and Losartan Treatment in Type 2 Diabetic Patients With Nephropathy

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ABSTRACT

Losartan treatment reduced renal outcomes in proteinuric patients with type 2 diabetes in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study. It is unknown whether an insertion (I)/deletion (D) polymorphism in the angiotensin I-converting enzyme (ACE) gene predicts renal outcomes and death and influences the effect of losartan in these patients. Pharmacogenetic analyses were performed comparing losartan with placebo administered with conventional blood pressure-lowering therapy in 1435 (95%) of the 1513 RENAAL study patients. The primary endpoint was the composite of doubling of baseline serum creatinine concentration, end-stage renal disease (ESRD) or death. Cox regression models were stratified on baseline proteinuria and included treatment, geographic region, ACE/ID genotype, and treatment × genotype interaction. Within the placebo group, subjects with the ID or DD genotype were more likely than those with the II genotype to reach the composite endpoint (by 17.5% and 38.1%, respectively, $P = 0.029$) or its individual components. Within the losartan group, genotype did not correlate with reaching the composite endpoint. Compared with placebo, however, losartan reduced the risk of reaching the composite endpoint by 5.8% (95% confidence interval, −23.3, 28.0), 17.6% (3.8, 29.4), and 27.9% (7.0, 44.1) among those with the II, ID, and DD genotypes, respectively. Similar trends were demonstrated for the individual endpoints. In conclusion, proteinuric type 2 diabetic patients with the D allele of the ACE gene have an unfavorable renal prognosis, which can be mitigated and even improved by losartan.

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Activation of the renin-angiotensin-aldosterone system is involved in the initiation and progression of diabetic nephropathy.[1,2] An insertion (I)/deletion (D) polymorphism of the angiotensin-converting enzyme (ACE) gene influences the circulating and renal activity of this system.[3–5] Increased conversion of angiotensin I to angiotensin II, secondary to the higher ACE concentrations, has been suggested to be a mechanism underlying differences in cardiovascular and renal function/prognosis in subjects with the DD genotype compared with the II genotype.[4,5] Increased responses of arterial

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Table 1. Baseline characteristics of the patients according to the ACE insertion/deletion (ID) genotype

Characteristic	ID Genotype			*P*
	II (*n* = 370)	ID (*n* = 674)	DD (*n* = 399)	
Age, yr	60.3 (7.5)	60.0 (7.3)	60.2 (7.6)	0.833
Sex, no. (%)				
female	125 (23.7)	251 (47.5)	152 (28.8)	0.411
male	245 (26.8)	423 (46.2)	247 (27.0)	
Race or ethnic group, no. (%)				
Asian	99 (39.9)	115 (46.4)	34 (13.7)	<0.001
Black	57 (26.5)	79 (36.7)	79 (36.7)	
Hispanic	81 (30.6)	122 (46.0)	62 (23.4)	
White	128 (18.4)	349 (50.1)	219 (31.5)	
Other	5 (26.3)	9 (47.4)	5 (26.3)	
Body mass index[a]	28.75 (5.78)	29.78 (6.47)	30.36 (6.39)	0.002
Known duration of diabetes, yr				
≥5 yr	324 (25.0)	614 (47.3)	359 (27.7)	0.238
<5 yr	45 (31.5)	60 (42.0)	38 (26.6)	
not available	1	0	2	
Blood pressure, mmHg				
systolic	152.7 (20.0)	152.3 (19.2)	152.5 (19.2)	0.946
diastolic	82.3 (10.9)	82.3 (10.4)	82.8 (10.0)	0.738
Medical history, no. (%)				
use of antihypertensive drugs	346 (27.6)	626 (46.4)	367 (26.0)	0.629
angina pectoris	30 (22.1)	66 (48.5)	40 (29.4)	0.590
Myocardial infarction	46 (24.9)	76 (41.1)	63 (34.1)	0.105
coronary revascularization procedure	1 (50.0)	1 (50.0)	0	0.479
stroke	1 (100.0)	0	0	0.256
lipid disorder	130 (24.9)	247 (47.2)	146 (27.9)	0.876
amputation	32 (25.8)	65 (52.4)	27 (21.8)	0.255
neuropathy	186 (25.8)	348 (48.3)	187 (25.9)	0.317
retinopathy	248 (26.9)	427 (46.3)	248 (26.9)	0.333
current smoking	67 (26.0)	121 (46.9)	70 (27.1)	0.977
Laboratory variables				
urinary albumin/creatinine ratio, median mg/g (range)	1364 (60–9328)	1193 (34–12,208)	1166 (31–10,634)	0.626
serum creatinine, mg/dl[b]	1.85 (0.48)	1.87 (0.48)	1.85 (0.48)	0.791
glomerular filtration rate,[c] ml/min/1.73 m^2	40.5 (12.5)	39.2 (11.9)	40.4 (12.9)	0.159
Serum cholesterol, mg/dl[d]				
total	230.0 (56.8)	225.8 (55.1)	228.59 (54.3)	0.464
LDL	145.4 (47.4)	140.3 (44)	141.1 (45.6)	0.239
HDL	43.4 (15.0)	45.5 (15.3)	45.2 (14.6)	0.099
Serum triglycerides, mg/dl[e]	222.9 (197.1)	219.4 (207.3)	216.81 (158.0)	0.908
Hemoglobin, g/dl[f]	12.5 (1.8)	12.5 (1.8)	12.6 (1.9)	0.826
Glycosylated hemoglobin, %	8.6 (1.7)	8.5 (1.6)	8.3 (1.6)	0.054
Serum albumin, g/dl	3.8 (0.4)	3.8 (0.4)	3.8 (0.4)	0.227

Mean (SD) values are presented for all variables, except medical history, race or ethnic group, and sex (*n*, %) and urinary albumin/creatinine ratio (median (range)).

[a]Body mass index is the weight in kilograms divided by the square of the height in meters.

[b]To convert values to micromoles per liter, multiply by 88.4.

[c]Estimated using the modified equation from the Modification of Diet in Renal Disease study.

[d]To convert values to millimoles per liter, multiply by 0.02586.

[e]To convert values to millimoles per liter, multiply by 0.01129.

[f]To convert values to millimoles per liter, multiply by 0.6206.

blood pressure and renal hemodynamics to angiotensin I infusion have been demonstrated in diabetic subjects carrying the DD genotype.[5] A meta-analysis of 14,727 type 1 and type 2 diabetic patients revealed an enhanced risk for development of diabetic nephropathy in the DD genotype compared with the II genotype.[6] Originally, Parving *et al.* observed that the deletion allele of the ACE polymorphism reduces the long-term beneficial effect of ACE inhibition on the progression of overt diabetic nephropathy in patients with type 1 diabetes.[7,8] In contrast, long-term treatment with the angiotensin II-receptor blocker losartan induced a similar rate of decline in glomerular filtration rate (GFR) (surrogate endpoint) in a small group of

Table 2. Allele and genotype frequencies by geographic region and race

	n (%)	Allele Frequency		Genotype Frequency		
		D, %	I, %	D/D, %	D/I, %	I/I, %
Geographic region						
Asia	253 (17.5)	41.3	58.7	17.8	47.0	35.2
Europe	276 (19.1)	56.5	43.5	31.9	49.3	18.8
Latin America	263 (18.2)	47.2	52.9	24.0	46.4	29.7
North America	651 (45.1)	54.0	46.0	31.2	45.6	23.2
total	1443	51.0	49.0	27.7	46.7	25.6
Race						
Asian	248 (17.2)	36.9	63.1	13.7	46.4	39.9
black	215 (14.9)	55.1	44.9	36.7	36.7	26.5
Hispanic	265 (18.4)	46.4	53.6	23.4	46.0	30.6
white	696 (48.2)	56.5	43.5	31.5	50.1	18.4
other	19 (1.3)	50.0	50.0	26.3	47.4	26.3
total	1443	51.0	49.0	27.7	46.7	25.6

hypertensive type 1 diabetic patients with II and DD ACE genotypes.[9] The impact of the ACE/ID polymorphism on principal renal endpoints (*i.e.*, doubling of baseline serum creatinine and/or end-stage renal disease [ESRD] and death) in type 2 diabetic patients with nephropathy and the interaction with angiotensin II-receptor blockade is unknown. Consequently, we assessed these issues in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study.[10] Our pharmacogenetic study of the interaction between losartan and the I/D polymorphism of the ACE gene was prespecified and planned at exactly the same time as the main RENAAL protocol.

RESULTS

Baseline characteristics of the patients according to the ACE insertion/deletion genotypes were similar, except for race and

Table 3. Renal outcomes and death by ACE/ID genotype in the losartan group compared with the placebo group

	Losartan, *n/N* (%)	Placebo, *n/N* (%)	Adjusted[a] Risk Reduction Losartan *vs* Placebo (95% CI)	*P*[b]
Composite endpoint of doubling of serum creatinine, ESRD, or death				
II	72/170 (42.4)	85/200 (42.5)	5.8 (−23.3 to 28.0)	0.215
ID	155/352 (44.0)	151/317 (47.6)	17.6 (3.8 to 29.4)	
DD	81/189 (42.9)	106/207 (51.2)	27.9 (7.0 to 44.1)	
ESRD or death				
II	58/170 (34.1)	67/200 (33.5)	7.5 (−24.6 to 31.4)	0.149
ID	120/352 (34.1)	132/317 (41.6)	22.3 (7.6 to 34.6)	
DD	60/189 (31.7)	87/207 (42.0)	34.6 (13.0 to 50.9)	
Doubling of serum creatinine				
II	40/170 (23.5)	54/200 (27.0)	10.2 (−29.2 to 37.6)	0.250
ID	77/352 (21.9)	71/317 (22.4)	24.3 (6.2 to 38.9)	
DD	38/189 (20.1)	60/207 (29.0)	36.2 (8.5 to 55.5)	
ESRD				
II	39/170 (22.9)	42/200 (21.0)	3.1 (−41.0 to 33.3)	0.032
ID	67/352 (19.0)	84/317 (26.5)	30.5 (13.0 to 44.4)	
DD	30/189 (15.9)	56/207 (27.1)	50.1 (27.1 to 65.8)	
Death				
II	28/170 (16.5)	37/200 (18.5)	0.4 (−49.2 to 33.5)	0.927
ID	82/352 (23.3)	64/317 (20.2)	1.8 (−23.4 to 21.9)	
DD	38/189 (20.1)	49/207 (23.7)	3.2 (−39.9 to 33.1)	
Doubling of serum creatinine or ESRD				
II	56/170 (32.9)	65/200 (32.5)	2.3 (−33.7 to 28.6)	0.084
ID	105/352 (29.8)	104/317 (32.8)	21.6 (5.8 to 34.8)	
DD	52/189 (27.5)	79/207 (38.2)	37.1 (14.4 to 53.8)	

CI, confidence interval; ESRD, end-stage renal disease; *n/N*, number of patients who had an event/total number of patients.
[a]Adjusted with terms including treatment (losartan or placebo), geographic region, genotype, and the treatment × genotype interaction.
[b]*P* value for treatment × genotype interaction (*i.e.*, comparison of the losartan treatment effect between the 3 genotype groups).

body mass index (BMI) (Table 1). Asians had on average a lower BMI than other ethnic groups (Asians, 25.0; blacks, 32.0; Hispanics, 28.3; whites, 31.0; and others 32.3 kg/m^2, $P < 0.05$). The difference in BMI between genotypes was not significant after adjustment for race ($P = 0.557$), whereas the trend to a difference in glycosylated hemoglobin became significant after adjustment for race ($P = 0.020$).

Baseline characteristics by genotype were similar between the losartan and placebo groups (data not shown) except for an imbalance in baseline proteinuria in the II genotype group (median baseline losartan, 1496 mg/g, 36% >2000 mg/g; median baseline placebo, 1230 mg/g, 31% >2000 mg/g, $P = 0.298$ for the medians).

The overall distribution of patients by the ACE/ID polymorphism was 25.64% II, 46.71% ID, and 27.65% DD. The distribution of ACE genotypes in the different ethnic groups was in Hardy-Weinberg equilibrium except for the black patient group who represented about 15% of the total group (Table 2). Therefore, ACE genotype analyses were performed separately on the overall group and the group excluding the black patients. Because the results were similar, only the data from the overall group are presented.

The crude risk estimates of the outcomes by treatment group and genotype were as follows: composite endpoint–losartan II 42.4%, ID 44.0%, DD 42.9%, and placebo II 42.5%, ID 47.6%, DD 51.2%; ESRD or death losartan–II 34.1%, ID 34.1%, DD 31.7%, and placebo II 33.5%, ID 41.6%, DD 42.0%; doubling of serum creatinine–losartan II 23.5%, ID 21.9%, DD 20.1%, and placebo II 27.0%, ID 22.4%, DD 29.0%; ESRD–losartan II 22.9%, ID 19.0%, DD 15.9%, and placebo II 21.0%, ID 26.5%, DD 27.1%; death–losartan II 16.5%, ID 23.3%, DD 20.1%, and placebo II 18.5%, ID 20.2%, DD 23.7%; and doubling of serum creatinine or ESRD–losartan II 32.9%, ID 29.8%, DD 27.5%, and placebo II 32.5%, ID 32.8%, DD 38.2% (Table 3). For the placebo group only (Table 4), a similar Cox regression model was used in the primary genetic analyses, except the treatment term was dropped. For the ESRD endpoint, the genotypes approached significant difference (0.091). The composite endpoint was significantly increased in ID *versus* II (17.5%) and DD *versus* II (38.1%). The Kaplan-Meier curves illustrate that the event rates in the placebo group for the composite endpoint and ESRD were higher and similar in the DD and ID ACE groups and lower in the II genotype group (Figure 1). Furthermore, the individual components of this endpoint showed the same trend (Table 4). Interestingly, the losartan group showed no such differences in endpoints between the genotypes. Figure 1B shows overlapping Kaplan-Meier curves for the primary composite endpoint between the three genotypes and even a better outcome for the DD genotype when looking at the ESRD endpoint (Figure 1D). Table 3 and the Kaplan-Meier curves in Figures 1 and 2 show that the losartan treatment effects were greatest in the ACE/DD genotype group and intermediate in the ID group for all endpoints (except death in the ID group). Among the endpoints presented in Table 3, there was a statistically significant treatment × genotype interaction only for ESRD (Figure 2), a component of the primary composite endpoint. Relative to placebo, losartan was associated with adjusted ESRD risk reductions, averaging 3.1%, 30.5%, and 50.1% for II, ID, and DD genotypes, respectively (test of treatment × genotype interaction, $P = 0.032$)(Table 3). Treatment with losartan lowered the event rates of the above-mentioned endpoints in all groups, with the greatest effect in the DD followed by ID and II geno-

Table 4. Renal outcomes and death by ACE/ID genotype in the placebo group[a]

	n/N (%)	Risk Reduction[b] (95% CI)	*P*
Composite endpoint of doubling of serum creatinine, ESRD, or death			
ID *vs* II	151/317 (47.6) *vs* 85/200 (42.5)	−17.5 (−35.9 to −1.6)	0.029
DD *vs* II	106/207 (51.2) *vs* 85/200 (42.5)	−38.1 (−84.7 to −3.3)	
ESRD or death			
ID *vs* II	132/317 (41.6) *vs* 67/200 (33.5)	−16.4 (−36.3 to 0.7)	0.060
DD *vs* II	87/207 (42.0) *vs* 67/200 (33.5)	−35.4 (−85.8 to 1.3)	
Doubling of serum creatinine			
ID *vs* II	71/317 (22.4) *vs* 54/200 (27.0)	−14.6 (−39.7 to 6.0)	0.177
DD *vs* II	60/207 (29.0) *vs* 54/200 (27.0)	−31.4 (−95.1 to 11.6)	
ESRD			
ID *vs* II	84/317 (26.5) *vs* 42/200 (21.0)	−18.7 (−44.7 to 2.7)	0.091
DD *vs* II	56/207 (27.1) *vs* 42/200 (21.0)	−40.8 (−109.4 to 5.3)	
Death			
ID *vs* II	64/317 (20.2) *vs* 37/200 (18.5)	−12.0 (−39.3 to 9.9)	0.307
DD *vs* II	49/207 (23.7) *vs* 37/200 (18.5)	−25.5 (−94.0 to 18.8)	
Doubling of serum creatinine or ESRD			
ID *vs* II	104/317 (32.8) *vs* 65/200 (32.5	−17.5 (−94.5 to 1.9)	0.064
DD *vs* II	79/207 (38.2) *vs* 65/200 (32.5)	−38.0 (−39.4 to 1.0)	

CI, confidence interval; ESRD, end-stage renal disease; *n/N*, number of patients with an event/total number of patients pertaining to each approach.
[a]In endpoint trials, there is often a difference between the risk reduction as determined on the basis of the Cox regression model and the risk reduction as determined on the basis of the crude rates of events. The difference results in part from the fact that the Cox regression model accounts for the time at risk (*i.e.*, the longer average follow-up in the losartan group than in the placebo group).
[b]A negative risk reduction thus denotes an increased risk.

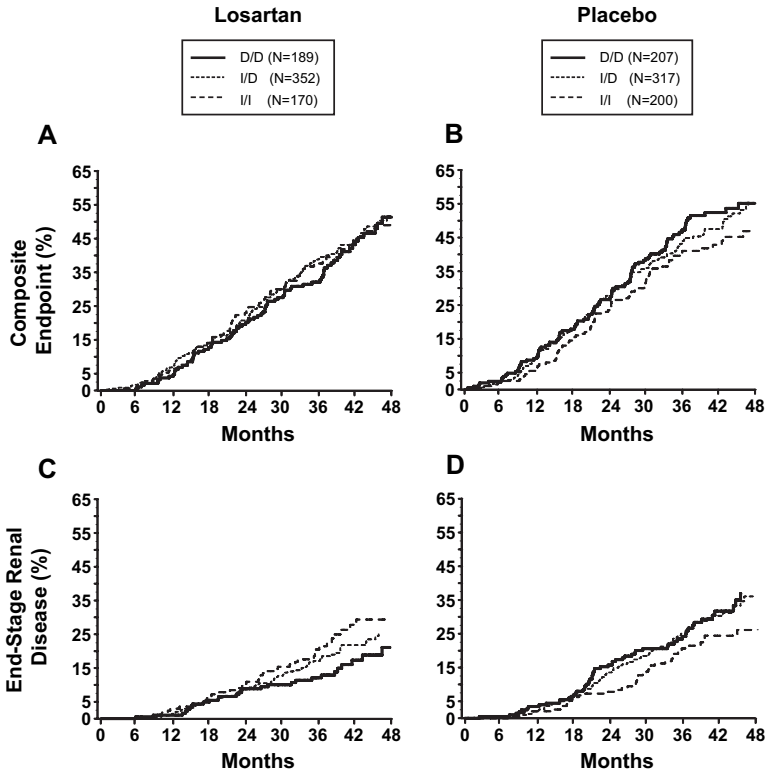

Figure 1. Kaplan-Meier curves of the percentage of patients with the primary composite endpoint in the losartan (A) and placebo (B) groups and one of its individual components, end-stage renal disease, in the losartan (C) and placebo (D) groups. The mean follow-up time was 3.4 yr (42 mo).

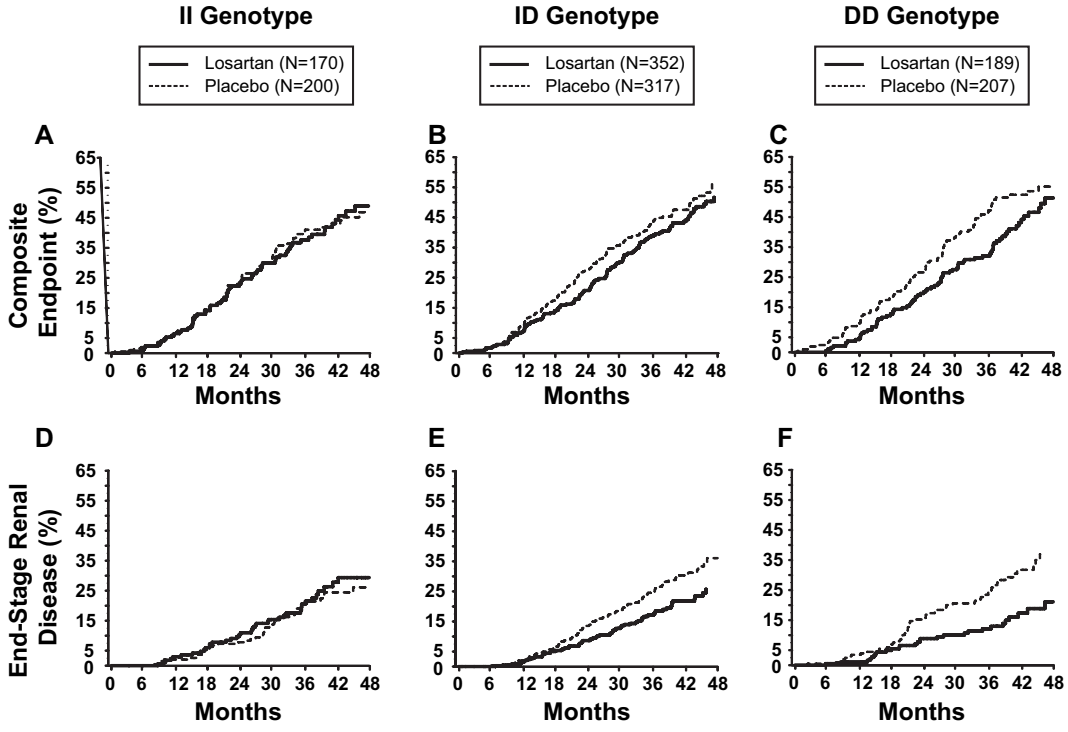

Figure 2. Kaplan-Meier curves of the percentage of patients with the primary composite endpoint in the II (A), ID (B), and DD (C) genotype groups and one of its individual components, end-stage renal disease, in the II (D), ID (E), and DD (F) genotype groups. The mean follow-up time was 3.4 yr (42 mo). The *P* values for treatment × genotype interaction analyses are shown in Table 4.

types (Figures 1 and 2). For the ESRD endpoint assuming the additive model, the ID *versus* II comparison for losartan *versus* placebo ($P = 0.026$) and the DD *versus* II for comparison for losartan *versus* placebo ($P = 0.002$) were both statistically significant, which is in line with the significant genotype × treatment interaction found for the inheritance pattern assumed. Our study did not reveal any statistical differences in the renoprotective effects of losartan between the different ethnic groups, and D allele carriers had the biggest treatment effect in all ethnic groups.

DISCUSSION

Based on a large, double-blind, randomized study comparing the renoprotective effects of losartan *versus* placebo on top of conventional blood pressure-lowering drugs in proteinuric type 2 diabetic patients, we demonstrated that the deletion allele of the ACE gene had a harmful impact on the composite endpoint of doubling of baseline serum creatinine concentration, ESRD, or death. The D allele carriers had the worst renal prognosis in all ethnic groups. It should be stressed that demographic data, clinical history, and laboratory variables at baseline by genotype were similar between the losartan and placebo groups with the exception of proteinuria in the II genotype group. The beneficial effects of losartan were greatest in the ACE/DD group and intermediate in the ID group for nearly all endpoints, a trend suggesting a quantitative interaction between losartan treatment and ACE/ID genotype on progression of renal disease. In agreement with a previous analysis of the RENAAL study, losartan exhibited renoprotection in all ethnic groups.[11] The treatment-ACE-genotype interaction was significant for the risk reduction of the ESRD endpoint. The major novel clinical importance of our study is that those patients who have the greatest need for renoprotective treatment have the best effect of losartan (DD and ID), whereas those patients with a better renal prognosis (II) also derived renal benefit. The finding of similar values for serum creatinine and albumin/creatinine ratio at baseline in the three genotype groups may well be due to previous renoprotective treatment, which according to the present analysis mitigated differences observed in patients receiving RAAS blockade.

The influence of ACE/ID polymorphism on renal progression in chronic nondiabetic nephropathies has been evaluated in the Ramipril Efficacy In Nephropathy study.[12–15] In this randomized, double-blind, placebo-controlled trial of 352 white patients with predominantly primary glomerular diseases, the ACE/ID polymorphism did not predict progression in GFR or risk of ESRD.[13] Furthermore, ACE inhibition was uniformly renoprotective in women regardless of the ACE polymorphism, and in men with the ACE/DD genotype, but lacked beneficial effect in men with II or ID genotype.[14] A *post hoc* analysis of 27 type 2 diabetic patients revealed no renoprotective effects of ACE inhibition with ramipril.[16]

Well-known nongenetic risk factors such as elevated blood pressure, albuminuria, increased serum creatinine, poor glycemic control, lipid abnormalities, and reduced hemoglobin explain approximately 30% to 50% of the interindividual variation in the loss of GFR in diabetic nephropathy.[1,17,18] Identification of additional risk factors is needed to further understand the progression of diabetic nephropathy, thus enhancing the ability to identify high-risk individuals and to improve treatment and overall prognosis.[19] Our genetic study revealed that each additional D allele of the ACE polymorphism increases the risk for the composite endpoint. Previous observational studies in Asian type 2 diabetic patients have demonstrated that the DD genotype is an independent predictor of doubling of baseline serum creatinine concentration and ESRD.[20,21] These findings strongly suggest that the enhanced progression of kidney function loss in DD and ID patients is the result of increased intrarenal activity of ACE.[5] In addition, the ACE/DD genotype has been shown to enhance the risk of developing diabetic glomerulopathy lesions in type 2 diabetic patients with microalbuminuria and macroalbuminuria.[22]

In addition to nongenetic factors, drug responses are also known to be influenced by inherited factors (*i.e.*, pharmacogenomics).[23,24] The inherited determinants generally remain stable throughout a person's lifetime. The interindividual differences in drug response can be due to sequence variants in genes encoding drug-metabolizing enzymes, drug transporters, or drug targets.[23,24] Previous long-term observational follow-up studies of ACE inhibition in type 1 diabetic patients with diabetic nephropathy revealed a reduced renoprotective effect in patients homozygous for the D allele of the ACE gene.[7,8] Furthermore, a reduced anti-albuminuric response to ACE inhibition has been demonstrated in type 1 diabetic patients with the D allele.[25] So *et al.*[26] recently reported a large long-term observational study of a consecutive cohort of 2089 Chinese type 2 diabetic patients genotyped for the ACE/ID gene polymorphism. The study revealed that DD genotype enhanced the risk of a doubling of baseline serum creatinine concentration and ESRD. ACE inhibition was renoprotective and improved survival, the greatest benefit being observed in the group homozygous for the I allele. In contrast to the abovementioned data, our randomized, double-blind study demonstrated that the proteinuric type 2 patients with the worse genetically determined renal outcome (DD patients) exhibited the most marked renoprotective effect of losartan. Several factors may explain the difference between the previous observational studies during ACE inhibition in type 1 and type 2 diabetes and the present study findings. Observational studies suffer from selection bias with regards to ACE inhibition, survival bias, and several other confounding factors, such as changes in antihypertensive treatment, blood pressure, and glycemic control, all of which can influence the progression of nephropathy.

Jacobsen *et al.* have previously demonstrated a potential synergistic interplay among ACE/ID, angiotensinogen-M235T, and angiotensin II type 1 receptor (A1186C) polymorphisms in type 1 diabetic patients with nephropathy, all result-

ing in adverse renal outcomes.[8] The deletion allele of the ACE polymorphism was presumed to be a primary susceptibility locus, with the other polymorphisms within loci reinforcing the impact of the D allele.[8] The blood pressure response to losartan treatment in diabetic patients is influenced by the CYP2C9 of the cytochrome P450 superfamily, which catalyzes the metabolism of the drug by creating moderate and high metabolizers.[27] Unfortunately, the original RENAAL study protocol and informed consent form do not allow us to investigate these other above-mentioned polymorphisms.

Baseline genotype–phenotype analyses revealed associations in relation to race or ethnic group and to BMI. The lower BMI in the II group is partially explained by the high I allele frequency in the Asian population, who have significantly lower BMI compared with the other race/geographic region groups (Table 2).

Previous studies have suggested that the effect of ACE inhibitors is modified by dietary sodium intake in DD patients but not in the other genotypes.[4] Unfortunately, data on dietary sodium intake were not available in the RENAAL study.

Finally, it should be recalled that long-term poor glycemic control during liberal sodium intake (200 mmol/24 h) enhances the angiotensin I-induced responses of arterial blood pressure and renal hemodynamics in diabetic patients carrying the DD genotype.[5] Poor long-term glycemic control was present in all three genotypes.

The main strengths of the RENAAL study include the double-blind, randomized design and a large multiracial group of proteinuric type 2 diabetic subjects with validated prespecified primary endpoints. However, several limitations should be noted. Even although the RENAAL study is the largest trial evaluating the association of the ACE/ID polymorphism on renal outcome and death during angiotensin II-receptor blockade in diabetic nephropathy, the study may well be underpowered in relation to the individual components of our composite endpoint. This is suggested by the finding that, although there were consistent trends between the ACE genotypes and renal outcomes, not all components were statistically significant. The second concern is the observation that the ID alleles were not in Hardy-Weinberg equilibrium in the black group. However, the results of the overall and the nonblack groups were similar, suggesting that the consistent trends from the overall analysis are valid. Lastly, despite randomization, higher baseline proteinuria was present in the II genotype in the losartan group. We have no biologic explanation for the higher baseline proteinuria in the II group. We adjusted the analyses by baseline proteinuria <2000 or ≥ 2000 mg/g; however, this may not have completely compensated for the imbalance.

In summary, proteinuric type 2 diabetic patients with the deletion allele of the ACE/ID polymorphism are shown to have an unfavorable renal prognosis, which can be mitigated and even improved by losartan treatment.

CONCISE METHODS

The RENAAL study was an investigator-initiated, multinational, double-blind, randomized, placebo-controlled study designed to evaluate the renoprotective effects of losartan in 1513 patients with type 2 diabetes and nephropathy, as described previously.[10,28] In 1443 patients (95%), the ACE/ID genotypes could be determined (Table 1). Of these, 716 received losartan and 727 received placebo. All analyses except those of baseline characteristics (Table 1) excluded 8 protocol violators (patients with eligibility and compliance violation as defined in the original study). Of these 8 patients, 5 were in the losartan group and 3 in the placebo group.

The primary outcome in this pharmacogenetic substudy was the time to the first event of the composite endpoint of doubling of baseline serum creatinine concentration, ESRD, or all-cause death, identical to the main RENAAL study.[10] Exploratory endpoints of interest included the individual components of the primary composite endpoint as well as time to ESRD or death and time to doubling of serum creatinine or ESRD.

A blood sample was collected from each patient on the day of randomization (day 1) for genotyping and transferred to the central laboratory (Centre National de Genotypage; Paris, France) at -80°C. Genomic DNA was isolated from leukocytes using standard methods. All genotyping was conducted in a manner that was blinded to clinical outcome and treatment status. PCR was used to detect the two alleles of the ACE/ID polymorphism. DNA was amplified using allele-specific primers and PCR-cycling conditions, as described previously.[29] Subjects were classified according to the presence or absence of a 287-bp insertion in intron 16 of the ACE gene, as homozygous II or DD or heterozygous for ID. All patients gave written informed consent to study only the polymorphism of the ACE gene, thus excluding exploratory studies of other candidate genes.

Statistical Methods

These analyses were prespecified in the RENAAL genetics substudy protocol. Statistical significance is on a per-comparison basis at the 2-tailed $\alpha = 0.05$. The censoring approach used for the time-to-event analyses included all follow-up that occurred before study termination. All patients with valid baseline and postrandomization values were included in the endpoint analyses.

The χ^2 tests for Hardy-Weinberg equilibrium were performed by treatment, geographic region, and race groups. Baseline risk factors were summarized by ACE/ID genotypes to explore the possible associations. Likelihood ratio tests were performed to assess the significance of the observed associations for discrete baseline risk factors; the significance of the observed association was assessed by the F test for the continuous variables.

All time-to-event endpoints were analyzed using a Cox regression model stratified according to patient baseline levels of urinary albumin/creatinine ratio <2000 or ≥ 2000 mg/g, with terms including treatment (losartan or placebo), geographic region, ACE/ID genotype, and the treatment $\times$ genotype interaction. Analyses in the main RENAAL study were also adjusted for geographic region: Asia (17% of patients), Europe (19.3%), Latin America (18.1%), and North America (45.5%).[10] Trends by race were similar (Table 2). Geographic region was used in the Cox regression models because it was the covariate used in the main study and presumed to be the stronger covariate for adjusting the model. Both additive and general models were run; however, tests for the mode of inheritance of the effect of the genotype

were performed and additive models preferred because there was insufficient evidence ($P \geq 0.05$) to reject them. The ratios and 95% confidence intervals were determined using the Cox regression models; risk reductions (equal to relative risk) were calculated as 100 percent $\times$ (1 $-$ hazard ratio). A negative risk reduction thus denotes an increased risk. Event curves were based on the Kaplan-Meier procedure. The relationship between the ACE/ID polymorphism and race was examined for the composite endpoint of doubling of serum creatinine, ESRD, or death and each component of the composite endpoint separately. No multiplicity adjustments were applied to the analyses of the multiple endpoints.

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DISCLOSURES

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