Long-Term Renoprotective Effects of Losartan in Diabetic Nephropathy

Interaction with ACE insertion/deletion genotype?

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OBJECTIVE — Several observational follow-up studies have found that the D allele of the insertion (I)/deletion (D) polymorphism of the ACE gene (ACE/ID) is associated with an increased risk of renal function loss, even during ACE inhibition. Therefore, we investigated the long-term effect of the angiotensin II subtype-1 (AT1) receptor antagonist losartan (100 mg o.d.) on kidney function in II and DD type 1 diabetic patients with diabetic nephropathy.

RESEARCH DESIGN AND METHODS — A total of 54 hypertensive type 1 diabetic patients with diabetic nephropathy homozygous for the insertion ($n = 26$) or the deletion ($n = 28$) allele were included in the study. After a 4-week washout, the patients received losartan (tablet, 100 mg o.d.) and were followed prospectively with a mean follow-up period of 36 months. Patients and investigators were blinded to ACE genotypes. At baseline, after 2 and 4 months and every 6 months thereafter, glomerular filtration rate (GFR), albuminuria, and 24-h blood pressure were determined.

RESULTS — At baseline, GFR, albuminuria, and blood pressure were similar in the two genotype groups, II versus DD: mean (SD), 86 (22) vs. 88 (24) ml · min^{-1} · 1.73 m^{-2}; median (interquartile range), 1,134 (598–2,023) vs. 1,451 (893–1,766) mg/24 h; and mean (SD), 156/82 (17/9) vs. 153/80 (17/11) mmHg, respectively. GFR decreased similarly in both genotype groups, versus DD, respectively ($P = 0.4$): geometric mean (95% CI), 2.9 (2.0–4.2) vs. 3.4 (2.3–5.1) ml · min^{-1} · year^{-1}. Albuminuria and arterial blood pressure were significantly reduced during the study; no differences were noted between groups. During follow-up, albuminuria was decreased by 75% (95% CI 59–85) and 73% (56–83) in the II and DD groups, respectively ($P < 0.01$ vs. baseline). Mean systolic and diastolic blood pressures were 139/74 mmHg (14/8) in both genotype groups during the study ($P < 0.01$ vs. baseline).

CONCLUSIONS — In contrast to previous observational studies with ACE inhibitors, long-term treatment with losartan has similar beneficial renoprotective effects on progression of diabetic nephropathy in hypertensive type 1 diabetic patients with ACE II and DD genotypes.

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The association between the insertion (I)/deletion (D) polymorphism of the gene coding for ACE (ACE/ID) in the development (1) and progression of diabetic and nondiabetic kidney disease has been thoroughly investigated through the last decade (2–12). Patients homozygous for the D allele are characterized by elevated plasma levels of ACE compared with patients homozygous for the I allele, which might explain a diversity in the response to ACE inhibition (13,14). Reduced long-term renoprotective effect of ACE inhibition has been demonstrated in type 1 diabetic patients with diabetic nephropathy homozygous for the D allele (3). Furthermore, the ACE/ID polymorphism has been suggested to play an important role in the individual antiproteinuric response to ACE inhibition in diabetic renal disease (4,15), whereas conflicting results have been reported in nondiabetic renal disease (2,11,12,16,17).

Blocking the action of angiotensin II at the site of the subtype-1 (AT1) receptor may overcome the interaction between the ACE/ID polymorphism and ACE inhibition. Therefore, we aimed to evaluate the long-term renoprotective effect of AT1 receptor blockade on progression of diabetic nephropathy in hypertensive type 1 diabetic patients homozygous for the I or D allele of the ACE/ID polymorphism.

RESEARCH DESIGN AND METHODS

— Since 1993, all patients with diabetic nephropathy treated at the Steno Diabetes Center have been invited to participate in a study of genetic risk factors including analysis of ACE/ID genotypes for the development and progression of diabetic nephropathy. For the present study, records of all patients homozygous for the I or D allele with type 1 diabetes and diabetic nephropathy in the registry at the Steno Diabetes Center were examined. A total of 85 patients (39 II, 46 DD) fulfilled inclusion and exclusion criteria and were invited to participate in the study. A total of 56 patients agreed to participate. Demographic data of patients who declined participation and those who were included in the study were similar (data not shown). Patients were blinded to ACE/ID genotypes, as were all investigators except one (L.T.), who had knowledge of genotypes and identified homozygous patients from the registry. Two patients were excluded during the

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Abbreviations: ACE/ID, insertion/deletion polymorphism of the ACE gene; AT1, angiotensin II subtype-1; GFR, glomerular filtration rate; MABP, mean arterial blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances

See accompanying editorial, p. 1631.

Table 1—*Baseline characteristics of 54 hypertensive type 1 diabetic patients with diabetic nephropathy and homozygosity for the I (n = 26) or D (n = 28) allele of the ACE/ID polymorphism*

	ACE genotype	
	II	DD
Sex (men/women)	16/10	17/11
Age (years)	44 ± 10	45 ± 8
Duration of diabetes (years)	34 ± 8	33 ± 10
Duration of nephropathy (years)	12 ± 7	12 ± 6
Retinopathy (simplex/proliferative) (%)	12/88	25/75
Albuminuria (mg/24 h)	1,134 (598–2,023)	1,451 (893–1,766)
24-h systolic blood pressure (mmHg)	156 ± 17	153 ± 17
24-h diastolic blood pressure (mmHg)	82 ± 9	80 ± 11
GFR (ml · min^{-1} · 1.73 m^{-2})	86 ± 22	88 ± 24

Data are means ± SD or median (interquartile range).

first month of the study, one because of malignant disease and one due to heart failure requiring ACE inhibition. One patient withdrew informed consent after 4 months because of social problems. A total of 53 patients homozygous for the I ($n = 26$) or the D ($n = 27$) allele of the ACE/ID polymorphism entered the long-term follow-up study with a minimum follow-up time of 18 months. Compliance was assessed by pill counts. All patients fulfilled the compliance criteria of >85%.

A total of 52 patients received antihypertensive medication before the study: 42 were treated with ACE inhibitors and 10 were treated with calcium antagonists. Before enrollment in the study, all antihypertensive medication was withdrawn for at least 4 weeks. All patients fulfilled the following inclusion criteria: diabetic nephropathy, glomerular filtration rate (GFR) >60 ml · min^{-1} · 1.73 m^{-2}, office blood pressure >135/85 mmHg, and age between 18 and 70 years. Diabetic nephropathy was diagnosed clinically in patients with persistent albuminuria (>300 mg/24 h), diabetic retinopathy, and absence of other evidence of kidney or renal tract disease (18). Patients were excluded if they had a history of malignant hypertension, congestive heart failure, myocardial infarction, or stroke within the past 3 months. The study was performed according to the principles of the Declaration of Helsinki and approved by the ethical committee of Copenhagen County. All patients gave their informed consent.

Patients were included from June 1998 to December 1999 and followed prospectively until December 2001. Mean follow-up time was 36 months (range 18–42). In the first 2 months, patients received losartan 50 mg daily, which was followed by 100 mg daily in all patients regardless of blood pressure in the next 2 months to evaluate the short-term antiproteinuric and antihypertensive effects (19). Hereafter, losartan 100 mg o.d. was continued throughout the study in all patients as well as additional antihypertensive treatment, i.e., diuretics, calcium channel blockers, and α-blockers given in an attempt to achieve a target blood pressure lower than 135/85 mmHg. Dietary intake of protein or salt was not restricted. Blood pressure measurements and adjustment of antihypertensive medication were performed every third month. Clinical investigations were performed every 6 months and included determination of GFR, 24-h blood pressure, and albuminuria.

ACE/ID genotyping was performed with allele-specific oligonucleotides and PCR (20,21).

GFR was measured after a single intravenous injection of 3.7 MBq ^{51}Cr-EDTA at 8:00 A.M. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (22,23). The results were standardized for 1.73 m^2 body surface area, using the patient's surface area at the start of the study. The mean coefficient of variation in GFR of each patient from day to day was 4%.

Albuminuria was determined as the geometric mean of at least two consecutive 24-h urine collections, completed immediately before each visit (Turbidimetry, Cobas Mira Plus; Roche, Montclair, NJ). Urinary excretion of sodium and urea (Cobas Mira Plus; Roche) was measured in all urine collections. The excretion of urea was used to calculate the protein intake from the nitrogen content of the urea and an estimated value of nonurea nitrogen of 31 mg · kg^{-1} · day^{-1} (24).

From venous blood samples, serum potassium, sodium, creatinine, and cholesterol concentrations were determined (Cobas Mira Plus; Roche) and HbA_{1c} was measured by high-performance liquid chromatography (normal range 4.1–6.4%) (Variant; Biorad, Richmond, CA). Blood samples for determination of angiotensin II levels were collected in prechilled tubes after 30 min supine rest and immediately centrifuged at 4°C, and plasma concentrations were measured radioimmunologically (25). Renin concentrations in plasma were determined according to the method of Deinum et al. (26).

Blood pressure values are based on 24-h ambulatory blood pressure measurements performed with the Takeda TM2420 device (version 7; A&D, Tokyo, Japan). Blood pressures were measured every 15 min during the day (7:00 A.M. to 11:00 P.M.) and every 30 min during the night (11:00 P.M. to 7:00 A.M.). Values were averaged for each hour before calculating the 24-h blood pressure.

Data are expressed as means ± SD (Table 1) and means ± SE except for albuminuria, renin, angiotensin II, aldosterone, ACE, and rate of decline in GFR, which were logarithmically transformed before statistical analysis owing to their skewed distribution and are given as geometric means (95% CI). At baseline, albuminuria is given as median (interquartile range). Comparisons of normally or log-normally distributed parameters were performed with a Student's *t* test. The rate of decline in kidney function was analyzed by regression lines for GFR over individually determined times during the treatment period. After comparison of II and DD genotype groups, data were combined to evaluate the correlation of putative predictors of progression and the rate of decline in GFR by multiple linear regression.

A prestudy sample size calculation was performed based on data from 40 hypertensive type 1 diabetic patients with diabetic nephropathy and GFR >40 ml · min^{-1} · 1.73^{-2}. The observed SD on rate of

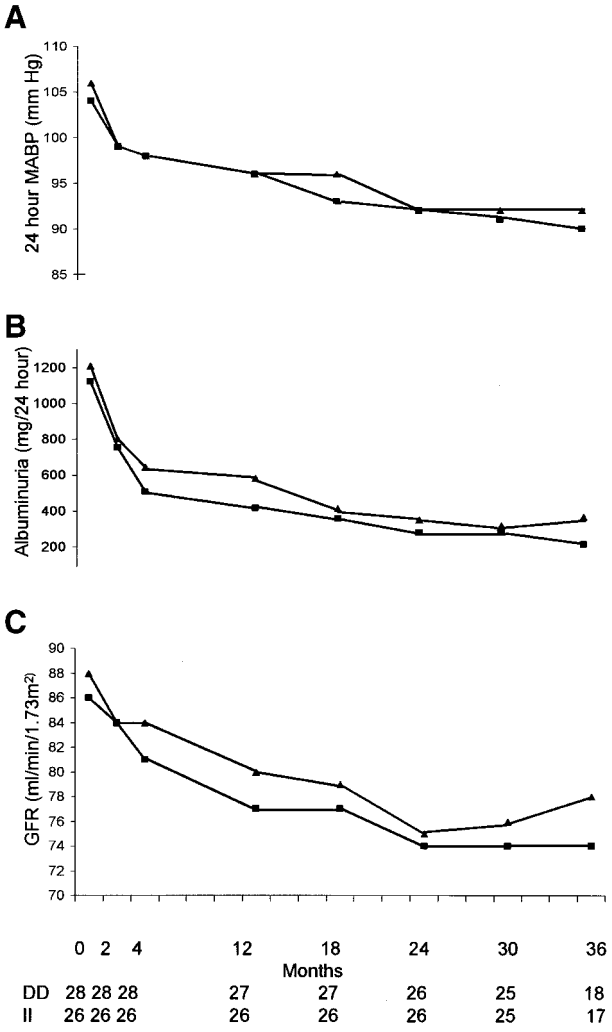

Figure 1—*24-h MABP* (A)*, albuminuria* (B)*, and GFR* (C) *in II* (■) *and DD* (▲) *patients.*

decrease in GFR in this group was 2.5 ml · min^{-1} · $year^{-1}$ during ongoing antihypertensive treatment. To detect a difference in rate of decline in GFR of 2 ml · min^{-1} · $year^{-1}$ (α = 5%, β = 20%), 48 patients were to be included in the present study. A *P* value <0.05 was considered significant (two-tailed). Data were analyzed using SPSS statistical software (version 10.0; SPSS, Chicago, IL).

RESULTS

Baseline clinical characteristics did not differ significantly by ACE genotype (Table 1). The 24-h mean arterial blood pressure (MABP) was significantly reduced in both genotype groups during the study. In the II group, MABP was lowered from 106 ± 2 mmHg (mean ± SE) at baseline to an average of 95 ± 1 mmHg during the study ($P < 0.01$). DD patients achieved a reduction from 104 ± 2 mmHg at baseline to 95 ± 1 mmHg during the investigation period ($P < 0.01$). There was no significant difference between the blood pressure reductions in the two genotype groups (Fig. 1).

There was a significant reduction in GFR in both groups during the study period ($P < 0.01$). GFR declined by 2.9 ml · min^{-1} · $year^{-1}$ (2.0–4.2) in the II group compared with 3.4 ml · min^{-1} · $year^{-1}$ (2.3–5.1) in the DD group ($P = 0.4$ II vs. DD).

Losartan significantly lowered albuminuria in both groups. After 4 months, albuminuria was reduced from baseline by 55% (95% CI 35–68; $P < 0.01$) in the II group and 46% (28–61; $P < 0.01$) in the DD group (NS between groups). Furthermore, after 30 months, albuminuria was similarly lowered by 75% (59–85) and 73% (56–83) in the II and DD groups, respectively (NS).

As expected, ACE levels were significantly higher in the patients homozygous for the D allele but remained unchanged during the investigation period in both groups. Renin and angiotensin II increased similarly in the II and DD groups, as shown in Table 2. Levels of renin, ACE, angiotensin II, and aldosterone during follow-up represent single determinations at the end of the study. Additional follow-up data in Table 2 represent averages of biannual determinations apart from HbA_{1c}, which was measured every third month.

When progression promoters of decline in GFR, including ACE/ID genotype, were examined in a multiple regression analysis, high levels of baseline albuminuria and systolic blood pressure during follow-up were significant predictors of decline in GFR ($P < 0.05$), whereas ACE/ID genotype was not.

One patient in the II group died of stroke after 26 months of follow-up. In one patient in the DD group, end-stage renal disease developed after 24 months, and one patient in the DD group was lost to follow-up after 18 months but was alive without end-stage renal disease at the end of study. Two patients reported orthostatic vertigo (one in each genotype group), but no other side effects related to the study medication were recorded.

At the end of the study, 40 patients (17 II and 23 DD; NS) received diuretic treatment, 12 patients (7 II and 5 DD; NS) received calcium channel blockers, and 3 patients (1 II versus 2 DD; NS) received an α-blocking agent. A total of 10 patients received lipid-lowering medication. All participants were given low-dose aspirin as primary or secondary prevention of cardiovascular disease.

CONCLUSIONS

— In our double-blind prospective intervention study with a mean follow-up of 36 months, the AT1 receptor antagonist losartan induced a similar long-term beneficial renoprotective effect on GFR and a comparable reduction in arterial blood pressure in albuminuric type 1 diabetic patients homozygous for the I or the D allele of the ACE polymorphism. Likewise, albuminuria was similarly and progressively reduced in both genotype groups. Our long-term data expand the information from our previous short-term study (19) in which albuminuria and arterial blood pressure were similarly reduced in both

Table 2—*Laboratory data in hypertensive type 1 diabetic patients with diabetic nephropathy and homozygosity for the I or D allele of ACE/ID polymorphism during 36 months of treatment with losartan 100 mg*

	Baseline		Follow-up	
	II	DD	II	DD
Hemoglobin (mmol/l)	8.6 ± 0.2	8.9 ± 0.2	8.3 ± 0.2†	8.4 ± 0.2†
HbA_{1c} (%)	8.7 ± 0.2	9.0 ± 0.2	9.1 ± 0.2	9.4 ± 0.2
Serum potassium (mmol/l)	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Serum creatinine (μmol/l)	103 ± 4	107 ± 7	118 ± 6†	114 ± 8†
Serum cholesterol (mmol/l)	5.0 ± 0.1	5.4 ± 0.2	5.0 ± 0.1	5.2 ± 0.2
Serum HDL cholesterol (mmol/l)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Serum albumin (g/l)	37 ± 1	36 ± 1	38 ± 1	38 ± 1
Serum renin (μU/ml)*	40 ± 27	38 ± 22	112 ± 25†	141 ± 52†
Serum ACE (IU/l)*	16 ± 1	25 ± 1‡	16 ± 1	25 ± 1‡
Plasma angiotensin II (pmol/l)*§	9 ± 1	16 ± 3	29 ± 9†	33 ± 1†
Plasma aldosterone (pg/ml)*	67 ± 20	97 ± 34	62 ± 19	72 ± 11
Dietary protein intake ($g \cdot kg^{-1} \cdot 24\ h$)	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Extracellular volume ($l/1.73\ m^2$)	14.0 ± 0.5	14.2 ± 0.4	13.2 ± 0.4	14.0 ± 0.3
Urinary sodium (mmol/24 h)	162 ± 9	139 ± 11	158 ± 9	156 ± 8

Data are means ± SE. *Geometric means ± SE. †$P < 0.05$ vs. baseline; ‡$P < 0.05$ II vs. DD; §$n = 18$ II + 13 DD.

genotype groups during 4 months of losartan treatment.

As expected, levels of ACE were ~50% higher in the DD group compared with the II patients, whereas renin, angiotensin II, and aldosterone levels were similar in the two groups, in keeping with previous data (27). Recently, attention has focused on the direct effects of angiotensin II on renal injury in diabetic nephropathy, some of which seem to be independent of hemodynamic changes (28). Even though systemic levels of angiotensin II are comparable in the II and DD groups, intrarenal and vascular levels, which may be more relevant, could be elevated in DD patients, as suggested from experimental and functional studies (29).

To obtain valid information about rate of decline in GFR in individual patients with chronic progressive kidney disease, the following requirements should be fulfilled (30): a precise method for determination of GFR, repeated GFR measurements (approximately every 6 months), and duration of observation of at least 2 years. All requirements were met in our study. Furthermore, a power calculation was performed according to data from a previous longitudinal study, in which we found a distinction of more than $3\ ml \cdot min^{-1} \cdot year^{-1}$ in rate of decline in GFR between II and DD patients during ACE inhibition (3). To support the probability to detect an even smaller difference between groups, $2\ ml \cdot min^{-1} \cdot year^{-1}$ was chosen.

All patients except two received antihypertensive treatment before the study. Furthermore, baseline clinical characteristics did not differ significantly between genotype groups. Therefore, past antihypertensive treatment cannot be expected to influence potential differences in progression of renal damage between II and DD patients.

A total of 15 type 1 diabetic patients heterozygous for the ACE/ID polymorphism with diabetic nephropathy and clinical characteristics comparable to the homozygous patients were followed according to a protocol similar to the present study. As expected, renoprotective effects of long-term losartan treatment in ID patients were comparable to homozygous patients. MABP was lowered to an average of 93 ± 2 mmHg, albuminuria was reduced by 67% (range 45–80), and the rate of decrease in GFR was $2.8\ ml \cdot min^{-1} \cdot year^{-1}$ (1.6 −4.1).

The natural course of diabetic nephropathy is characterized by a mean rate of decline in GFR of $10–15\ ml \cdot min^{-1} \cdot year^{-1}$ (range 0–25) (31–33). High levels of arterial blood pressure, albuminuria, HbA_{1c}, and serum cholesterol predict decrease in GFR in diabetic nephropathy (34). Accordingly, albuminuria and arterial blood pressure were found to be progression motors in the present study, whereas ACE/ID was not. In our recent observational study of 301 type 1 diabetic patients with diabetic nephropathy followed for 7 years (3–14), the mean rate of decrease in GFR was $4.0\ ml \cdot min^{-1} \cdot year^{-1}$ and MABP was 102 mmHg (34). In the present study, with a mean follow-up of 3 years, the annual loss of GFR was $3.2\ ml \cdot min^{-1} \cdot year^{-1}$ (2.2–4.7) on average, considering the entire cohort with a MABP of 95 ± 1 mmHg during AT1 receptor blockade. MABP of 95 mmHg corresponds to a rate of decrease in GFR of $\sim 2.5\ ml \cdot min^{-1} \cdot year^{-1}$ in the observational study described above. In the largest intervention study of type 1 diabetic patients with diabetic nephropathy (35), the average rate of decrease in creatinine clearance was considerably higher than in the present study: $8.0\ ml \cdot min^{-1} \cdot year^{-1}$ in the captopril group and $10.8\ ml \cdot min^{-1} \cdot year^{-1}$ in the patients treated with antihypertensive drugs other than ACE inhibitors or calcium channel blockers. Recently, two large studies in type 2 diabetes, RENAAL and IDNT, have investigated the long-term renoprotective effect of AT1 receptor blockade in hypertensive type 2 diabetic patients with diabetic nephropathy (36,37). AT1 receptor blockade compared with conventional antihypertensive treatment conferred beneficial renoprotective effects in both studies on rate of decline in GFR. In RENAAL, the estimated rate of decline in GFR was $4.4\ ml \cdot min^{-1} \cdot year^{-1}$ in the losartan group compared with $5.2\ ml \cdot min^{-1} \cdot year^{-1}$ in the conventionally treated group ($P = 0.01$). Similarly, in the IDNT study, rate of decline in GFR as evaluated by creatinine clearance was 5.5

ml · min^{-1} · year^{-1} in the irbesartan group compared with 6.5 ml · min^{-1} · year^{-1} in the conventional group. No information of a possible influence of ACE/ID polymorphisms is available from these studies.

Pharmacogenetics is the study of influence of genetic differences on patients' response to pharmacologic intervention. Uncovering gene polymorphisms in patients with similar disease phenotypes may identify groups of patients who are more likely to benefit more from a particular drug. The Euclid Study Group included 530 normotensive normoalbuminuric or microalbuminuric type 1 diabetic patients in a randomized trial lasting 2 years and demonstrated, in a prospective design, a significantly greater beneficial effect of ACE inhibition on urinary albumin excretion rate in the II group as compared with the DD genotype (4). We have previously investigated the association between the ACE/ID polymorphism and the initial antiproteinuric response to ACE inhibition (15). We found that type 1 diabetic patients suffering from diabetic nephropathy homozygous for the I allele have a significantly larger antialbuminuric response to ACE inhibitor treatment as compared with the DD genotype patients, which may suggest a superior long-term beneficial outcome for the II group (38).

Several longitudinal follow-up studies in diabetic kidney disease have found that the D allele is associated with an increased risk of renal function loss (3,5,7,8,10). These data have been supported by recent morphological studies of early diabetic glomerulopathy (39,40). A study from our group in type 1 diabetic patients suffering from diabetic nephropathy showed an accelerated initial and sustained rate of decline in GFR during ACE inhibition in patients homozygous for the D allele compared with II homozygous patients (3). Conflicting data have been reported in nondiabetic renal disease (11,12). Application of pharmacogenetics in medical treatment may expand the population of patients who can be optimally treated. The renoprotective effect of AT1 receptor blockade demonstrated in the present study may be an important advance when influence of the ACE/ID genotype is eliminated. DD patients may obtain a specific blockade of the renin-angiotensin system by AT1 receptor blockade, compared to a potential incomplete inhibition of the system during ACE inhibition. However, suggestions of selective renoprotective therapy for different genotypes require further prospective studies, ideally a randomized prospective comparison of ACE inhibition and AT1 receptor blockade in II and DD patients. Furthermore, pharmacogenetic studies related to other genetic polymorphisms may also be considered.

In summary, we investigated the long-term effect of the losartan 100 mg o.d. on kidney function in II and DD type 1 diabetic patients with diabetic nephropathy. In contrast to previous observational studies with ACE inhibitors, long-term treatment with losartan has similar beneficial renoprotective effects on progression of diabetic nephropathy in hypertensive type 1 diabetic patients with ACE II and DD genotypes.

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