Original Articles

Lack of Relationship Between an Insertion/Deletion Polymorphism in the Angiotensin I–Converting Enzyme Gene and Diabetic Nephropathy and Proliferative Retinopathy in IDDM Patients

Lise Tarnow, François Cambien, Peter Rossing, Flemming S. Nielsen, Birgitte V. Hansen, Laure Lecerf, Odette Poirier, Sergei Danilov, and Hans-Henrik Parving

Genotypic abnormalities of the renin-angiotensin system have been suggested as a risk factor for the development of diabetic nephropathy and proliferative retinopathy. We studied the relationship between an insertion(I)/deletion (D) polymorphism in the angiotensin-converting enzyme (ACE) gene in insulin-dependent diabetes mellitus (IDDM) patients with diabetic nephropathy (121 men and 77 women, age 40.9 ± 10 years, diabetes duration 27 ± 8 years) and in IDDM patients with normoalbuminuria (118 men and 74 women, age 42.7 ± 10 years, diabetes duration 26 ± 8 years). A total of 155 patients (40%) had proliferative retinopathy, and 67 patients (17%) had no diabetic retinopathy. There was no difference in genotype distribution between IDDM patients with diabetic nephropathy and those with normoalbuminuria: 63 (32%)/95 (48%)/40 (20%) vs. 67 (35%)/77 (41%)/46 (24%) had DD/ID/II genotypes, respectively. Patients with nephropathy had higher plasma ACE levels (609 [151–1,504] μg/l) compared with patients with normoalbuminuria (428 [55–1,630] μg/l) ($P < 0.001$). Multiple linear regression analysis revealed that the plasma ACE level in patients with nephropathy is partially determined by ACE/ID polymorphism, mean arterial blood pressure, and glomerular filtration rate ($r^2 = 0.30$, $P < 0.001$). There was no difference in genotype distribution between IDDM patients with proliferative retinopathy and those without diabetic retinopathy: 52 (34%)/74 (48%)/29 (19%) vs. 26 (39%)/25 (37%)/16 (24%) had DD/ID/II genotypes, respectively. There was also no difference in plasma ACE concentration detected among patients with no, simplex, or proliferative retinopathy. We conclude that the ACE/ID polymorphism does not contribute to the genetic susceptibility to diabetic nephropathy and proliferative retinopathy, whereas the raised plasma ACE concentration may play a role in the initiation and progression of diabetic nephropathy in Caucasian IDDM patients. *Diabetes* 44:489–494, 1995

From the Steno Diabetes Center (L.T., P.R., F.S.N., B.V.H., H.-H.P.), Gentofte, Denmark; and Institut National de la Santé et de la Recherche Médicale SC7 (F.C., L.L., O.P) and U367 (S.D.), Paris, France.

Address correspondence and reprint requests to Dr. Lise Tarnow, Steno Diabetes Center, Niels Steensens Vej 2, DK-2820 Gentofte, Denmark.

Received for publication 10 October 1994 and accepted in revised form 19 January 1995.

ACE, angiotensin-converting enzyme; BMI, body mass index; BP, blood pressure; CI, confidence interval; dBP, diastolic blood pressure; D, deletion; I, insertion; IDDM, insulin-dependent diabetes; OR, odds ratio; PCR, polymerase chain reaction; sBP, systolic blood pressure; UAER, urinary albumin excretion rate.

Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria, relentless decline in glomerular filtration rate, raised arterial blood pressure (BP), and high relative morbidity and mortality from cardiovascular disease (1–3). The pathogenesis of this complication is not fully understood, but present evidence suggests a multifactorial origin with contributions from genetic factors (4,5), hemodynamic alterations (6,7), metabolic abnormalities (8,9), and various growth factors, e.g., angiotensin II, as reviewed by Parving et al. (10). Angiotensin-converting enzyme (ACE) is of key importance in regulating systemic and glomerular circulation by converting angiotensin I into angiotensin II and inactivating bradykinin (11). Pharmacological inhibitors of ACE have a beneficial effect on the initiation and progression of diabetic nephropathy (12–17). Furthermore, an increased plasma ACE level has been associated with diabetes per se and microvascular complications in particular (18–21). A recent study has demonstrated that an insertion(I)/deletion (D) polymorphism of the ACE gene (ACE/ID) is strongly associated with the level of circulating ACE and increased risk of coronary heart disease in nondiabetic patients (22). Based on these findings, the ACE gene has been suggested as a candidate gene predisposing to diabetic nephropathy. Two studies from 1994 dealing with relatively small numbers of micro- and macroalbuminuric patients with insulin-dependent diabetes mellitus (IDDM) have yielded conflicting results regarding the ACE/ID polymorphism (21,23).

We have studied the relationship between ACE/ID polymorphism and plasma ACE concentration in large groups of IDDM patients with and without diabetic nephropathy. Since previous studies have suggested a potential role for the renin-angiotensin system in the development of proliferative retinopathy (24), we assessed the possible links among ACE/ID polymorphism, plasma ACE concentration, and proliferative diabetic retinopathy.

RESEARCH DESIGN AND METHODS

We examined the records of all albuminuric patients attending the outpatient clinic at Steno Diabetes Center in 1993 who had IDDM and

TABLE 1
Clinical characteristics of 198 IDDM patients with diabetic nephropathy and 190 IDDM patients with normoalbuminuria

	Nephropathy	Normoalbuminuria	*P* value
n	198	190	
Sex (M/W)	121/77	118/72	NS
Age (years)	40.9 ± 9.6	42.7 ± 10.2	NS
Duration of diabetes (years)	26.7 ± 7.9	25.8 ± 8.5	NS
BMI (kg/m^2)	24.0 ± 3.3	23.6 ± 2.5	NS
HbA_{1c} (%)	9.6 ± 1.5	8.5 ± 1.1	<0.001
Smokers (%)	50	42	NS
UAER (mg/24 h)	796 (16–14,545)	8 (1–30)	—
Serum creatinine (μmol/l)	103 (54–684)	76 (40–116)	<0.001
Glomerular filtration rate ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	75 (10–143)	—	—
sBP (mmHg)	151 ± 23	132 ± 18	<0.001
dBP (mmHg)	86 ± 13	76 ± 10	<0.001
Prevalence of antihypertensive treatment (%)	76	12	<0.001
Retinopathy	—	—	<0.001
Nihil	0	67 (35)	—
Simplex	61 (31)	105 (55)	—
Proliferative	137 (69)	18 (10)	—

Data are means ± SD, median (range), or *n* (%). Some patients with previously persistent albuminuria receiving antihypertensive medication had UAER <300 mg/24 h.

diabetic nephropathy and had their glomerular filtration rate measured during the same year. There were 242 Caucasian patients >18 years old identified and invited to participate in the study, and 200 patients (83%) accepted the invitation and were enrolled. ACE genotyping was performed in 198 patients (Table 1). No additional exclusion criteria were applied. Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent albuminuria of ≥300 mg/24 h in at least two of three consecutive 24-h urine collections, presence of retinopathy, and no clinical or laboratory evidence of kidney or renal tract disease other than diabetic glomerulosclerosis (25,26).

The 201 previously normoalbuminuric (urinary albumin excretion rate [UAER] ≤30 mg/24 h) IDDM patients who were matched for sex, age, and duration of diabetes were recruited from our outpatient clinic (Table 1). At baseline all patients had their UAERs measured: 18 patients had a UAER between 30 and 300 mg/24 h (these patients were asked to collect three additional urine samples), and 10 patients had persistent microalbuminuria. ACE genotyping was performed in 190 patients with persistent normoalbuminuria.

All patients had been dependent on insulin treatment from the time of diagnosis and received at least two daily injections of insulin. The experimental design was approved by the local ethics committee, and all patients gave their informed consent.

Investigations were performed in the morning after an overnight fast. No antihypertensive treatment was received by 24% of the nephropathic patients and 88% of the normoalbuminuric patients. All of the remaining patients were asked to stop their antihypertensive and diuretic treatment 8 days before examination. In patients with nephropathy and normoalbuminuria, 69 (46%) and 9 (39%), respectively, did not want to stop their antihypertensive treatment. Plasma ACE levels were determined by an enzyme-linked immunosorbent assay method using a sandwich combination of monoclonal and polyclonal antibodies (S.D., F. Savoie, B. Lenoir, and F. Alhenc-Gelas, unpublished data) in all patients (129 patients with nephropathy and 181 normoalbuminuric patients) not receiving antihypertensive medication during the last 8 days before blood samples were drawn.

Lymphocytes were isolated from peripheral blood, and DNA was prepared by standard techniques (27). Polymerase chain reaction (PCR) was used to detect the two alleles of the ID polymorphism. DNA was amplified using primers and PCR-cycling conditions as described previously (28). 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.

Urinary albumin concentration was determined by enzyme immunoassay (29) from 24-h urine collections. Arterial BP was measured twice in the right arm after at least 10 min of rest in the supine position, and the results were averaged. The measurements were performed with a Hawksley random zero sphygmomanometer (Hawksley & Sons, Lancing, Sussex, U.K.) and appropriate cuff size. Diastolic BP (dBP) was recorded at the disappearance of Korotkoff sounds (phase V). The glomerular filtration rate was measured after a single intravenous injection of 3.7 MBq ^{51}Cr-EDTA at 9 A.M. by determining the radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (30). The results were standardized for 1.73 m^2 body surface area. Retinopathy was assessed by fundus photography after pupillary dilatation and graded: nihil, simplex, or proliferative diabetic retinopathy.

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). From venous blood samples, HbA_{1c} was determined by high-performance liquid chromatography (DIAMAT analyzer, Bio-Rad, Richmond, CA). The normal range of HbA_{1c} in our laboratory is 4.1–6.1%. Serum creatinine concentration was assessed by a kinetic Jaffe method. Smokers were defined as subjects smoking more than one cigarette, cigar, or pipe per day; all others were classified as nonsmokers.

Statistical analysis. UAER and serum creatinine and plasma ACE concentrations were non-normally distributed and values are given as medians (range); all other values are given as means ± SD.

For normally distributed variables, comparison between groups was performed by an unpaired Student's *t* test or analysis of variance. For non-normally distributed continuous variables, a Mann-Whitney *U* test or Kruskal-Wallis test was used for comparison between groups.

A χ^2 test was used to compare the distribution of ACE genotypes and alleles in case patients and control subjects and was also used for comparison between groups of noncontinuous variables. Allele frequencies were estimated by the gene-counting method, and Hardy-Weinberg equilibrium was checked by a χ^2 test. Multivariate regression analyses were performed by introducing all variables showing significant association ($P < 0.05$) with plasma ACE in univariate analyses. The R^2 value is adjusted for the number of variables introduced into the model.

A *P* value (two-sided) of <0.05 was considered statistically significant. All calculations were performed with a commercially available program, Statgraphics (Manugistics, Rockville, MD).

RESULTS

The group of patients with nephropathy and the normoalbuminuric group were well matched with regard to sex, age, and duration of diabetes. Clinical data for these patients are shown in Table 1.

Patients with nephropathy had elevated systolic BP (sBP) and dBP, raised serum creatinine concentration ($P < 0.001$), and reduced glomerular filtration rate (74 ± 34 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) compared with the normoalbuminuric group. HbA_{1c} was elevated in the nephropathic group compared

TABLE 2
Distribution of ACE genotypes in 198 IDDM patients with diabetic nephropathy and 190 IDDM patients with normoalbuminuria

Genotype	IDDM patients with nephropathy	IDDM patients with normoalbuminuria
DD	63 (32)	67 (35)
ID	95 (48)	77 (41)
II	40 (20)	46 (24)

Data are *n* (%)

with patients with normoalbuminuria ($P < 0.001$). Patients with nephropathy had a higher prevalence of proliferative retinopathy (137 [69%]) compared with patients with normoalbuminuria (18 [10%]; $P < 0.001$).

There was no difference between the ACE genotype distribution in IDDM patients with diabetic nephropathy and IDDM patients with normoalbuminuria (Table 2). The risk of developing nephropathy given an II genotype assessed by odds ratio (OR) (95% confidence interval [CI]) was 0.80 (0.51–1.27). The frequency of D allele/I allele was 0.56/0.44 for both patients with nephropathy and patients with normoalbuminuria. The distribution of ACE genotypes in case patients and control subjects was in Hardy-Weinberg equilibrium (NS).

Plasma ACE levels were 597 (130–1,630), 489 (122–1,625), and 391 (55–1,285) μg/l in patients with DD, ID, and II genotypes, respectively ($P < 0.001$). Irrespective of genotype, nephropathic patients had higher plasma ACE concentrations (609 [151–1,504] μg/l) compared with normoalbuminuric patients (428 [55–1,630] μg/l; $P < 0.001$) (Table 3).

Plasma ACE concentrations were significantly higher in nephropathic patients with sBP ≥140 mmHg ($n = 89$) (685 [151–1,504]) μg/l) compared with nephropathic patients with sBP <140 mmHg ($n = 40$) (519 [202–916] μg/l; $P < 0.001$). In the same group of patients, plasma ACE concentration was elevated with dBP ≥90 mmHg ($n = 58$) (716 [151–1,504] μg/l) compared with a dBP <90 mmHg ($n = 71$) (566 [235–1,134] μg/l; $P < 0.001$). No difference in plasma ACE concentration was observed between nephropathic patients with HbA_{1c} <9.4% (median value) compared with patients with HbA_{1c} >9.4%.

In patients with nephropathy, univariate analyses showed that plasma ACE concentration is correlated with UAER ($r^2 = 0.08, P < 0.002$), sBP ($r^2 = 0.12, P < 0.001$), dBP ($r^2 = 0.09$, $P < 0.001$), mean arterial BP ($r^2 = 0.12$, $P < 0.001$), glomerular filtration rate ($r^2 = 0.15, P < 0.001$), and ACE/ID polymorphism ($r^2 = 0.13$, $P < 0.001$). Subsequent multiple linear regression analysis with these parameters revealed that the plasma ACE level is partially determined by the ACE/ID polymorphism and also increases with rising mean arterial BP and declining glomerular filtration rate (R^2 [adjusted] = 0.30, $P < 0.001$), while UAER does not contribute to the variation in plasma ACE concentration.

TABLE 3
Plasma ACE in 312 IDDM patients with and without diabetic nephropathy

	Plasma ACE (μg/l) with ACE/ID genotype		
	DD	ID	II
Nephropathy ($n = 129$)	659 (256–1,504)	609 (336–1,113)	475 (151–874)
Normoalbumiuria ($n = 181$)	479 (130–1,630)	428 (122–1,625)	363 (55–1,285)
P value	<0.006	<0.001	0.06

All patients had stopped their antihypertensive medication at least 8 days before investigation. The *P* value compared patients with nephropathy to patients with normoalbuminuria, stratified according to ACE/ID genotypes.

TABLE 4
Clinical characteristics of 155 IDDM patients with proliferative retinopathy and 67 patients with no signs of diabetic retinopathy

	Proliferative retinopathy	No signs of diabetic retinopathy	*P* value
n	155	67	
Sex (M/W)	89/66	41/26	NS
Age (years)	41.0 ± 8.6	42.0 ± 9.3	NS
Duration of diabetes (years)	28.2 ± 7.9	25.2 ± 9.1	<0.01
BMI (kg/m^2)	23.9 ± 3.3	23.4 ± 2.4	NS
HbA_{1c} (%)	9.6 ± 1.6	8.4 ± 0.9	<0.001
Smokers (%)	45	50	NS
UAER (mg/24 h)	733 (4–14,545)	9 (2–30)	<0.001
Serum creatinine (μmol/l)	99 (59–684)	76 (40–103)	<0.001
sBP (mmHg)	151 ± 22	132 ± 18	<0.001
dBP (mmHg)	86 ± 13	76 ± 11	<0.001
Prevalence of antihypertensive treatment (%)	72	7	<0.001

Data are means ± SD, median (range), or *n* (%).

Plasma ACE concentration was similar in normoalbuminuric patients with sBP <140 mmHg, dBP <90 mmHg, and HbA_{1c} <8.4% (median value) compared with patients with HbA_{1c} >8.4%.

When patients were stratified according to retinopathy status, we found no difference regarding sex, age, and BMI between patients with proliferative retinopathy and patients without retinopathy (Table 4). Patients with proliferative retinopathy had a longer duration of diabetes ($P = 0.01$), higher HbA_{1c} ($P < 0.001$), elevated sBP and dBP ($P < 0.001$), raised UAER ($P < 0.001$), and increased creatinine concentration ($P < 0.001$). Diabetic nephropathy was present in 137 (88%) patients with proliferative retinopathy.

There was no difference in ACE genotype distribution and no difference in allele frequency (D/I, 0.57/0.43) between patients with proliferative retinopathy and without diabetic retinopathy (Table 5). No difference in plasma ACE concentration in patients with no, simplex, or proliferative retinopathy was observed either, irrespective of presence or absence of diabetic nephropathy.

DISCUSSION

Our cross-sectional case-control study of Caucasians revealed no difference in the I/D polymorphism in the ACE

TABLE 5
Distribution of ACE genotypes in 155 IDDM patients with proliferative retinopathy and 67 IDDM patients without diabetic retinopathy

Genotype	IDDM patients with proliferative retinopathy	IDDM patients with no retinopathy
DD	52 (34)	26 (39)
ID	74 (48)	25 (37)
II	29 (19)	16 (24)

Data are *n* (%).

gene between a large group of IDDM patients with diabetic nephropathy and a large normoalbuminuric group of IDDM patients matched for sex, age, and duration of diabetes. The distributions of the ACE/ID genotypes in case patients and control subjects were in Hardy-Weinberg equilibrium and in accordance with the allele frequencies (0.54/0.46, D allele/I allele) found in the Etude Cas-Témoin de l'Infarctus du Myocarde study control group of Caucasian subjects (n = 733) (22). Furthermore, the allele frequencies in 150 nondiabetic patients measured in the Danish part of the Monitoring of Cardiovascular Diseases study (31) revealed allele frequencies (D/I, 0.55/0.45; T. Hansen, Steno Diabetes Center, personal communication) comparable to our findings of 0.56/0.44 (D/I). Plasma ACE concentration was related to genotype and was elevated in the nephropathic IDDM patients, with higher values in patients with advanced diabetic kidney disease (elevated mean arterial BP and decreased glomerular filtration rate). This finding confirms and extends previous findings from studies of IDDM and non-insulin-dependent diabetes mellitus patients in which increased plasma ACE level is associated with micro- and macroalbuminuria (19–21). No correlation between plasma ACE concentration and metabolic control was observed in our study. Plasma ACE concentration was only measured in patients who had stopped their antihypertensive treatment at least 8 days before the investigation because previous studies have demonstrated that plasma concentrations of the various components of the renin-angiotensin system returned to pretreatment values within 1 week after withdrawal of ACE inhibition (32).

Demographic data for the 17% of patients with diabetic nephropathy who did not wish to participate in the present study were similar to data for the patients studied, except for glomerular filtration rate, which was lower in nonparticipants (61 [10–158] vs. 75 [10–143] $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$; $P <$ 0.03), suggesting an unbiased sample. The risk of development of diabetic nephropathy in our normoalbuminuric group must be considered to be very low because of the long-standing diabetes.

Originally, Marre et al. (21) reported that the II genotype of the ACE gene is a marker for reduced risk for diabetic nephropathy in IDDM patients. Patients with the II genotype had the lowest level of plasma ACE, in agreement with a previous study (33) and the present study. The OR (95% CI) of diabetic nephropathy given a II genotype was 0.22 (0.07–0.65) in the original study (21), 0.72 (0.33–1.56) in a recent study by Doria et al. (23), and 0.80 (0.51–1.27) in the present study. Since the methods applied to measuring ACE/ID genotype were identical and the statistical analyses were very similar, the discrepancy between the three studies must be ascribed to differences in the patients studied or to random fluctuation.

Marre et al. (21) studied 62 IDDM patients with incipient (n = 37) or overt nephropathy (n = 25) compared with 62 normoalbuminuric IDDM patients. The patients with incipient nephropathy all had persistent microalbuminuria (at least two of three consecutive measurements) and normotension, and 43% lacked diabetic retinopathy. The majority will develop diabetic nephropathy. However, a sizable proportion of this group of patients will remain microalbuminuric, and some will spontaneously return to normoalbuminuria (34,35). Furthermore, patients with a number of other conditions, including stroke or myocardial infarction during the preceding 6 months, were excluded from the study.

Doria et al. (23) investigated 74 IDDM patients with evidence of nephropathy ranging from microalbuminuria (n = 33) to overt proteinuria (n = 41) compared with 77 normoalbuminuric IDDM patients. The renal status of each patient was based on only one measurement of albumin excretion rate during a 3-h urine collection period. Because the intra-individual coefficient of variation is very high (30–50%), repeated measurements of UAER are requested to classify patients correctly (26,36,37). The importance of this recommendation is clearly documented, since 33% (95% CI 24–42) of IDDM patients classified as microalbuminuric based on a single determination of UAER will be spontaneously normoalbuminuric 5 years later (38). Regression toward the mean will also play a role in patients characterized as having overt nephropathy based on a single determination of UAER. The diagnosis of diabetic nephropathy in our study was based on generally accepted clinical criteria as described above (25,26,36,37).

It should be mentioned that no difference in the ACE/ID genotype distribution or allele frequency was detected between 175 patients undergoing hemodialysis (46 case patients with diabetic nephropathy) and 136 healthy control subjects (39). From the present study, we cannot exclude the possibility that other DNA sequence differences in the ACE gene may contribute to genetic susceptibility to diabetic nephropathy in IDDM patients (23).

ACE is an endothelial ectoenzyme that plays a key role in the renin-angiotensin and kallikrein-kinin systems by activating angiotensin I into angiotensin II and inactivating bradykinin (11). Rigat et al. (33) found that the ID polymorphism in the ACE gene accounts for half of the phenotypic variance of plasma ACE and that the plasma ACE level in DD subjects was about twice that of II subjects, with ID subjects having intermediate levels. The ACE/ID polymorphism has been shown to be a potent risk factor for myocardial infarction in men formerly considered to be at low risk for cardiovascular complications (22). The ACE gene polymorphism might exert its effect through modulation of the circulating and local levels of angiotensin II and bradykinin. These two peptide hormones have opposite effects on vascular tonus and smooth muscle cell proliferation. The renin-angiotensin system has been suggested to play a role in the development of diabetic retinopathy and nephropathy (10,24,40–42). Although the ACE/ID polymorphism is probably not the locus directly involved in regulating the variability of plasma ACE concentration, it is a strong marker for a nearby functional locus. In our study of patients with diabetic nephropathy, only 29% of variation in plasma ACE concentration could be explained by ACE/ID polymorphism, BP, and glomerular filtration rate, suggesting that other genetic and nongenetic factors are involved. The observed elevation of plasma ACE level in patients with diabetic microalbuminuria (19,21) may suggest that plasma ACE plays a role in the initiation of diabetic nephropathy or at least serves as a risk marker for later development of overt diabetic nephropathy. On the other hand, our results suggest that plasma ACE is involved in the progression of diabetic nephropathy. However, the question of cause or consequence in this matter can only be answered by prospective longitudinal studies in normoalbuminuric IDDM patients

followed until development of incipient and overt diabetic nephropathy.

In diabetic patients, retinopathy is associated with elevated plasma prorenin (24,41), and patients with retinopathy have higher serum ACE levels when compared with nondiabetic subjects (43). These findings have led to the thesis that elevated serum ACE (angiotensin II) levels may be a potential cause of retinal vascular damage in diabetes (24,42).

In accordance with previous studies (20,21), we found that proliferative diabetic retinopathy was associated with a long duration of diabetes and poor metabolic control but not with ACE/ID polymorphism or plasma ACE concentration. The confounding effects of incipient and overt nephropathy were not taken into account in the previous studies that reported an association between diabetic retinopathy and plasma ACE concentration (18,43).

Because of our method of selection, half of our patients had diabetic nephropathy, and since proliferative retinopathy is more prevalent among these patients compared with other IDDM patients, there was a preponderance of patients with diabetic nephropathy among patients with proliferative retinopathy. This preponderance contributes to the differences in UAER, serum creatinine concentration, and BP between patients with proliferative retinopathy and patients without retinopathy. In the normoalbuminuric group, 18 patients had proliferative retinopathy. The ACE/ID gene distribution in these patients was not different from the distribution in normoalbuminuric patients without retinopathy, but the numbers were small.

We conclude that the ACE/ID polymorphism does not contribute to the genetic susceptibility to diabetic nephropathy and proliferative retinopathy, whereas the raised plasma ACE concentration may play a role in the initiation and progression of diabetic nephropathy in Caucasian IDDM patients.

ACKNOWLEDGMENTS

We acknowledge the assistance of B.R. Jensen, U.M. Smidt, and F. Savoie in conducting this study. We thank Dr. Francois Alhenc-Gelas and Dr. Knut Borch-Johnsen for their contributions to the discussion.

REFERENCES

1. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 14:363–370, 1978
2. Parving H-H, Smidt UM, Friisberg B, Bonnevie-Nielsen V, Andersen AR: A prospective study of glomerular filtration rate and arterial blood pressure in insulin-dependent diabetics with diabetic nephropathy. *Diabetologia* 20:457–461, 1981
3. Borch-Johnsen K, Andersen PK, Deckert T: The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 28:590–596, 1985
4. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence of genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161–1165, 1989
5. Borch-Johnsen K, Nørgaard K, Hommel E: Is diabetic nephropathy an inherited complication. *Kidney Int* 41:719–722, 1992
6. Hostetter TH, Rennke HG, Brenner BM: The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med* 72:375–380, 1982
7. Parving H-H, Viberti GC, Keen H, Christiansen JS, Lassen NA: Hemodynamic factors in the genesis of diabetic microangiopathy. *Metabolism* 32:943–949, 1983
8. Wang PH, Lau J, Chalmers TC: Meta-analysis of effects of intensive blood-glucose control on late complications of type I diabetes. *Lancet* 341:1306–1309, 1993
9. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
10. Parving H-H, Østerby R, Anderson PW, Hsueh WA: Diabetic nephropathy. In *The Kidney*. Brenner BM, Rector FC, Eds. Philadelphia, Saunders, 1995. In press
11. Erdos EG: Angiotensin I-converting enzyme and the changes in our concepts through the years. *Hypertension* 16:363–370, 1990
12. Marre M, Chatellier G, Leblanc H, Guyenne T-T, Ménard J, Passa Ph: Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. *Br Med J* 297:1092–1095, 1988
13. Mathiesen ER, Hommel E, Giese J, Parving H-H: Efficacy of captopril in postponing nephropathy in normotensive diabetic patients with microalbuminuria. *Br Med J* 303:81–87, 1991
14. Viberti GC, Mogensen CE, Groop L, Pauls JF: Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. *JAMA* 271:275–279, 1994
15. Parving H-H, Hommel E, Smidt UM: Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *Br Med J* 297:1086–1091, 1988
16. Björck S, Mulec H, Johnsen SA, Nordén G, Aurell M: Renal protective effect of enalapril in diabetic nephropathy. *Br Med J* 304:339–343, 1992
17. Lewis E, Hunsicker L, Bain R, Rhode R: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 329:1456–1462, 1993
18. Lieberman J, Sastre A: Serum angiotensin-converting enzyme: elevations in diabetes mellitus. *Ann Intern Med* 93:825–826, 1980
19. Hallab M, Bled F, Ebran JM, Suraniti S, Girault A, Fressinaud P, Marre M: Elevated serum angiotensin I converting enzyme activity in type I, insulin-dependent diabetic subjects with persistent microalbuminuria. *Acta Diabetol* 29:82–85, 1992
20. van Dyk J, Erman A, Erman F, Chen-Gal B, Sulkes J, Boner G: Increased serum angiotensin converting enzyme activity in type I insulin-dependent diabetes mellitus: its relation to metabolic control and diabetic complications. *Eur J Clin Invest* 24:463–467, 1994
21. Marre M, Bernadet P, Gallois Y, Savagner F, Guyenne T-T, Hallab M, Cambien F, Passa Ph, Alhenc-Gelas F: Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. *Diabetes* 43:384–388, 1994
22. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359:641–644, 1992
23. Doria A, Warram JH, Krolewski AS: Genetic predisposition to diabetic nephropathy: evidence for a role of the angiotensin I–converting enzyme gene. *Diabetes* 43:690–695, 1994
24. Danser AHJ, van den Dorpel MA, Deinum J, Derkx FHM, Franken AAM, Peperkamp E, de Jong PTVM, Schalekamp MADH: Renin, prorenin, and immunoreactive renin in vitreous fluid from eyes with and without diabetic retinopathy. *J Clin Endocrinol Metab* 68:160–167, 1989
25. Parving H-H, Andersen AR, Smidt UM, Svendsen PA: Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* i:1175–1179, 1983
26. Mogensen CE, Chachati A, Christensen CK, Close CF, Deckert T, Hommel E, Kastrup J, Lefevre P, Mathiesen ER, Feldt-Rasmussen B, Schmitz A, Viberti GC: Microalbuminuria: an early marker of renal involvement in diabetes. *Uremia Invest* 9:85–95, 1985
27. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual*. Plainview, NY, Cold Spring Harbor Laboratory Press, 1991
28. Rigat B, Hubert C, Corvol P, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP 1) (dipeptidyl-carboxy peptidose 1). *Nucleic Acids Res* 20:1433–1433, 1992
29. Feldt-Rasmussen B, Dinesen B, Deckert M: Enzyme immunoassay: an improved determination of urinary albumin in diabetics with incipient nephropathy. *Scand J Clin Lab Invest* 45:539–544, 1985
30. Bröchner-Mortensen J, Rödbro P: Selection of routine method for determination of glomerular filtration rate in adult patients. *Scand J Clin Lab Invest* 36:35–45, 1976
31. Jørgensen T: An epidemiological investigation. *Dan Med Bull* 37:336–346, 1990
32. Gadsbøll N, Leth A, Giese J, Nielsen MD, Rasmussen S: Blood pressure response to withdrawal of converting enzyme inhibition. *J Hypertens* 4 (Suppl. 6):S474–S476, 1986
33. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346, 1990
34. Feldt-Rasmussen B, Mathiesen ER, Deckert T: Effect of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. *Lancet* ii:1300–1304, 1986
35. Feldt-Rasmussen B, Mathiesen ER, Jensen T, Lauritzen T, Deckert T: Effect of improved metabolic control on loss of kidney function in type 1 (insulin-dependent) diabetic patients: an update of the Steno studies. *Diabetologia* 34:164–170, 1991
36. Feldt-Rasmussen B: Microalbuminuria and clinical nephropathy in type 1

(insulin-dependent) diabetes mellitus: pathophysiological mechanisms and intervention studies. *Dan Med Bull* 36:405–415, 1989
37. Mathiesen ER: Prevention of diabetic nephropathy: microalbuminuria and perspectives for intervention in insulin-dependent diabetes. *Dan Med Bull* 40:273–285, 1993
38. Almdal T, Nørgaard K, Feldt-Rasmussen B, Deckert T: The predictive value of microalbuminuria in IDDM. *Diabetes Care* 17:120–125, 1994
39. Nomura H, Koni I, Michishita Y, Morise T, Takeda R: Angiotensin-converting enzyme gene polymorphism in haemodialysis patients. *Lancet* 343:482–483, 1994
40. Fernandez LA, Twickler J, Mead A: Neovascularization produced by angiotensin II. *J Lab Clin Med* 105:141–145, 1985
41. Wilson DM, Luetscher JA: Plasma prorenin activity and complications in children with insulin-dependent diabetes mellitus. *N Engl J Med* 323: 1101–1106, 1990
42. Franken AAM, Derkx FHM, Man in't Veld AJ, Hop WCJ, van Rens GH, Peperkamp E, de Jong PTVM, Schalekamp MADH: High plasma prorenin in diabetes mellitus and its correlation with some complications. *J Clin Endocrinol* 71:1008–1015, 1990
43. Toop MJ, Dallinger KJC, Jennings PE, Barnett AH: Angiotensin-converting enzyme (ACE): relationship to insulin-dependent diabetes and microangiopathy. *Diabetic Med* 3:455–457, 1986