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Evaluation of risk factors for the development of nephropathy in patients with IDDM: insertion/deletion angiotensin converting enzyme gene polymorphism, hypertension and metabolic control

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Summary Diabetic nephropathy represents a major complication in patients with insulin-dependent diabetes mellitus (IDDM). Intervention trials using angiotensin-converting enzyme (ACE) inhibitors have pointed towards the important pathogenetic role of the renin-angiotensin system. Recently an insertion/deletion (I/D) polymorphism for the gene encoding the ACE has been described, the deletion type being associated with higher plasma ACE levels. As the intrarenal renin-angiotensin system might also be activated in this setting, we determined the ACE genotype together with other risk factors for the development of diabetic nephropathy in 122 patients with IDDM from a single centre with ($n = 63$) and without ($n = 59$) nephropathy. Long-term glycaemic control was evaluated using mean HbA_{1c} values from the last 10 years. The two patient group were comparable with regard to duration of diabetes and glycaemic control as assessed by current HbA_{1c} values. However, mean long-term HbA_{1c} values were significantly higher in patients with diabetic nephropathy as was systemic blood pressure. The DD genotype was more prevalent in patients with renal disease. In the subgroup of patients who had had diabetes for more than 20 years ($n = 90$), the DD genotype was even more frequent in patients with nephropathy, and blood pressure and long-term HbA_{1c} values were also higher in patients with renal disease. Logistic regression analysis revealed long-term glycaemic control, blood pressure and the ACE genotype to be independent risk factors for the prevalence of diabetic nephropathy. [Diabetologia (1997) 40: 327–331]

Keywords Diabetic nephropathy, risk factors, ACE polymorphism, glycaemic control, hypertension.

Diabetic nephropathy develops in about 30 % of patients with insulin-dependent diabetes mellitus (IDDM) [1]. The pathogenesis is considered to be multifactorial and genetic, and other factors such as metabolic control and haemodynamic alterations resulting in systemic and intrarenal hypertension might also contribute [2]. The results of several trials using angiotensin-converting enzyme (ACE) inhibitors suggest a renoprotective potency of these drugs which seems to be even independent of the effects on systemic blood pressure [3–5], indicating an important role of the intrarenal renin-angiotensin system [6].

Recently, an insertion/deletion (I/D) polymorphism of the ACE gene located on intron 16 was shown to account for 44 % of the interindividual variability of plasma ACE levels [7]. The DD genotype is associated with higher plasma ACE levels [8, 9] and therefore a higher activity of the intrarenal renin-angiotensin system resulting in potentially deleterious effects on a diseased kidney might be expected in these subjects.

Although several groups [10, 11] found plasma ACE levels to be higher in patients with diabetes and nephropathy when compared to patients without

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; ACE, angiotensin converting enzyme; I/D, insertion/deletion.

renal disease, a clear correlation between the ACE polymorphism and the prevalence of diabetic nephropathy has not been established. After a first positive report controversial data were published recently [11, 12].

We therefore analysed the frequency distribution of the various genotypes of the ACE gene together with other risk factors for the development of diabetic nephropathy in a homogeneous group of patients with IDDM.

Subjects and methods

We enrolled 122 Caucasian patients (78 male, 44 female) with IDDM for the study. Age, sex, onset of diabetes and prevalence of retinopathy (background or proliferative) were assessed. Blood pressure, glycosylated haemoglobin (HbA_{1c}), serum creatinine, creatinine clearance and 24-h albumin excretion were measured on two consecutive visits. Of the patients 43 were on antihypertensive therapy, 29 of them were treated with an ACE inhibitor. Blood pressure was measured twice at the end of each visit, after at least 10 min rest and the mean value of these two measurements was used for analyses.

All patients were recruited from a single outpatient clinic and therefore HbA_{1c} values from the last 10 years (HbA_{1c} analyses had been carried out in this department using an identical HPLC procedure (Diamat; Bio Rad, Labs., Hercules, CA 94547) during the complete study period) could be used for evaluation of long-term glycaemic control. For all analyses, mean HbA_{1c} values from each year (of three values on average) were calculated. The mean of these values (four on average) was used for logistic regression analyses and comparisons with recent HbA_{1c} values.

Patients were diagnosed as suffering from diabetic nephropathy if they showed any of the following characteristics: microalbuminuria of 30–300 mg/day on both occasions, overt albuminuria, an increase in serum creatinine or if they needed any kind of renal replacement therapy.

The ACE I/D polymorphism analysis was performed by isolating DNA from peripheral blood cells, followed by a PCR procedure using standard techniques [13]. Subsequently the genotype of DD patients was confirmed using an insertion specific primer pair as described by Lindpaintner et al. [14].

ACE genotyping was also performed in an age-matched group of local Caucasian healthy control subjects.

Statistical analysis was performed by using the unpaired *t*-test for comparison of data between the two patient groups, while the chi-square test was used to compare frequency distribution of the ACE gene polymorphism. The paired *t*-test was used to compare current HbA_{1c} values with the mean value of the previous years. Univariate logistic regression analysis was done for the prognostic factors (blood pressure, long-term glycaemic control, duration of diabetes (all of them used as continuous factors) and ACE genotype) to evaluate their unadjusted influence on the prevalence of diabetic nephropathy in various patient populations. In a second step we calculated the multiple regression model to analyse the influence of each covariate adjusted for the other factors. All mentioned covariates were included at the same time (no stepwise mode was used). The dependent variable of these models was therefore the prevalence of nephropathy (yes vs no). In order to analyse the risk associated with the various ACE genotypes, the genotype was supposed to be ordinal scaled, with II at the lowest level, followed by ID and DD. This assumption was based on an initial statistical analysis using ACE I/D as qualitative factors, which showed effects increasing from ID vs II to DD vs II. As this is also in good agreement with the corresponding ACE plasma levels published elsewhere [8, 9], we used the ordinal scale to achieve a higher statistical power in the multiple logistic regression model. To control whether the influence of one parameter considered was dependent on the level of the others, we also included interaction terms in the multiple logistic regression model.

All values are given as mean ± SD.

Results

No clinical sign of renal disease was seen in 59 patients, whereas 63 patients were allocated to the nephropathy group. Of these, 42 had normal renal excretory function but were positive in terms of microalbuminuria, corresponding to stage 3 according to Mogensen et al. [15], 9 patients suffered from chronic renal failure (stage 4), 2 patients were on regular haemodialysis, 5 were on peritoneal dialysis and 5 had a functioning kidney allograft (stage 5).

The two groups did not differ with regard to age, sex, duration of diabetes and current glycaemic control (Table 1). The patients with nephropathy had higher long-term HbA_{1c} values and systolic, diastolic and mean arterial blood pressure although the prevalence of antihypertensive therapy was much higher in this group (37 patients on antihypertensive treatment in the group with nephropathy vs 6 patients in the group without nephropathy. ACE inhibitors were used in 29 patients, all of whom were in the nephropathy group). Retinopathy was significantly more common in patients with nephropathy; 33 patients of the nephropathy group had background and 29 patients proliferative retinopathy compared to 32 patients with background and 6 with proliferative retinopathy in the non-nephropathy group. In the patient group with renal dysfunction, the prevalence of the DD genotype was higher and the prevalence of II was lower, although this did not reach statistical significance (27% DD genotype, 56% ID and 17% II as compared to 12% DD, 58% ID and 30% II in patients

Table 1. Patient characteristics

Nephropathy	Yes	No
n (male/female)	63 (46/17)	59 (32/27)
Age (years)	46 ± 11	43 ± 12
Duration of diabetes (years)	27.6 ± 10	24 ± 10
Current HbA_{1c} (%)	8.5 ± 1.3	8.2 ± 1.1
Long-term mean HbA_{1c} (%)	8.3 ± 1.1	7.8 ± 1[a, b]
Systolic blood pressure (mm Hg)	138 ± 19	123 ± 15[a]
Diastolic blood pressure (mm Hg)	82 ± 9	76 ± 10[a]
Mean arterial pressure (mm Hg)	100 ± 11	92 ± 10[a]
Retinopathy	62 (98%)	38 (64%)[a]

Data are mean ± SD

[a] $p < 0.05$ nephropathy yes vs no; [b] $p < 0.05$ current vs long-term HbA_{1c}

without nephropathy, $p = 0.057$). When performing a contingency analysis between nephropathy yes/no and the allelic frequencies, we found a significantly higher D allele frequency in the nephropathy group than in control subjects (0.55 % D in the nephropathy group vs 0.41 % in the control group, $p < 0.05$).

Since nephropathy usually becomes clinically evident 15 to 20 years after manifestation of IDDM [1], a fact which might result in patients with shorter disease duration to be misplaced into the "no-disease" group, we performed a further analysis that comprised only those patients with a diabetes duration of at least 20 years ($n = 90$). Again, the patient groups were not different regarding age, sex, current glycaemic control and duration of diabetes (Table 2). Long-term mean HbA_{1c} values, systolic, diastolic and mean arterial blood pressure remained higher in patients with diabetic nephropathy. All the patients in this group showed signs of retinopathy. In these patients we found significantly different ACE genotype frequencies between patients with and without nephropathy (Table 3).

As in patients with IDDM other factors might also predispose to nephropathy we performed a univariate and multiple logistic regression analysis in both patient groups using several other risk factors. For this analysis, differences between long-term glycaemic control and recent values were assessed. Long-term glycaemic control values were significantly lower in the entire patient group than recent HbA_{1c} values (8.07 ± 1.1 vs 8.37 ± 1.22 %). Moreover, after having divided patients on the basis of "good" or "poor" glycaemic control (mean HbA_{1c} value below or above 8.1 %, respectively according to Krolewski et al. [16]), we found that only 73 % of the patients were allocated to the same group for both parameters. We therefore used mean long-term HbA_{1c} values for all logistic regression analyses.

Moreover, we included additional parameters such as diabetes duration as well as ACE genotype. All parameters, i.e. diabetes duration, metabolic control and the ACE genotype were assessed as independent risk factors in the analysis of the total study population. In the presence of diabetes for more than 20 years, poor long-term glycaemic control and presence of the DD genotype vs ID and ID vs II, respectively were associated with an increased prevalence of diabetic renal disease (Table 4).

In order to determine whether blood pressure is also a risk factor for the development of diabetic nephropathy, all patients with overt nephropathy were excluded because renal disease per se could increase blood pressure. In 77 microalbuminuria-positive patients, logistic regression showed mean arterial pressure to be an independent risk factor for the prevalence of diabetic nephropathy (Table 4).

The odds ratios of the covariates adjusted for the other factors (multiple logistic regression) of all three populations are given in Table 4. These estimated odds ratios in the case of continuous factors describe the relative odds for developing nephropathy for each increment by one unit. In the case of ACE genotype the odds ratio describes the relative odds for developing nephropathy for ID patients compared to II patients and DD patients compared to ID patients, respectively.

Table 2. Characteristics of the patients with a minimum diabetes duration of 20 years ($n = 90$)

Nephropathy	Yes	No
n (male/female)	50 (35/15)	40 (22/18)
Age (years)	47 ± 11	47 ± 12
Duration of diabetes (years)	31 ± 8.5	29 ± 8
Current HbA_{1c} (%)	8.4 ± 1.3	8.0 ± 1.2
Long-term mean HbA_{1c} (%)	8.1 ± 1[b]	7.6 ± 1[a, b]
Systolic blood pressure (mm Hg)	139 ± 20	123 ± 17[a]
Diastolic blood pressure (mm Hg)	81 ± 10	76 ± 9[a]
Mean arterial pressure (mm Hg)	101 ± 11	92 ± 11[a]
Retinopathy	50 (100 %)	31 (78 %)[a]

Data are mean ± SD
[a] $p < 0.05$ nephropathy yes vs no; [b] $p < 0.05$ current vs long-term HbA_{1c}

Table 3. Distribution of the ACE gene polymorphism in patients with a minimum diabetes duration of 20 years ($n = 90$)

	ACE polymorphism		
	DD	ID	II
Patients (%)			
With nephropathy	14 (28)	27 (54)	9 (18)
Without nephropathy	4 (10)	21 (53)	15 (37)

Data are *n* (%)
$\chi^2 = 6.78$, $p < 0.05$

Table 4. Odds ratios (OR) and 95 % confidential intervals (CI) in the multiple regression models in all three patient populations

Whole popultion ($n = 122$)	OR	95 % CI
ACE : ID vs II	2.15	1.16–3.95
HbA_{1c} mean	1.69	1.16–2.46
Diabetes duration	1.05	1.01–1.10
Population with diabetes > 20 years ($n = 90$)	OR	95 % CI
ACE : ID vs II	2.26	1.13–4.52
HbA_{1c} mean	1.77	1.09–2.88
Microalbuminuria positive patients ($n = 77$)	OR	95 % CI
ACE : ID vs II	2.58	1.13–5.9
HbA_{1c} mean	1.65	0.97–2.8
Mean arterial pressure	1.06	1–1.12

Discussion

In our population of 122 patients with IDDM the DD genotype was more common in patients with nephropathy although the allele frequency distribution in

the whole study population was not significantly different from that obtained in a local healthy control group (in 168 persons we found a DD/ID/II distribution of 47/81/40, D-allele frequency = 0.52) or in the large control groups published by Schunkert or Cambien [17, 18]. However, in order to correctly allocate patients to the nephropathy or non-nephropathy group it is necessary to keep in mind the fact that in our study microalbuminuria was used as the earliest clinical marker of nephropathy, which usually develops after a diabetes duration of 15–20 years [1]. We therefore performed a further analysis that included only those patients who had been suffering from diabetes for at least 20 years. An analysis of this subpopulation revealed an even stronger co-dominant effect of the D-allele on the presence of diabetic renal disease, with a 2.25-fold increase in risk in patients with the DD genotype when compared to ID and a 5-fold increase in risk when compared to II genotype patients (values adjusted for glycaemic control).

Poor metabolic control is also known to be a major risk factor for the development of diabetic nephropathy [16]. Tarnow et al. [11] compared patients with significantly different HbA_{1c} values (8.5 and 9.6%), and found that the ACE polymorphism did not have any effect on the prevalence of diabetic nephropathy. Based on the fact that mean HbA_{1c} levels were significantly higher in patients with nephropathy than in those without, one could argue that in this group, an effect of the I/D polymorphism on nephropathy may be obscured by the metabolic situation. In order to objectively evaluate the impact of the ACE genotype, the patient groups should be well matched with regard to metabolic control.

In contrast to recently published studies dealing with ACE polymorphism and diabetic nephropathy, we assessed all available HbA_{1c} values since the beginning of analyses in this outpatient department. The long-term values definitely provide a better overview of glycaemic control and were statistically different from recent HbA_{1c} values. Krolewski et al. [16] who were the first to assess the exact impact of glycaemic control on diabetic nephropathy, found a cut-off point of HbA_{1c} of 8.1% above which there was an increased risk for diabetic nephropathy. Accordingly, we divided our patients in terms "good" and "poor" glycaemic control (above or below 8.1%). The current HbA_{1c} values were found to correctly allocate only 73% of the total patient population. Using the long-term follow-up values, glycaemic control was shown to be an independent risk factor for the prevalence of diabetic nephropathy and should be considered in all analyses.

Our findings are comparable with the data published by Marre et al. [10] who described similar results in a comparable study population. However, these results were not confirmed by Schmidt et al. [12]. One possible explanation for these contrasting findings might be that the study by Schmidt et al. [12] was a multicentre trial with an inhomogeneous study population as suggested by the low number of heterozygote patients. In genetic analyses, selection of patients is of great importance and single centre analyses are preferable [19]. Conclusions from multicentre studies should be drawn with caution.

Owing to the fact that our population included patients with chronic renal failure and renal replacement therapy, and the fact that the origin of hypertension may be multifactorial in these cases, blood pressure could not be included in the logistic regression analyses. We therefore performed a further analysis, that included only microalbuminuria positive patients from the nephropathy group. In these patients, mean arterial pressure was found to be a further independent risk factor for the prevalence of diabetic nephropathy, in addition to the effect of ACE genotype. This is in striking contrast to more recent findings that point towards the importance of the renin-angiotensin system for the development of hypertension and cardiovascular complications thereof in IDDM [20–22]. Nonetheless, as mean arterial pressure is a predictive factor for the prevalence of diabetic nephropathy even in patients under antihypertensive treatment, one can argue that intensified blood pressure lowering therapy might be beneficial.

In conclusion, poor glycaemic control, high blood pressure and a co-dominant effect of the ACE-D allele are major risk factors for the development of diabetic nephropathy. A 5-fold increase in risk was registered in DD genotype patients when compared to II genotype patients. An analysis of ACE polymorphism is of great interest because it permits early identification of high-risk patients.

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