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Genetic variants of the renin-angiotensin system, diabetic nephropathy and hypertension

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Summary Recent studies have suggested an association between a deletion (D) variant of the angiotensin-converting-enzyme (ACE) gene and diabetic nephropathy. However, this finding has not been confirmed by all investigators. Furthermore, an M235T variant of the angiotensinogen (AGT) gene has been associated with hypertension, an important risk factor for the development and progression of diabetic nephropathy. The objective of our study was therefore to examine the relationship between these genetic variants of the renin-angiotensin system and diabetic nephropathy and hypertension, respectively, in a large (n = 661) group of Caucasian patients with insulin-dependent (n = 360) or non-insulin-dependent (n = 301) diabetes mellitus. The study had a power of 0.8 to detect a doubling of risk of nephropathy or hypertension in patients with the ACE-DD or AGT-235TT genotype, respectively. Allelic frequencies of the ACE-D and AGT-235T alleles were similar between patients with and without nephropathy in either type of diabetes, and accordingly, there was no significant association between diabetic nephropathy and the ACE or AGT genotype. Likewise, there was no significant association between the ACE or AGT genotype and hypertension. Thus, our data, in this large and ethnically homogeneous group of patients, do not support the hypothesis that these genetic variants of the renin-angiotensin system are strongly associated with either nephropathy or hypertension in patients with insulin-dependent or non-insulin-dependent diabetes mellitus. These genetic markers are therefore unlikely to serve as clinically useful predictors of either nephropathy or hypertension in Caucasian patients with diabetes. [Diabetologia (1997) 40: 193–199]

Keywords Angiotensinogen, angiotensin-converting enzyme, hypertension, genetic, genes, diabetes mellitus, insulin-dependent, non-insulin-dependent, nephropathy.

Diabetic nephropathy, usually preceded by hypertension and persistent albuminuria, eventually develops in 30 to 50 % of patients with insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) diabetes mellitus, and is one of the leading causes of end-stage renal failure in most industrialized countries. Based on the finding that both diabetic nephropathy [1] and hypertension [2] tend to cluster in families, it is anticipated that genetic markers can be identified which could allow the early detection of diabetic individuals prone to the development of this devastating complication.

The renin-angiotensin system plays a central role in blood pressure regulation and renal function not only as a key regulator of sodium homeostasis, but also as a modulator of vascular tone and possibly vascular structure [3]. These effects are primarily mediated by angiotensin II which is liberated from angiotensinogen (AGT) by the sequential action of renin

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Abbreviations: ACE, Angiotensin-converting enzyme; AGT, angiotensinogen; BMI, body-mass index; CAD, coronary artery disease; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; I, insertion; D, deletion; M, methionine; T, threonine; ESRD, end-stage renal disease.

Table 1. Characteristics of diabetic patients

	IDDM (n = 360)		NIDDM (n = 301)	
	Without nephropathy (n = 226)	With nephropathy (n = 134)	Without nephropathy (n = 140)	With nephropathy (n = 161)
Gender (female/male)	96/130	58/76	71/69	77/84
Age (years)	35.7 ± 11.4	38.9 ± 13.1	58.6 ± 9.6	61.4 ± 10.6
Body-mass index (kg/m^2)	24.2 ± 3.1	24.3 ± 3.8	27.8 ± 4.9	27.8 ± 5.1
Duration of diabetes (years)	14.8 ± 10.3	18.8 ± 10.5	11.6 ± 7.9	14.4 ± 9.4
Hypertension (%)	10	23[b]	35	60[c]
Coronary artery disease (%)	2	4	16	25
Stroke (%)	1	1	1	12[c]
Retinopathy (%)	20	41[b]	15	35[c]
Laser therapy (%)	3	16[b]	4	17[c]
HbA_{1C} (%)	8.5 ± 2.1	8.6 ± 2.0	9.0 ± 2.2	8.7 ± 2.3
Serum creatinine (mmol/l)[a]	63.7 ± 8.8	87.5 ± 70.7	65.4 ± 17.7	107.0 ± 150.3
Total serum cholesterol (mmol/l)	5.2 ± 1.1	5.6 ± 1.3	5.9 ± 1.1	6.0 ± 1.3
Serum triglycerides (mmol/l)	1.06 ± 0.62	1.34 ± 0.75	2.01 ± 1.25	2.46 ± 1.58

Data are means ± SD. [a] Non-oliguric patients; [b] $p < 0.005$ vs IDDM patients without nephropathy; [c] $p < 0.005$ vs NIDDM patients without nephropathy

and angiotensin-converting-enzyme (ACE). The importance of the renin-angiotensin system for the development of diabetic nephropathy is supported by several studies indicating a beneficial effect of inhibition of this system on the development and progression of this complication [4, 5].

Recent studies have identified several molecular variants of genes encoding for components of the renin-angiotensin system. One such variant of the ACE gene, involving an intronic deletion (D) of a 289 base pair (bp) *Alu* sequence, is associated with increased plasma and tissue activity of this enzyme [6, 7], and has been implicated as a risk factor for left ventricular hypertrophy [8], myocardial infarction [9], and stroke [10]. Similarly, a variant of the AGT gene, resulting in the substitution of a threonine for a methionine at position 235 (M235T), is associated with increased circulating levels of AGT, and has been reported to be more common in hypertensive than in normotensive control subjects [11].

Several recent studies have also examined the role of these genetic variants of the renin-angiotensin system on the development of diabetic nephropathy [12–18] and other renal diseases [19–21], but the results remain controversial. Thus, while several studies have reported an association between the ACE-D allele and diabetic nephropathy both in patients with IDDM [12] and NIDDM [14, 17, 18], other investigators have failed to confirm this relationship [13, 16]. Although one preliminary report in a small group of patients with NIDDM (n = 76) failed to demonstrate a relationship between the AGT 235T-variant and diabetic nephropathy [15], the issue remains to be addressed in a larger population.

The aim of the current study was therefore to examine the relationship between the ACE-I/D and AGT 235 M/T genotype and the prevalence of diabetic nephropathy and hypertension in a large group (n = 661) of ethnically homogeneous patients with IDDM and NIDDM.

Subjects and methods

The protocol of the study was approved by the ethics committee of our hospital and informed consent for genetic studies was obtained from all participants.

Patient selection and clinical investigation. Diabetic patients (n = 661) of Caucasian ethnicity were enrolled in the study from one large diabetes clinic and four dialysis centres in Berlin. On selected days, determined by laboratory capacity, all patients with diabetes presenting in the diabetes clinic were approached, of whom more than 70 % agreed to participate in the study. In the dialysis centres, 55 patients with end-stage-renal failure due to diabetic nephropathy were identified, of whom 51 agreed to participate. Classification of diabetes as IDDM or NIDDM was based on World Health Organization (WHO) criteria [22]. The diagnosis of nephropathy was based on repeated evidence of albumin excretion of more than 30 mg/24 h or 20 μg/min in non-oliguric patients (n = 610) or terminal renal failure necessitating renal replacement therapy (dialysis or transplantation) in patients with end-stage renal failure (n = 51). Other causes of increased albumin excretion were excluded by appropriate clinical investigation. Hypertension was defined as a systolic blood pressure over 140 mmHg and a diastolic blood pressure over 95 mmHg noted in the medical records on at least two separate occasions or the prescription of antihypertensive medication excluding diuretics. Past medical history regarding coronary heart disease, stroke, and retinopathy was obtained by review of the medical records of each patient by an investigator unaware of the patient's genotype. Coronary heart disease was defined as a history of myocardial infarction, coronary angioplasty or bypass surgery, positive coronary angiography, treatment with nitrates or clinical history of angina pectoris. Stroke was defined as a history of stroke in medical records. Retinopathy was defined as stage III or IV on fundoscopy and history of laser therapy was used as a surrogate marker for the presence of proliferative retinopathy. Serum creatinine, serum lipids and HbA_{1c} were determined by standard laboratory techniques.

Table 2. Characteristics of patients according to ACE I/D genotype

	IDDM (n = 360)						NIDDM (n = 301)					
	Without nephropathy (n = 226)			With nephropathy (n = 134)			Without nephropathy (n = 140)			With nephropathy (n = 161)		
	II (n = 39)	ID (n = 130)	DD (n = 57)	II (n = 31)	ID (n = 68)	DD (n = 35)	II (n = 36)	ID (n = 69)	DD (n = 35)	II (n = 33)	ID (n = 84)	DD (n = 44)
Gender (female/male)	15/24	55/75	26/31	15/16	26/42	17/18	17/19	38/31	16/19	19/14	39/45	19/25
Age (years)	38.4 ± 12.2	35.8 ± 11.6	33.6 ± 10.3	39.0 ± 11.9	41.0 ± 13.5	33.8 ± 10.9	58.1 ± 8.3	59.3 ± 9.9	57.5 ± 10.4	63.6 ± 10.9	60.9 ± 10.6	60.9 ± 10.3
Body-mass index (kg/m^2)	24.4 ± 2.9	24.2 ± 3.1	24.0 ± 3.1	24.2 ± 3.6	24.2 ± 3.9	24.6 ± 3.2	28.1 ± 5.3	27.7 ± 4.7	27.6 ± 5.0	27.7 ± 5.8	27.4 ± 4.3	27.8 ± 5.6
Duration of diabetes (years)	18.2 ± 13.6	14.3 ± 10.4	13.5 ± 9.5	18.4 ± 9.5	20.5 ± 11.2	16.2 ± 9.7	11.1 ± 6.2	11.7 ± 8.6	11.9 ± 8.3	15.1 ± 10.4	13.8 ± 9.5	15.0 ± 8.6
Hypertension (%)	15	9	9	32	27	57	39	36	29	61	67	45
Coronary artery disease (%)	3	2	2	10	6	3	25	16	6	15	29	27
Stroke (%)	0	1	0	0	2	3	3	0	0	12	12	14
Retinopathy (%)	26	19	19	45	43	34	19	16	9	39	35	34
Laser therapy (%)	3	3	2	26	13	11	8	3	0	21	15	28
HbA_{1C} (%)	7.6 ± 0.9	7.9 ± 1.4	8.3 ± 1.5	8.7 ± 1.5	8.5 ± 1.4	8.3 ± 1.6	8.4 ± 1.6	8.3 ± 1.5	8.3 ± 1.7	8.4 ± 1.7	8.4 ± 1.9	8.5 ± 1.6

Data are means ± SD

Genotyping. Genomic DNA from each patient was prepared from peripheral leukocytes separated from a 20 ml blood sample using a DNA-selective preparation method (Quiagen, Hilden, Germany). Subsequently, the ACE I/D variant region was amplified with the polymerase chain reaction technique using a flanking primer pair [7]. In order to exclude mistyping of heterozygotes, amplification was performed in the presence of 5 % dimethylsulphoxide (DMSO) [23]. The AGT M235T-genotype was determined using the mutagenetically separated polymerase chain reaction technique using allele-specific primers as described previously [24].

Statistical analysis

All data are presented as means ± SD or as proportions. Continuous variables were compared by two-sided Student's *t*-test for independent samples and categorical data were assessed by two-sided chi-square statistics. Stepwise logistic regression analysis was performed to identify predictors of nephropathy (SPSS-PC + 6.0). Sample size and power were calculated as described for unequal case-control studies [25]. A p value of less than 0.05 was considered as statistically significant.

Results

The groups of patients with IDDM (n = 360) and NIDDM (n = 301) with and without nephropathy, respectively, were similar with regard to gender distribution, age, body-mass index and known duration of diabetes (Table 1). As expected, hypertension and retinopathy were more common in patients with nephropathy than in non-albuminuric patients. The history of stroke was most common in patients with NIDDM and nephropathy. Coronary artery disease was also more common in patients with NIDDM and tended to be associated with nephropathy in this group. HbA_{1c} levels were comparable among the different groups.

Allelic frequencies of the ACE-D allele was not different between patients with and without nephropathy in patients with IDDM (qD = 0.54 vs 0.51) or NIDDM (qD = 0.50 vs 0.53), and distribution of the ACE genotype was similar to those reported by other investigators in Caucasians [6, 8] (Table 2). There was also no consistent relationship between genotype distribution and the prevalence of hypertension, stroke, retinopathy or coronary artery disease in patients with either IDDM or NIDDM with or without nephropathy.

Similarly, frequencies of the AGT-235T allele was not different between patients with and without nephropathy in IDDM (qT = 0.43 vs 0.46) or NIDDM (qT = 0.46 vs 0.44), and distribution of the AGT genotype was comparable to those reported by us [24] and other investigators [11] in Caucasians (Table 3). There was also no consistent relationship between AGT genotype distribution and the prevalence of hypertension, stroke, retinopathy or coronary artery disease in patients with either IDDM or NIDDM with or without nephropathy.

Table 3. Characteristics of patients according to AGT 235 T/M genotype

	IDDM (n = 360)						NIDDM (n = 301)					
	Without nephropathy (n = 226)			With nephropathy (n = 134)			Without nephropathy (n = 140)			With nephropathy (n = 161)		
	MM (n = 71)	MT (n = 115)	TT (n = 40)	MM (n = 42)	MT (n = 61)	TT (n = 31)	MM (n = 45)	MT (n = 62)	TT (n = 33)	MM (n = 46)	MT (n = 88)	TT (n = 27)
Gender (female/male)	33/38	44/71	20/20	19/23	27/34	13/18	21/24	35/27	15/18	28/18	36/52	13/14
Age (years)	35.2 ± 11.1	35.2 ± 11.5	38.2 ± 12.0	36.9 ± 12.4	40.7 ± 13.9	37.1 ± 10.8	57.4 ± 10.3	60.0 ± 8.4	57.3 ± 10.5	61.1 ± 10.5	61.1 ± 10.7	63.2 ± 10.3
Body-mass index (kg/m^2)	23.4 ± 2.6	24.5 ± 3.2	24.6 ± 3.2	24.1 ± 4.3	24.6 ± 3.4	24.1 ± 3.6	27.1 ± 4.8	27.7 ± 4.8	28.6 ± 5.4	27.3 ± 5.4	27.7 ± 5.0	27.4 ± 4.0
Duration of diabetes (years)	14.4 ± 12.1	14.6 ± 9.2	15.9 ± 10.2	17.8 ± 9.7	20.2 ± 11.4	17.7 ± 9.9	10.8 ± 7.7	11.3 ± 8.0	13.1 ± 8.2	15.1 ± 8.9	14.2 ± 10.2	13.7 ± 7.7
Hypertension (%)	10	11	8	17	26	23	27	42	33	59	60	59
Coronary artery disease (%)	1	3	3	10	5	3	18	18	9	37	20	22
Stroke (%)	0	1	0	0	3	0	0	2	0	17	11	7
Retinopathy (%)	24	18	15	36	44	42	11	15	21	37	33	41
Laser therapy (%)	0	3	3	5	21	19	4	3	3	17	18	11
HbA_{1c} (%)	8.1 ± 1.8	8.0 ± 1.2	8.0 ± 1.3	8.4 ± 1.3	8.6 ± 1.7	8.3 ± 1.3	8.2 ± 1.5	8.5 ± 1.5	8.3 ± 1.9	8.6 ± 2.0	8.4 ± 1.6	8.0 ± 1.5

Data are means ± SD

Stepwise logistic regression analysis, both in IDDM and NIDDM patients, revealed retinopathy ($p < 0.01$), HbA_{1c} ($p < 0.01$), and hypertension ($p = 0.05$), but not ACE ($p > 0.2$) or AGT ($p > 0.2$) genotype, as significant predictors for nephropathy.

Subgroup analysis of the 51 patients with end-stage renal failure on renal replacement therapy likewise revealed similar frequencies of the ACE-D (qD = 0.47) and the AGT-235T (qT = 0.43) alleles to those in the whole population, and genotype distribution in this group was also not related to progression of renal failure or the prevalence of hypertension, retinopathy or coronary artery disease (Table 4). Similarly, subgroup analysis of the 178 patients with retinopathy did not reveal significant differences in allele frequencies of either the ACE-D or AGT-235T alleles in patients with retinopathy compared to those in patients with both retinopathy and nephropathy (Table 5). When IDDM and NIDDM patients were classified with regard to presence or absence of hypertension, again no difference was found between the frequency distribution of the ACE-D or AGT 235T alleles between the hypertensive and normotensive subjects in each group (Table 6).

Discussion

In contrast to previous reports that the ACE-DD genotype may be a risk factor for the development of diabetic nephropathy in patients with IDDM [12] or NIDDM [14, 17, 18], we found no evidence to support this in our large group of Caucasian diabetic patients. This finding is thus in line with the recent report of Schmidt et al. [13], who likewise failed to find an association between the ACE-D allele and nephropathy in patients with IDDM and NIDDM recruited in Germany and Poland. Similarly, Tarnow et al. [16], in a smaller study in patients with IDDM, found no relationship between the ACE-I/D polymorphism and diabetic nephropathy or retinopathy. Thus, together with our findings, it appears unlikely that the ACE-DD genotype will serve as a clinically useful marker of a genetic predisposition for diabetic nephropathy in Caucasian diabetic patients.

Similarly, although the AGT-235TT genotype has been reported as a marker for genetic predisposition to the development of essential hypertension [11, 24] we found no evidence supporting a relationship between this variant and hypertension in patients with either IDDM or NIDDM. Furthermore, the AGT-235TT genotype was not associated with diabetic nephropathy or other vascular complications in these patients. These findings therefore do not support a role for the AGT-235TT genotype as a clinically useful marker for a genetic predisposition for the development of hypertension in Caucasian diabetic patients.

Table 4. Characteristics of patients with end-stage renal disease (ESRD) (n = 51)

	ACE I/D Genotype			AGT 235 M/T Genotype		
	II (n = 16)	ID (n = 22)	DD (n = 13)	MM (n = 18)	MT (n = 22)	TT (n = 11)
IDDM (n)	4	3	2	3	3	3
NIDDM (n)	12	19	11	15	19	8
Time until ESRD (years)	14.6 ± 8.8	16.2 ± 10.4	16.8 ± 9.3	17.3 ± 9.8	16.9 ± 11.5	16.6 ± 9.6
Hypertension (%)	38	23	62	44	36	27
Coronary artery disease (%)	31	68	38	67	45	36
Retinopathy (%)	82	82	85	95	68	82
Laser therapy (%)	50	46	62	44	50	55

Data are means ± SD

Table 5. Characteristics of patients with diabetes and retinopathy with and without nephropathy

	IDDM		NIDDM	
	Retinopathy (n = 45)	Retinopathy and nephropathy (n = 55)	Retinopathy (n = 21)	Retinopathy and nephropathy (n = 57)
Gender (female/male)	26/19	35/20	10/11	32/25
Age (years)	43.6 ± 11.8	42.2 ± 13.2	60.4 ± 8.1	64.9 ± 9.2
Body-mass index (kg/m^2)	24.1 ± 2.9	23.9 ± 3.4	27.9 ± 3.7	26.4 ± 4.6
Duration of diabetes (years)	25.2 ± 9.8	25.2 ± 8.8	19.2 ± 8.8	19.7 ± 8.8
Systolic blood pressure (mm Hg)	132.0 ± 23.9	136.0 ± 22.1	148.0 ± 25.7	151.0 ± 24.6
Diastolic blood pressure (mm Hg)	77.2 ± 11.8	78.7 ± 11.6	79.0 ± 11.5	84.0 ± 17.1
ACE-genotype (II/ID/DD)	10/24/11	14/29/12	7/11/3	13/29/15
AGT-genotype (MM/MT/TT)	17/22/6	15/27/13	5/9/7	17/29/11

Data are means ± SD

Table 6. Characteristics of normotensive and hypertensive diabetic patients

	IDDM		NIDDM	
	Normotensive (n = 306)	Hypertensive (n = 54)	Normotensive (n = 156)	Hypertensive (n = 145)
Gender (female/male)	137/169	17/37	69/87	79/66
Age (years)	34.9 ± 10.8	48.4 ± 12.9[a]	57.9 ± 10.5	62.5 ± 9.4
Body-mass index (kg/m^2)	24.14 ± 3.30	24.97 ± 3.92	27.8 ± 5.4	27.5 ± 4.4
Duration of diabetes (years)	14.8 ± 9.6	24.7 ± 11.7	14.9 ± 8.7	11.3 ± 8.5
Systolic blood pressure (mm Hg)	125.5 ± 14.9	149.0 ± 22.6[a]	132.1 ± 19.0	153.9 ± 23.8[b]
Diastolic blood pressure (mm Hg)	76.4 ± 9.4	83.2 ± 13.1[a]	75.6 ± 8.9	84.3 ± 15.0[b]
ACE-genotype (II/ID/DD)	54/167/85	16/31/7	34/72/49	34/81/30
AGT-genotype (MM/MT/TT)	99/146/61	14/30/10	52/71/33	39/79/27

Data are means ± SD. [a] $p < 0.01$ vs normotensive patients with IDDM; [b] $p < 0.01$ vs normotensive patients with NIDDM

Given the negative nature of these findings several methodological and statistical aspects of our study must be considered. One concern is clearly whether or not albuminuria is indeed a valid marker of diabetic nephropathy. Not all albuminuric diabetic patients ultimately proceed to end-stage renal failure [26], while on the other hand some patients with diabetes may have glomerular lesions without albuminuria [27]. Furthermore, a substantial proportion of patients with NIDDM may have albuminuria for other reasons including congestive heart failure or urinary tract infection [28] which we tried to eliminate in our patients by clinical and laboratory investigation. Nevertheless, even when we analysed subgroups meeting more stringent selection criteria such as patients with albuminuria, hypertension, and retinopathy, or included only those on renal replacement therapy there was still no significant difference in the allelic frequencies of either the ACE-D or AGT-235T variants in these subgroups compared to non-albuminuric control subjects.

Another critical issue regards the statistical power of our study. Based on an α-error of 0.05 and a β-error of 0.2 our study had the power to detect a doubling of the risk (odds ratio of 2.0) of nephropathy associated with the ACE-DD genotype, or a doubling of the risk of hypertension associated with the AGT-235TT genotype, in both IDDM and NIDDM patients, respectively. Detection of a lower risk was not considered clinically important, given the multifactorial nature of these complications. It is therefore unlikely that our negative findings can be attributed to a lack of statistical power.

Previous studies have also implicated the ACE-D allele as a risk factor for the development of coronary artery disease [9, 29] and stroke [30]. However, in our study there was no apparent relationship between the presence of this allele and either coronary artery disease or stroke at least based on clinical evidence for the presence of these complications. We of course fully realise that clinical findings especially with regard to angina may be particularly unreliable in diabetic patients [31], and therefore this question may have to be addressed specifically by future studies based on more stringent diagnostic criteria.

Obviously, the lack of a relationship between the development of nephropathy or hypertension and the studied variants of the renin-angiotensin system does not rule out a role for this system for the development of either complication in diabetic patients. Thus, other, hitherto unrecognised or unstudied variants of the ACE and AGT genes could well be associated with either nephropathy and/or hypertension in diabetic patients, and these genes therefore certainly remain attractive candidate genes. Furthermore, given that both diabetic nephropathy and hypertension are probably due to complex interactions between a variety of genetic and environmental factors [32], our study clearly does not rule out the role of these genetic variants of the renin-angiotensin system in certain subsets of diabetic patients or under certain environmental conditions. Lastly, based on the finding that the studied variants of the ACE and AGT genes are associated with increased activity or expression of these components of the renin-angiotensin-system [6, 7, 11], it may still be of interest to ascertain whether individuals with a certain genotype will profit more from therapeutic blockade of this system by ACE-inhibitors or angiotensin receptor type 1-antagonists than other individuals.

In summary, our study does not support the hypothesis that the ACE-DD or the AGT-235TT genotypes are strongly associated with either nephropathy or hypertension, respectively, in patients with IDDM or NIDDM. These markers are therefore unlikely to serve as clinically useful predictors of either of these important complications in Caucasian patients with diabetes.

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