Angiotensin I–Converting Enzyme and Angiotensinogen Gene Polymorphisms in Non–Insulin-Dependent Diabetes Mellitus. Lack of Relationship With Diabetic Nephropathy and Retinopathy in a Caucasian Mediterranean Population

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Genotypic abnormalities of the renin-angiotensin system have been suggested as a risk factor for the development of microangiopathic complications in diabetic patients. We studied the relationship of either an insertion-deletion polymorphism in the angiotensin-converting enzyme (ACE) gene and the M235T and T174M variant polymorphisms of the angiotensinogen (AGT) gene in non-insulin-dependent diabetes mellitus (NIDDM) patients and its relationship with cardiovascular complications. A total of 193 NIDDM patients (89 men and 104 women aged 59.2 ± 10.0 years; diabetes duration, 13.2 ± 6.2 years) and 90 control subjects (42 men and 48 women aged 45.4 ± 12.6 years) were recruited for the association study. Distribution of the genotype or allelic frequencies for all the studied polymorphisms did not differ significantly between controls and NIDDM patients. ACE and AGT genes did not display any difference in clinical or metabolic parameters according to each gene's genotype for either the control or the NIDDM group. For evaluation of nephropathy and retinopathy, NIDDM patients were matched with subjects not having microangiopathic complications. Thus, a total of 60 patients had diabetic nephropathy and were compared with 100 patients with normoalbuminuria. Sixty-eight NIDDM patients had diabetic retinopathy, and 92 patients presented no signs of retinopathy. There were no differences in genotypic or allelic distribution between NIDDM patients for either the presence or absence of retinopathy or nephropathy. We conclude that the ACE and AGT polymorphisms do not contribute to the genetic susceptibility to diabetic nephropathy and retinopathy in a caucasian Mediterranean population.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM) is a disease frequently associated with an increase in cardiovascular risk. The greater incidence of cardiovascular risk factors in diabetes (obesity, hypertension, and dyslipidemia), as well as long-term complications such as retinopathy and nephropathy, affect both the quality of life and survival of diabetic patients.[1-3]

The development of these vascular complications displays strong interindividual differences. In addition to the greater incidence of classic cardiovascular risk factors in diabetes, genetic factors may contribute to the development of these complications. Several genetic markers have been studied, but a main genetic locus has not yet been identified.

The extensive knowledge of physiological systems implicated in the regulation of blood pressure offers the opportunity to examine the possible role of some candidate genes in the pathogenesis of human hypertension, one of the cardiovascular risk factors related to NIDDM, which in turn would be involved in the outcome of NIDDM long-term complications.

The renin-angiotensin system plays a central role in salt and water homeostasis and maintenance of vascular tone.[4] Recent studies have shown that human cardiovascular tissues contain several components of the renin-angiotensin system: angiotensinogen (AGT), renin, angiotensin I–converting enzyme (ACE), chymase, and angiotensin II receptors. It seems that locally produced angiotensin II may play an important role in cardiovascular homeostasis.[5] Consequently, each component of this system represents a potential candidate in the etiology of hypertension. One of the genes examined in the present study is the ACE gene, whose insertion/deletion (I/D) polymorphism (due to a 287-bp deletion) is strongly associated with the level of its encoded circulating enzyme. This, in turn, plays a key role in the production of angiotensin II and in the catabolism of bradykinin, two peptides involved in the modulation of vascular tone and proliferation of smooth muscle cells.[6] Homozygous DD subjects show higher ACE plasma levels than the remaining genotypes, homozygous II subjects being those who display the lowest ACE plasma values.[7] A greater DD frequency has been observed in patients with myocardial infarction compared with the healthy population.[6]

Another candidate gene is the AGT gene in its two variants M235T (threonine for methionine at position 235) and T174M (methionine for threonine at position 174). The TT genotype of the variant that has a functional effect, M235T, has been associated with higher AGT plasma levels.[8] Correlations between plasma AGT concentration and blood pressure have been reported either in the general population,[9] in hypertensive young adults,[10] or in the offspring of hypertensive parents.[11] Both linkage and association of AGT molecular variants (M235T and T174M) with hypertension have also been reported, suggesting that these AGT polymorphisms may represent markers of an inherited predisposition to essential hypertension in humans.[12] Few such studies on diabetic subjects have been performed, which makes this gene a candidate for the study of cardiovascular or chronic complications in diabetic patients.

We report the findings of an investigation of population frequencies of the ACE and AGT genes and their relationships with nephropathy and retinopathy in NIDDM subjects.

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SUBJECTS AND METHODS

Participants

A case-control study was conducted in the Endocrinology Outpatient Department of the University Hospital Joan XXIII, Tarragona, Spain, on NIDDM subjects with or without diabetic nephropathy or retinopathy. The study population consisted of 193 NIDDM Mediterranean patients aged 31 to 82 years (104 women and 89 men) and 90 randomly selected control subjects aged 20 to 72 years (48 women and 42 men). For evaluation of nephropathy and retinopathy, NIDDM patients were matched with subjects without microangiopathic complications according to gender, age, body mass index (BMI), duration of diabetes, and variables known to be associated with increased urinary albumin concentration, including hemoglobin A_{1c} (HbA_{1c}) and the lipidic profile.

All subjects provided informed consent, and the study was approved by the ethics committee of the University Hospital Joan XXIII of Tarragona. Control subjects had fasting plasma glucose levels less than 6 mmol/L, and those who had an immediate relative with diabetes were excluded.

Blood pressure, height, and weight were measured with a standardized protocol. Blood pressure was always measured on the right arm with subjects in the sitting position, using an appropriately sized cuff. Systolic and diastolic blood pressure (SBP and DBP) were recorded as the mean of three values (with an interval of 2 minutes between each and after 10 minutes of initial rest) to the nearest 2 mm Hg as the first and fifth Korotkoff phases using a standard mercury manometer. Hypertensive subjects were individuals with a SBP of at least 140 and/or DBP of at least 90 mm Hg or who were taking antihypertensive medication. BMI was calculated as the measured weight in kilograms divided by height in meters squared. Retinopathy was evidenced by funduscopy examination by an independent ophthalmologist. Microalbuminuria was defined as at least two urinary albumin excretion (UAE) measurements between 30 and 300 mg/d in a 6-month period. Macroproteinuria was determined by UAE levels higher than 300 mg/d.

Blood and Urine Collection and DNA Extraction

After overnight fasting, blood samples for determination of HbA_{1c}, plasma lipids, lipoprotein(a) [Lp(a)], insulinemia (immunoreactive insulin [IRI]), and C-peptide were obtained. Plasma levels of cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured by established techniques.[13] Low-density lipoprotein cholesterol was obtained by ultracentrifugation.[14] Lp(a) level was measured by enzyme immunoassay (Behring Institute, Marburg, Germany) with a monoclonal antibody against Lp(a) and a polyclonal anti-Lp(a). Intraassay and interassay coefficients of variation for the method were 5% and 10%, respectively. IRI and C-peptide were analyzed by radioimmunoassay only on diet-treated diabetic patients. HbA_{1c} level was measured by a chromatographic method (Glico Hb Quick Column Procedure; Helena Laboratories, Beaumont, TX). The normal range for HbA_{1c} values in our laboratory is 6% to 8%.

A 10-mL sample of venous blood was collected in an EDTA vacutainer. Within 1 hour of sampling, the buffy coat was separated from the blood by centrifugation at 800 to 900 × *g* for 10 minutes. Genomic DNA was isolated from the buffy coat using QiaAMP spin columns (Qiagen, Chatsworth, CA). One hundred nanograms of DNA was used for polymorphism analysis by the polymerase chain reaction (PCR).

Subjects were instructed to collect a 24-hour urine sample for measurement of UAE, determined by nephelometry (Behring; Behringwerke, Marburg, Germany).

ACE Gene Polymorphism Analysis

Intron 16 of the ACE gene was amplified; 100 ng of the extracted DNA was used as a template. The primers used were 5′ CTGGAGACCACTCCCATCCTTTCT 3′ and 5′ GATGTGGCCATCACATTCGTCAGAT 3′. The reaction was performed on a final volume of 50 μL containing 3 mmol/L $MgCl_2$, 0.5 mmol/L of each dNTP (Boehringer, Mannheim, Germany), 0.2 μmol/L of each primer, and 1 U Taq polymerase (Boehringer). DNA was amplified during 30 cycles with 1 minute of denaturation at 94°C, 1 minute of annealing at 58°C, and 2 minutes of extension at 72°C. PCR products were detected by bromide staining in a 1.2% agarose gel.

Because of the preferential D allele amplification, each DD sample underwent a second independent PCR amplification with a primer pair that recognized an insertion-specific sequence: 5′ TGGGACCAGAGCGCCCGCCACTAC 3′ and 5′ TCGCCAGCCCTCCCATGCCCATAA 3′ with identical PCR conditions except for an annealing temperature of 67°C.

RFLP AGT Gene Analysis

The second exon of the AGT gene was amplified. This gene shows two variants, M235T at position +704 and T174M at position +521. For the M235T variant, the primers used were 5′ CAGGGTGCTGTCCACACTGGACCCC 3′ and 5′ CCGTTTGTGCAGGGCCTGGCTTCT 3′. The reaction was performed on a final volume of 50 μL containing 1.5 mmol/L $MgCl_2$, 0.5 mmol/L of each dNTP, 0.2 μmol/L of each primer, and 1 U Taq polymerase. DNA was amplified during 10 cycles with 1 minute of denaturation at 94°C, 1 minute of annealing at 68°C, and 1 minute of extension at 72°C, and 30 cycles with 30 seconds of denaturation at 90°C, 1 minute of annealing at 68°C, and 30 seconds of extension at 72°C. PCR products were digested with 10-fold excess *Asp*I restriction enzyme at 37°C for 2 to 4 hours and electrophoresed on a 4.5% low-melting agarose gel. *Asp*I RFLP was detected by ethidium bromide staining.

For the T174M variant, the primers used were 5′ TACAGGCAATCCTGGGTGTTCCTTG 3′ and 5′ AGCAGAGAGGTTTGCCTTACCTTG 3′. The reaction was performed on a final volume of 50 μL containing 5 mmol/L $MgCl_2$, 0.5 mmol/L of each dNTP, 0.2 μmol/L of each primer, and 1 U Taq polymerase. DNA was amplified during 30 cycles with 1 minute of denaturation at 94°C, 1 minute of annealing at 58°C, and 1 minute of extension at 72°C. PCR products were digested with 10-fold excess *Nco*I restriction enzyme at 37°C for 2 to 4 hours and electrophoresed on a 2.5% agarose gel. *Nco*I RFLP was detected by ethidium bromide staining.

Statistical Analysis

Statistical calculations were performed with the statistical package SPSS/PC+ (SPSS, Chicago, IL). Differences in the distribution of genotypes and alleles were determined by the chi-square procedure, as was the association between genotype frequencies and chronic complications. Results are expressed as the mean ± SD or median (quartile deviation) for nonparametric distributions. The Student *t* test was used when comparing clinical or metabolic characteristics between the study groups (control and NIDDM). One-way ANOVA or Kruskall-Wallis test (for nonparametric distribution) were performed when comparing parameters according to the genotype distribution of each of the studied genes. Statistical significance occurred if a computed two-tailed probability value was less than 5% ($P < .05$). We also used the Bonferroni correction when necessary, and in this case a *P* value less than .01 was considered significant.

RESULTS

ACE gene insertion/deletion polymorphism analysis resulted in a PCR product of 190 bp in the absence of an insertion (Dallele) and a 490-bp fragment in the presence of an insertion (Iallele). The D-allele–specific PCR amplification yielded a 335-bp fragment only in the presence of an I allele, and none in homozygous DD samples. This procedure identified 4% to 5% of ID samples that would have been misclassified with the first amplification.

RFLP-M235T AGT analysis revealed a two-allele polymorphism that produced three bands of different sizes. A 165-bp band corresponded to the T allele (absence of restriction site), and a set of 141- and 24-bp bands corresponded to the C allele (restriction site present). RFLP-T174M AGT analysis disclosed another two-allele polymorphism: a 405-bp fragment corresponding to the C allele (restriction site absent) and a set of 259- and 146-bp fragments corresponding to the T allele (restriction site present).

The observed genotypic frequency for the all the studied polymorphisms did not differ from that predicted by Hardy-Weinberg equilibrium in the control or the diabetic group. Heterozygosity of the different polymorphisms in control subjects was 0.487, 0.493, and 0.241 for ACE, M235T-AGT, and T174M-AGT genes, respectively. For each of the previous genes, the respective polymorphism information content in the control subjects was 0.368, 0.371, and 0.211.

Distribution of the genotype or allelic frequencies for all the studied polymorphisms did not differ significantly between controls and NIDDM patients (Table 1).

Table 2 displays clinical characteristics of the participants. Among diabetic patients, 43% were only diet-treated, 33% were treated with insulin, and 24% were treated with hypoglycemic agents. As a result of the sampling strategy, NIDDM patients were older and more obese and had higher SBP and DBP than the control subjects ($P < .0001$ in every parameter comparison).

ACE and AGT genes did not display any difference in clinical or metabolic parameters according to each gene's genotype in the NIDDM group (Table 3). Moreover, the proportion of antihypertensive-treated and nontreated patients is similar in each genotype.

Analysis of chronic complications (retinopathy and nephropathy) according to each gene's polymorphism was performed in 160 NIDDM patients.

Patients with retinopathy had elevated SBP ($P = .002$; 95% confidence interval [CI], 4.0 to 17.2 mm Hg) compared with the group without retinopathy (Table 4). There was no difference between ACE, M235T-AGT, and T174M-AGT genotype distribution in NIDDM patients with diabetic retinopathy and patients without retinopathy.

Table 5 shows clinical characteristics of the NIDDM patients according to nephropathy status. Patients with established nephropathy have higher SBP ($P = .006$). Distribution of the studied genotypes did not display any difference among microalbuminuric, macroproteinuric, and NIDDM patients without nephropathy (Table 6).

Table 1. Genotype and Allele Frequencies for ACE and AGT Gene Polymorphisms

Gene	Genotype	Allele	Control Subjects	NIDDM Patients
ACE	II		0.12	0.14
	DD		0.28	0.34
	ID		0.60	0.52
		I	0.42	0.40
		D	0.58	0.60
M235T-AGT	TT		0.35	0.31
	CC		0.24	0.15
	TC		0.41	0.54
		T	0.56	0.58
		C	0.44	0.42
T174M-AGT	TT		0.03	0.02
	CC		0.74	0.77
	TC		0.23	0.21
		T	0.14	0.12
		C	0.86	0.88

Table 2. Clinical Characteristics of the Participants

Variable	Control Subjects	NIDDM Patients	P
No.	90	193	
Sex (M/F)	42/48	89/104	NS
DT, D/I/OH (%)	—	43/33/24	—
Age (yr)	45.4 ± 12.6	59.2 ± 10.0*	<.0001
DD (yr)	—	13.2 ± 6.2	—
BMI (kg/m²)	25.2 ± 4.1	29.1 ± 5.5†	<.0001
SBP (mm Hg)	125.4 ± 22.1	148.0 ± 21.6‡	<.0001
DBP (mm Hg)	74.4 ± 13.5	84.8 ± 11.2§	<.0001

NOTE. Results are expressed as the mean ± SD. All statistical comparisons were performed with the Student *t* test.

Abbreviations: M, males; F, females; DT, diabetes treatment; D/I/OH, diet/insulin/oral hypoglycemic agents; DD, duration of diabetes.

*95% CI, 10.8 to 16.7 years.

†95% CI, 2.6 to 5.1 kg/m².

‡95% CI, 16.5 to 28.76 mm Hg.

§95% CI, 6.8 to 14.1 mm Hg.

DISCUSSION

There are many studies confirming that inherited factors play an important role in predisposing to diabetic nephropathy.[15] On the other hand, hypertension is an important contributing factor in the development and evolution of diabetic nephropathy. Recently, several common possible genetic markers of essential hypertension and diabetic nephropathy have been reported. Genes involved in the renin-angiotensin system have been the object of the majority of studies.[16-19]

In this study, we did not find any association between ACE and AGT alleles and diabetic microangiopathy. No relationship was found between the classic cardiovascular risk factors usually displayed in the diabetic population and the genotypes analyzed. Some studies have found an association between high blood pressure and AGT genotypes in the general population. This association mainly occurs in lean subjects, suggesting a genetically different type of hypertension in overweight versus lean subjects.[20] Hypertension is more frequent in NIDDM patients than in the general population, and there must be

Table 3. Comparison of Several Variables Between ACE and M235T-AGT Genotypes in NIDDM Patients

Variable	ACE Genotype			M235T-AGT Genotype		
	II	DD	ID	TT	CC	TC
No.	27	66	100	60	29	104
SBP (mm Hg)	147.0 ± 19.3	146.2 ± 23.5	148.3 ± 20.1	146.6 ± 21.3	148.0 ± 19.7	149.1 ± 23.0
DBP (mm Hg)	82.8 ± 12.9	83.5 ± 10.5	85.3 ± 10.1	83.8 ± 11.9	82.7 ± 7.1	84.8 ± 11.5
BMI (kg/m^2)	29.8 ± 5.7	29.2 ± 6.6	28.5 ± 4.7	28.6 ± 4.8	28.1 ± 4.2	29.3 ± 6.7
Cholesterol (mg/dL)	5.7 ± 1.1	5.8 ± 1.2	5.9 ± 1.3	5.7 ± 1.4	5.6 ± 1.1	5.8 ± 1.2
Triglycerides (mg/dL)	1.6 ± 0.6	2.0 ± 1.2	1.9 ± 1.2	1.8 ± 1.2	1.7 ± 0.7	1.9 ± 1.0
HDL-C (mg/dL)	1.4 ± 0.4	1.3 ± 0.5	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.4	1.3 ± 0.5
HbA_{1c} (%)	7.8 ± 1.9	7.7 ± 2.0	7.8 ± 2.2	8.1 ± 2.2	7.0 ± 1.8	7.8 ± 2.1

NOTE. Results are expressed as the mean ± SD. All statistical comparisons were performed with 1-way ANOVA.

complex causes of its development.[21] Patients with NIDDM usually have associated obesity, and the mechanisms responsible for hypertension may be more similar to those observed in obese patients than in lean subjects. In our study, there was no relationship between high blood pressure and ACE and AGT genotypes. These results did not show variations when patients were analyzed taking BMI into account. The possible interpretation may raise several hypotheses. Hypertension in NIDDM patients has a different etiology from that in the rest of the population. Diabetes may predispose to hypertension by promoting sodium retention, increasing vascular tone, and contributing to nephropathy.[22] These differences may reflect genetically different types of hypertension in the general and diabetic population. Another possible explanation is that diabetes acts as a modulating factor in the role that ACE or AGT genes play in hypertension.

The presence of nephropathy has been widely analyzed in association studies for these genetic markers. Nephropathy shows marked interindividual variation, and its presence increases cardiovascular morbidity in both IDDM and NIDDM patients. We have not found any association between ACE and AGT genotypes and the presence or absence of nephropathy. The majority of studies have been performed in IDDM patients, and there is not a general agreement for ACE genotype to confer a greater risk of developing diabetic nephropathy. For the AGT gene, a recent report describes an association with the M235T variant.[23] Some studies have found an association between the DD genotype and the presence of microangiopathic complications and major coronary heart disease[6,16] associated with greater ACE plasma levels, but others have failed to confirm this association.[17,18]

Few studies have been performed in NIDDM. In the Japanese population, Ohno et al[19] found the ACE DD genotype to be associated with the presence of microproteinuria and macroproteinuria in NIDDM Japanese subjects, with a similar number of patients affected by nephropathy as in our study,[19] but when analyzing the M235T variant of the AGT gene, there was no association. Another study in the same racial population attributed a prognostic value for the progressive deterioration of renal function to the ACE gene.[24] However, other studies in caucasians did not find such an association,[25] in agreement with our results. Only one study found a weak association between the DD genotype and high urinary albumin levels among patients with increased albumin excretion.[26]

It is noteworthy that ACE genotype frequencies show a different distribution according to race. Thus, genotype II frequency is greater in Japanese NIDDM subjects[20] (DD:ID:II, 13%:39%:48%) than in caucasian NIDDM patients (DD:ID:II, 34%:52%:14% for our NIDDM population), which agrees with the values obtained in other caucasian studies.[25] This different genotype distribution observed in Japanese patients makes the association matchless with the results obtained for the caucasian population. Other problems arise when studying nephropathy in the NIDDM population. Survival bias associated with early mortality in patients with increased albuminuria or the different microalbuminuric specificity found in NIDDM and IDDM

Table 4. Clinical Characteristics of NIDDM Patients With and Without Retinopathy

Variable	Retinopathy	No Signs of Retinopathy	*P*
No.	68	92	
Sex (M/F)	29/39	45/47	NS
DD (yr)	14.8 ± 5.7	12.1 ± 6.3	NS
Age (yr)	61.9 ± 9.1	59.6 ± 10.3	NS
BMI (kg/m^2)	29.9 ± 5.3	28.2 ± 5.7	NS
SBP (mm Hg)	155.6 ± 22.0*	145.0 ± 19.0	.002
DBP (mm Hg)	85.3 ± 11.2	84.1 ± 9.1	NS
HbA_{1c} (%)	7.7 ± 2.1	7.0 ± 1.6	NS

NOTE. Results are expressed as the mean ± SD. All statistical comparisons were performed with the Student *t* test.

Abbreviations: M, males; F, females; DD, duration of diabetes.

*95% CI, 4.0 to 17.2 mm Hg.

Table 5. Clinical Characteristics of NIDDM Patients According to Nephropathy Status

Variable	Microalbuminuria	Macroproteinuria	No Nephropathy	*P*
N	40	20	100	
Sex (M/F)	17/23	11/9	47/53	NS
DD (yr)	15.1 ± 6.2	16.1 ± 7.1	12.0 ± 5.5	NS
Age (yr)	60.1 ± 10.6	64.2 ± 9.2	59.7 ± 9.9	NS
BMI (kg/m^2)	29.7 ± 5.4	30.8 ± 6.2	28.3 ± 5.5	NS
SBP (mm Hg)	156.9 ± 21.4	154.2 ± 19.9	145.3 ± 20.3	.006
DBP (mm Hg)	85.5 ± 8.5	84.5 ± 13.8	84.5 ± 9.9	NS
HbA_{1c} (%)	7.4 ± 2.1	7.4 ± 1.9	7.3 ± 1.7	NS

NOTE. Results are expressed as the mean ± SD. All statistical comparisons were performed with 1-way ANOVA.

Abbreviations: M, males; F, females; DD, duration of diabetes.

Table 6. Chronic Complications Associated With Allelic Frequencies in NIDDM Patients

Gene	Allele	Nephropathy			Retinopathy	
		Micro-albuminuria	Macro-proteinuria	No	Yes	No
ACE	I	0.39	0.32	0.38	0.31	0.39
	D	0.61	0.68	0.62	0.69	0.61
M235T-AGT	T	0.55	0.55	0.59	0.59	0.57
	C	0.45	0.45	0.41	0.41	0.43
T174M-AGT	T	0.12	0.16	0.14	0.14	0.13
	C	0.88	0.84	0.86	0.86	0.87

patients are some of the factors that can interfere in association studies. Unlike IDDM patients, microalbuminuria is not well correlated with the histological changes observed in renal biopsies of NIDDM patients; and consistently, the possibility of obtaining renal biopsies is not feasible for making a diagnosis of diabetic nephropathy in a large sample of patients.

In conclusion, our findings do not support the use of ACE and AGT genes as markers to confer susceptibility to microvascular complications in NIDDM patients. Further investigations in caucasian patients are required to clarify the role of renin-angiotensin system genes in diabetic complications.

REFERENCES

1. Andersen AR, Christiansen JS, Andersen JK, et al: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: An epidemiological study. Diabetologia 14:363-370, 1978
2. Parving HH, Smidt UM, Friisberg B, et al: 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. Hall JE, Guyton AC: Control of sodium excretion and arterial pressure by intrarenal mechanisms and the renin angiotensin system, in Laragh JH, Brenner BM (eds): Hypertension: Pathophysiology, Diagnosis and Management. New York, NY, Raven, 1990, pp 1105-1129
5. Urata H, Nishimura H, Ganten D: Chymase-dependent angiotensin II forming systems in humans. Am J Hypertens 9:277-284, 1996
6. Cambien F, Poirier O, Lecerf L, et al: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 359:641-644, 1992
7. Rigat B, Hubert C, Alhenc-Gelas F, et al: An insertion/deletion polymorphism in the angiotensin I–converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343-1346, 1990
8. Bloem LJ, Manatunga AK, Tewksbury DA, et al: The serum angiotensinogen concentration and variants of the angiotensinogen gene in white and black children. J Clin Invest 93:948-953, 1995
9. Walker WG, Whelton PK, Saito H, et al: Relationship between blood pressure and renin, renin substrate, angiotensin II, aldosterone and urinary sodium and potassium in 574 ambulatory subjects. Hypertension 1:287-291, 1979
10. Watt GCM, Harrap SB, Foy CJW, et al: Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: A four corners approach to the identification of genetic determinants of blood pressure. J Hypertens 10:473-482, 1992
11. Fasola AF, Martz BL, Helmer OM: Plasma renin activity during supine exercise in offspring of hypertensive parents. J Appl Physiol 25:410-415, 1968
12. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al: Molecular basis of human hypertension: Role of angiotensinogen. Cell 71:169-180, 1992
13. Muls E, Blaton V, Rosseneu M, et al: Serum lipids and apolipoprotein A1, A2 and B in hyperthyroidism before and after treatment. J Clin Endocrinol Metab 55:459-464, 1982
14. Lipids Research Clinics: Manual of Laboratory Operations. Lipids Research Clinics Program, vol 1. Lipid and Lipoprotein Analysis. DHEW Publication No. NIH 75-628. Washington, DC, US Government Printing Office, 1984
15. Seaquist ER, Goetz FC, Rich S, et al: Familial clustering of diabetes kidney disease. N Engl J Med 320:1161-1165, 1989
16. Marre M, Bernadet P, Gallois Y, et al: Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. Diabetes 43:384-388, 1994
17. Tarnow L, Cambien F, Rossing P, et al: Insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene is associated with coronary heart disease in IDDM patients with diabetic nephropathy. Diabetologia 38:798-803, 1995
18. Tarnow L, Cambien F, Rossing P, et al: Lack of relationship between an insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. Diabetes 44:489-494, 1995
19. Ohno T, Kawazu S, Tomono S: Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non–insulin-dependent diabetics. Metabolism 45:218-222, 1996
20. Tiret L, Ricard S, Porier O, et al: Genetic variation at the angiotensinogen locus in relation to high blood pressure and myocardial infarction: The ECTIM Study. J Hypertens 13:311-317, 1994
21. World Health Organization Multinational Study: Prevalence of small vessel and large vessel disease in diabetic patients from 14 centres. Diabetologia 28:615-640, 1985 (suppl 1)
22. Weidmann P, Beretta-Piccoli C, Trost BN: Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. Hypertension 7:33-42, 1985 (suppl 2)
23. Fogarty DG, Harron JC, Hughes AE, et al: A molecular variant of angiotensinogen is associated with diabetic nephropathy in IDDM. Diabetes 45:1204-1208, 1996
24. Yoshida H, Kuriyama S, Atsumi Y, et al: Angiotensin I converting enzyme gene polymorphism in non–insulin dependent diabetes mellitus. Kidney Int 50:657-664, 1996
25. Panagiotopoulos S, Smith TJ, Aldred GP, et al: Angiotensin-converting enzyme (ACE) gene polymorphism in type II diabetic patients with increased albumin excretion rate. J Diabetes Complications 9:272-276, 1995
26. Dudley CRK, Keavney B, Stratton IM, et al: U.K. Prospective Diabetes Study XV: Relationship of renin-angiotensin system gene polymorphisms with microalbuminuria in NIDDM. Kidney Int 48:1907-1911, 1995