Influence of Angiotensin-Converting Enzyme and Angiotensin II Type 1 Receptor Gene Polymorphisms on Aortic Stiffness in Normotensive and Hypertensive Patients

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Background Clinical and experimental studies have demonstrated a major role of the renin-angiotensin system in the functional and structural changes of the large arteries in hypertension. Because genetic studies may help us to understand the mechanisms underlying the involvement of this system in arterial regulation, the present study was designed to assess the contribution of polymorphisms of the *ACE* insertion/deletion (*I/D*) and angiotensin II type 1 receptor ($AGTR_1$ A^{1166}C) genes on aortic stiffness regulation.

Methods and Results This study included 311 untreated hypertensive and 128 normotensive subjects. Aortic stiffness was evaluated by measurement of the carotid-femoral pulse-wave velocity (PWV). In normotensive subjects, the two polymorphisms did not influence any of the studied parameters. In hypertensive subjects, there was a decreasing trend of mean PWV with the number of *ACE D* alleles, but this association became significant only after adjustment for blood pressure ($P<.05$). Conversely, the $AGTR_1$ A^{1166}C polymorphism was independently associated with aortic stiffness. Mean values of PWV were 11.6±2.7 m/s in $AGTR_1$ AA homozygotes, 13.3±3.3 m/s in AC heterozygotes, and 15.3±4.3 m/s in CC homozygotes ($P<.0001$ and $P<.00001$ after adjustment for age and mean blood pressure, respectively). The percentage of variance of PWV explained by $AGTR_1$ A^{1166}C polymorphism (11.6%) was much larger than that of *ACE I/D* polymorphism (1.7%).

Conclusions These results suggest that in hypertensive but not normotensive subjects, the $AGTR_1$ and *ACE* genotypes are involved in the regulation of aortic rigidity. The presence of the $AGTR_1$ C allele is a strong independent determinant of aortic stiffness, whereas presence of the *ACE I* allele is weakly associated with increased stiffness. (***Circulation.* 1996;94:698-703.**)

Key Words • angiotensin • aorta • genetics • hypertension

It is generally accepted that hypertension and aging increase stiffness of the large arteries by inducing several structural alterations of the arterial wall, including hypertrophy and changes in extracellular matrix. An increased mechanical stress caused by high blood pressure is a major determinant of arterial wall stiffness in hypertension.[1-3] In addition, local hormonal factors such as angiotensin II acting through $AGTR_1$[4,5] may play a pressure-independent role on the arterial wall, mainly by modifying cell growth or synthesis of the extracellular matrix.[5-9] In humans, higher levels of ACE have been observed in subjects with increased thickness of the carotid wall.[10] Clinical and experimental pharmacological studies have shown that ACE inhibitors can prevent and/or beneficially affect hypertension-induced structural and functional alterations of the arterial wall independent of blood pressure changes.[11,12]

Therefore, the *ACE* and $AGTR_1$ genes, which have been shown to be polymorphic,[13,14] could be candidate genes for large-artery stiffness. In the present study, we tested the involvement of the *ACE I/D* and the $AGTR_1$ A^{1166}C gene polymorphisms in 311 untreated hypertensive and 128 normotensive subjects. Our group recently published a preliminary report concerning the role of the $AGTR_1$ polymorphism in a subgroup of 134 never-treated hypertensive subjects.[15] In that study, we found that the $AGTR_1$ gene was involved in the regulation of aortic stiffness. The aim of the present study was to test the role of *ACE I/D* and $AGTR_1$ A^{1166}C gene polymorphisms and their interactions with age and arterial pressure on aortic distensibility. Aortic stiffness was assessed in hypertensive and normotensive subjects by measurement of carotid-femoral PWV, a noninvasive, accurate, safe, and reproducible method suited to the screening of large populations.[1,16-18]

Methods

In this study, 311 white hypertensive patients 18 to 74 years of age and 128 normotensive subjects 19 to 72 years of age were analyzed.

Normotensive subjects were selected according to the following criteria: (1) no history of hypertension and coronary artery disease; (2) SBP ≤145 mm Hg and DBP ≤90 mm Hg as measured with both a sphygmomanometer and an automatic oscillometric device (Dinamap model 845, Critikon) on the morning of the study; and (3) no recent symptoms of coronary artery disease, heart failure, stroke, and lower-limb arterial disease.

Hypertensive subjects were selected according to the following criteria: (1) a history of hypertension; (2) no antihypertensive or

Received December 18, 1995; revision received February 8, 1996; accepted February 16, 1996.

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Selected Abbreviations and Acronyms

$AGTR_1$ = angiotensin II type 1 receptor
BMI = body mass index
DBP = diastolic blood pressure
I/D = insertion/deletion
PWV = pulse-wave velocity
SBP = systolic blood pressure

vasodilator treatments for at least 3 weeks before the start of the study; (3) SBP >145 mm Hg and/or DBP >90 mm Hg as measured with both a sphygmomanometer (mean of three measurements made with patients in the supine position, Korotkoff V sound) and an automatic oscillometric device (DINAMAP 845) on the morning of the study[19]; (4) no clinical or biological signs of secondary hypertension; and (5) no recent symptoms of coronary artery disease, heart failure, stroke, and lower-limb arterial disease.

Normotensive subjects were recruited in our outpatient clinic from 180 consecutive volunteers without histories of hypertension who were undergoing systematic annual health checkups. Among these, 37 subjects were eliminated for high blood pressure levels, whereas 13 more patients were not included because at least one parameter—age, sex, SBP, DBP, PWV, or DNA extraction—was missing.

Hypertensive subjects were recruited in the hospital and our outpatient clinic. Between November 1993 and August 1995, we identified 465 consecutive patients who had not received antihypertensive or vasodilator treatment for at least 3 weeks. Among these, 106 were not included because they did not fulfill the blood pressure criterion. From the 359 remaining patients, 18 were excluded for missing values, and 28 were eliminated for secondary hypertension discovered after the end of our examination. Patients whose preliminary results were reported previously were included in the present study (except 7 who were eliminated for missing values).

All the participants were examined in the morning after a fast of at least 12 hours, and all underwent the same procedure after providing written informed consent. After 15 minutes of rest in the supine position, carotid-femoral PWV was evaluated with two pressure probes. This method has been extensively analyzed.[16,18] Briefly, two pressure waves were recorded transcutaneously at two sites (at the base of the neck for the common carotid artery and over the right femoral artery). PWV was determined as the foot-to-foot velocity. The foot of the pressure wave was identified as the beginning point of the sharp systolic upstroke. Pulse transit time was determined to be the average of 10 consecutive beats, and the mean value of two different observers' readings was retained for the statistical analysis. The distance traveled by the pulse wave was measured over the body surface as the distance between the two recording sites. Aortic PWV was calculated as the ratio of distance to transit time. The reproducibility of the measurement for aortic PWV (expressed as percent variation of the mean value) has been found to be 5.3±3.6%.[16]

During the procedure and for 5 minutes afterward, blood pressure was measured automatically every 2 minutes with a DINAMAP device. The mean of five consecutive measurements was calculated. An automatic method was chosen for the statistical analysis to avoid interobserver variations and to diminish the "white coat" reactivity. At the end of this procedure, plasma was drawn for determination of standard biochemical measurements and DNA extraction. Plasma glucose and serum lipids (total and HDL cholesterol and triglycerides) were considered only in patients not receiving pharmacological treatment for diabetes and dyslipidemia, respectively.

Genotype Investigations

Blood was drawn for DNA extraction. The *ACE I/D* polymorphism was determined by DNA amplification by the polymerase chain reaction as previously described.[20] The $AGTR_1$ $A^{1166}C$ polymorphism was assayed by allele-specific oligonucleotide hybridization.[14] The primers used to amplify the $AGTR_1$ region encompassing the $A^{1166}C$ polymorphism were 5′-AATGCTTGTAGCCAAAGTCACCT-3′ and 5′-GGCTTTGCTTTGTCTTGTTG-3′. For technical reasons, *ACE* was not genotyped in one normotensive subject, whereas $AGTR_1$ was not genotyped in one normotensive and two hypertensive subjects.

Statistical Analysis

The statistical analysis was performed only in patients without any missing values, ie, in 311 hypertensive and 128 normotensive subjects.

Both polymorphisms were tested as three class variables or as codominant 0, 1, or 2 ordinal variables (presence of 0, 1, or 2 *C* or *D* alleles). Interaction tests were performed to assess the homogeneity of genotype-phenotype association in normotensive and hypertensive subjects. Multiple regression analyses were performed separately in normotensive and hypertensive subjects to evaluate the effects of the *ACE I/D* and $AGTR_1$ $A^{1166}C$ polymorphisms on PWV after adjustment for covariables. The Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 *df*. Interactions between the two polymorphisms on the studied variables were tested with a two-way ANOVA or by regression analysis in which both polymorphisms were analyzed as codominant. Statistical analysis was performed with the SAS software package.

Results

Among hypertensive patients, 53 (17%) were treated for dyslipidemia, and 19 (6%) received an oral antidiabetic treatment; 60 (19%) were smokers. Time from discovery of hypertension was 6.9±8.1 (mean±SD) years. Among normotensive subjects, 15 (12%) were treated for dyslipidemia, 11 (9%) received oral antidiabetic treatment, and 29 (23%) were smokers. Of the hypertensive patients, 177 had never before been treated for hypertension.

Table 1 gives the main characteristics of the hypertensive and normotensive subjects. Normotensive subjects were slightly younger and thinner than hypertensive patients. The differences in SBP between hypertensive and normotensive subjects were 33 and 37 mm Hg in men and women, respectively, whereas for DBP the differences were 20 and 19 mm Hg, respectively. Mean PWV was

TABLE 1. Main Characteristics of Hypertensive and Normotensive Subjects

	Hypertensive Patients		Normotensive Subjects		*P* by ANOVA		
	Men (n=214)	Women (n=97)	Men (n=80)	Women (n=48)	Men vs Women	Hypertensive vs Normotensive Subjects	Interaction
Age, y	49.2 (10.3)	48.8 (11.3)	45.3 (11.1)	43.4 (11.4)	NS	<.0001	NS
BMI, kg/m²	26.2 (3.1)	24.9 (4.0)	24.9 (3.4)	22.8 (3.6)	<.0001	<.0001	NS
SBP, mm Hg	157.4 (12.5)	158.1 (14.7)	124.4 (9.7)	121.1 (10.3)	NS	<.0001	NS
DBP, mm Hg	97.2 (9.4)	93.5 (11.0)	77.0 (7.4)	74.6 (6.7)	<.001	<.0001	NS
PWV*, m/s	12.3 (3.0)	12.5 (3.5)	9.4 (1.7)	8.8 (1.7)	NS	<.0001	NS

*Statistical tests were performed on log PWV.

TABLE 2. Distribution of *ACE I/D* and *AGTR*$_1$ A^{1166}C Genotypes in Normotensive and Hypertensive Subjects

	Normotensive Subjects		Hypertensive Patients	
	n (%)	Men/Women	n (%)	Men/Women
ACE				
II	25 (19.5)	15/10	46 (14.8)	31/15
ID	56 (43.7)	31/25	159 (51.1)	107/52
DD	47 (36.7)	34/13	106 (34.1)	76/30
I/D	0.41/0.59		0.40/0.60	
AGTR$_1$				
AA	77 (60.2)	48/29	186 (59.8)	131/55
AC	41 (32.0)	24/17	109 (35.1)	73/36
CC	10 (7.8)	8/2	16 (5.1)	10/6
A/C	0.76/0.24		0.77/0.23	

No deviation from Hardy-Weinberg equilibrium.
The distribution of *ACE* and *AGTR*$_1$ genotypes do not differ between normotensive and hypertensive subjects.

similar in the two sexes and was higher in hypertensive than in normotensive subjects (2.9 and 3.7 m/s in men and women, respectively).

Table 2 gives the distributions of the *ACE I/D* and *AGTR*$_1$ A^{1166}C genotypes and allele frequencies in hypertensive and normotensive subjects. In both hypertensive and normotensive subjects, genotype frequencies did not deviate significantly from the Hardy-Weinberg expectation, and there were no significant differences in genotypes and allele frequencies between normotensive and hypertensive individuals.

Table 3 lists the main characteristics of the hypertensive and normotensive subjects according to *AGTR*$_1$ A^{1166}C genotypes. In normotensive subjects, the mean values of age, BMI, SBP, DBP, and PWV did not significantly differ according to *AGTR*$_1$ genotype. In hypertensive patients, PWV was the only variable to differ between genotypes. The presence of the ^{1166}C alleles was associated with a codominant increase in PWV by 1.6 and 3.7 m/s in heterozygotes and ^{1166}C homozygotes, respectively ($P<.0001$). There was a significant heterogeneity in the association between PWV and *AGTR*$_1$ A^{1166}C genotype in normotensive and hypertensive subjects ($P<.001$), suggesting that the presence of hypertension was necessary for the genetic effect to be expressed. Serum levels of total and HDL cholesterol, triglycerides, glucose, and potassium were similar in the three genotype categories in both normotensive and hypertensive subjects (data not shown).

Table 4 shows the main characteristics of normotensive and hypertensive subjects according to *ACE I/D* genotype. In normotensive subjects, there was a weak and inconsistent association between the *ACE I/D* polymorphism and BMI. In hypertensive patients, BMI was not associated with the *ACE I/D* polymorphism, but mean SBP and DBP were significantly higher in *ID* heterozygotes compared with the *II* and *DD* homozygotes ($P<.003$ and $P<.005$, respectively). In univariate analysis, there was a decreasing trend of mean PWV with the number of *ACE D* alleles, but this association became significant only after adjustment for SBP and age. Biological parameters were similar in the three genotype categories in both normotensive and hypertensive subjects (data not shown).

A multiple regression analysis was conducted to assess the independent effects of different factors and the *AGTR*$_1$ A^{1166}C and *ACE I/D* polymorphisms on PWV (Table 5). In normotensive subjects, age and SBP were the primary determinants of PWV; the two factors explained 16% and 4.2% of the variance of PWV, respectively. In hypertensive patients, age and BMI were significant predictors of PWV, but SBP was the primary contributor, explaining 14.1% of the variance of the trait ($P<.0001$). Interestingly, DBP was negatively associated with PWV after adjustment for SBP ($P<.004$); the higher the PWV was, the lower the DBP was for a given level of SBP. In hypertensive patients, after adjustment for age, BMI, SBP, and DBP, both the *AGTR*$_1$ A^{1166}C and the *ACE I/D* polymorphisms were independently associated with PWV. However, the percentage of variance of PWV explained by the *AGTR*$_1$ A^{1166}C polymorphism (11.6%) was much larger than that explained by the *ACE* polymorphism (1.7%). There was no significant interaction between the two polymorphisms and PWV.

The homogeneity of effect of the *AGTR*$_1$ A^{1166}C and *ACE I/D* polymorphisms on PWV, according to sex, the median of age and BMI, and the presence or absence of previous antihypertensive treatment, also were investigated (Table 6). Overall, the effect of the *AGTR*$_1$ A^{1166}C polymorphism on PWV was quite similar and strongly significant across the different categories. However, there was a tendency for a stronger effect in older patients (heterogeneity according to median of age, $P=.05$). This effect of the *AGTR*$_1$ A^{1166}C polymorphism on PWV in never-treated patients was discussed previously.[15] The rest of the recruited patients for this study had already been treated for hypertension. The similarity of effect of the polymorphism is striking. Concerning the *ACE I/D* polymorphism, the negative association between the presence of the *D* allele and PWV was present only in men ($P<.02$), but there was no statistical heterogeneity between sexes. Conversely, there was significant heterogeneity across classes of BMI ($P<.02$), indicating that the *ACE* gene polymorphism influenced PWV only in overweight hypertensive patients.

TABLE 3. Mean Characteristics of Normotensive and Hypertensive Subjects According to *AGTR*$_1$ A^{1166}C Genotypes

	Normotensive Subjects				Hypertensive Patients			
	AA (n=77)	AC (n=41)	CC (n=10)	*P* by ANOVA	AA (n=186)	AC (n=109)	CC (n=16)	*P* by ANOVA
Age, y	43.9±10.9	45.6±11.4	45.5±13.2	NS	48.5±10.5	49.7±10.8	51.8±10.1	NS
BMI, kg/m^2	24.3±4.0	23.9±2.9	24.1±3.3	NS	25.6±3.4	26.2±3.4	25.6±4.9	NS
SBP, mm Hg	122.0±10.4	124.9±8.9	125.5±11.0	NS	157.7±13.5	157.4±12.6	158.2±13.8	NS
DBP, mm Hg	75.4±7.4	77.0±7.1	78.0±6.2	NS	96.6±10.2	95.1±9.9	96.6±8.9	NS
PWV, m/s	9.1±1.8	9.3±1.6	8.9±1.5	NS	11.6±2.7	13.2±3.3	15.3±4.3	<.0001

Values are mean±SD. The ANOVA was performed on log PWV; test of codominant effect (genotypes coded as 0, 1, 2 ordinal variables), $P<.0001$. The effect of the polymorphism on log PWV in normotensive and hypertensive subjects was significantly heterogeneous (interaction term including hypertension status and genotypes coded as 0, 1, or 2 ordinal variable), $P<.001$.

TABLE 4. Mean Characteristics of Normotensive and Hypertensive Subjects According to *ACE I/D* Genotypes

	Normotensive Subjects				Hypertensive Patients			
	II (n=25)	*ID* (n=56)	*DD* (n=47)	*P* by ANOVA	*II* (n=46)	*ID* (n=159)	*DD* (n=106)	*P* by ANOVA
Age	46.9±10.0	42.4±12.5	46.0±9.8	NS	48.9±10.2	49.2±10.7	49.0±10.7	NS
BMI, kg/m²	24.5±3.9	23.2±3.5	25.0±3.4	<.05*	25.6±3.7	25.7±3.6	26.0±3.2	NS
SBP, mm Hg	122.0±10.8	121.8±10.1	125.5±9.2	NS	153.6±11.9	160.1±14.4	155.6±11.0	<.003*
DBP, mm Hg	75.6±7.1	75.1±7.6	77.6±6.8	NS	93.5±11.7	97.8±10.5	94.5±7.8	<.005*
PWV†, m/s	9.1±1.7	9.4±1.9	9.0±1.5	NS	13.1±3.7	12.4±3.2	12.0±2.7	NS‡

Values are mean±SD.
*Test of codominant effect, P=NS.
†ANOVA was performed on log PWV.
‡Test of codominant effect, P<.05 after adjustment for SBP and DBP.

Discussion

The aim of this cross-sectional study was to assess the role of two genes coding for components of the renin-angiotensin system, the *ACE* and $AGTR_1$ genes, on the elastic properties of the aorta of normotensive and hypertensive subjects. Arterial stiffness was assessed by measuring aortic PWV with a noninvasive, accurate, safe, and reproducible method suited to the screening of large populations.[16-18]

Our study confirms that age and blood pressure are major determinants of aortic stiffness.[18,21] The negative relationship observed between PWV and DBP (after adjustment for SBP) in hypertensive patients in this study suggests that increased aortic stiffness can decrease diastolic pressure and probably diastolic blood flow and coronary perfusion.[22,23]

However, the main result of the present study is that in hypertensive subjects the $AGTR_1$ $A^{1166}C$ polymorphism is the second-most-important determinant of aortic stiffness, explaining 11.6% of PWV variance, just after SBP (14.1%) and before age (6.1%).

$AGTR_1$ $A^{1166}C$ Polymorphism

The $AGTR_1$ $A^{1166}C$ polymorphism is located at the 5′ end of the 3′ untranslated region of the gene.[14,24] This polymorphism is probably not functional but might be in linkage disequilibrium with an unidentified functional variant. It has been shown that the frequency of the $AGTR_1$ C allele is increased in patients with severe hypertension.[14] In our study, no relationship was observed between the $AGTR_1$ $A^{1166}C$ polymorphism and hypertension. Moreover, in both normotensive and hypertensive subjects, mean blood pressure levels were similar in the different genotypes.

In hypertensive subjects, the presence of the $AGTR_1$ C allele was associated with increased aortic stiffness in both sexes, independent of blood pressure levels, and this polymorphism could explain 11.6% of the PWV variance. This effect appears codominant; ie, mean PWV values in $AGTR_1$ AC heterozygotes were intermediate between those of the two groups of homozygotes. The fact that the $AGTR_1$ polymorphism is associated with aortic stiffness in hypertensive but not normotensive subjects may be related to a potentiation of angiotensin II effects in the presence of hypertension. Noda et al[25] suggested that increased cyclic mechanical stretch synergizes with angiotensin II in the activation of intracellular signaling pathways in rat vascular smooth muscle cells. Sudhir et al[26] reported that angiotensin II–induced vascular smooth muscle proliferation in vitro was strongly enhanced by increased mechanical stretch and concluded that hypertension-associated mechanical or structural alterations may potentiate the $AGTR_1$-mediated actions of angiotensin II.

The strong association between $AGTR_1$ $A^{1166}C$ genotype and PWV was observed in both young and old patients, but this effect was more pronounced in the latter subgroup (Table 6), showing that this polymorphism could amplify the effects of age on arterial stiffness.

TABLE 5. Multiple Regression Analysis of PWV* on the $AGTR_1$ $A^{1166}C$ and *ACE I/D* Genotypes and Covariables

Independent Variable	Normotensive Subjects		Hypertensive Patients	
	Partial R^2	*P*	Partial R^2	*P*
Sex	.021	.07	···	NS
Age	.160	<.0001	.061	<.0001
BMI (kg/m²)	···	NS	.014	<.01
SBP (mm Hg)	.042	<.02	.141	<.0001
DBP (mm Hg)	···	NS	.019	<.004
ACE I/D		NS	.017	<.006
$AGTR_1$ A/C†	···	NS	.116	<.0001
Model R^2†	.222	<.0001	.368	<.0001

*Log-transformed PWV was used as a dependent variable.
†Both polymorphisms were tested as codominant 0, 1, or 2 ordinal variables (presence of 0, 1, or 2 C or D alleles). The interaction term involving both polymorphisms was not statistically significant.

ACE I/D Polymorphism

Our results are consistent with those of previous studies showing a similar distribution of the *ACE I/D* genotypes in normotensive and hypertensive subjects.[27] In hypertensive subjects, however, the heterozygote *I/D* patients had significantly higher pressure levels than the two homozygote groups. We have no explanation for this surprising observation, which may be artifactual because patients were selected according to blood pressure levels.

ACE activity was not determined in this study, but previous investigations have shown that plasma and tissue ACE activity are increased in proportion to the number of *D* alleles.[28-30] The direct role of ACE activity and angiotensin II levels on vascular hypertrophy has been demonstrated unambiguously by in vivo transfer of the human *ACE* gene into intact rat carotid arteries.[6] In these experiments, the local increase in ACE activity was followed by an increase in vascular DNA content and by vascular hypertrophy, and these effects were abolished by an angiotensin II receptor blocker.

In humans, increased medial-intimal thickness of the carotid artery has been found to be associated with higher plasma ACE levels[10] and the presence of the *ACE D* allele.[31] Thus, it was anticipated that the *ACE D* allele would be associated with increased aortic stiffness. This hypothesis has not been confirmed in the

TABLE 6. Effects of the *AGTR*$_1$ A^{1166}C and *ACE I/D* Genotypes on PWV According to Sex, Median of Age and BMI, and Presence of Previous Treatment

Grouping Variable	Genotype				
	AA	AC	CC	P*	Homogeneity, P
Sex					
Men	11.7±2.8	13.0±2.7	15.5±3.7	<.0001	NS
Women	11.5±2.2	13.5±4.2	15.1±5.6	<.001	
Age					
<Median	11.2±2.4	12.0±2.0	12.9±2.2	.01	.05
≥Median	12.1±2.9	14.1±3.7	16.7±4.7	<.0001	
BMI					
<Median	11.7±2.9	12.6±2.7	15.6±4.8	<.001	NS
≥Median	11.5±2.3	13.6±3.6	14.8±3.9	<.0001	
Treatment for hypertension					
Never†	11.2±2.3	12.7±2.9	14.7±4.0	<.0001	NS
Stopped	11.9±2.9	13.5±3.5	15.9±4.7	<.0001	
	II	ID	DD		
Sex					
Men	13.4±3.3	12.3±2.9	11.9±2.8	<.02	NS
Women	12.4±4.4	12.6±3.8	12.2±2.2	NS	
Age					
<Median	12.1±2.8	11.4±2.2	11.4±2.3	NS	NS
≥Median	13.9±4.2	13.3±3.7	12.6±2.9	NS	
BMI					
<Median	12.7±3.8	11.9±3.1	12.4±2.8	NS	<.02
≥Median	13.7±3.6	13.0±3.3	11.6±2.5	<.002	
Treatment for hypertension					
Never†	12.3±3.3	12.1±2.7	11.7±2.8	NS	NS
Stopped	13.6±3.9	12.6±3.5	12.2±2.6	NS	

Values are mean±SD.
*Test of codominant effect
†The results concerning the effect of the *AGTR*$_1$ polymorphism on PWV in never-treated patients were published previously (Reference 15).

present study. In normotensive subjects, aortic stiffness was similar in the three *ACE I/D* genotypes, whereas a slight inverse effect was observed in hypertensive subjects. These apparently conflicting results could suggest that the mechanisms involved in the development of arterial hypertrophy and stiffness are different. Indeed, it was shown recently that wall thickening of the large arteries is not necessarily associated with increased stiffness, indicating that other structural changes occur to regulate arterial elastic properties.[32,33]

From the present study, however, we cannot draw conclusions on the causes of increased stiffness in the presence of the *ACE I* allele, especially in overweight patients. One could suggest that chronically lower plasma and tissue ACE levels could be responsible for an upregulation of AGTR$_1$ that could be associated in some categories of hypertensive patients with increased aortic stiffness. Nevertheless, because of the low incidence of the *II* genotype, larger studies are needed to draw conclusions on this issue.

Finally, if the *AGTR*$_1$ A^{1166}C polymorphism seems to have a strong and independent involvement in aortic stiffness regulation in hypertensive patients, several questions are unanswered by this study. Further studies are required to identify intermediate phenotypes such as a modification of the number or affinity of the AGTR$_1$ on accessible cells. In a previous study, no mutation in the coding sequence of the gene was detected in a group of 50 hypertensive patients.[14] Therefore, it is likely that the neutral marker we have used in this study is in linkage disequilibrium, with a variant affecting *AGTR*$_1$ gene expression through transcriptional regulation or mRNA stability.

Conclusions

The results of the present study suggest that in hypertensive subjects the *AGTR*$_1$ A^{1166}C genotype is a strong, independent determinant of aortic stiffness that explains >10% of PWV variance. On the other hand, the presence of the *ACE I* allele is associated with increased aortic stiffness, but the *ACE I/D* genotype explains <2% of PWV variance.

Acknowledgments

This study was supported in part by INSERM, the Claude Bernard Association, and the Groupement de Recherche et d'Etudes sur les Génomes. We thank the Servier and Hoechst pharmaceutical companies for support. We are grateful to Drs Anne Charru and Xavier Jeunemaitre for their advice and help. We thank Isabelle Gautier for her excellent technical assistance.

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