Influence of Angiotensin II Type 1 Receptor Polymorphism on Aortic Stiffness in Never-Treated Hypertensive Patients

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Abstract Several clinical and experimental studies have suggested a significant role of angiotensin II in the development of alterations of small and large arteries. The present study was designed to assess the contribution of polymorphism (corresponding to an $A^{1166}\rightarrow C$ transversion) of the angiotensin II type 1 receptor (AT_1) gene to aortic stiffness. One hundred thirty-four never-treated hypertensive patients were included in the study. Aortic distensibility was evaluated by measuring carotid-femoral pulse wave velocity. Age, systolic and diastolic pressure, and metabolic parameters were similar in the three genotypes. Pulse wave velocity was 11.4±2.5 m/s in AT_1 AA homozygotes, 12.5±3.2 m/s in AC heterozygotes, and 14.7±4.0 m/s in CC homozygotes ($P=.003$, $P<.001$ after adjustment for age, blood pressure, and body mass index). Moreover, an interaction was found between AT_1 genotype and the ratio of total to high-density lipoprotein cholesterol in terms of the development of aortic stiffness. Thus, a positive correlation was observed between the ratio of total to high-density lipoprotein cholesterol and pulse wave velocity in AC and CC ($r=.42$, $P<.001$) but not AA patients. These results suggest that the AT_1 gene is involved in the development of aortic stiffness in hypertensive patients and could modulate the effects of lipids on large arteries. (***Hypertension*. 1995;26:44-47.**)

Key Words • renin-angiotensin system • polymorphism (genetics) • receptors, angiotensin

The two major causes of increased stiffness in the large arteries are age and high blood pressure. Increased stiffness is associated with several structural changes of the arterial wall, including arterial thickening and changes in extracellular matrix. In addition to age and high blood pressure, local hormonal factors may play a role in the modification of the arterial wall, mainly by modifying cell growth or synthesis of the extracellular matrix.[1] Among these factors angiotensin II might be of particular importance, since it induces hypertrophy of vascular smooth muscle cells in culture and increases collagen production by fibroblasts.[2,3] In vivo administration of nonpressor doses of angiotensin II induces arterial thickening in rats,[4] whereas in humans, higher levels of angiotensin I–converting enzyme have been observed in subjects with increased thickness of the carotid wall.[5] Most of the actions of angiotensin II are mediated by the effects of this peptide on the angiotensin II type 1 receptors (AT_1),[6,7] and the recent identification of polymorphic DNA markers on the AT_1 gene[8] offers a good opportunity to study the involvement of the AT_1 gene in arterial rigidity.

In the present cross-sectional study we investigated whether in never-treated hypertensive patients this genetic polymorphism is associated with aortic stiffness evaluated by the method of carotid-femoral pulse wave velocity (PWV).[9-11]

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Methods

One hundred thirty-four white never-treated hypertensive patients aged 21 to 72 years were included in this study after they provided written informed consent. Subjects were recruited from the Broussais Hospital in Paris at the Hypertension Department during an ambulatory checkup for hypertension. They were selected according to the following criteria: (1) systolic blood pressure the morning of the study greater than 145 mm Hg and/or diastolic blood pressure greater than 90 mm Hg with both sphygmomanometer (mean of three measurements in the supine position, Korotkoff phase V sound) and an automatic oscillometric device (DINAMAP 845)[12]; (2) absence of clinical or biological signs of secondary hypertension; and (3) lack of any antihypertensive or vasodilatory treatment in the past.

The mean duration of hypertension was 5.1±6.0 years (range, 0 to 30 years). No subject had recent symptoms of coronary artery disease. Patients with diabetes type I or II were excluded from the study.

All the participants were examined in the morning after a fast of at least 12 hours, and all underwent the same procedure. After 15 minutes of rest in the supine position, aortic PWV was evaluated with the use of two pressure probes. This method has been extensively analyzed previously.[13] 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). Pulse transit time was determined as the average of 10 consecutive beats. 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%.[13]

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 measure-

TABLE 1. Distribution of AT_1 A/C Polymorphism in Male and Female Patients

	Men	Women	Both
AA	57	18	75 (56%)
AC	37	14	51 (38%)
CC	6	2	8 (6%)
C allele	24.5%	26.5%	25.0%

AT_1 indicates angiotensin II type 1 receptor.

ments was calculated. An automatic method was chosen for the statistical analysis to avoid interobserver variations and diminish "white coat" reactivity.

At the end of this procedure, plasma was drawn for determination of serum potassium, plasma glucose, triglycerides, and total and high-density lipoprotein (HDL) cholesterol levels according to the standard methods. Metabolic parameter determination was not performed in patients receiving treatment for dyslipidemia.

Genotype Investigations

DNA was extracted according to standard methods.[8] The $A^{1166}\rightarrow C$ polymorphism of the AT_1 gene was determined as previously described[8] in all but three subjects in whom genotyping was unsuccessful for technical reasons.

Statistical Analysis

One-way ANOVA was used to test differences in the means of quantitative variables between genotypes before and after adjustment of covariables (age, blood pressure, body mass index). The comparison of the effects of age, blood pressure, and metabolic parameters on PWV in the different genotypes was performed with an interaction test (ANCOVA). Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 *df*.

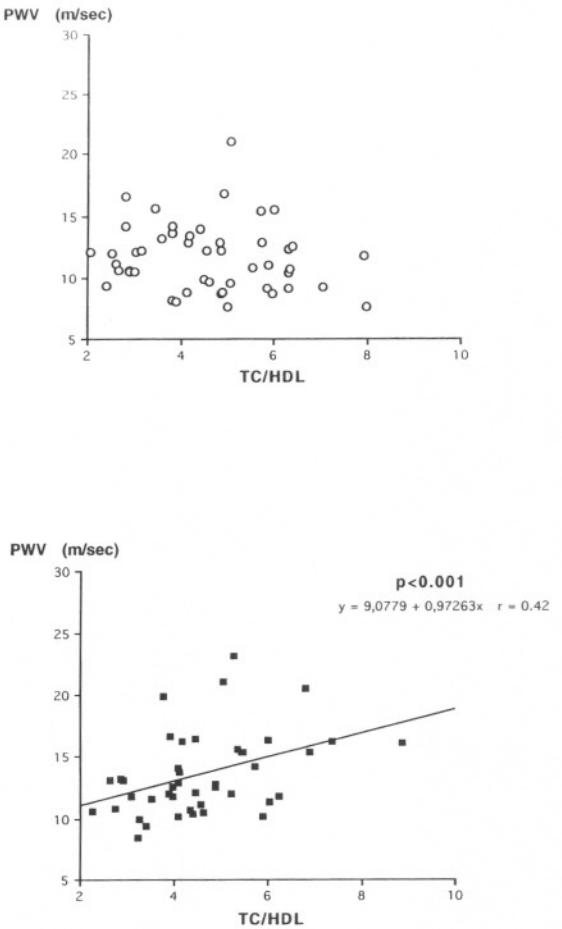
Results

The distributions of the AT_1 A/C genotypes and allele frequencies were similar in men and women (Table 1). Age, blood pressure, duration of hypertension, body mass index, and metabolic parameters were similar in the three genotypes, whereas the mean values of PWV were significantly influenced by this polymorphism ($P=.003$) (Table 2). This effect was even stronger after adjustment for age, body mass index, and systolic or diastolic blood pressure ($P<.001$). Thus, AA patients showed lower, CC patients showed higher, and AC patients showed intermediate values of PWV (AA versus AC, $P<.02$; AA versus CC, $P<.005$; AC versus CC, $P<.05$). Interactions were also tested to establish whether the AT_1 polymorphism might influence the relationship between known determinants of PWV (age, systolic blood pressure, diastolic blood pressure, body mass index, and the ratio of total to HDL cholesterol) and PWV. The only significant interaction ($P<.02$) involved the ratio of total to HDL cholesterol; the association between this ratio and PWV existed only in the presence of the AC or CC genotypes (Figure).

TABLE 2. Mean Characteristics of Subjects According to AT_1 A/C Genotypes

	AA	AC	CC	*P* (ANOVA)
Male, %	75	73	75	NS
Age, y	48.4±10.2	49.8±10.6	52.6±11.9	NS
BMI, kg/m^2	26.0±3.5	26.6±4.0	25.2±5.6	NS
Serum K^+, mmol/L	4.3±0.3	4.3±0.3	4.1±0.2	NS
Cholesterol, mmol/L	6.1±1.1	6.0±0.81	6.5±1.3	NS
HDL, mmol/L	1.39±0.38	1.40±0.39	1.46±0.41	NS
Glucose, mmol/L	6.1±1.0	6.1±1.4	6.3±0.8	NS
SBP, mm Hg	156±11	155±11	155±11	NS
DBP, mm Hg	98±10	95±10	98±8	NS
PWV, m/s	11.4±2.5	12.5±3.2	14.7±4.0	.003

AT_1 indicates angiotensin II type 1 receptor; BMI, body mass index; HDL, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; and PWV, pulse wave velocity. Values are mean±SD.



Scatterplots show relationship between aortic pulse wave velocity (PWV) and ratio of total to high-density lipoprotein cholesterol (TC/HDL) in angiotensin II type 1 receptor (AT_1) AA genotype ($r=-.03$, P=NS) (top) or AC and CC genotypes ($r=.41$, $P<.001$) (bottom). The symbol ○ indicates AA patients; ■, AC and CC patients. The interaction of TC/HDL and AT_1 polymorphism on PWV was statistically significant ($P=.003$).

Discussion

In contrast to previous human studies focusing on the genetic determination of blood pressure, this cross-sectional study assessed the role of genetic factors in the development of large-artery stiffness in never-treated hypertensive patients. Arterial stiffness was assessed by measuring aortic PWV, a noninvasive, accurate, safe, and reproducible method suited to the screening of large populations. Aortic PWV is well correlated with left ventricular hypertrophy,[13,14] general atherosclerosis,[15] and especially coronary artery disease,[11] so that patients

with coronary heart disease have abnormally rigid aortas.[16,17]

As reported previously, aortic stiffness is positively correlated with age and systolic blood pressure in both normotensive and hypertensive subjects.[18,19] The independent role of the renin-angiotensin system in the regulation of aortic stiffness in hypertensives has been suggested in pharmacological studies by comparing the effects of converting-enzyme inhibitors to other antihypertensive drugs. However, these results are not consistent and could be influenced by nonspecific effects of the different drugs. Moreover, anterior treatments may have residual effects on arterial structure and function, thereby modifying aortic distensibility.

The main result of our study is that the AT_1 polymorphism seems to be involved in the regulation of aortic stiffness in never-treated hypertensive patients. This effect is codominant, ie, AT_1 AC heterozygotes have a mean PWV that is intermediate between that of both types of homozygotes. Moreover, this association remains significant after adjustment for covariables strongly affecting PWV.

The AT_1 A/C polymorphism corresponds to an $A^{1166} \rightarrow C$ transversion located at the 5′ end of the 3′ untranslated region of the gene.[8] This polymorphism does not seem to be functional but might be in linkage disequilibrium with an unidentified functional variant. It has been shown that the frequency of the C allele is increased in patients with severe hypertension from 28% to 40%.[8] In our study no relationship was observed between the AT_1 A/C polymorphism and blood pressure level, and the frequency of the C allele was 25%, which is comparable to the frequency observed by Bonnardeaux et al[8] in normotensives. The lack of association of the AT_1 C^{1166} allele with hypertension in this study could be due to the noninclusion of patients with severe hypertension. Although the physiological role of the AT_1 is well defined, the mechanism of PWV increase associated with this genetic polymorphism is unclear. First, because of the tissular localization of the receptor, there is no modification of an intermediate phenotype described in association with this polymorphism. Further studies will be required to identify intermediate phenotypes such as a modification of the number or affinity of this receptor on accessible cells, such as platelets. In a previous study no mutation in the coding sequence of the gene was detected in a group of 50 hypertensive patients.[8] Therefore, it is more likely that the nonfunctional marker we used in this study is in linkage disequilibrium with a variant affecting either the stability of the mRNA or the regulation of the expression of the gene. It is conceivable that an increased number of receptors could increase the effect of angiotensin II on the arterial wall and hence increase arterial wall stiffness.

In our study an interaction was found between AT_1 genotype and the ratio of total to HDL cholesterol in terms of the development of aortic stiffness. Thus, in hypertensives a positive correlation was observed between ratio of total to HDL cholesterol and PWV in AC and CC but not AA patients. The role of the metabolic parameters, and especially plasma cholesterol, in the development of aortic stiffness is controversial. Some studies have shown that patients with lipid disturbances have decreased aortic elasticity, but these results were disputed by others.[20,21] In the present study we did not find any significant relationship between PWV and ratio of total to HDL cholesterol when the whole population of patients was considered. These results suggest that the effects of lipids on large arteries may vary as a function of the AT_1 genotype.

If these results are confirmed, AT_1 gene polymorphism appears to be a particularly important risk predictor for arterial stiffness in hypertensive subjects. It is therefore important to identify the variant of the gene responsible for the biological effect. The implication of the renin-angiotensin system in this process, through the AT_1 gene polymorphism, also suggests that blockade of the system might have beneficial effects on arterial wall structure and function.

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