Angiotensin II type 1 receptor $^{-153}$A/G and ^{1166}A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects

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Objectives **Arterial stiffness is associated with excess morbidity and mortality, independently of other cardiovascular risk factors. Age is the main determinant responsible for arterial wall changes leading to arterial stiffening. Environmental and genetic factors may however influence the magnitude of the effects of age on large artery stiffness.**

Design and methods **The present study assessed whether or not the relationship between age and aortic stiffness was influenced by genetic variants of angiotensinogen (AGT ^{174}T/M, ^{235}M/T), angiotensin converting enzyme (ACE I/D), angiotensin II type 1 receptor (AT_1 ^{1166}A/C, $^{-153}$A/G) and aldosterone synthase ($CYP11B_2$ $^{-344}$T/C). This study was realized in 441 untreated hypertensive subjects of European origin (aged 18–74 years). Aortic stiffness was assessed by carotid–femoral pulse wave velocity (PWV).**

Results **Carriers of the angiotensin II type 1 receptor $^{-153}$G allele showed a steeper age/PWV relationship than the AT_1 $^{-153}$AA subjects. The effect of the AT_1 $^{-153}$A/G polymorphism on aortic stiffness became apparent after the age of 55 years. In subjects with the AT_1 ^{1166}C allele, the relationship age/PWV is shifted upward, indicating higher values of aortic stiffness at any age compared to the AT_1 ^{1166}AA patients. Carriers of both the AT_1 ^{1166}C and $^{-153}$G alleles presented the additive effects of these 2 genotypes on aortic stiffness. Angiotensinogen, ACE and $CYP11B_2$ genotypes did not influence the effects of age on PWV.**

Conclusions **AT_1 receptor genotypes could influence arterial ageing in hypertensive subjects. These results also show that the association between genotypes and arterial stiffness may manifest itself later in life.** ***J Hypertens*** **19:407–413 © 2001 Lippincott Williams & Wilkins.**

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Introduction

The mechanical properties of large arteries play an essential role in cardiovascular haemodynamics through the buffering of stroke volume and the propagation of pulse pressure. Clinical and epidemiological studies strongly suggest that subjects with stiffer arteries have wide pulse pressure, and that stiffening of large arteries is associated with excess morbidity and mortality, independently of other cardiovascular risk factors [1–4].

Ageing is the main determinant responsible for structural and functional changes of the arterial wall (hypertrophy, extracellular matrix accumulation, calcium deposits) [5,6]; leading to an increase in arterial stiffness [7]. Arterial stiffness is also influenced by the activity of several vasoactive systems. The renin–angiotensin–aldosterone system (RAAs) activity seems to play a key role in the regulation of cardiovascular homeostasis [5,8,9]. Genetic variants of the RAAs can influence large artery structure and function [10,11]. It has been shown that in hypertensives, the angiotensin II type 1 (AT_1 ^{1166}A/C) receptor [10] and aldosterone synthase ($CYP11B_2$ $^{-344}$T/C) gene variants [11] are significant determinants of arterial stiffness. Studies have also shown that genetic variants of the RAAs interact with environmental factors such as obesity and physical exercise in determining cardiovascular phenotypes [12]. To our knowledge, no study has ever evaluated the possible influence of genetic variants of the RAAs on age-related arterial stiffness.

In the present study, we examined the influence of genetic variants of angiotensinogen (AGT ^{174}T/M and ^{235}M/T), angiotensin converting enzyme (ACE I/D), angiotensin II type 1 receptor (AT_1 ^{1166}A/C, $^{-153}$A/G)

and aldosterone synthase ($CYP11B_2$ $^{-344}$T/C) on the relationship between age and aortic stiffness, assessed by carotid–femoral pulse wave velocity (PWV), in 441 untreated subjects of European origin.

Methods

Study population

The study sample included 441 consecutive untreated subjects (293 men, 148 women) who were examined for hypertension at the Broussais Hospital outpatient clinic (Paris, France). All were of European ancestry, aged 18 to 74 years. Among these patients, 179 had never been treated for hypertension and the remaining 262 had been. For these patients, treatment was interrupted 3 to 5 weeks prior to the examination.

Hypertensive subjects were selected according to the following criteria: (1) personal history of hypertension; (2) no anti-hypertensive or vasodilatory treatments for at least 3 weeks before the start of the study; (3) systolic blood pressure (SBP) $>$ 145 mmHg or diastolic blood pressure (DBP) $>$ 90 mmHg as measured with a sphygmomanometer (mean of three measurements made with subjects in the supine position, Korotkoff phase V sound); (4) no clinical or biological signs of secondary hypertension; and (5) no recent symptoms of coronary artery disease, heart failure, stroke, and low-limb arterial disease. All participants were examined in the morning after a $\geqslant$ 12 h fast and all underwent the same procedures after providing written informed consent.

After 15 min of rest in the supine position, the carotid–femoral PWV was measured with the use of an automatic device, the Complior (Colson), which allowed an online pulse wave recording and an automatic calculation of PWV. Two transducers were used, one positioned at the base of the neck for the common carotid artery and the other over the femoral artery, as previously described [13]. The validation of the Complior has previously been described, with an intra-observer repeatability coefficient of 0.935 and an inter-observer reproducibility of 0.890 [13].

During the procedure, and for an additional 5 min following the procedure, blood pressure was measured automatically every 2 min with a DINAMAP device. The mean of five consecutive measurements was calculated. An automatic method was chosen in order to avoid inter-observer variations and diminish 'white coat' reactivity. At the end of this procedure, blood was drawn for DNA extraction and standard biochemical tests.

Genotyping

Genotyping of all subjects for AT_1 $^{-153}$A/G, ^{1166}A/C, AGT ^{174}T/M, ^{235}M/T and $CYP11B_2$ $^{-344}$T/C polymorphisms was performed using allele-specific oligonucleotides as previously described [10,14,15]. For the ACE I/D polymorphism, genotype was determined by DNA amplification by the polymerase chain reaction (PCR) as previously described [16]. AT_1 ^{1166}A/C and ACE I/D gene variants from the first 310 hypertensive patients have previously been reported [10]. The primers used to amplify the AT_1 region encompassing the $^{-153}$A/G polymorphism were 5′-CCTCAC GACCCCTCGCTAGG-3′ for the upper and 5′-TGTCAGGCGCTGGAATCATT-3′ for the lower. After enzymatic amplification, one-fifth of the PCR product was denatured and blotted onto nylon membranes. After incubation with specific oligonucleotide probes, each AT_1 $^{-153}$A/G allele was detected: for $^{-153}$A, 5′-TGCCGTCAATATCCCGA-3′; for $^{-153}$G, 5′-TCGGGATACTGACGGCA-3′ [15]. The hybridization temperatures were 47 and 49°C, respectively. The washing temperatures in 0.5 SSC were 49 and 51°C for $^{-153}$A and $^{-153}$G probes, respectively. For technical reasons, genotyping was unsuccessful in 7, 15, 9, 31, 33 and 12 subjects for the ACE I/D, AT_1 $^{-153}$A/G, ^{1166}A/C, AGT ^{174}T/M, ^{235}M/T, and $CYP11B_2$ $^{-344}$ T/C polymorphisms, respectively.

Data analysis

Data from hypertensive patients with no missing clinical values ($n = 441$) were statistically analysed. Hardy-Weinberg equilibrium and allele frequencies were tested using a χ^2 test. Genotypes were tested by one way analysis of variance (ANOVA) on crude values. Results are expressed as mean $\pm$ SD.

In order to determine the more appropriate regression model of the age/PWV relationship in our population, we compared the residual sum of the squares of several nested regression models by ANOVA (F-test) [17,18]. Age was included in these models as a continuous variable. The influence of genotype on the age/PWV relationship was evaluated by comparing the residual sum of the squares from the regression models both with the age/genotype interaction included and without it. In the Results section, we represented the specific P value of each F-test (comparison of the residual sum of the squares). A P-value of < 0.05 was considered as significant.

Results

Blood pressure and PWV levels according to RAAs genotypes

Mean age of the 293 men and 148 women was 49 ± 11 (mean $\pm$ SD). Mean values for SBP and DBP were 152 ± 17 and 93 ± 12 mmHg, respectively, and for PWV the mean value was 12.3 ± 3.0 m/s (data not shown). No association between RAAs polymorphisms and age, SBP, DBP was found (Table 1). Body mass index (BMI), glycemia, total cholesterol, high-density

Table 1 Main characteristics of hypertensive subjects according to AT_1 receptors (^{1166}A/C, $^{-153}$A/G), angiotensin-converting enzyme (ACE I/D), aldosterone synthase (CYP11B_2 $^{-344}$T/C) and angiotensinogen (^{174}T/M and ^{235}M/T) genotypes

Genotypes		Age (years)	SBP (mmHg)	DBP (mmHg)	PWV (m/s)
	AA (254)	49 ± 11	153 ± 18	93 ± 12	11.9 ± 2.7
AT_1 ^{1166}A/C	AC (157)	49 ± 10	152 ± 16	92 ± 11	12.8 ± 3.1
	CC (21)	51 ± 10	156 ± 15	94 ± 12	14.4 ± 4.1*
	AA (291)	49 ± 11	153 ± 16	93 ± 12	12.3 ± 2.8
AT_1 $^{-153}$A/G	AG (119)	50 ± 10	152 ± 20	92 ± 12	12.4 ± 3.5
	GG (16)	48 ± 9	152 ± 15	91 ± 12	12.3 ± 2.4
	DD (144)	49 ± 11	152 ± 17	92 ± 11	12.1 ± 2.7
ACE I/D	ID (218)	50 ± 11	155 ± 18	94 ± 12	12.4 ± 3.1
	II (72)	48 ± 11	148 ± 15	89 ± 13	12.7 ± 3.2
	TT (141)	48 ± 11	152 ± 17	94 ± 12	11.9 ± 2.5
CYP11B_2 $^{-344}$T/C	TC (208)	50 ± 11	152 ± 18	91 ± 12	12.8 ± 3.5**
	CC (80)	49 ± 10	155 ± 16	94 ± 11	11.9 ± 2.3
	TT (322)	48 ± 11	153 ± 18	93 ± 12	12.4 ± 3.1
AGT^{174}T/M	TM (85)	50 ± 10	151 ± 16	92 ± 12	12.7 ± 2.7
	MM (3)	54 ± 2	151 ± 15	92 ± 15	12.7 ± 3.1
	MM (116)	49 ± 11	152 ± 17	91 ± 12	12.7 ± 3.0
AGT^{235}M/T	MT (192)	49 ± 10	153 ± 17	94 ± 12	12.1 ± 2.7
	TT (100)	47 ± 11	152 ± 20	91 ± 12	12.8 ± 3.6

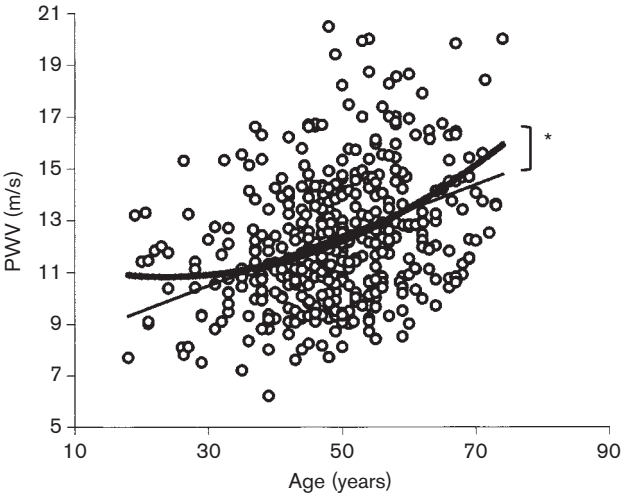
SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity. Values are means ± SD. Genotypes were tested by one way analysis of variance on crude values.* $P < 0.001$; for AT_1 ^{1166}A/C, CC versus AA or AC; ** $P < 0.005$; for CYP11B_2 $^{-344}$T/C, TC versus TT or CC.

lipoprotein (HDL) cholesterol, triglycerides, potassium and 24 h urinary sodium were also similar between genotypes (data not shown). PWV was influenced by AT_1 ^{1166}A/C and CYP11B_2 $^{-344}$T/C genotypes. Carriers of the AT_1 ^{1166}C allele and CYP11B_2 $^{-344}$C allele had higher PWV compared to the AT_1 AA ($P < 0.001$) and CYP11B_2 TT ($P < 0.005$) genotypes, respectively. These two polymorphisms were introduced in a multivariate analysis, which included age and mean arterial pressure (MAP). This analysis showed that the AT_1 ^{1166}A/C polymorphism was a significant independent determinant of PWV, explaining 4.8% of its variability ($P < 0.001$), whereas the CYP11B_2 $^{-344}$T/C polymorphism was not. Age and MAP were the main independent determinants of PWV, explaining 13.1% ($P < 0.0001$) and 5.3% ($P < 0.0001$) of the variability, respectively.

Multiple variable regression models for age/PWV relationship

Figure 1 shows the relationship between PWV and age for the population as a whole. First, we tested whether the age/PWV relationship was represented by a linear regression model. Using the F-test to compare different relationships, it appeared that the linear regression model was significant ($y = 0.097\text{age} + 7.55$, $r = 0.350$, $P < 0.01$). However, the plot distribution of the age/PWV relationship suggested that quadratic or exponential regression models could explain more of the variance of PWV than first-order equations with only the linear term of age. In order to determine the more appropriate regression model for the age/PWV relationship in our population, we compared several nested regression models to linear models by ANOVA (Table 2), using the residual sum of the squares (RSS). This analysis showed that in the entire population studied, the second-order model (quadratic) provided the best fit for the age/PWV relationship compared to both linear (RSS: 3396.4 versus 3446.2; Table 2, $P = 0.01$) and exponential (RSS: 3396.4 versus 3428.5; Table 2, $P = 0.04$) equations, and explained more of the variance of PWV than the other models (Table 2). The residual sum of the squares determined by this quad-

Fig. 1



Relationship between pulse wave velocity (PWV) and age in 441 hypertensive subjects. ○ indicates individual subjects. –– Linear regression model: $y_1 = 0.0974x + 7.5545$; $r = 0.350$, $P < 0.01$. ▬ Quadratic regression model: $y_2 = 0.002x^2 - 0.0968x + 11.97$; $r = 0.368$, $P < 0.01$. *age/PWV relationship was better represented by a quadratic model than by a linear model ($F = 8.91$; $P = 0.003$).

Table 2 Strength of first-order, second-order, third-order and exponential models for age/pulse wave velocity (PWV) relationship

First-order model (linear)	
r	0.350**
Variance explained (%)	12.3
Second-order model (quadratic)	
r	0.368**
Variance explained (%)	13.6
F(age^2, age, K versus age, K)†	8.91***
Third-order model (cubic)	
r	0.367**
Variance explained (%)	13.5
F (age^3, age^2, age, K versus age^2, age, K)†	−0.42
Exponential model	
r	0.356**
Variance explained (%)	12.7
F (age^2, age, K versus exp^{age})§6	4.14*

K, the constant; age, linear term of age; age^2, quadratic terms of age; age^3, cubic terms of age; exp^{age}, exponential terms of age. †, *F*-ratio for addition of higher order model (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$), § quadratic model versus exponential model: the age/PWV relationship was better described by a

ratic model was the lowest (RSS = 3396.4), and the beta value of the quadratic term (age^2) was significant ($P = 0.01$). The quadratic regression equation obtained are illustrated in Figure 1 (PWV = 0.002age^2 – 0.0968age + 11.97; $r = 0.368$; $P < 0.01$). The third-order model, which in addition included the cubic term of age, did not further improve the correlation between PWV and age (Table 2; RSS: 3399.7, $P = 0.51$, compared to quadratic equation). This approach also showed that gender had no significant effect on the quadratic age/PWV relationship (data not shown, $P = 0.40$).

Influence of RAAs genotypes on the age/PWV relationship

Univariate analyses were conducted to assess the influence of RAAs genotypes on the age/PWV relationship. For these analyses, homozygotes with the less frequent allele were pooled with heterozygote subjects. The age/PWV relationship was not influenced by the genetic variants of the ACE I/D, AGT ^{174}T/M and ^{235}M/T, and CYP11B$_2$ polymorphisms (data not shown). When determining PWV, a significant age/genotype interaction was observed with the AT$_1$ $^{-153}$A/G polymorphism. As shown in Figure 2a, the age/PWV slope was steeper in $^{-153}$G carriers than in homozygous AA subjects (interaction term, $P = 0.046$). The effect of the AT$_1$ $^{-153}$A/G polymorphism becomes apparent only after the age of 55 years. According to the AT$_1$^{1166}A/C genotypes, the age/PWV relationship showed that carriers of the^{1166}C allele presented higher PWV values as compared to ^{1166}AA subjects (Fig. 2b, gene effect, $P = 0.00002$), whereas the age/AT$_1$^{1166}A/C genotype interaction was not significant.

The combined effects of the two AT$_1$ polymorphisms ($^{-153}$A/G, ^{1166}A/C) were studied. A chi-square analysis showed that the AT$_1$$^{-153}$A/G gene variant was not in linkage disequilibrium with the AT$_1$^{1166}A/C gene polymorphism (data not shown, $P = 0.90$). The four haplotypes formed from the combination of the two polymorphisms had the same age, BMI and blood pressure levels (data not shown). PWV was greater in subjects with the 'AT$_1$$^{-153}$G-AT$_1$^{1166}C' haplotype (13.3 ± 4.3 m/s) versus the other three haplotypes: 11.9 ± 2.6 m/s in 'AT$_1$$^{-153}$AA-AT$_1$^{1166}AA'; 11.9 ± 3.0 m/s in 'AT$_1$$^{-153}$G-AT$_1$^{1166}AA'; 12.6 ± 2.6 m/s in 'AT$_1$$^{-153}$AA-AT$_1$^{1166}C' (interaction 'AT$_1$$^{-153}$A/G-AT$_1$^{1166}A/C': $P = 0.05$).

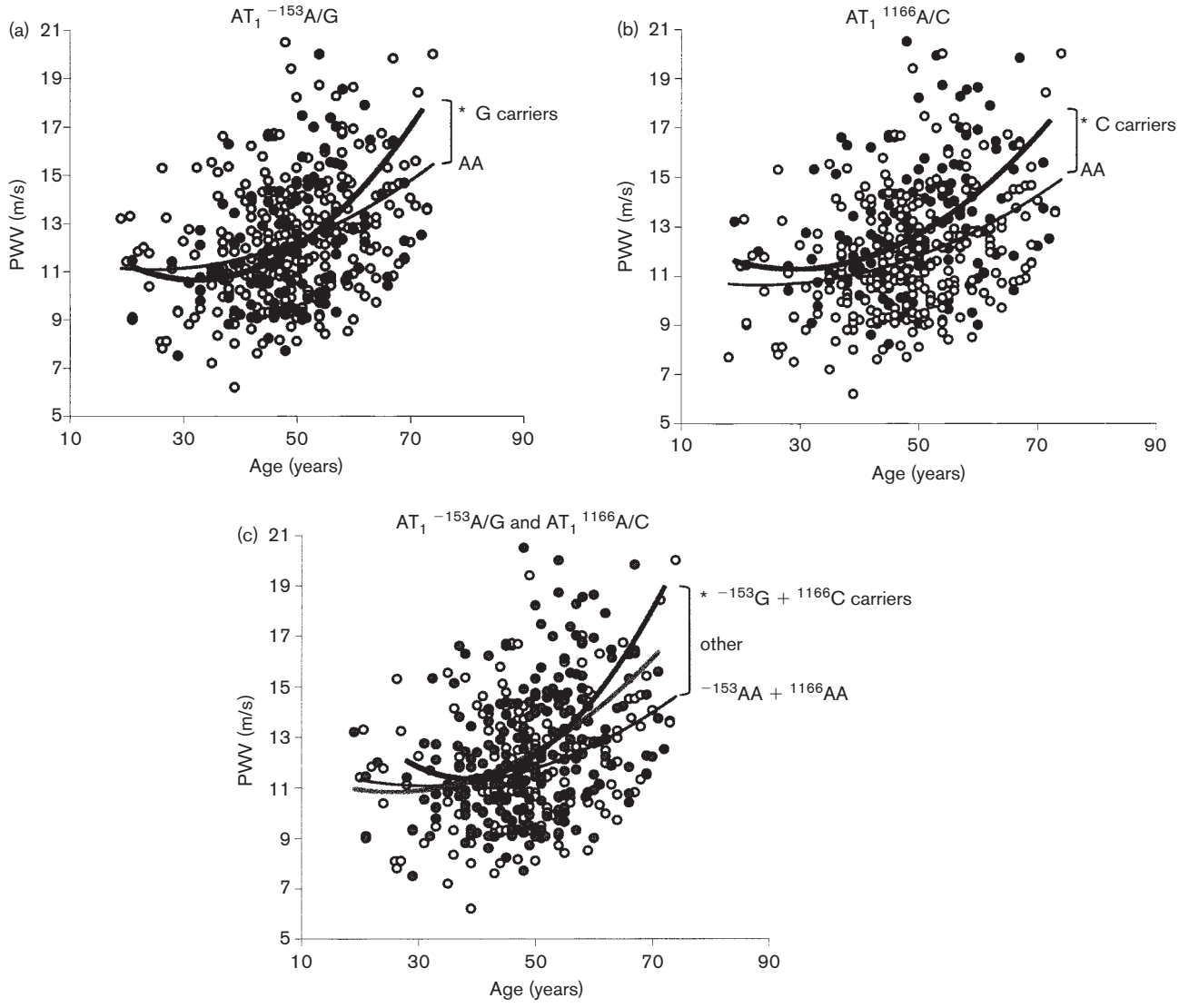
As shown in Figure 2c, the age/PWV relationship in subjects with the '^{1166}C-^{153}G' haplotype shifted upward (haplotype effect, $P = 0.0017$) and presented a steeper slope (interaction term, $P = 0.022$) than the 'AT$_1$$^{-153}$AA-AT$_1$^{1166}AA' haplotype. Subjects with the other genotype combinations (subgroups II in Fig. 2c) showed intermediate age/PWV slopes. Interaction tests showed an additive effect with age of the two AT$_1$ polymorphisms in determining PWV ($P = 0.011$). The combined effects of AT$_1$ genotypes on the age/PWV relationship were the same in both genders (data not shown).

Discussion

The main result of the present study is that both AT$_1$ genotypes influence PWV in hypertensive subjects. Subjects with the AT$_1$ ^{1166}C and $^{-153}$G haplotype have the additive effects of these two genotypes on aortic stiffening: higher PWV values at any age observed in AT$_1$ ^{1166}C carriers and a steeper increase in age/PWV observed in AT$_1$$^{-153}$G carriers.

Quadratic regression models have often been used in epidemiological studies in order to evaluate the effects of risk factors on target organ damage [19]. In our study, the quadratic regression model describes the association between age and PWV more appropriately than the conventional approach of a linear logistic model. Our results show a steeper increase in PWV with age, after 50 years. This result corroborates the well-known epidemiological observation of an increasing prevalence of systolic hypertension, the main clinical manifestation of large artery stiffness, after the age of 55 years [20]. Many research groups using various assessment techniques observed the age-dependent increase in arterial stiffness in both healthy and diseased populations. The effects of ageing are different on proximal, predominantly elastic arteries, compared to distal, predominantly muscular arteries [21,22]. Central arteries stiffen progressively with age, whereas stiffness of muscular arteries changes little with age [21]. Changes with ageing have been explained on the basis of fatigue and fracture of the elastin fibres after repetitive stress cycles [22] and structural changes of the extracellular matrix, mainly collagen [23].

Fig. 2



Relationship between pulse wave velocity (PWV) and age in hypertensive subjects according to $^{-153}$A/G (a), ^{1166}A/C (b) angiotensin II type 1 receptor genotypes and to combined AT_1 receptor genotypes (c). (a) ○ indicates AT_1 $^{-153}$AA ● indicates AT_1 $^{-153}$G carriers which correspond to AG and GG subjects —— AT_1 $^{-153}$AA subjects: $y_1 = 0.0018x^2 - 0.0871x + 12.142$; $r = 0.343$, $n = 291$, $P < 0.01$. ▬ AT_1 $^{-153}$G carriers: $y_2 = 0.0044x^2 - 0.2856x + 15.239$; $r = 0.469$, $n = 135$, $P < 0.01$. *significant difference in the slopes of the 2 groups (interaction term; $F = 3.10$, $P = 0.046$). (b) ○ indicates AT_1 ^{1166}AA subjects; ● indicates AT_1 ^{1166}C carriers which correspond to AG and CC subjects. —— AT_1 ^{1166}AA subjects: $y_1 = 0.0021x^2 - 0.1168x + 12.48$; $r = 0.352$, $n = 254$, $P < 0.01$. ▬ AT_1 ^{1166}C carriers: $y_2 = 0.0032x - 0.188x + 14.006$; $r = 0.420$, $n = 178$, $P < 0.01$. *significant difference in the intercepts of the 2 groups ($F = 8.29$, $P = 0.00002$). (c) ○, indicates subgroup I ("$^{-153}$AA − ^{1166}AA"); ◎, subgroup II ('$^{-153}$AA − ^{1166}C and $^{-153}$G − ^{1166}AA'); ●, subgroup III ('$^{-153}$G − ^{1166}C'). AT_1 ^{1166}C and $^{-153}$G carriers correspond to subjects with AC or CC and AG or GG genotypes, respectively. —— Subgroup I; ▬ Subgroup II; ▬ Subgroup III. $y_I = 0.0019x^2 - 0.1183x + 12.912$; $r = 0.314$, $n = 171$, $P < 0.01$; $y_{II} = 0.0027x^2 - 0.1373x + 12.582$; $r = 0.420$, $n = 197$, $P < 0.01$; $y_{III} = 0.0066x^2 - 0.5042x + 20.989$; $r = 0.483$, $n = 56$, $P < 0.01$. *significant difference in the slopes ($F = 3.84$, $P = 0.022$) and the intercepts ($F = 5.11$, $P = 0.0017$) between subgroups I and III.

The effects of age on arterial stiffness in various populations are influenced by ethnic differences in addition to environmental and geographical factors [24]. Rywik *et al.* [25] recently showed that aortic-femoral PWV increased more rapidly in African Americans than in Caucasians. Environmental factors such as salt intake or garlic consumption have been reported to have independent effects on arterial wall properties and to modify the effects of age on large artery stiffness [26]. Genetic variants could also influence the large artery structure and function. In fact, we previously reported that in hypertensive subjects, the AT_1 ^{1166}A/C receptor gene variant was a significant determinant of arterial stiffness development [10] and of its regression with anti-hypertensive treatment [27]. This polymorphism is located in an untranslated region of the gene but might

be in linkage disequilibrium with a functional variant. In a recent study, we found no association between this polymorphism and the number or functionality of platelet AT_1 receptors [28]. These results however cannot eliminate possible changes in function or expression of AT_1 receptors at the vascular tissue level. The results of recent human studies showing that the AT_1 ^{1166}C allele was associated with increased vascular reactivity to several vasoconstrictors *in vivo* [29–31] and *in vitro* [32] support this hypothesis. In the present study, we observed that the presence of the AT_1 $^{-153}$G allele was a significant determinant of age-related aortic stiffening in hypertensive subjects. Based on the AT_1 $^{-153}$A/G gene variants, the increase in the PWV slope with age varies, especially after the age of 55 years. Our results showed that hypertensive subjects with '$^{-153}$G-^{1166}C' haplotype had both the highest level of PWV and the steepest increase after the age of 50 years, leading to an age/haplotype '$^{-153}$G-^{1166}C' interaction.

The present results clearly show that arterial ageing is influenced by an important number of parameters (certainly both genetic and environmental). Unfortunately, due to the relatively low number of studied subjects, the present study could not evaluate the effects of the combination of more than two polymorphisms or the interaction between environmental factors and gene variants. An interesting finding from our study is that one of these parameters could be the AT_1 genotypes.

The recently identified polymorphism $^{-153}$A/G was not in linkage disequilibrium with the ^{1166}A/C and our results suggest that these two polymorphisms influence the variability of aortic stiffness independently. Poirier *et al.* [15] found no association between the $^{-153}$A/G polymorphism of AT_1 receptor gene and the ^{1166}A/C with blood pressure levels in control subjects from the ECTIM study. More recently, Tiret *et al.* [33] also showed no association between these polymorphisms and the risk or severity of idiopathic dilated cardiomyopathy. The influence of the AT_1 $^{-153}$A/G gene variant on the level of PWV and its changes with age still need to be confirmed by other studies. Our findings illustrate the influence of genetic variants on the effects of chronological age on aortic stiffening, a strong indicator of arterial ageing.

In conclusion, the development of aortic stiffness can be influenced by AT_1 genotypes. Abnormal aortic elastic properties may indicate a disease process prior to its clinical manifestation and observing this process may be useful for studying the progression of the disease and in preventing it. The results of the present study could also contribute to understanding some controversial results obtained in several studies dealing with genotype-phenotype associations since the effect of genotypes on some phenotypes only become apparent at a relatively old age.

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