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$_1$ $^{1166}$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.

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 (CYP11B2 $^{-344}$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 ≥ 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 AT1 $^{-153}$A/G, $^{1166}$A/C, AGT $^{174}$T/M, $^{235}$M/T and CYP11B2 $^{-344}$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]. AT1 $^{-153}$A/G and ACE I/D gene variants from the first 310 hypertensive patients have previously been reported [10]. The primers used to amplify the AT1 region encompassing the $^{-153}$A/G polymorphism were 5'-CCTGAC GACCCCCCTCGGTAGG-3' for the upper and 5'-TGTCAGGGCGCTGGAATCATTT-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 AT1 $^{-153}$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, AT1 $^{-153}$A/G, AGT $^{174}$T/M, $^{235}$M/T, and CYP11B2 $^{-344}$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 $\chi^2$ test. Genotypes were tested by one way analysis of variance (ANOVA) on crude values. Results are expressed as mean ± 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 ± 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
lipoprotein (HDL) cholesterol, triglycerides, potassium and 24 h urinary sodium were also similar between genotypes (data not shown). PWV was influenced by AT11166A/C and CYP11B2344T/C genotypes. Carriers of the AT11166C allele and CYP11B2344C allele had higher PWV compared to the AT1 AA (P < 0.001) and CYP11B2 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 AT11166A/C polymorphism was a significant independent determinant of PWV, explaining 4.8% of its variability (P < 0.001), whereas the CYP11B2344T/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 = 0.0979age + 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-
The combined effects of the two AT1 polymorphisms (−153A/G, 1166A/C) were studied. A chi-square analysis showed that the AT1−153A/G gene variant was not in linkage disequilibrium with the AT11166A/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 ‘AT1−153G-AT11166C’ haplotype (13.3 ± 4.3 m/s) versus the other three haplotypes: 11.9 ± 2.6 m/s in ‘AT1−153AA-AT11166AA’; 11.9 ± 3.0 m/s in ‘AT1−153G-AT11166AA’; 12.6 ± 2.6 m/s in ‘AT1−153AA-AT11166C’ (interaction ‘AT1−153A/G-AT11166A/C’: \( P = 0.05 \)).

As shown in Figure 2c, the age/PWV relationship in subjects with the ‘1166C-153G’ haplotype shifted upward (haplotype effect, \( P = 0.0017 \)) and presented a steeper slope (interaction term, \( P = 0.022 \)) than the ‘AT1−153AA-AT11166AA’ 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 AT1 polymorphisms in determining PWV (\( P = 0.011 \)). The combined effects of AT1 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 AT1 genotypes influence PWV in hypertensive subjects. Subjects with the AT11166C and −153G haplotype have the additive effects of these two genotypes on aortic stiffening: higher PWV values at any age observed in AT11166C carriers and a steeper increase in age/PWV observed in AT1−153G 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].
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 AT1 1166A/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\(_1\) 1166C 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-1166C 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-1166C 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 1166A/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 1166A/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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**References**


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