Influence of the AGTR1 A1166C Genotype on the Progression of Arterial Stiffness: A 16-Year Longitudinal Study

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BACKGROUND
We examined the influence of the AGTR1 A1166C genotype on the 16-year evolution of pulse wave velocity (PWV) in a middle-aged population. In a cross-sectional study, we reported that the presence of the AGTR1 A1166C allele was associated with higher aortic stiffness compared with the AGTR1 1166AA genotype.

METHODS
The study was conducted in 259 subjects who underwent 3 health check-ups over 16 years at the Centre IPC–Paris: an initial visit in 1992–1993, an intermediate visit in 1998–1999, and a final visit in 2007–2008. Aortic stiffness was assessed during the 3 visits by measuring carotid–femoral PWV. AGTR1 A1166C polymorphism was assayed by allele-specific oligonucleotide hybridization.

RESULTS
AGTR1 1166C allele carriers (AC + CC genotypes) had a 35% more pronounced increase in PWV over this 16-year period when compared with the AGTR1 1166AA subjects (3.01 ± 0.32 vs. 1.92 ± 0.23 m/s; P < 0.001). This increase remained significant after adjustment for age, sex, initial PWV values, and changes in blood pressure (+37%; P < 0.05). The genotype-related differences in PWV were only observed at the last visit (i.e., later in life, after the age of 55 years). The effects of this genotype on PWV were not related to the presence of antihypertensive treatment.

CONCLUSIONS
This is the first long-term longitudinal study indicating that AT1 1166C carriers are at increased risk of pronounced arterial stiffening during aging especially after the age of 55.

Keywords: aging; angiotensin receptors; arterial stiffness; blood pressure; genetic polymorphism; hypertension.

doi:10.1093/ajh/hpt141

Increased central arterial stiffening is a hallmark of the aging process and the consequence of many disease states, such as diabetes, atherosclerosis, and chronic renal compromise. Clinically, the gold-standard parameter is pulse wave velocity (PWV), which is an estimation of the velocity of the propagation of the forward and backward pressure waves between two points of the arterial tree. Several clinical studies have shown an independent association between PWV and morbidity and mortality in various populations.1 As a result, a number of studies are now focused on determining PWV reference values for various populations.2,3 There is also growing interest in defining the factors and markers able to influence the rhythm of arterial aging, which could help identify those individuals at increased risk for cardiovascular complications.

Several studies support the presence of genetic factors that can modulate the pace of arterial stiffness. A first study showed the influence of the A1166C polymorphism of the angiotensin II type 1 receptor gene (AGTR1) on the regulation of aortic stiffness in hypertensive subjects.4 The AGTR1 1166C allele was, in most cases, associated with higher aortic stiffness, even after adjustment for age and blood pressure (BP). In this cross-sectional study, presence of the AGTR1 1166C allele was associated with a positive relationship between age and PWV, which was shifted upwards (i.e., was linked to higher values of aortic stiffness, compared with the AGTR1 1166AA genotype). Furthermore, the role of this genotype on collagen synthesis and arterial stiffness has been confirmed by some but not all studies.5–8 However, all of these data stem from

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Initially submitted March 28, 2013; date of first revision July 1, 2013; accepted for publication July 1, 2013; online publication August 13, 2013.
cross-sectional studies and thus require confirmation by longitudinal studies.

Hence, the aim of our longitudinal study was to assess the influence of the AGTR1 A1166C polymorphism on the long-term evolution of PWV in a middle-aged population. This genotype/phenotype analysis was performed in the subgroup of the Evolution de la Rigidité Artérielle (ERA) cohort9 who underwent sequential PWV measurements over a period of 16 years.

METHODS

This study was conducted in a subset of a population of 675 subjects examined at the Centre IPC–Paris and previously described by our group. Briefly, the aim of this initial cohort of 675 subjects was to study the evolution of PWV over a period of 6 years (i.e., from the initial visit (1992–1993) to the intermediate (1998–1999) visit). Our study includes 259 subjects who underwent a third final visit (2007–2008) 10 years after the intermediate visit and in whom all sequential PWV measurements and genotype characterization were performed.

Among those who did not come to the final visit, 10 died between the intermediate and final visits (identified by the National Statistic Service, INSEE), whereas the remainder did not answer our request to return. We hypothesize that the majority of these subjects had changed address during this long period of time. As compared with those who came to the final visit, those who did not come were older (53±11 years vs. 50±10 years; P<0.001), were more frequently hypertensive (56% vs. 46%; P<0.01), and showed a slight elevation in PWV (10.9±2.1 vs. 10.6±2.0; P<0.05). No other difference between these 2 groups was observed at the initial visit.

Carotid–femoral pulse wave velocity was measured to assess aortic stiffness. During the 1992–1993 examination (initial visit), PWV was calculated manually, whereas during the 1998–1999 examination (intermediate visit), an automatic device was used (Compilior, Colson, Vincennes, France). The procedure used for PWV measurements has been described in detail in our previous publication of this cohort. The same device used for the intermediate visit was also used for the 2007–2008 examination (final visit).

All of the clinical, biological, and arterial measurements were performed at the same site (Centre IPC–Paris) during a half-day (morning) examination. The same procedure was used for all 3 visits. Details regarding this procedure are provided in our previous publication. As was the case for the initial and intermediate visits, the ethics committee of Cochin Hospital approved the study protocol, and written informed consent was obtained from all study participants.

Blood was drawn for DNA extraction. The AGTR1 A1166C polymorphism was assayed by allele-specific oligonucleotide hybridization. The primers used to amplify the AGTR1 region encompassing the A1166C polymorphism were 5′-AAT-GCTTGTAGCCAAAGTCACCT-3′ and 5′-GGGCTTTGGCTTTG-TCTTGTG-3′.

Data analysis

Subjects were divided according to the presence or absence of the AGTR1 1166C allele (AA vs. AC + CC). In separate analyses, our population was also analyzed according to the 3 genotypes. Values presented in Table 1 are expressed as means ± SD. For each genotype, mean values of the initial and final visits were compared using a paired Student t test.

Sample sizes were consistent (power = 88%) with the ability to display 1 m/s PWV differences between groups, which is considered clinically relevant.

Multivariable analysis of variance and generalized linear models were performed. For some analyses, subjects were classified according to the presence or absence of antihypertensive treatment as follows: nontreated in either of the initial and final visits (for hypertension); untreated during the initial visit but treated at the final visit; treated during both visits.

Data have been further analyzed by multiple marginal regression models for repeated measures using the generalized estimating equations method. This procedure is especially suited for testing between-group and within-group comparisons of longitudinal analyses of repeated measures.

Statistical analyses were carried out using the NCSS 2000 statistical software package (NCSS, Kaysville, UT) and STATA software package (StataCorp, College Station, TX). P<0.05 was considered to be statistically significant.

RESULTS

Table 1 summarizes the demographic and clinical characteristics of the subjects during the initial visit and last visit (16 years later), according to the presence or absence of the AGTR1 1166C allele. The characteristics during the initial visit were the same in the 2 genotypes with the exception of the presence of a lower percentage of women in the AC + CC genotypes (P<0.02). Genotype frequencies did not deviate significantly from the Hardy–Weinberg expectation. Genotype distribution in subjects who showed up for the final visit was identical to those who did not return (Table 2, upper panel).

By the last visit however, several of the clinical characteristics had changed. Specifically, there was an observed increase in body mass index, triglycerides, serum creatinine, and the percentage of subjects with hypertension and with antihypertensive treatment, and there was an increase in body mass index, triglycerides, serum creatinine, and the percentage of subjects with hypertension and with antihypertensive treatment, and there was a decrease in total and high-density lipoprotein (HDL) cholesterol and glycemia. Plasma High Sensitivity C-reactive protein (CRP) values measured at the final visit were not different between the 2 genotypes. No changes in BP and heart rate were observed between the 2 visits. All these modifications were similar in both carriers and noncarriers of the AGTR1 1166C allele. We have also recorded the major cardiovascular events (myocardial infarction, Coronary Artery Bypass Graft (CABG), strokes, and aneurisms) that occurred in each of the 2 genotype groups during the period between the intermediate and the final visits. We recorded 17 events in the AGTR1 1166AA group vs. 13 events in the AGTR1 1166C group (not significant).
Table 1. Clinical characteristics during the initial and final visits according to the presence or absence of the AGTR1 1166C allele

<table>
<thead>
<tr>
<th>AGTR1 A1166C genotypes</th>
<th>Initial visit</th>
<th>Final visit</th>
<th>Initial visit</th>
<th>Final visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC/CC</td>
<td>AA</td>
<td>AC/CC</td>
</tr>
<tr>
<td>No.</td>
<td>138</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, %</td>
<td>38</td>
<td>24#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>49.7±10.6</td>
<td>65.7±10.5***</td>
<td>50.6±9.3</td>
<td>66.5±9.3***</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4±3.8</td>
<td>26.7±4.5***</td>
<td>25.4±3.3</td>
<td>26.4±3.5***</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>140.4±18.1</td>
<td>142.0±19.2</td>
<td>139.7±18.5</td>
<td>139.4±18.9</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>86.0±11.9</td>
<td>85.2±8.5</td>
<td>85.1±12.7</td>
<td>84.2±8.9</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>104.2±13.6</td>
<td>104.1±10.5</td>
<td>103.3±14.1</td>
<td>102.5±10.7</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>54.4±9.3</td>
<td>56.8±16.7</td>
<td>54.6±9.9</td>
<td>55.2±15.9</td>
</tr>
<tr>
<td>HR, bmp</td>
<td>68.6±13.2</td>
<td>68.8±10.5</td>
<td>69.3±12.1</td>
<td>70.9±11.5</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>10.6±1.9</td>
<td>12.5±3.2***</td>
<td>10.5±2.1</td>
<td>13.5±4.1***</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>222.2±43.0</td>
<td>213.9±40.8 (0.06)</td>
<td>227.0±33.3</td>
<td>216.3±35.1**</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>60.2±15.5</td>
<td>51.4±14.7***</td>
<td>59.5±17.5</td>
<td>50.3±13.2***</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>93.4±57.4</td>
<td>107.2±49.2**</td>
<td>98.9±53.0</td>
<td>114.2±55.2**</td>
</tr>
<tr>
<td>Glycemia, mg/dl</td>
<td>106.7±19.0</td>
<td>100.9±13.7***</td>
<td>108.3±12.9</td>
<td>101.4±10.8***</td>
</tr>
<tr>
<td>Creatinine, mg/l</td>
<td>9.5±1.6</td>
<td>10.4±2.8***</td>
<td>9.8±1.6</td>
<td>10.6±1.9***</td>
</tr>
<tr>
<td>hsCRP, µg/ml</td>
<td>-</td>
<td>2.62±5.22</td>
<td>-</td>
<td>2.62±6.33</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>62 (45)</td>
<td>78 (57)***</td>
<td>55 (45)</td>
<td>75 (62)***</td>
</tr>
<tr>
<td>Treated for HT, no. (%)</td>
<td>32 (23)</td>
<td>75 (54)***</td>
<td>28 (23)</td>
<td>66 (55)***</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless otherwise noted.
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HR, heart rate; HT, hypertension; PP, pulse pressure; PWV pulse wave velocity; SBP, systolic blood pressure.

*P < 0.05; **P < 0.01; ***P < 0.001 initial vs. final visit; #P < 0.05; ##P < 0.001 AA vs. AC+CC.

Table 2. Distribution of AGTR1 A1166C genotypes and pulse wave velocity increase (ΔPWV) during the 16-year follow-up period in the subgroup with final visit

<table>
<thead>
<tr>
<th>AGTR1 A1166C genotypes, no. (%)</th>
<th>A1166C allele carriers, %</th>
<th>CC carriers, %</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGTR1 A1166C genotypes, no. (%)</td>
<td>AA</td>
<td>AC/CC</td>
<td></td>
</tr>
<tr>
<td>No final visit</td>
<td>208 (53%)</td>
<td>151 (38%)</td>
<td>39 (9%)</td>
</tr>
<tr>
<td>With final visit</td>
<td>138 (53%)</td>
<td>99 (38%)</td>
<td>22 (9%)</td>
</tr>
<tr>
<td>ΔPWV, m/s</td>
<td>Unadjusted 1.92±0.23</td>
<td>3.05±0.36*</td>
<td>2.85±0.78</td>
</tr>
<tr>
<td>Adjusted* 2.08±0.25</td>
<td>2.85±0.29*</td>
<td>2.93±0.60</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Abbreviation: ANOVA, analysis of variance.
*Adjusted for age, sex, change in mean arterial pressure, and initial value of pulse wave velocity.
**P < 0.05 vs. AA; not significant between AC and CC.

Only PWV exhibited a different evolution according to genotype between the 2 visits. Indeed, although PWV was the same in the 2 genotypes during the first visit, it was significantly higher at the last visit in AGTR1 1166C allele carriers compared with AA subjects (P < 0.01) (Table 1). This difference remained significant after adjustment for current mean arterial pressure (MAP), heart rate, sex, and antihypertensive drug use (P < 0.05). Thus, presence of the 1166C allele was associated with a 35% more pronounced increase in PWV (ΔPWV) over the 16-year period (3.01±0.32 vs 1.92±0.23 m/s; P < 0.001).

This difference in ΔPWV still persisted after adjustment for age, sex, ΔMAP, and baseline PWV values. (+37%; P < 0.05) (Figure 1). Similar results were observed after further adjustment for current MAP, heart rate, and antihypertensive treatment (P < 0.01). The effect of the AGTR1 A1166C allele was also assessed by considering the 3 genotypes separately (Table 2). These results clearly demonstrate that AC and CC had a similar increase in PWV (ΔPWV) over the follow-up period. DPWV was significantly higher in the AC and CC genotypes than in the AA genotype.

No sex/genotype interaction was observed for PWV changes from Hardy–Weinberg expectations over the 16-year period (P = 0.92).

To analyze the kinetics of this increase in PWV, we also took into consideration the intermediate visit performed 6 years after the initial visit. Figure 2 shows that the genotype-related differences in PWV were not observed during either the first or intermediate visit but only at the last visit.

Finally, the overall generalized estimating equation analyses of PWV in a model including age, MAP and genotype group (in interaction with age) displayed significant results
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for age ($P < 0.001$), MAP ($P < 0.001$), and genotype group ($P < 0.007$), thus indicating a significantly different response between the 2 genotype groups.

The upper panel of Figure 3 shows the influence of the AGTR1 A1166C genotype in the evolution of PWV in 3 subgroups of patients (nontreated; untreated during the first visit but treated during the second; treated at both visits) according to the presence or absence of antihypertensive treatment during the initial and final visits. In all 3 groups and after adjusting for age and sex, PWV increased between the 2 visits in a more pronounced manner in the AGTR1 1166C allele carriers (genotype effect, $P < 0.008$). No genotype/group interaction was observed ($P = 0.99$). By contrast, changes in MAP were not influenced by genotype in any of the 3 groups studied (Figure 3, lower panel). As expected, there was a strong group effect on the changes in MAP ($P < 0.0001$); those who received treatment between the initial and the final visit showed a more pronounced decrease in MAP. This effect was

Figure 1. Changes in pulse wave velocity (PWV) between the initial and final visits in the AGTR1 1166AA and the AGTR1 1166C allele carriers. Values are expressed as mean ± SEM and adjusted for age, sex, initial PWV values, and changes in mean arterial pressure (MAP). Abbreviation: ΔPWV, changes in PWV. *$P < 0.05$ AC + CC vs. AA AGTR1 A1166C genotype.

Figure 2. Pulse wave velocity (PWV) vs. age during the initial, intermediate, and final visits in the 2 AGTR1 A1166C genotypes. Results are expressed as mean ± SEM. **$P < 0.01$ AC + CC vs. AA AGTR1 A1166C genotype.
not influenced by genotype. In an additional analysis focused on the interaction between the AGTR1 1166 genotype and treatment with renin-angiotensin-aldosterone system (RAAS) blockers on the PWV or BP long-term evolution, we did not find any significant result (data not shown). However, among patients treated with either angiotensin converting-enzyme inhibitor and/or Angiotensin II Receptor, type 1 (AT1R) antagonists at the final visit, only 17 in the AGTR1 1166 C genotype and 17 in the AGTR1 1166 AA genotype had this treatment for a long period (>10 years).

**DISCUSSION**

This longitudinal study is, to our knowledge, the first to assess the long-term evolution of PWV according to the AGTR1 A1166C genotype. The 2 major findings of this study are (i) that this effect is clinically relevant because presence of the AGTR1 1166C allele is associated with more pronounced aortic stiffening by 35% as compared with the AA genotype and (ii) that it appears later in life (i.e., after the age of 55 years). Hence, our data clearly indicate an accelerated arterial aging in subjects with presence of the AGTR1 1166C allele. The effect of this genotype on PWV is not related to any effect on mean BP levels because BP and changes in BP over this period of time were exactly the same in the 2 genotypes. In addition, this genotype effect on PWV evolution was not influenced by the presence of antihypertensive treatment.

The aging of the arterial tree, as expressed in PWV, is a dynamic process. It reflects the genesis of age-dependent arterial stiffening, which is accelerated after the fifth decade. The differences among individuals in an aging-related phenotype such as PWV may not necessarily be apparent at a younger age. One would anticipate that already at birth or childhood, individuals harboring the AGTR1 1166C allele would show increased PWV. In fact, our findings underscore the power of a long-term, longitudinal study to capture the genotype-phenotype relationship of an aging-dependent phenomenon that might not otherwise be detected in a cross-sectional model. Indeed, the genotype of the individual does not change with age, but many phenotypes,
including PWV, do change with age. We believe that the 16-year follow-up of our study is a major advantage in this regard. This effect has been clearly demonstrated using also a generalized estimating equation, which is an appropriate method to assess the association with longitudinal changes in PWV.

In this study, subjects with or without the presence of the AGTR1 1166C allele had similar PWV levels during the initial visit as well as during the second visit 6 years later. The observed increases in aortic PWV during the follow-up period were clearly higher in subjects carrying the C allele, when considering heterozygote or homozygote subjects either all together or separately. The absence of difference between AC and CC genotypes suggests a dominant effect of the C allele. However, such conclusion should be interpreted with caution given the relative small number of CC subjects.

The mean age of our population, which approximated 50, 56, and 66 years at the initial, intermediate, and final visits, respectively, explains the higher aortic PWV at the final visit. Indeed, previous cross-sectional studies have shown that the increase in PWV is not linear and is accelerated after the age of 55. Similar findings were also observed longitudinally in the previous report of this study, whereby the increase in PWV over a period of 6 years (i.e. between the initial and the second visit) was more pronounced in subjects aged >55 years of age compared with the younger group. The present long-term longitudinal analysis is able to provide further insight into this process. We can now report, in the presence of the AGTR1 1166C allele, an acceleration of PWV after the second visit (i.e. after the age of 55 years).

These findings observed in a long-term, longitudinal design confirm the hypothesis previously put forward in a cross-sectional analysis in a different population of 441 untreated hypertensive subjects that the presence of the 1166C allele is associated with more pronounced PWV values later in life. These considerations may also explain discrepancies in the results of different studies regarding the role of the AT1R on PWV. For example, Levy et al. showed no association, although their cross-sectional study examined younger subjects (mean age = 52 years), which corresponds to the age of our population during the first visit where there was no effect of this genotype on PWV.

It is now well established that there is an activation of the intravascular RAAS in the elderly, leading to a proinflammatory phenotype, an intimal and medial thickening, and, ultimately, vascular wall stiffening. These effects in turn enhance the levels or activity of factors such as matrix metalloproteinase 2 (MMP-2), and local angiotensin-converting enzyme and angiotensin II in aortas of elderly monkeys in comparison with the same tissues in younger animals. More recently, it has been shown that increased signaling of constitutive mineralocorticoid receptors may promote and amplify age-associated inflammation that accompanies arterial aging through increased angiotensin II–stimulated expression of mineralocorticoid receptors.

The crucial question remains whether the AGTR1 A1166C polymorphism located in a noncoding region may influence expression of this receptor and consequently the evolution of cardiovascular disease. An answer to this question has partly been provided by Martin et al. who demonstrated that, in the presence of the 1166C allele, base-pairing complementarity between miR-155 and the AT1R mRNA is reduced such that miR-155 can no longer attenuate translation as efficiently, thus leading to an increase in AT1R density. These results could explain the observed clinical associations between the AGTR1 1166C allele with enhanced vascular reactivity and increased renovascular sensitivity to angiotensin II as well as with cardiovascular diseases such as essential hypertension, cardiac hypertrophy, aortic stiffness, myocardial infarction, and increased oxidative stress levels in human heart failure. The absence of association between the AGTR1 1166C allele and cardiovascular outcomes in our population may be related to the age range (50–67 years) of the population over the follow-up period.

Taken together, the above and present findings indicate that the 1166C allele is associated with overactivity of the AT1R responsible for structural and functional arterial remodeling, thus leading to more pronounced increase in PWV with age and therefore accelerating arterial aging. Previous controlled prospective studies have shown that the presence of the AGTR1 1166C allele is associated with a more pronounced BP and PWV response to drugs blocking the RAAS. However, in this analysis we did not find any significant drug treatment/genotype interaction on long term PWV evolution. This was also observed when the presence of RAAS blockers was taken into account. As we mentioned in the Results section, the absence of such effect may be related to the small number of individual treated with RAAS blockers during a long period.

This study does carry certain limitations. The number of women in the C allele genotype group was smaller than the number in AA genotype group. However, this unexplained difference cannot be responsible for the principal result of the study because no genotype/sex interaction on PWV evolution was observed. Moreover, a separate analysis in the male population showed similar results (i.e., a higher increase in PWV in the presence of the C allele, as compared with the AA male population).

Also, the single polymorphism approach may certainly be more limited in explaining the PWV evolution than a single nucleotide polymorphism approach, which would be preferable as the “functional” variant in the AGTR1 gene. However, this polymorphism has been extensively studied in several studies in the past, showing interesting functional effects as commented above.

Another limitation concerns the absence of any circulating markers of RAAS activity or inflammatory response explaining the effects of this genotype on PWV. The long-term follow-up of our study does not allow such sequential measurements for technical reasons. In addition, the biological significance of 1 single measurement of these markers at the end of a clinical process over a period of >15 years is difficult to interpret.

In conclusion, the clinical interest of our study is that subjects with the presence of AGTR1 1166C allele are at high risk of having more accelerated arterial aging by one-third as compared with noncarriers of this allele. Such a demonstration with regard to an aging-related process can only be performed in long-term longitudinal studies. This study can
contribute to identifying subjects at high risk for developing age-related cardiovascular diseases.

ACKNOWLEDGMENTS

We thank Mr Pierre Pothier for stimulating discussions and language corrections.

This work was supported by the Fondation pour la recherche Medicale (FRM DCV-20070409250), the Caisse Nationale d’Assurance Maladie des Travailleurs Salariés (CNAM-TS, France), the Caisse Primaire d’Assurance Maladie des Travailleurs Salariés recherche Medicale (FRM DCV-20070409250), the Caisse age-related cardiovascular diseases.

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