

Gender-Specific Association of the Plasminogen Activator Inhibitor-1 4G/5G Polymorphism With Central Arterial Blood Pressure

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Gender-specific association of the plasminogen activator inhibitor-1 4G/5G polymorphism with central arterial blood pressure

Running headline: PAI-1 and central blood pressure

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Abstract

Background. The functional plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism has previously been associated with hypertension. In recent years, central blood pressure, rather than brachial has been argued a better measure of cardiovascular damage and clinical outcome. The aim of this study was to investigate the possible influence of the 4G/5G polymorphism on central arterial blood pressure in a cohort of elderly individuals.

Methods. We studied 410 individuals, 216 men and 194 women, aged 70-88. Central pressures and pulse waveforms were calculated from the radial artery pressure waveform by the use of the SphygmoCor system and a generalized transfer function. Brachial pressure was recorded using oscillometric technique (Dinamap). PAI-1 antigen was determined in plasma.

Results. The results showed that central pressures were higher in women carrying the PAI-1 4G/4G genotype compared to female carriers of the 5G/5G genotype, ($P=0.025$, $P=0.002$ and $P=0.002$ for central systolic-, diastolic- and mean arterial pressure, respectively). The association remained after adjustment for potentially confounding factors related to hypertension. No association of the PAI-1 genotype with blood pressure was found in men. Multiple regression analysis revealed an association between PAI-1 genotype and plasma PAI-1 levels ($P=0.048$).

Conclusions. Our findings show a gender-specific association of the PAI-1 4G/5G polymorphism with central arterial blood pressure. The genotype effect was independent of other risk factors related to hypertension, suggesting that impaired fibrinolytic potential may play an important role in the development of central hypertension in women.

Introduction

Hypertension is a major risk factor for the development of cardiovascular disease. Plasminogen activator inhibitor-1 (PAI-1) is the principal inhibitor of the fibrinolytic system, interfering with the activation of plasminogen and subsequent matrix degradation. Circulating PAI-1 influence smooth muscle cell proliferation as well as cell migration^{1,2}, and has previously been associated with increased risk of thrombotic events^{3,4}. Furthermore, plasma PAI-1 has been positively correlated with brachial blood pressure^{5,6} and plasma PAI-1 activity is significantly increased in hypertensive patients compared to normotensive subjects⁷. The functional 4G/5G polymorphism in the PAI-1 promoter is associated with variations in plasma PAI-1 levels, 4G/4G individuals having higher levels of circulating PAI-1 than 5G carriers^{8,9}. Consequently, the PAI-1 4G/4G genotype has been associated with with higher relative risk of hypertension¹⁰, as well as with an increased risk of myocardial infarction (reviewed by Boekholdt et al¹¹).

In late years, central blood pressure has been argued a better measure of left ventricular load than brachial pressure, and suggested as an independent predictor of cardiovascular damage and clinical outcome¹²⁻¹⁴. No study so far has however investigated the effect of the PAI-1 4G/5G polymorphism on central hemodynamics. The aim of this study was to examine the possible influence of the PAI-1 4G/5G polymorphism on central arterial blood pressure in a cohort of elderly individuals. Gender-dependent effects of the polymorphism were of specific interest as plasma PAI-1 levels previously have been shown to differ between genders^{15,16}.

Methods

Study population

A total of 410 individuals (216 men and 194 women), aged 70-88 were studied. All were members of a previous longitudinal study involving elderly inhabitants in Kinda municipality, South East of Sweden¹⁷. The present study population has been described in detail elsewhere¹⁸. In brief, in the original study, all inhabitants in Kinda municipality aged 65-82 (n=1130) were invited, 876 of whom agreed to participate. During a follow-up study on 675 individuals in years 2003-2005, all participants were asked to take part in the present study. A total of 452 individuals accepted, of which pulse wave analysis (PWA) and PAI-1 genotyping were successful in 410 subjects. All examinations were performed by the same two investigators on one single occasion. Subjects were requested to refrain from tobacco, coffee and tea 4 hours prior to assessment. Each participant provided written informed consent and the study protocol was approved by the Regional Ethical Review board in Linköping, Sweden. The study was conducted in accordance with the principles stated in the Declaration of Helsinki.

Biochemical measurements and variable definitions

Blood samples were drawn following overnight fasting and collected in pre-chilled plastic vacutainer tubes containing EDTA (Terumo EDTA K-3). Plasma was prepared by centrifugation at 3000 g for 10 minutes at 4°C. All samples were stored at -70°C pending analyses. Body mass index (BMI) was calculated from height and weight measurements. Fasting glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), creatinine and C-reactive protein (CRP) were determined in plasma. Glomerular filtration rate (GFR) was calculated according to the Cockcroft-Gault formula¹⁹. Information on conventional cardiovascular risk factors, history of cardiovascular events and medications were obtained by

a standardized interview during the examination phase. Diabetes was defined as use of anti-diabetic medication and/or a fasting glucose concentration equal to or above 7.0 mmol L⁻¹. Hypertension was defined as a blood pressure equal or above 140/90 mmHg or a previous diagnosis of hypertension. Ischemic heart disease was defined as a history of angina pectoris or a verified myocardial infarction.

Determination of PAI-1 antigen

Levels of PAI-1 antigen were analyzed in plasma using TriniLIZE PAI-1 antigen (T6003) assay (Trinity Biotech, NY, USA), according to the manufacturer's instructions. The same positive control was included in all analyses to control for inter-assay variation.

Blood pressure measurements

Brachial blood pressure was recorded following 15 minutes of rest with an oscillometric device (Dinamap model PRO 200 Monitor; Critikon, Tampa, FL, USA). The subjects were in the supine position with the left arm in a relaxed position and the cuff in the same position as estimated position of the left atrium of the heart.

Measurement of intima-media thickness and lumen diameter in the abdominal aorta

Intima-media thickness (IMT) of the posterior wall was measured in diastole. Diastolic lumen diameter and pulsatile diameter change were measured between the posterior and the anterior wall and used for calculation of systolic lumen diameter. The aorta was examined at the midpoint between the renal arteries and the aortic bifurcation. Measurements were carried out using an ultrasound scanner (Esaote AU5, Esaote Biomedica, Florence, Italy) equipped with a 7.5 MHz linear transducer or a 7.3 MHz curved transducer. The ultrasound system was connected to a PC equipped with the Wall Track System software (WTS2, Pie Medical,

Maastricht, The Netherlands). Details of the ultrasound technique have been described elsewhere^{20,21}. All measurements were performed with subjects in the supine position, directly following brachial blood pressure registrations. Mean values from three consecutive recordings were used for statistical analysis.

Calculations

Distensibility coefficient (DC) (10^{-3} kPa^{-1}) and compliance coefficient (CC) ($\text{mm}^2 \text{ kPa}^{-1}$) were calculated as measures of arterial stiffness²². DC $((2 \times Dd \times \Delta D + \Delta D^2)/(Dd^2 \times \Delta P))$ is the relative change in arterial diameter for a given increase in pressure. CC $(\pi(2 \times Dd \times \Delta D + \Delta D^2)/(4 \times \Delta P))$ is the absolute increase in cross-sectional area for a given increase in arterial pressure, assuming that the vessel length is constant during the pulse wave²². Dd is the end-diastolic diameter (mm), ΔD is the diameter change between systole and diastole (mm) and ΔP is the pressure change between systole and diastole (kPa). There is a nonlinear pressure-diameter relationship of the aortic wall, meaning that distensibility characteristics of the abdominal aorta are somewhat dependent on blood pressure. However, the index stiffness β seems to be less sensitive to pressure changes²³. Stiffness β was calculated as follows; Stiffness $\beta = \ln(P_{\text{sys}}/P_{\text{dia}})/(\Delta D/Dd)^{24}$. P_{sys} and P_{dia} are the maximal systolic and end-diastolic blood pressure levels (mmHg). Details of all measures of arterial stiffness have been described previously¹⁸.

Applanation tonometry

Radial artery pulse waves were obtained noninvasively by the use of the SphygmoCor system (version 7.0, Model MM3, AtCor Medical, Sydney, Australia), equipped with a Millar pressure tonometer. For PWA, the central pressure waveform was derived using a generalized transfer function, calculated from an 11 seconds recording of the radial artery pressure

waveform. Brachial systolic- and diastolic blood pressure were measured prior to pulse wave recordings and used for calibration of pressure waves. All pulse wave registrations were repeated at least three times, and average data from three registrations were used for statistical analysis. Only recordings with high quality curves were used for PWA. Augmentation index (AIx) and pressure augmentation (AugP) were calculated from the central pressure waveform. AIx is the pressure augmentation expressed as percentage of the central pulse pressure (PP):

$$\text{AIx} = (\text{AugP}/\text{PP}) \times 100.$$

Determination of PAI-1 4G/5G genotype

Genomic DNA was prepared from peripheral blood using the QIAamp[®] DNA Mini Kit (Qiagen, Hilden, Germany), and stored at -20°C pending analysis. PAI-1 4G/5G genotype was determined using PCR and endonuclease digestion, as described elsewhere²⁵. A total of 20 ng was amplified in 20 µl reaction volumes.

Statistical analysis

All data are presented as mean values ± standard deviation (SD) unless otherwise stated. Hardy-Weinberg equilibrium was evaluated for the PAI-1 4G/5G genotype using Pearson's chi-squared test; allele-frequencies were determined by gene counting. Differences in continuous data were analyzed using one-way ANOVA followed by Bonferroni post hoc test, or independent-samples t-test. Categorical data was evaluated by the use of Pearson's chi-squared test. Adjustments for potentially confounding factors were made using general linear models. Linear regression analysis was performed to evaluate the relationship between plasma PAI-1 level and blood pressure. Multiple regression analysis was used to assess determinants of plasma PAI-1 levels. To address whether significant associations were gender-specific, two-way ANOVA including PAI-1 genotype, gender and a gender-genotype interaction were

performed. Logarithmic transformation was performed on skewed data before analysis were made. A two-tailed P -value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS 15.0 for Windows software (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study population

Basic characteristics of participants are given in Table 1. The study population consisted of 216 men and 194 women with a mean age of 78.7 ± 3.1 and 78.8 ± 3.7 years, respectively. Compared to men, women had higher levels of plasma PAI-1 antigen ($P=0.006$), LDL and HDL ($P<0.001$ for both) (Table 1), and a higher frequency of hypertension ($P=0.039$). Further, women had higher peripheral and central systolic blood pressure ($P=0.002$ and $P<0.001$, respectively), mean arterial pressure ($P<0.001$ for both) and pulse pressure ($P<0.001$ for both) (Table 2) than men. There was no difference in stiffness β between genders, however, women had higher IMT ($P=0.002$), AIx and AugP ($P<0.001$ for both) (Table 2) and lower CC ($P<0.001$) (data not shown) than men. In addition, linear regression analysis showed no association between blood pressure and plasma PAI-1 antigen, in either men or women (data not shown). A total of 65% of the study subjects were on anti-hypertensive treatment. The percentages of cardiovascular medications were as follows: ACE inhibitors (ACEi) 21%, angiotensin II receptor blockers (ARBs) 4%, beta receptor blockers 37%, calcium channel blockers (CCBs) 17%, diuretics 36% and statins 24%. There was no significant difference in medications between genders (data not shown).

Prevalence of genotypes

Since plasma PAI-1 levels previously have been shown to be influenced by gender, all additional analyses were performed in men and women separately^{15,16}. The genotype distribution of the PAI-1 4G/5G polymorphism in men/women was as follows: 56/45 subjects were 4G/4G homozygous, 117/108 were heterozygous and 43/41 were carriers of the 5G/5G genotype. The allele frequency for the 5G allele was 0.48 (males 0.47, females 0.49). All

genotypes and calculated allele frequencies were compatible with Hardy-Weinberg equilibrium and similar to previous findings²⁶.

TABLE 1. Characteristics of study population and groups

	All	Men	Women	P (♂ vs. ♀)
N	410	216	194	
Age, years	78.7 (3.4)	78.7 (3.1)	78.8 (3.7)	0.839
Body mass index, $kg\ m^{-2}$	26.5 (4.0)	26.1 (3.3)	26.9 (4.7)	0.061
Laboratory Data				
p CRP, $mg\ L^{-1}$	4.6 (11.0)	5.2 (14.0)	4.0 (6.3)	0.313
p LDL, $mmol\ L^{-1}$	3.1 (1.0)	2.9 (0.9)	3.3 (1.1)	<0.001
p HDL, $mmol\ L^{-1}$	1.3 (0.3)	1.2 (0.3)	1.4 (0.4)	<0.001
p Creatinine, $\mu mol\ L^{-1}$	78.5 (23.6)	86.0 (23.8)	70.1 (20.5)	<0.001
p glucose, $mmol\ L^{-1}$	5.8 (2.3)	5.9 (2.1)	5.7 (2.5)	0.594
p PAI-1 antigen, $ng\ mL^{-1}$	34.1 (16.3)	32.0 (14.8)	36.5 (17.6)	0.006
History				
Ischemic heart disease	98 (24)	60 (28)	38 (20)	0.052
Hypertension	343 (84)	173 (80)	170 (87)	0.039
Diabetes	94 (23)	51 (24)	43 (22)	0.728
Smoking	37 (9)	28 (13)	9 (5)	0.003

Values are mean (SD) or number of subjects in group (%). p CRP: plasma C-reactive protein; p LDL: plasma low-density lipoprotein; p HDL: plasma high-density lipoprotein; p PAI-1: plasma plasminogen-activator inhibitor-1.

TABLE 2. Pressure and stiffness characteristics

	Men	Women	P
Peripheral BP, mmHg			
Systolic	143 (20)	152 (24)	<0.001
Diastolic	75 (10)	76 (12)	0.388
Mean arterial pressure	97 (12)	103 (15)	<0.001
Pulse pressure	68 (17)	77 (19)	<0.001
Central BP, mmHg			
Systolic	132 (20)	142 (23)	<0.001
Diastolic	75 (10)	77 (12)	0.216
Mean arterial pressure	97 (12)	103 (15)	<0.001
Pulse pressure	56 (17)	65 (18)	<0.001
Augmentation index, %	30.4 (9.5)	35.0 (8.8)	<0.001
Pressure augmentation, mmHg	18.1 (9.4)	23.4 (10.4)	<0.001
Wall properties abdominal aorta			
Intima-media thickness, mm	0.55 (0.14)	0.60 (0.22)	0.002
Stiffness β	28.4 (20.5)	27.0 (23.3)	0.493

Values are mean (SD). BP: blood pressure.

Association of PAI-1 4G/5G polymorphism with blood pressure and abdominal aortic wall mechanics

Associations between the PAI-1 4G/5G polymorphism and clinical characteristics are presented in Table 3. Peripheral and central blood pressures (systolic, diastolic and mean arterial pressure) were significantly higher in women carrying the 4G/4G genotype compared to female carriers of the 5G/5G genotype, while no differences were found in men. The association between PAI-1 genotype and blood pressures were gender-specific, as shown by

TABLE 3. Characteristics according to PAI-1 4G/5G genotype

	Men					Women				
	4G/4G	4G/5G	5G/5G	P	P _{Adj} ^a	4G/4G	4G/5G	5G/5G	P	P _{Adj} ^a
N	56	117	43			45	108	41		
Age, years	78.7 (2.9)	78.8 (3.3)	78.4 (2.9)	0.832		78.7 (2.9)	78.8 (3.9)	78.6 (3.9)	0.929	
Body mass index, kg m ⁻²	26.2 (3.6)	25.9 (3.2)	26.3 (2.8)	0.774		28.0 (5.0)	26.6 (4.6)	26.2 (4.5)	0.170	
Plasma PAI-1 antigen, ng mL ⁻¹	32.2 (14.2)	33.1 (15.8)	28.9 (12.7)	0.285	0.893 ^b	40.0 (16.2)	35.5 (17.3)	35.0 (19.5)	0.300	0.123 ^b
Peripheral BP, mmHg										
Systolic	138 (21)	145 (20)	143 (19)	0.088	0.184	160 (22)	152 (24)	145 (25)	0.011 ^d	0.034
Diastolic	74 (9)	75 (10)	75 (10)	0.635	0.727	80 (11)	75 (12)	72 (11)	0.003 ^e	0.007
Mean arterial pressure	95 (12)	98 (12)	98 (12)	0.291	0.508	109 (14)	103 (15)	98 (16)	0.003 ^f	0.010
Pulse pressure	64 (18)	70 (16)	69 (16)	0.094	0.181	80 (17)	77 (20)	73 (18)	0.233	0.293
Central BP, mmHg										
Systolic	126 (21)	134 (20)	132 (19)	0.062	0.152	148 (21)	142 (23)	134 (23)	0.031 ^g	0.048
Diastolic	74 (9)	76 (10)	75 (10)	0.663	0.751	81 (11)	76 (12)	73 (11)	0.003 ^{h,i}	0.006
Mean arterial pressure	95 (12)	98 (12)	98 (12)	0.293	0.510	109 (14)	103 (15)	98 (16)	0.003 ^j	0.010
Pulse pressure	52 (18)	58 (16)	57 (16)	0.066	0.147	66 (17)	65 (19)	62 (17)	0.449	0.348
Augmentation index, %	27.9 (8.9)	31.5 (9.6)	30.8 (9.6)	0.068		33.0 (10.1)	35.3 (8.4)	36.1 (8.3)	0.227	
Pressure augmentation, mmHg	15.4 (8.9)	19.2 (9.7)	18.5 (8.5)	0.043		22.7 (10.6)	24.0 (10.9)	22.6 (8.9)	0.671	
Wall properties abdominal aorta										
Intima-media thickness, mm	0.53 (0.12)	0.55 (0.14)	0.55 (0.16)	0.446		0.59 (0.20)	0.60 (0.19)	0.62 (0.29)	0.816	
Stiffness β	31.9 (25.6)	25.7 (18.2)	31.1 (18.1)	0.231	0.294 ^c	35.2 (35.1)	25.1 (19.1)	22.8 (13.7)	0.090	0.129 ^c
CC, mm ² kPa ⁻¹	2.03 (1.68)	1.97 (8.54)	1.81 (1.26)	0.603		1.21 (0.93)	1.49 (0.96)	1.61 (1.11)	0.121	
DC, 10 ⁻³ kPa ⁻¹	9.43 (8.54)	9.04 (6.10)	7.28 (5.41)	0.298		7.39 (5.93)	9.12 (6.24)	9.29 (5.22)	0.074	

Values are mean (SD) or number of subjects in group. PAI-1: Plasminogen activator inhibitor-1; BP: blood pressure; CC: compliance coefficient; DC: distensibility coefficient. ^a Adjusted for age, body mass index, low-density lipoprotein, high-density lipoprotein, intima-media thickness, glomerular filtration rate, diabetes and smoking; ^b 4G/4G subject vs. 5G-carriers; ^c Adjusted for age and mean arterial pressure.

Bonferroni Post hoc test

^d P-value (0.008) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

^e P-value (0.003) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

^f P-value (0.002) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

^g P-value (0.025) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

^h P-value (0.042) of subjects with 4G/4G genotype vs. subjects with 4G/5G genotype

ⁱ P-value (0.002) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

^j P-value (0.002) of subjects with 4G/4G genotype vs. subjects with 5G/5G genotype

significant interactions between PAI-1 genotype and gender (e.g. $P=0.006$ for central systolic pressure). Adjustment for potentially confounding factors related to hypertension (age, BMI, LDL, HDL, IMT, GFR, diabetes and smoking) had no or little effect on the associations. There was no difference in plasma PAI-1 antigen between genotypes, although women carrying the 4G/4G genotype showed a tendency towards higher antigen levels compared to 5G carriers (4G/4G: 40.0 ± 16.2 ng/ml; 5G carriers: 35.4 ± 17.9 ng/ml; $P=0.123$). However, multiple regression analysis controlling for a large number of potentially confounding factors (sex, age, BMI, diabetes, smoking, LDL-cholesterol and CRP) showed a weak but significant association between PAI-1 genotype and plasma PAI-1 antigen ($P=0.048$). In addition, BMI ($P<0.000$), sex ($P=0.004$) and current smoking ($P=0.002$) were shown to be strong determinants of plasma PAI-1 antigen. No significant difference in distribution of medications (ACEi, ARBs, beta receptor blockers, CCBs and diuretics) was shown between genotype groups (Table 4), and the association remained significant after additional adjustment for medications (data not shown). Women carrying the 4G/4G genotype showed a tendency towards higher stiffness of the abdominal aorta compared to 5G/5G carriers, although the difference did not reach statistical significance ($P=0.090$). There was no association between PAI-1 genotype and IMT of the abdominal aorta in either men or women.

TABLE 4. Distribution of medications according to PAI-1 4G/5G genotype

	Men				Women			
	4G/4G	4G/5G	5G/5G	P	4G/4G	4G/5G	5G/5G	P
N	56	117	43		45	108	41	
Medication, <i>n</i> (%)								
ACE inhibitor	13 (23)	18 (15)	12 (28)	0.164	8 (18)	25 (23)	10 (24)	0.712
ARB	2 (4)	5 (4)	2 (5)	0.962	4 (9)	4 (4)	1 (2)	0.287
Beta receptor blocker	24 (43)	41 (35)	17 (40)	0.595	18 (40)	36 (33)	16 (39)	0.668
Ca ²⁺ -channel blocker	8 (14)	26 (22)	7 (16)	0.405	7 (16)	14 (13)	7 (17)	0.792
Diuretic	15 (27)	40 (34)	15 (35)	0.578	21 (47)	41 (38)	15 (37)	0.545

Values are number of subjects in group (%). ARB: Angiotensin receptor blocker.

Discussion

The PAI-1 gene has previously been suggested as a candidate gene predisposing peripheral hypertension. In the present study, the possible influence of the single 4G/5G guanosine polymorphism on central arterial blood pressure was investigated. We show that the PAI-1 4G/4G genotype is associated with higher central systolic-, diastolic- and mean arterial blood pressure in women, whereas no association was found in men. The associations remained after adjustment for potentially confounding factors, i.e. age, BMI, LDL, HDL, IMT, GFR, diabetes and smoking.

The present study highlights two major findings. Firstly, the PAI-1 4G/5G polymorphism is associated not only with peripheral blood pressure, but more importantly also with central blood pressure. Secondly, the 4G/4G genotype-phenotype association was only found in women, suggesting a gender-specific biology of PAI-1.

The brachial artery has for many years been the standard site for measurement of blood pressure. However, it is the central blood pressure to which the major organs affected by hypertension are exposed. Therefore, central pressure has lately been argued as a better measure of left ventricular afterload than peripheral pressure, and has been shown to be an independent predictor of end organ damage¹²⁻¹⁴. In addition, central and peripheral pressures are differently affected by antihypertensive drugs, and large-scale trials have emphasized central hemodynamics as a meaningful target of treatment²⁷. Increased central pressure is associated with elevated aortic stiffness, also a predictor of cardiovascular events. In the present study, a tendency towards higher stiffness of the abdominal aorta in 4G/4G women, compared to women carrying the 5G/5G genotype was shown. The difference did not however reach statistical significance.

Central pressure can be reliably assessed by a range of different noninvasive techniques. In the present study, a generalized transfer function was used to calculate aortic pressure from non-invasive calibrations of the radial artery pressure waveform. Although the use of this technique may introduce errors, such as underestimations of the central pressure^{28,29}, it will most likely not affect comparative studies between different groups of subjects.

The present study suggests a gender-specific biology of PAI-1 as the 4G/4G genotype-phenotype association only was found in women. Gender has previously been associated with plasma PAI-1 levels, men having higher levels of circulating PAI-1 than women^{30,31}. Surprisingly, in the present study the association with central pressure was only seen in women, which might seem counterintuitive. However, previous reports have shown that gender differences in PAI-1 levels disappear to a large extent with age, increasing with age in women but stay roughly the same in men⁵. In our study, with a population mean age of ~80 years, women had significantly higher plasma PAI-1 antigen levels compared to men. An increase in plasma PAI-1 could possibly result in elevated blood pressure, however, linear regression analysis showed no association between blood pressure and plasma PAI-1 antigen.

The synthesis of PAI-1 is transcriptionally regulated by the 4G/5G promoter polymorphism. The 4G allele binds an activator, whereas the 5G allele binds an activator and a repressor. This results in increased transcription and higher plasma PAI-1 levels in individuals carrying the 4G allele^{8,9}. The allele-specific increase in plasma PAI-1 has been shown in several populations and in both men and women^{9,32,33}. This indicates that the gender-related genotype-phenotype association found in the present investigation may be a consequence of later events rather than regulation on a transcriptional level. However, arguing against this is

the finding that the 4G/5G polymorphism previously been implicated in an allele-specific response to plasma triglycerides³⁴, instead suggesting a gene-environmental interaction. Dyslipidemia, as a component of the metabolic syndrome, is associated with increased arterial stiffness, and this association is particularly pronounced in women³⁵. Further, human adipose tissue has previously been suggested an important source of plasma PAI-1, especially under obese conditions³⁶, and BMI has been strongly related to plasma PAI-1 levels in both men and women^{15,16}. In the present study, women had higher LDL ($P<0.001$) and slightly higher BMI ($P=0.061$) than men. Unfortunately, triglyceride measurements were not available, leaving the genotype-phenotype relationship to be further elucidated.

Based on previous findings, it might be reasonable to believe that the genotype-phenotype association, found in the present study is mediated by variations in plasma PAI-1. Although no difference in plasma PAI-1 antigen was seen between genotypes, multiple regression analysis controlling for a large number of potential confounders revealed a weak but significant association between PAI-1 genotype and plasma PAI-1 antigen ($P=0.048$). In addition, sex, BMI and current smoking were shown to be strong determinant of plasma PAI-1 antigen. Hence, there seem to be an association between PAI-1 4G/5G genotype and plasma PAI-1 antigen levels, although in an elderly population like ours other factors may interfere.

A number of medical therapies, commonly used in an elderly population have been shown to influence plasma PAI-1 levels. Blood pressure lowering drugs such as ACEi reduce plasma PAI-1 antigen levels, in postmenopausal women³⁷, in patients with hypertension³⁸, and in subjects with chronic activation of the renin-angiotensin-aldosterone system³⁹. Additional adjustment for ACEi, ARBs, beta receptor blockers, CCBs or diuretics had no significant effect on the association between PAI-1 genotype and central pressures. Furthermore,

hormonal replacement therapy (HRT) is effective in lowering PAI-1 levels in postmenopausal women⁴⁰. There are no data available on HRT use in the present study. However, as all women included in this study live in a rural community, and are relatively old, the usage of HRT is most likely negligible.

Limitations of study

Due to shortage of plasma we could not measure triglyceride levels. Such data would potentially further disentangle the allele- and gender-dependent association found in the present investigation. In addition, a larger sample of subjects would possibly have strengthened our findings.

In summary, the study presents a gender-specific association of the PAI-1 4G/5G polymorphism with peripheral, and more importantly, central arterial blood pressure. The genotype-phenotype association remained after correction for potentially confounding factors. These findings may provide valuable insight into the understanding of the development of hypertension, however, further studies are required to disentangle the molecular mechanism underlying the association.

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Disclosure

No conflict of interest.

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