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Male–female differences in the genetic regulation of t-PA and PAI-1 levels in a Ghanaian population

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Abstract

Tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) directly influence thrombus formation and degradation, and have been identified as risk factors for thromboembolic disease. Prior studies investigated determinants of t-PA and PAI-1 expression, but mainly in Caucasian subjects. The aim of this study was to identify the contributions of genetic and other factors to inter-individual variation in plasma levels of t-PA and PAI-1 in a large-scale

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population-based sample from urban West Africa. t-PA, PAI-1 and several demographic, anthropometric, and metabolic parameters were measured in 992 residents of Sunyani, the capital of the Brong-Ahafo region of Ghana. In addition, nine gene polymorphisms associated with components of the renin-angiotensin and fibrinolytic systems were determined. We found that BMI, systolic and diastolic blood pressure, total cholesterol, glucose, and triglycerides were all significant predictors of t-PA and PAI-1 in both females and males. In addition, a significant relationship was found between the PAI-1 4G/5G (rs1799768) polymorphism on PAI-1 levels in females, the TPA I/D (rs4646972) polymorphism on t-PA and PAI-1 in males, the renin (rs3730103) polymorphism on t-PA and PAI-1 in males, the ethanolamine kinase 2 (rs1917542) polymorphism on PAI-1 in males, and the renin (rs1464816) polymorphism on t-PA in females and on PAI-1 in males. This study of urban West Africans shows that t-PA and PAI-1 levels are determined by both genetic loci of the fibrinolytic and renin-angiotensin systems and other factors often associated with cardiovascular disease, and that genetic factors differ between males and females.

Introduction

Hemostasis requires a balance between thrombus formation and dissolution. Fibrinolysis defines the latter of these processes. Biochemical regulators of fibrinolysis include tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1). Elevated plasma concentrations of t-PA and PAI-1 are associated with early atherosclerosis (Salomaa et al. 1995) and are predictive of first myocardial infarction (Hamsten et al. 1987; Ridker et al. 1993; Thogersen et al. 1998), recurrent myocardial infarction (Juhan-Vague et al. 1996), and mortality after myocardial infarction (Collet et al. 2003; Jansson et al., 1993). Given the profound worldwide burden of atherothrombotic disease, we believe clinical studies to identify determinants of t-PA and PAI-1 expression and therapeutic strategies to limit t-PA and PAI-1 activity may have considerable societal benefit.

Plasma concentrations of t-PA and PAI-1 are controlled by both genetic as well as traditional cardiovascular risk factors. Although the demographic, anthropometric, and metabolic correlates of t-PA and PAI-1 have been well characterized in high-risk subjects, few studies have examined the genetic and non-genetic determinants of these proteins' expression in large-scale population-based cohorts. We have sought to fill this void with complementary population-based studies in the Netherlands (Asselbergs et al. 2006, 2007a, b, c) and Ghana (Williams et al. 2007).

Despite initiatives intended to reduce health disparities, significant racial differences exist in the prevalences of cardiovascular risk factors and outcomes in the US. Indeed, cardiovascular disease remains significantly more common among black Americans than white Americans (Mensah et al. 2005). This may be attributable in part to racial differences in expression of hemostatic factors such as t-PA and PAI-1 (Lutsey et al. 2006). Therefore, in order to define the genetic architecture of t-PA and PAI-1 for black Americans, we chose to study the population of Sunyani, Ghana (Williams et al. 2007). This offered the advantage of reducing admixture, which has been estimated at 10–20% among black Americans (Parra et al. 1998), thereby reducing complexity due to locus heterogeneity. Given the importance of West Africa as the site of embarkation for the involuntary African diaspora of the eighteenth and nineteenth centuries, results obtained in Ghana should be applicable to black Americans. In addition, previous studies of the patterns of genetic variation within and between Ghana and Nigeria indicate little genetic differentiation between these two West Africa populations that were major sources for much of the slave trade to North America. Therefore, studies in either these two countries may represent a substantial portion of genetic variation in parts of West Africa that were the source gene pool for African Americans.

For our genetic analyses, we selected two polymorphisms located in non-coding regions of the t-PA and PAI-1 genes and seven polymorphisms associated with components of the renin-angiotensin system (RAS). The t-PA I/D polymorphism (rs4646972) is defined by the absence or presence of an Alu repeat in the eighth intron of the t-PA gene (Ludwig et al. 1992). The PAI-1 4G/5G polymorphism (rs1799768) is defined by the length of a 4/5-guanine tract in the promoter of the PAI-1 gene (Eriksson et al. 1995). Both the t-PA an dPAI-1 variants have been previously associated with plasma concentrations of the respective proteins and have been argued to be functional (Eriksson et al. 1995; Festa et al. 2003; Jern et al. 1999; Morange et al. 2007). Polymorphisms in the RAS were analyzed in deference to mounting evidence that RAS activation impairs fibrinolysis by stimulating PAI-1 production and t-PA release (Vaughan 2002). RAS polymorphisms included two within the renin gene (rs3730103, rs1464816); two within the ethanolamine kinase 2 gene, which were chosen because they are adjacent to renin on chromosome 1q32 (rs2293337, rs1917542) and may represent promoter variation in the renin gene. Variants rs2293337 and rs1464816 are the farthest apart of these variants and are separated by approximately 15 kb, and are in a region of relatively low LD in the Yoruba population. In addition, one variant each in the angiotensin converting enzyme [ACE insertion/deletion in intron 16 (rs4646994)], angiotensinogen (rs699), and angiotensin II type 1 receptor genes (rs5186) that have been previously associated with a variety of cardiovascular endpoints were assayed.

With these guiding principles in mind, the aim of this study was to identify the contributions of genetic and traditional cardiovascular risk factors to inter-individual variation in plasma levels of t-PA and PAI-1 in a large-scale population-based sample of urban West Africa. First, we evaluated the relationships between demographic, anthropometric, and metabolic variables and plasma levels of t-PA and PAI-1. Second, we evaluated the effects of t-PA, PAI-1, and RAS polymorphisms on the plasma levels of these proteins. Finally, we determined which of these polymorphisms contributed independently to the prediction of plasma levels of t-PA and PAI-1 after accounting for the non-genetic factors.

Materials and methods

Study population and non-genetic measurements

Enrollment of the population-based sample analyzed in this study has been described previously (Williams et al. 2007). In summary, 992 residents of Sunyani, the capital of the Brong-Ahafo region of Ghana, were recruited by word-of-mouth during 2002–2003. Exclusion criteria included age less than 18, prior enrollment of a first or second degree relative, and any sign of acute illness that may impact t-PA or PAI-1 levels. An excess of females (57.3%) was enrolled, though not by design. Fasting glucose, lipid, t-PA, and PAI-1 levels were measured using commercially available techniques as described elsewhere (Williams et al. 2007). Blood samples were obtained between 8:00 and 10:00 a.m. in all subjects to minimize variability due to circadian rhythms of t-PA and PAI-1 antigen levels, but day-to-day variability of t-PA and PAI-1 levels was not addressed in the current study. Briefly, black top tubes (Stabilyte tubes, Biopool, Umea, Sweden) were drawn and chilled on ice. Within 20 min of the blood being drawn, cells were centrifuged for 20 min at 3000 Gs at 0°C. After centrifugation plasma was transferred to a cryotube with a sterile transfer pipette and immediately frozen in liquid nitrogen. Plasma samples were stored in liquid nitrogen and shipped to Nashville, TN, in IATA approved Cryo shippers (MVE Products). Plasma levels of PAI-1 and t-PA antigen were measured at Vanderbilt University using a commercially available enzyme-linked immunoassay (ELISA, Biopool AB, Umea). Protocols and consent forms were approved by both US and Ghanaian authorities in accordance with the Declaration of Helsinki.

Measurement of genetic polymorphisms

DNA was isolated on-site in Ghana using PureGene DNA purification kits (Gentra Systems). All polymorphisms were analyzed using TaqMan probes and PCR primers, designed through the Assay-by-Design service of Applied Biosystems. TaqMan assays were carried out according to the manufacturers recommendations on an ABI 7900HT apparatus, and results were analyzed with SDS 2.0 genotype calling software (Applied Biosystems).

Statistical analysis

The overall objective of the data analysis was to assess the roles of non-genetic factors, namely age, body mass index (BMI), systolic blood pressure, diastolic blood pressure, fasting glucose, total cholesterol, triglycerides, smoking status, and genetic polymorphisms in the prediction of plasma levels of t-PA and PAI-1.

Our first goal was to determine whether the distributions of the preceding non-genetic factors and t-PA and PAI-1 were dependent on gender. In addition, we stratified females by expected menopausal status to investigate whether these distributions change after menopause. Because menopausal status was not assessed at time of enrollment, we used age less than 48 versus age equal to or greater than 48 as a proxy for pre- versus post-menopausal status. This cut-off value was based on a prior study of 123 Ghanaian women for whom the mean and median ages of menopause were found to be 48 ± 3.6 years (Kwawukume et al. 1993), and was in agreement with other menopausal surveys in Morocco and Nigeria (Okonofua et al. 1990;Reynolds and Obermeyer 2003). Re-analysis after exclusion of peri-menopausal women within two standard deviations of this mean age of menopause (i.e., comparing women ≤40 vs. ≥55 years) provided very similar results (data not shown), and added confidence to our acceptance of age as a proxy for menopausal status. t-PA, PAI-1, glucose and triglycerides levels were transformed to normality by taking the natural logarithm (ln). Continuous variables were compared between males and females using a univariate general linear model adjusted for age. Discrete variables, e.g., smoking, were compared between males and females by contingency testing.

Our second goal was to assess the relationships between the preceding non-genetic factors and plasma levels of t-PA and PAI-1. Within each gender, we fit univariate regression models for t-PA and PAI-1 with each non-genetic variable separately. The significant univariate predictors were then included in a complete model. The R^2 and P values from the F test for these models were calculated. In addition, we performed a partial F test for homogeneity of regression among genders for each univariate model.

Our third goal was to determine whether each genetic polymorphism was predictive of plasma t-PA and PAI-1 levels. Prior to analysis we tested the null hypothesis that each polymorphism was in Hardy–Weinberg equilibrium using a chi-square goodness-of-fit test. We then determined whether each polymorphism was associated with inter-individual variation in plasma t-PA and PAI-1, levels using a one-way analysis of variance (ANOVA). We carried out three separate analyses for each polymorphism using different genetic models—additive, recessive, and dominant. For those polymorphisms that were statistically significant we selected the most significant models and then evaluated whether the polymorphisms were still associated with t-PA or PAI-1 after adjusting for the significant non-genetic factors in the prior linear regression analysis.

Results were considered statistically significant if the P value for the test statistic was less than or equal to the set type I error rate (α) of 0.05. Results were considered marginally significant at the 0.10 level. No adjustment for multiple comparisons was performed because there were few statistical tests and there is good biological evidence that each of the systems under investigation is involved in the regulation of t-PA and PAI-1 expression either directly or

indirectly, suggesting the universal null hypothesis does not apply to these data (Rothman 1990).

All calculations were performed with SPSS version 14.0 software (SPSS).

Results

Impact of gender on demographic, anthropometric, and metabolic factors

In total, 569 females (mean age 42.7 ± 10.9 years) and 423 males (mean age 44.0 ± 12.7 years) were enrolled in the present study. There was no significant difference between the age distributions of the male and female participants ($P = 0.081$). Table 1 summarizes the mean ± SD of ln t-PA, ln PAI-1, BMI, systolic blood pressure, diastolic blood pressure, ln glucose, total cholesterol, and ln triglycerides for males and females separately. While males had significantly higher systolic blood pressure ($P < 0.001$) and ln triglycerides ($P = 0.016$), females had significantly higher BMI, ln glucose, and total cholesterol ($P \leq 0.003$ for all three). Females also had significantly higher levels of ln PAI-1 ($P = 0.005$), but equivalent levels of ln t-PA ($P = 0.537$).

After stratifying females by predicted menopausal status, mean systolic blood pressure and ln triglycerides were significantly lower in pre-menopausal females ($P < 0.001$ and $P = 0.009$, respectively), but not in post-menopausal females ($P = 0.180$ and $P = 0.712$, respectively) as compared with males. Mean ln PAI-1, BMI, ln glucose and total cholesterol levels were significantly higher in pre-menopausal females as compared to males ($P < 0.05$) and significance was comparable between post-menopausal females as compared to males for these traits, except for glucose ($P = 0.08$). Except for BMI which increased with age ($P = 0.004$), no significant differences were observed between pre- and post-menopausal females.

Taken together, these results demonstrated that the continuous distributions of the preceding cardiac risk factors, as well as ln PAI-1, are gender-dependent. All additional analyses were therefore performed in males and females separately.

Prediction of plasma t-PA and PAI-1 levels by traditional cardiac risk factors

Table 2 summarizes the regression relationships of ln t-PA and ln PAI-1 with age, BMI, systolic and diastolic blood pressure, total cholesterol, ln glucose, ln triglycerides, and smoking for females and males separately.

For females, we found that age, BMI, systolic and diastolic blood pressure, total cholesterol, ln glucose, ln triglycerides, and ln PAI-1 were all significant predictors of ln t-PA both individually ($P \leq 0.001$) and together in a complete model ($P < 0.001$). For males, except for age which was marginally significant, all of the same factors were significant univariate predictors of ln t-PA ($P < 0.05$) and the multivariate complete model was highly significant ($P < 0.001$).

For both females and males, we found that BMI, systolic and diastolic blood pressure, total cholesterol, ln glucose, ln triglycerides, and ln t-PA were significant predictors of ln PAI-1 individually and in a complete model ($P \leq 0.01$). Age was found to be a significant predictor of ln PAI-1 in females ($P = 0.019$), but not males.

Among 992 study participants, there were only 14 smokers, all of whom were male (1.4% of all participants, 3.3% of males). Comparing male smokers to male non-smokers, we found no significant impact of smoking on either ln t-PA or ln PAI-1 levels.

The tests of homogeneity of regression revealed that the slopes of the regression lines relating BMI and ln triglycerides to ln PAI-1 were significantly different between females and males ($P < 0.05$). No significant gender differences were found between the slopes of the regression lines relating the preceding cardiac risk factors to ln t-PA.

Prediction of plasma t-PA and PAI-1 levels by polymorphisms in genes from the fibrinolytic and renin-angiotensin systems

Table 3 summarizes the genotype frequencies for each measured polymorphism by gender. Only TPA I/D gene polymorphism was marginally significantly different between gender ($P = 0.077$). Except for TPA I/D, which was marginally out of Hardy–Weinberg equilibrium in males ($P = 0.074$), no significant deviation from Hardy–Weinberg equilibrium was observed for the other polymorphisms in females or males.

Starting with an additive genetic model, we found a significant effect of the PAI-1 4G/5G polymorphism on ln PAI-1 levels in females ($P = 0.029$), the TPA I/D polymorphism on ln t-PA in males ($P = 0.026$), the renin (rs3730103) on ln t-PA in males ($P = 0.019$), and a marginally significant relation between the ethanolamine kinase 2 (rs1917542) and ln PAI-1 in males ($P = 0.066$).

Continuing with recessive and dominant genetic models, we found a significant recessive effect of the TPA D variant on ln PAI-1 levels in males ($P = 0.044$), but not in females. Both renin polymorphisms were found to influence regulators of fibrinolysis, as the renin rs1464816 T variant had a significant dominant effect on ln t-PA in females ($P = 0.049$) and a significant dominant effect on ln PAI-1 in males ($P = 0.046$). The renin rs3730103 C variant had a significant recessive effect on ln PAI-1 levels in males ($P = 0.040$).

The ACE I/D (rs4646994), angiotensinogen M235T (rs699), and the angiotensin II type 1 receptor (rs5186) gene polymorphisms had no significant relationship with ln t-PA or ln PAI-1 levels in females or males.

We next wanted to know whether the alleles found to predict ln t-PA and ln PAI-1 levels in our additive, recessive, and dominant genetic models were capable of explaining inter-individual variance in ln t-PA and ln PAI-1 after adjusting for the effects of the traditional cardiac risk factors found to be significant in our prior linear regression analysis. Table 4 summarizes these results. We found that the TPA I/D polymorphism was a significant predictor of ln t-PA and ln PAI-1 levels in males ($P < 0.02$) and the PAI-1 4G/5G polymorphism was a significant predictor of ln PAI-1 levels in females ($P = 0.001$) after adjusting for non-genetic factors. In addition, renin polymorphisms were significant predictors of ln t-PA levels in females and males ($P = 0.02$) after adjusting for non-genetic factors. Renin and renin-linked polymorphisms were also marginally significant predictors of ln PAI-1 in males ($P < 0.08$) after adjusting for non-genetic factors.

Discussion

This large population-based study from urban West Africa shows that plasma levels of t-PA and PAI-1 are determined by both traditional cardiovascular risk factors and genetic loci of the fibrinolytic and renin-angiotensin systems. The relationships between these genetic loci and plasma t-PA and PAI-1 levels are independent of traditional cardiac risk factors and differ between genders.

Prevalences of cardiac risk factors in Sunyani, Ghana

We sited this study in Sunyani, Ghana, for multiple reasons. First, from a genetic standpoint, Sunyani provided us with a relatively homogeneous population since there is evidence of little

genetic differentiation between ethnic groups in Ghana (Adeyemo et al. 2005). Additionally, Sunyani is inhabited predominantly by a single ethnic group, the Brong. Given this ethnic homogeneity, we expected the genetic determinants of t-PA and PAI-1 levels to be similar across individuals in our sample. Second, because the populations of West Africa includes the source gene pool from which many African–Americans are descended, it provided increased opportunity to detect fundamental genetic relationships by limiting admixture. Third, because Sunyani is an urban regional capital, it is home to many of the environmental pressures that have been associated with cardiovascular disease in Western society.

Despite these considerations, we were nonetheless impressed by the high prevalences of cardiac risk factors in Sunyani. Most striking were the high rates of obesity (BMI >30) among males (8.3%) and especially females (23.6%). These figures were somewhat greater than those previously reported for Kumasi, the capital of the Ashanti region of Ghana (males 2.7%, females 15.9%), and substantially greater than those previously reported for rural villages in the Ashanti region (males 0.4%, females 5.9%) (Agyemang 2006), but much less than those reported for African–Americans (males 27.8%, females 48.8%) (Mensah et al. 2005). These statistics support the prevailing hypothesis that urbanization and Westernization is accompanied by a high-energy diet and sedentary lifestyle that leads to obesity and cardiovascular disease (Yusuf et al. 2001).

Hypertension, diabetes, and hypercholesterolemia are also common in Sunyani. In our sample, hypertension as defined by the JNC 7 classification (Chobanian et al. 2003) was significantly more common among males (26.0%) than females (19.7%), in keeping with the trend reported for Kumasi (males 33.4%, females 28.9%) (Agyemang 2006). Among African–Americans, however, the opposite is true, with hypertension being more prevalent among females (46.6%) than males (42.6%) (Mensah et al. 2005). Reflecting the female predominance of obesity in our sample, elevated glucose and cholesterol levels were both more common among females than among males in Sunyani, similar to the pattern seen in African–Americans (Mensah et al. 2005). This correlation was not surprising, given the recognized ability of BMI to predict both diabetes and hypercholesterolemia.

Taken together, significant inter-individual and gender differences were observed with respect to each of the preceding cardiac risk factors, spanning the spectrum between health and disease. Despite the rising threat of cardiovascular disease in Africa, there remains a wide gap between the burden of cardiac risk shouldered by Africans and that shouldered by African-Americans. These demographics confirm the validity of studying the influences of non-genetic factors on fibrinolytic proteins in Sunyani, and indeed suggest that it may be better to study relationships between genetic loci and plasma levels of t-PA and PAI-1 in urban West Africa where environmental pressures are present but not as overwhelming as in North America.

Non-genetic determinants of t-PA and PAI-1

In Sunyani, we found that gender, age, BMI, blood pressure, cholesterol, glucose, and triglycerides are predictive of t-PA and PAI-1 levels. Our results are mostly in agreement with the Framingham Offspring Study, a prospective population-based study exclusively of white Americans that also found significant relationships between estrogen status (Gebara et al. 1995), age (Tofler et al. 2005), BMI (Rosito et al. 2004), blood pressure (Poli et al. 2000), LDL cholesterol (Welty et al. 1997), glycemic control (Meigs et al. 2000), and plasma levels of t-PA and PAI-1. In addition, similar results were found by our group in the PREVEND study, a large population-based cohort from the Netherlands (Asselbergs et al. 2006, 2007b). In PREVEND, BMI and waist hip ratio were strongly related to t-PA and PAI-1 levels in both females and males. BMI alone explained almost 14% of PAI-1 levels in this Ghanaian population and 17% in the predominantly Caucasian PREVEND study (Asselbergs et al. 2006). Prior studies demonstrated that adipose tissue produces PAI-1 and plasma PAI-1 are

increased in obese humans (Skurk and Hauner 2004). Interestingly, experimental studies suggest that overexpression of PAI-1 might also be involved in the development of obesity (Lijnen et al. 2003).

Next to BMI, blood pressure, cholesterol, glucose, and triglyceride levels were all significantly associated with t-PA and PAI-1 levels in this Ghanaian population as in PREVEND. The common pathway for these associations might be the presence of insulin resistance in adipose tissue. The presence of insulin resistance results in an increased release of free fatty acids to non-adipose tissue such as liver and skeletal muscle, leading to hyperinsulinemia, glucose intolerance, and increased very low density lipoprotein, with the latter leading to hypertriglyceridemia. Several of these factors such as insulin, triglycerides, and free fatty acids can stimulate PAI-1 expression by adipocytes, hepatocytes, and endothelial cells (Eriksson et al. 1998; Morange et al. 1999; Nilsson et al. 1998; Schneider and Sobel 1991). Hypertension is strongly related to insulin resistance, which may explain the observed association between blood pressure and t-PA and PAI-1 (Ferrannini et al. 1987), but hypertension may also influence t-PA and PAI-1 levels more directly through shear stress or endothelial dysfunction (Poli et al. 2000).

Genetic architecture of t-PA and PAI-1

Our study indicates that the genetic factors that predict t-PA and PAI-1 levels are for the most part different in males and females, supporting the results of previous studies that have found differences in risk profiles of cardiovascular disease in males and females (Bairey Merz et al. 2006; Shaw et al. 2006). These results are also consistent with our previous study from the Netherlands that found comparable male–female differences in t-PA and PAI-1 genetic risk factors (Asselbergs et al. 2006). Specifically, in our present study plasma levels of t-PA are affected by the t-PA polymorphism only in males, and although renin SNPs affect t-PA in both male and females, the specific variants differ. A similar dichotomy exists for PAI-1; the 4G5G polymorphism only predicts PAI-1 levels in females and only in males do the renin and renin-linked polymorphisms associate with PAI-1 levels. These results support the conclusion that the genetic architectures of t-PA and PAI-1 are different between males and females.

Of note is the fact that the R^2 values for all of these genetic variants is generally small (<2%) even when significant, suggesting that genes in other pathways may also play a role in the regulation of these proteins. Previous results in US populations provide similar findings to ours in terms of the amount of the variance explained by the 4G/5G PAI-1 variant (1–2%) (Festa et al. 2003). However, our study represents to our knowledge the first such study in an African population and can serve as a foundation for understanding the genotype-phenotype relationship in African–Americans. In addition, future studies need to take into account the environmental and genomic context of genetic effects (i.e., gene–environment and gene–gene interactions) (Asselbergs et al. 2007a, c). Thus, it is clear that both PAI-1 and t-PA antigenic variation is affected by genetic variations, but that genetic variation does not act equally in the two genders and that these variants do not explain most of the phenotypic variation.

Conclusions

In a relatively large survey of Ghanaians we have been able to identify several factors, both genetic and non-genetic that account for as much as 20–25% of the variation in plasma t-PA and PAI-1. Most of the variations in t-PA and PAI-1 were explained by traditional cardiovascular risk factors such as BMI, blood pressure, cholesterol, glucose, and triglyceride levels. Interestingly, these factors are consistently related to t-PA and PAI-1 among the two genders and different races. Of note are the different genetic factors that affect plasma expression in our study as compared to previous ones, and the differences that we have observed between males and females in genetic determinants of t-PA and PAI-1. These differences

emphasize the need to carefully assess each study population with respect to a variety of co-variates, both genetic and non-genetic in terms of elucidating the role that specific genetic variants play in regulation of plasma PAI-1 and t-PA.

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Table 1

Characteristic by gender and menopausal status

	All female (*n* = 569)	Pre-menopausal female (*n* = 395)	Post-menopausal female (*n* = 174)	Male (*n* = 423)	*P* value female versus male[b]	*P* value pre- versus post-menopausal female[b]	*P* value pre-menopausal female versus male[b]	*P* value post-menopausal female versus male[b]
t-PA (ln ng/ml)[a]	1.80 ± 0.67	1.77 ± 0.64	1.86 ± 0.72	1.78 ± 0.80	0.537	0.057	0.345	0.748
PAI-1 (ln ng/ml)[a]	1.27 ± 1.33	1.21 ± 1.33	1.41 ± 1.32	1.03 ± 1.49	0.005	0.816	0.022	0.032
Body mass index (kg/m^2)	26.6 ± 5.34	26.5 ± 5.33	26.8 ± 5.37	23.8 ± 4.15	<0.001	0.004	<0.001	<0.001
Systolic blood pressure (mmHg)	126 ± 18.9	121 ± 16.5	136 ± 19.9	132 ± 20.8	<0.001	0.265	<0.001	0.180
Diastolic blood pressure (mmHg)	77.3 ± 10.5	76.2 ± 10.9	79.8 ± 9.2	78.0 ± 13.1	0.701	0.221	0.404	0.310
Glucose (mg/dl)[a]	94.5 ± 24.8	92.0 ± 15.4	100.0 ± 37.8	91.6 ± 21.1	0.003	0.159	0.003	0.082
Total cholesterol (mg/dl)	182 ± 42.7	175 ± 40.4	197 ± 43.7	170 ± 45	<0.001	0.194	<0.001	<0.001
Current smoker	0%	0%	0%	3.3%	<0.001	NA	<0.001	0.015
Triglycerides (mg/dl)[a]	85.4 ± 44.4	79.6 ± 40.5	98.8 ± 49.6	96.3 ± 60.3	0.016	0.639	0.009	0.712

Data are expressed as mean ± SD

[a] Natural log transformed prior to analysis

[b] Age-adjusted

Table 2

Regression relationships of plasma t-PA and PAI-1 with concomitants among females and males

Dependent variable	Independent variable	Females (n = 569)		Males (n = 423)		P value, homogeneity of regression
		R^2 ×100	P value	R^2 ×100	P value	
t-PA[a]	Age (years)	2.3	<0.001	0.7	0.078	0.326
	BMI (kg/m^2)	12.8	<0.001	9.0	<0.001	0.170
	Systolic blood pressure	2.0	0.001	1.3	0.018	0.839
	Diastolic blood pressure	2.8	<0.001	3.1	<0.001	0.939
	Cholesterol	3.5	<0.001	4.4	<0.001	0.452
	Glucose[a]	2.9	<0.001	2.7	0.001	0.631
	Triglycerides[a]	4.7	<0.001	2.9	<0.001	0.710
	PAI-1[a]	7.3	<0.001	9.9	<0.001	0.276
	Smoking	NA	NA	0.3	0.279	NA
	Complete	18.4	<0.001	15.7	<0.001	
PAI-1[a]	Age (years)	1.0	0.019	0.3	0.257	0.468
	BMI (kg/m^2)	13.8	<0.001	13.6	<0.001	0.032
	Systolic blood pressure	1.2	0.009	3.8	<0.001	0.162
	Diastolic blood pressure	3.7	<0.001	5.0	<0.001	0.873
	Cholesterol	3.9	<0.001	4.1	0.001	0.828
	Glucose[a]	2.8	<0.001	4.1	<0.001	0.336
	Triglycerides[a]	3.4	<0.001	10.8	<0.001	0.011
	t-PA[a]	7.3	<0.001	9.9	<0.001	0.720
	Smoking	NA	NA	0.4	0.104	NA
	Complete	19.0	<0.001	23.5	<0.001	

BMI body mass index

[a]Natural log transformed

Table 3

Genotype frequencies by gender

	Female (*n* = 569)	Male (*n* = 423)	Total (*n* = 992)	*P* value for genotype distributions (Chi-square)
ACE insertion/deletion (rs4646994)				0.290
DD	36.2	31.9	34.4	
ID	45.7	47.0	46.3	
II	18.1	21.0	19.4	
Angiotensin II receptor, type 1 (rs5186)				0.386
AA	96.0	94.8	97.5	
AC	4.0	5.2	4.5	
CC	0.0	0.0	0.0	
Angiotensinogen m235t (rs699)				0.564
GG	83.0	85.3	85.8	
GA	16.2	13.7	13.3	
AA	0.9	0.9	0.9	
Ethanolamine kinase 2 (rs2293337)				0.362
CC	74.2	72.1	73.3	
CT	23.6	24.1	23.8	
TT	2.3	3.8	2.9	
Ethanolamine kinase 2 (rs1917542)				0.859
AA	36.2	34.8	35.6	
AG	48.2	49.9	48.9	
GG	15.6	15.4	15.5	
Renin (rs3730103)				0.363
TT	44.6	40.7	45.2	
TC	45.9	42.3	44.4	
CC	9.5	11.8	10.5	
Renin (rs1464816)				0.796
GG	39.9	40.2	40.0	
GT	48.2	46.6	47.5	
TT	12.0	13.2	12.5	
TPA I/D (rs4646972)				0.077
DD	42.4	38.5	40.7	
DI	44.5	51.3	47.4	
II	13.2	10.2	11.9	
PAI 4G/5G (rs1799768)				0.371
5G5G	61.2	65.5	63.0	
5G4G	32.7	29.3	31.3	
4G4G	6.2	5.2	5.7	

Table 4

Univariate and Multivariate Regression relationship of genetic polymorphism with ln (t-PA) and ln (PAI-1) including outliers

Gender	Dependent variable	Independent variables					
		Polymorphisms	$R^2 \times 100$	*P* value	Change $R^2 \times 100\%$[a]	Full model $R^2 \times 100\%$	*P* value for *F* change[a]
Female	t-PA[b]	Renin (rs1464816)[e]	0.7	0.049	0.8	19.1	0.020
	PAI-1[b]	PAI 4G5G (rs1799768)[c]	0.8	0.029	1.7	19.4	0.001
Male	t-PA[b]	TPA I/D(rs4646972)[c]	1.2	0.026	1.6	17.3	0.004
		Renin (rs3730103)[c]	1.3	0.019	1.1	16.8	0.020
	PAI-1[b]	Renin (rs1464816)[e]	0.9	0.046	0.6	24.1	0.069
		Ethanolamine kinase 2 (Rs1917542)[c]	0.8	0.066	0.6	24.1	0.079
		Renin (Rs3730103)[d]	1.0	0.040	0.6	24.1	0.064
		TPA I/D (rs4646972)[d]	1.0	0.044	1.1	24.6	0.014

[a] Adjusted for full model (Table 1)

[b] Natural log transformed

[c] Additive encoding

[d] Recessive encoding

[e] Dominant encoding