A Population-Based Study in Ghana to Investigate Inter-Individual Variation in Plasma t-PA and PAI-1

Scott M. Williams, PhD; Shelli Stocki, BS; Lan Jiang, MS; Kwabena Brew, BA; Seth Gordon, MD; Douglas E. Vaughan, MD; Nancy J. Brown, MD; Kwabena A. Poku, PhD; Jason H. Moore, PhD

INTRODUCTION

Thrombosis resulting in loss of blood flow to vital organs is one of the key steps in the progression of cardiovascular disease (CVD) and is an underlying mechanism of acute myocardial infarction, stroke and venous thromboembolism.1 Prevention and treatment of thrombosis will likely decrease the incidence of CVD and reduce the healthcare burden of this chronic disease. Previous studies have found that thrombosis susceptibility has a significant genetic component with a heritability of approximately 60%,2 suggesting that the identification of genetic risk factors will benefit public health strategies for thrombosis prevention and treatment. Success in this endeavor will depend critically on our ability to delineate the relationship between genetic variation, variation in intermediate risk factors and variation in risk of thrombosis, in the context of clinically relevant covariates such as sex, smoking, and body mass index. Our study is designed to examine two plasma fibrinolytic proteins that directly influence thrombus formation and degradation, tissue-type plasminogen activator (t-PA) (OMIM 173370) and plasminogen activator inhibitor-1 (PAI-1) (OMIM 173360), with the ultimate goal of understanding how genetic factors can affect expression of these proteins and the subsequent risk of thrombosis.

Thrombosis is initiated by the physical disruption of blood vessels that leads to stimulation of hemostatic responses involving the vascular endothelium, platelets, coagulation and fibrinolysis.3 Thrombus formation following damage to the endothelium can be reversed by fibrinolysis or the degradation of fibrin into soluble fragments. Inhibition of fibrinolysis occurs either through the inactivation of t-PA by PAI-1 or the inactivation of plasmin by α2-antiplasmin. Thus, one of the key biological factors influencing thrombus progression and regression is the relationship between t-PA and PAI-1. Plasma levels of both of these molecules have significant genetic components. The long-term goal of this population-based study is to understand the role of genetic variation in inter-individual differences in plasma levels of t-PA and PAI-1.

The ultimate goal of our study is to elucidate the genetic architecture of t-PA and PAI-1. While the genetic bases of t-PA and PAI-1 expression have not yet been fully elucidated, there is accumulating evidence suggesting that inter-individual variation in these plasma enzymes is significantly influenced by polymorphisms in more than one candidate gene.4–13

Herein, we describe a large, population-based study of t-PA and PAI-1 that will be used to study the genetic architecture of t-PA and PAI-1 expression in Blacks. The subjects were recruited from Sunyani, the regional capital of Brong Ahafo in Ghana, without regard to chronic disease status. We provide the details of the study design, the biological samples collected, the medical and demographic information collected, the biological measures (eg, t-PA and PAI-1 levels, BMI, fasting glucose and lipid levels), and the scientific questions that will be addressed. The population in Ghana was selected for several reasons. First, it will have less admixture than US populations. Less admixture will improve our ability to effectively evaluate the genetic basis of plasma t-PA and PAI-1 by reducing complexity due to locus...
heterogeneity. Second, Ghana was a primary source of slaves for the Americas. As such, it represents one of the major populations from which the African American gene pool is derived. Thus, results from studying Blacks from West Africa are likely to play an important role in understanding health and disease in African Americans. This study represents one of the largest population-based studies of inter-individual variation in plasma t-PA and PAI-1 levels for any ethnic group.

METHODS

Subject Ascertainment

The goal of this study is to establish a population-based sample of 2000 Blacks from Sunyani, Ghana. In this paper, we describe the first 1000 subjects ascertained as part of this study who were recruited between May 2002 and October 2003. Unrelated subjects were recruited into the study, without regard to chronic disease status, from the Brong Ahafo regional capital of Sunyani, Ghana. Subjects learned about the study by word-of-mouth at their local churches or at the local market. Subjects were excluded from the study if they had signs of acute illness such as malaria that might influence t-PA and PAI-1 levels, were <18 years of age, or were a first or second degree relative of someone already enrolled in the study. The ethnic self-description of the subjects and their parents was recorded. Subjects who wanted to participate in the study came to the project office at the Regional Hospital, Sunyani and made appointments for future dates. When the appointments were made the subjects were instructed to return on the appointed date at 8:00 AM without having eaten for at least the previous 10 hours. They were also asked how they heard about the study, and whether they knew of any relatives who had participated. If relatives had participated, samples were still taken but were not used for the analysis in the present study.

Subjects were recruited almost exclusively from the Sunyani District of the Brong Ahafo Region (Figure 1), which is predominantly rural (62.6%). However, the Sunyani District within this rural region has a population of approximately 180,000 residents and is predominantly urban (73.8%). Males represent 49.7% of the district population (Ghana Statistical Service, 2002).

Informed Consent

Study protocols and human subject consent forms were generated in collaboration with the US and Ghanaian investigators, and were approved by institution review boards (IRB) at both Vanderbilt University and Regional Hospital, Sunyani. A Federal Wide Assurance (FWA) was issued to the University of Ghana and Regional Hospital (FWA 00004718). The Regional Hospital IRB was also registered with the US National Institutes of Health Office of Human subjects Research (IRB 00003452). This NIH-funded project was approved by the US Department of State.

Prior to entry into the study, written informed consent was obtained from each participant. If a participant could not write, a fingerprint was obtained on the consent form. As part of the consent process, a good faith effort was made to explain the purpose of the study either in the local language or English and to answer any questions that potential participants may have had prior to obtaining consent. English is the official language of Ghana.

Collection of Medical and Demographic Information

All participants provided information on previous medical history as well as standard demographic data. The information included age, sex, education, smoking status, alcohol consumption, and current medications. It also included previous diagnoses for cardiovascular disease, diabetes, and several types of cancer.

Height, weight, and blood pressure were measured. Blood pressures were measured using an Omron HEM-705c instrument (Omron Healthcare Corp., Bannockburn, Illinois, USA). Participants were seated in a quiet location and two measurements were taken from their left arm. Blood pressure values were taken twice and the mean of the two measurements was used in the
analysis. All blood pressure measurements were taken prior to blood draws. Height and weight were measured with shoes removed and using a height scale on a wall in the examining room and a commercial scale calibrated for accuracy.

Collection and Processing of Biological Samples

Three tubes of blood were drawn from each subject. One purple top tube (EDTA) was drawn and used to measure fasting lipid levels. Both total cholesterol and triglycerides were measured using an Élan ATAC 8000 Random Access Chemistry System (Élan Diagnostics, Smithfield, Rhode Island, USA). Cholesterol was measured using the Cholesterol Reagent kit and triglycerides were measured using the triglycerides reagent kit, both from Élan Diagnostics and both according to the manufacturer’s protocol. Glucose levels were measured using a hand-held Sure Step glucose monitor by LifeScan with blood drops from the blood draw needles (LifeScan, Milpitas, California, USA).

Two black top tubes (Stabilyte tubes, Biopool, Umeå, Sweden) were drawn between the hours of 8:00 AM and 10:00 AM for measurement of t-PA and PAI-1 antigen levels. Tubes were chilled on ice prior to being used and kept on ice until centrifugation. Within 20 minutes of the blood being drawn, cells were centrifuged for 20 minutes at 3000 Gs at 0 degree 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 then shipped to Nashville, Tennessee, USA, in IATA Cryo shippers (MVE Products). Cells were stored for DNA isolation at 4 degrees C for up to 5 days. DNA was isolated at Regional Hospital, Sunyani equal. Variances were significantly different between females and males separately. Box plots are presented for male and female measurements of PAI-1 and t-PA in Figure 2. Males and females did not statistically differ with respect to age, t-PA levels, or DBP. All other statistical comparisons of the means revealed significant (P<.05) sex differences. Males and females differed significantly in BMI with an average female BMI of 26.61 kg/m² and an average male BMI of 23.85 kg/m² (P<.001). Average female BMI in this sample was consistent with being overweight (>25 kg/m²) as was the median BMI (26.19 kg/m²). Of the women in our study, 59.9% had a BMI>25 but only 32.9% of males had a BMI>25. Women also had significantly higher TC (181.25 mg/dL vs 169.93 mg/dL, P<.001) and fasting glucose levels (94.63 mg/dL vs 91.52 mg/dL, P=.001). Females had also had significantly higher PAI-1 levels (6.92 ng/mL vs 5.73 ng/mL, P=.001). Females were also significantly higher (97.03 mg/dL vs 85.53 mg/dL, P=.007) and SBP (131.67 mm Hg vs 125.78 mm Hg, P<.001). These numbers represent substantial mean differences between sexes for most measured variables.

Table 2 summarizes the variances for the same biological and clinical measures in females and males separately. Variances were significantly different between females and males for all traits.

Plasma t-PA and PAI-1 - Williams et al

University using a commercially available enzyme-linked immunoassay (ELISA, Biopool AB, Umeå).

Data Management

The medical history and demographic data questionnaires were formatted as scannable forms. Data was entered directly from each scannable form into a secure Oracle database. All entries were checked for accuracy and corrected by hand. The PAI-1 and t-PA data were recorded on a spreadsheet at the time of assay and entered into the database using a Perl script. Genotype data was recorded and entered in the same manner.

Data Analysis

Prior to statistical analysis, we tested the null hypothesis that plasma t-PA and PAI-1 levels are normally distributed using the Shapiro-Wilk test. When the null hypothesis was rejected (P<.05), we adjusted t-PA and/or PAI-1 levels to normality using Box-Cox power transformations. Statistical outliers were identified using the interquartile range test and were removed prior to analysis. This was done to reduce the impact of extreme outliers on the results in terms of understanding the factors that affect the distribution of PAI-1 and t-PA.

The goal of the initial statistical analysis was to compare the means and variances of age, body mass index (BMI), PAI-1, t-PA, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, glucose, and triglycerides among females (n=573) and males (n=427). The two-sample t test was used to compare means when the traits were normally distributed. Satterthwaite’s correction to the t test was applied when the variances were not equal. The Wilcoxon rank-sum test was used to compare means when one or both of the traits were not normally distributed and could not be transformed to normality. Variances were compared using the folded-F test. All analyses were done using SAS. All results were considered statistically significant at the .05 level.

RESULTS

Comparison of Biological and Clinical Measures between Females and Males

Table 1 summarizes the mean age, body mass index (BMI), PAI-1 level, t-PA level, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), glucose, and triglycerides (trig) in females and males separately. Box plots are presented for male and female measurements of PAI-1 and t-PA in Figure 2. Males and females did not statistically differ with respect to age, t-PA levels, or DBP. All other statistical comparisons of the means revealed significant (P<.05) sex differences. Males and females differed significantly in BMI with an average female BMI of 26.61 kg/m² and an average male BMI of 23.85 kg/m² (P<.001). Average female BMI in this sample was consistent with being overweight (>25 kg/m²) as was the median BMI (26.19 kg/m²). Of the women in our study, 59.9% had a BMI>25 but only 32.9% of males had a BMI>25. Women also had significantly higher TC (181.25 mg/dL vs 169.93 mg/dL, P<.001) and fasting glucose levels (94.63 mg/dL vs 91.52 mg/dL, P=.001). Females had also had significantly higher PAI-1 levels (6.92 ng/mL vs 5.73 ng/mL, P=.001). Females were also significantly higher (97.03 mg/dL vs 85.53 mg/dL, P=.007) and SBP (131.67 mm Hg vs 125.78 mm Hg, P<.001). These numbers represent substantial mean differences between sexes for most measured variables.

Table 2 summarizes the variances for the same biological and clinical measures in females and males separately. Variances were significantly different between females and males for all traits.
except TC. In contrast to the mean values, men had significantly higher variances for most of the traits (age, PAI-1, t-PA, SBP, DBP, and triglycerides, \( P < .001 \) for all comparisons except systolic blood pressure, \( P = .031 \)). Variances were greater in the female samples only for BMI and glucose level (\( P < .001 \) for both). The difference in variance may ultimately translate into very different disease risk, if thresholds define clinical risk. This is because even with identical means, if the variance is different between two groups, the percent of a population above a critical threshold will be defined by the variance.

### DISCUSSION

We describe the study design and preliminary analyses of a project that is intended to assess the role of genetic variation in t-PA and PAI-1 plasma levels. These two fibrinolytic proteins, the balance of which affects risk of thrombosis, both have high heritability, thus justifying the study of the genetics of their expression. To accomplish this goal, we have selected a population that is predominantly from a single ethnic group, Brong, from Ghana and from an area where even among ethnic groups there is little evidence of genetic differentiation. The choice of this relatively homogeneous population serves multiple purposes. First, because of the ethnic homogeneity we expect the genetic basis of the plasma levels to be similar across individuals in our study population. Second, this population should be related to the source gene

---

**Table 1. Comparison of the means of each biological and clinical measure between females and males**

<table>
<thead>
<tr>
<th></th>
<th>Females (n=573)</th>
<th>Males (n=427)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.73</td>
<td>44.04</td>
<td>.15</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26.61</td>
<td>23.85</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PAI-1* (ng/mL)</td>
<td>6.92</td>
<td>6.73</td>
<td>.016</td>
</tr>
<tr>
<td>t-PA (ng/mL)</td>
<td>7.25</td>
<td>7.53</td>
<td>.66</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>125.78</td>
<td>131.67</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>77.24</td>
<td>78.01</td>
<td>.895</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>181.25</td>
<td>169.93</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>94.63</td>
<td>91.52</td>
<td>.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>85.53</td>
<td>97.03</td>
<td>.007</td>
</tr>
</tbody>
</table>

* – PAI-1, plasminogen activator inhibitor 1.  
† – t-PA, tissue type plasminogen activator

---

**Fig 2.** Box and Whisker plot of t-PA and PAI-1 levels by sex. The line in each box is the median, the edges of the boxes are the quartiles surrounding the median. The dashed lines define the lower and upper extremes. Outliers, as defined in the text, are marked as circles.
American women have higher cholesterol levels than men (NHANES Health United States 2002). In contrast to these findings men had higher triglyceride levels. Higher glucose levels women were also expected because it is well-known that high BMI correlates with high glucose levels \(^{38}\) and risk of type 2 diabetes. \(^{27,29}\) Our results indicate that substantial variation exists for t-PA and PAI-1 levels and for several clinical and demographic variables that will be important in subsequent analyses of the role that genetic variation plays.

Our measured PAI-1 levels are lower than levels in several previous studies of African Americans, where values range from 9.8 ng/mL to 48.1 ng/mL. \(^{30-34}\) However, even though the BMI values are high in our subjects, especially for women, they are lower than those of these other studies where mean BMI values range from 25.8 to 34.3. The PAI-1 levels in the earlier publications are also based on much smaller samples, some of which are family-based and should therefore not represent independent samples. \(^{30,32}\) In addition, several of the other studies selected for specific distributions or over-representations of clinical endpoints such as glucose intolerance and diabetes that we did not. \(^{31,35}\) The t-PA levels in studies of African Americans range from 1.4 ng/mL to 8.3 ng/mL, \(^ {32,34}\) with BMI values higher than our study as well. Our means of t-PA are within these published ranges. The differences in our results for PAI-1, but not necessarily t-PA, probably reflect the role that increasing BMI plays in augmenting PAI-1 levels.

The high BMI values, especially among Sunyani women, of which 59.9% are overweight (BMI ≥ 25.0) and 23.6% are obese (BMI ≥ 30.0), is similar to recent findings of Black Africans in South Africa where 31.8% of women were obese and an additional 26.7% were overweight. \(^ {36}\) Similar, but more extreme, patterns exist among African Americans, where prevalence of obesity is 48.8% and 27.8% for women and men, respectively (National Center for Health Statistics 2004). These data suggest that the Sunyani population is likely to be representative of other populations of sub-Saharan origin, but that it may be better to study genetic factors regulating t-PA and PAI-1 because the environment is not yet as likely to have overwhelmed these genetic factors.

The large number of differences between men and women for the basic parameters we measured suggest that it is important to evaluate women and men separately in subsequent analyses of factors that affect t-PA and PAI-1. Although it is possible that sex differences can be accounted for with other independent variables such as BMI, this possibility cannot be assumed to be true but must be explicitly tested in all genetic analyses. The important role of sex in cardiovascular diseases has been recognized for some time. \(^ {32,37}\)

In summary, we have presented a study design to evaluate genetic factors that affect protein expression of PAI-1 and t-PA. The data demonstrate several significant differences between males and females for traits that can affect risk of thrombosis, most importantly PAI-1. We successfully recruited subjects at our study site and have provided descriptive analyses of the first 1000 people of a 2000-person study. Most importantly, our data demonstrate consistency with other similar but smaller studies, supporting the conclusion that the Sunyani
population will serve as an excellent cohort to study the genetic regulation of PAI-1 and t-PA plasma levels.

ACKNOWLEDGMENTS
This work was supported by NIH grants HL65234 (SMW and JHM) and HL60906 (DEV).

REFERENCES

AUTHOR CONTRIBUTIONS
Design concept of study: Williams, Jiang, Brew, Gordon, Vaughan, Brown, Poku, Moore
Acquisition of data: Williams, Stocki, Brew, Gordon, Brown, Poku, Moore
Data analysis and interpretation: Williams, Jiang, Moore
Manuscript draft: Williams, Stocki, Brew, Gordon, Vaughan, Poku, Moore
Statistical expertise: Williams, Stocki, Jiang, Moore
Acquisition of funding: Williams, Vaughan, Moore
Administrative, technical, or material assistance: Stocki, Vaughan, Brown, Moore

Supervision: Williams