Genetic effects on the correlation structure of cardiovascular disease risk factors in a Ghanaian population

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Abstract

Plasma concentration of plasminogen activator inhibitor-1 (PAI-1) is highly correlated with several cardiovascular disease (CVD) risk factors. It also plays a direct role in cardiovascular diseases, including myocardial infarction and stroke, by impeding the dissolution of thrombi in the blood. Insofar as PAI-1 “links” the risk factors of CVD to its endpoints, genetic variants modulating the relationship between PAI-1 and risk factors may be of particular clinical and biological interest. The high heritability of PAI-1, which has not been explained by genetic association studies, may also, in large part, be due to this relationship with CVD risk factors. Using exome-wide data from 1032 Ghanaian study participants, we tested for heterogeneity of correlation by genotype between PAI-1 and four CVD risk factors (BMI, triglycerides, mean arterial pressure, and fasting glucose), under the hypothesis that loci involved in the relationship between PAI-1 and other risk factors will also modify their correlational structure. We found more significant heterogeneities of correlation by genotype than we found marginal effects, with no evidence of Type I inflation. The most significant result among all univariate and multivariate tests performed in this study was the heterogeneity of correlation between PAI-1 and mean arterial pressure at rs10738554, near SLC24A2, a gene previously associated with high blood pressure in African Americans.
Introduction

Cardiovascular disease (CVD) is responsible for almost one-half of all non-communicable disease-related deaths worldwide.\(^1\) It comprises multiple disorders of the circulatory system, among which venous and arterial thrombotic disorders are the most common\(^2\). The enzyme plasminogen activator inhibitor-1 (PAI-1) plays a major role in the etiology of thrombosis by impeding fibrinolysis, or clot breakdown.\(^3\) Elevated plasma PAI-1 is accordingly a major risk factor for thrombotic events, such as deep vein thrombosis, myocardial infarction, and stroke\(^4\).

Plasma PAI-1 concentration has been considered a promising endophenotype for CVD, because it is linked etiologically to correlated clinical endpoints. Endophenotypes are likely to have simpler genetic architectures than the complex diseases with which they associate. They can also be defined unambiguously and measured precisely, making them potentially valuable targets for genome-wide association studies (GWAS)\(^5\). These advantages were recently demonstrated by a GWAS on serum transferrin (a biomarker for iron deficiency) that identified two loci explaining 40% of the genetic variation in this protein.\(^6\) Similar studies on PAI-1, however, have not been nearly as successful. A recent meta-analysis identified only three genome-wide significant loci, which together explained less than 3% of the genetic variance.\(^7\) The inability to identify any major genetic factors beyond the well-documented 4G/5G variant in the PAI-1 promoter is rather puzzling\(^8\), particularly because the heritability of PAI-1 has been estimated to be as high as 0.83.\(^9\) Furthermore, the small number of variants that are associated with PAI-1 do not appear to be associated with CVD-related outcomes,\(^10\) despite the fact that high PAI-1 levels are.

One possible explanation for this paradox is that indirect genetic effects are responsible for the high heritability of PAI-1. For example, even if the genes directly involved in PAI-1 production
were devoid of any variation, PAI-1 would still be a heritable trait, because many conditions which associate with PAI-1, such as obesity, hypertriglyceridemia, hypertension, and even dietary habits, are themselves heritable\textsuperscript{11-13}, and PAI-1 concentration increases steadily across the entire distribution of these and most other cardio-metabolic risk factors\textsuperscript{14}. While increasing GWAS sample sizes can improve the likelihood of detecting such indirect associations, the purpose of detecting them would not be entirely clear. In fact, variables such as BMI and triglycerides are adjusted for in most PAI-1 association studies\textsuperscript{7; 15; 16}

There are, however, potentially more important ways in which the positive association between cardiovascular risk factors and PAI-1 can help explain its missing heritability. In particular, there may be genetic variants that have direct but conditional effects on the concentration of PAI-1, dependent upon other specific risk factors. For example, a (hypothetical) variant may directly increase \textit{PAI-1} expression only when adiposity exceeds a certain threshold; such a variant would be difficult to detect at a genome-wide level of significance, owing to its purely conditional effect, despite its likely contribution to the heritability of PAI-1. Another kind of context-dependent variant may be involved in a (hypothetical) homeostatic pathway that either raises or lowers PAI-1 as adiposity increases or decreases. Genetic effects of this type would be difficult if not impossible to detect in conventional GWAS.

If a large portion of the missing heritability of PAI-1 is due to indirect genetic effects and/or to context-dependent genetic effects, we would expect to observe small effect sizes in association studies, unpredictable attempts at replication, and highly variable heritability estimates, as we do\textsuperscript{17; 18}. While increasing sample size can improve the power to detect variants whether they have indirect or context-dependent effects, culling the biologically meaningful results from the scores of trivially indirect associations in GWAS will become increasingly difficult\textsuperscript{19}. However, the
context-dependent variants, many of which likely remain to be found\textsuperscript{20, 21}, may be particularly important from a clinical perspective\textsuperscript{22}.

With the potential importance of context-dependency in mind, we chose to explicitly test whether the correlation between PAI-1 and associated risk factors differs by genotype, complementing conventional tests for differences in mean alone. The motivation for our analysis is the hypothesis that genetic variants that increase PAI-1 in response to another risk factor (and vice versa) will also modify the correlational structure between the two. Few studies have directly sought to identify genetic variants associated with changes in correlation. Two decades ago, Reilly et al. found that the correlation structure between various apolipoproteins varied with apolipoprotein E (ApoE) genotype, and in a gender-specific manner.\textsuperscript{23} This ability of ApoE to modulate lipid trait relationships was again demonstrated in a 2013 study, which concluded that the ApoE isoform genotype not only influenced the correlation between triglycerides and total cholesterol, but changed the relationship between both those traits and incident coronary heart disease, but in a population-specific manner.\textsuperscript{24} However, no high-throughput study of genes influencing the covariance among traits has been performed.
Methods

Study Population

The study population has been previously described\textsuperscript{25}. Briefly, unrelated participants were identified from Sunyani, the capital of the Brong Ahafo region of Ghana, population \sim 250,000 as of the 2012 census. Recruitment for the study began in 2002 and ended in 2007. Participants learned about the study at public venues, including local churches and markets. Individuals were excluded from analyses if they had signs of acute illness (e.g. malarial infection), were under 18 years of age, or were a first or second degree relative of someone already enrolled in the study. Participants provided information via questionnaire regarding their previous medical histories and other demographic and socio-economic variables, including age, sex, education, smoking status, alcohol consumption, and current medications. All participants provided informed consent. Institutional review boards at Vanderbilt University, Dartmouth College, and Regional Hospital, Sunyani approved all protocols.

Anthropometric measurements and biochemical analysis

Standing height and weight were measured to calculate body mass index (BMI). Blood pressure was measured twice; the means for both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were used in subsequent statistical analyses\textsuperscript{26}. Mean arterial pressure (MAP) was calculated using the formula: $\text{MAP} = \text{DBP} + \left(\frac{(\text{SBP}-\text{DBP})}{3}\right)$, which approximates the average arterial pressure during a single cardiac cycle. Blood was drawn between the hours of 8:00 AM and 10:00, after a minimum of 8 hours fast. These samples were used to assess fasting glucose, lipid, and PAI-1 levels. Fasting glucose levels were measured using a hand-held
Sure Step glucose monitor by LifeScan, using blood drops from the blood draw needles (LifeScan, Milpitas, California, USA). Plasma samples were stored in liquid nitrogen prior to shipment to Vanderbilt University, where concentrations of PAI-1 antigen were measured using a commercially available enzyme-linked immunoassay (ELISA, Biopool AB, Umea).

**Genotyping**

A subset of 1105 urban participants from the Ghanaian HeART cohort was selected for genotyping. DNA was genotyped using the Illumina Infinium HumanExome BeadChip platform (Illumina Inc., San Diego, CA). This platform interrogates strictly exonic variants, covering ~240,000 markers.

**Quality Control**

We removed all SNPs with a genotyping call rate < 95%. Individuals for whom < 95% of variants were called were removed from analyses. Variants with a minor allele frequency < 20% were also removed, as were variants with a Hardy Weinberg p value < 0.001. Cryptic relatedness was assessed in the data, and one participant in each pair of related individuals (pi-hat > 0.2) was randomly removed. Following quality control, 1032 of the 1105 participants and 15,890 variants remained for analyses. All quality control procedures were performed in PLINK (version 1.07)\textsuperscript{27}. 
**Statistical Analyses**

**Test for homogeneity of correlation by genotype**

Individuals were grouped by genotype, the correlations between two traits were calculated for each group, and a test of homogeneity of correlation among the three genotypic groups (0,1,2) was applied to assess whether the three sample correlation coefficients could have been drawn from the same population.

The variance of $\rho$, the population parameter of correlation between two traits, decreases as its absolute value approaches 1. Fisher’s $r$-to-$z$ transformation $z = \frac{1}{2} \ln \left( \frac{1+r}{1-r} \right)$ stabilizes the variance at $\frac{z^2}{n} = \frac{1}{n-3}$, and makes the distribution approximately normal, enabling conventional statistical tests. If we estimate correlation, $r_i$, for $k=3$ genotypic groups, and transform each to $z_i$, the weighted sum of squares is then distributed approximately as $\chi^2$ with $k-1$, or 2 degrees of freedom:

$$z = \frac{1}{n} \left( \sum_{i=1}^{k} \left( n_i - 3 \right) (z_i - z) \right)$$

We wrote R-code for this approach that allows for the adjustment for any number of covariates, making it essentially a test for partial correlation as well. The code also allows Spearman’s rank sum correlations to be used when deviation form normality is an issue; the variance term for the Z-transforms is simply adjusted by a factor of 1.06.$^{28}$ Although not implemented in this study, the code also allows for tests of dominant and recessive effects on correlation. In this study we chose the more conservative metric of correlation, Spearman’s rho, to minimize Type 1 error, which we assessed using QQ-plots (Figures 1-4). PAI-1 was paired with each of four cardiometabolic risk factors, namely, BMI, triglycerides (TG), fasting glucose
(GLUC), and mean arterial pressure (MAP). These risk factors were chosen based on a previous partial correlational analysis showing that they had the strongest independent relationships with PAI-1\textsuperscript{26}. All models were adjusted for age and sex. A joint multivariate test (MultiPhen\textsuperscript{29}), which assesses the association between genotype and two quantitative risk factors simultaneously, was also performed for each of the risk factor-PAI-1 pairs, to allow for pleiotropy.

Gene functions were ascertained using a literature search. Associations with p-values below the 1x10\textsuperscript{-4} level in any model were annotated using SNPinfo\textsuperscript{30} and are presented in the results. We chose this reasonable but not definitive threshold because this was an exploratory analysis, with a reduced number of SNPs, and because the SNPs were not pruned for LD\textsuperscript{31}. We recognize that these analyses will need to be repeated in other cohorts.
Results

In the single-trait tests, no association remained significant at the p<0.05 level after Bonferroni correction for multiple testing. Even at p<10^{-4}, there was only one association with PAI-1 (rs10266732 in MGAM), and none with glucose; two SNPs were associated with MAP (rs3736582 and rs16907312, in PSTK and OR51G2, respectively), and one with BMI (rs1420101 in IL1RL1) (Tables 1-3). Four SNPs (in MOG, ANP32A, EDIL3, and ZFP57) were associated with TG (Table 4). The bivariate tests, which modeled genotype as a function of PAI-1 and another risk factor, yielded no new associations at p<10^{-4}, and only two in total (both with PAI-1 and TG); one of these associations was stronger in the single-trait test of TG (Table 4).

The correlations by genotype between PAI-1 and each risk factor were tested for homogeneity. Overall, there were more significant differences in correlation by genotype than there were differences in mean by genotype for PAI-1, MAP, BMI, and glucose, (Tables 1-3). The most significant p-value in this study was for heterogeneity of correlation between MAP and PAI-1, at rs10738554 in SLC24A2 (Table 1, Figure 5). Among all tests in this study that included fasting glucose, only tests for heterogeneity of correlation with PAI-1 yielded significant results (p<10^{-4}). These were for rs1649292, rs63111160, and rs404890, the latter two within proximity of SETBP1 and NOTCH4, respectively (Table 2). Similarly, two of the three significant results reported in this study for BMI were heterogeneities of correlation (Table 3). QQ-plots for all tests revealed no reason to suspect Type I inflation (Figures 1-4).
Table 1. Associations \( (p<10^{-4}) \) with mean arterial pressure and PAI-1 in 1032 Ghanaian participants. Using univariate and multivariate methods, 15,890 exonic SNPs (MAF≥0.20) were tested for association; all models were adjusted for age and sex.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Single Trait MAP</th>
<th>Single Trait PAI-1</th>
<th>Bivariate</th>
<th>Homogeneity of Correlation</th>
<th>Gene</th>
</tr>
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<tr>
<td>rs10738554</td>
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<td>0.116</td>
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<td>SLC24A2*</td>
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<td>3.78E-04</td>
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<td>0.075</td>
<td>4.11E-04</td>
<td>0.857</td>
<td>OR51G2</td>
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<td>2.60E-04</td>
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<td>MGAM*</td>
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*previously associated with cardiovascular disease or hypertension

Column abbreviations: Chr.=chromosome; MAF=minor allele frequency; MAP=mean arterial pressure; Bivariate= MultiPhen, which models genotype as a function of MAP and PAI-1.
**Table 2. Associations (p<10^{-4}) with glucose and PAI-1 in 1032 Ghanaian participants.** Using univariate and multivariate methods, 15,890 exonic SNPs (MAF≥0.20) were tested for association; all models were adjusted for age and sex.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Single Trait Glucose</th>
<th>Single Trait PAI-1</th>
<th>Bivariate</th>
<th>p value</th>
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<td>0.04</td>
<td>3.59E-05</td>
<td></td>
<td><em>SETBP1</em></td>
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<td>rs404890</td>
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<td>0.465</td>
<td>6.88E-05</td>
<td></td>
<td><em>NOTCH4</em></td>
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</table>

*previously associated with cardiovascular disease, type 1 or type 2 diabetes mellitus

**rs10266732, which associated with PAI-1, is presented in Table 1.

Column abbreviations: Chr.=chromosome; MAF=minor allele frequency; Bivariate= MultiPhen, which models genotype as a function of glucose and PAI-1.
### Table 3. Associations (p<10^{-4}) with body mass index and PAI-1 in 1032 Ghanaian participants.

Using univariate and multivariate methods, 15,890 exonic SNPs (MAF≥0.20) were tested for association; all models were adjusted for age and sex.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>p value</th>
<th>p value</th>
<th>p value</th>
<th>Gene</th>
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<tr>
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<td>1.69E-04</td>
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<td>0.858</td>
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<td>rs9880989</td>
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<td>0.076</td>
<td>0.058</td>
<td>0.102</td>
<td>IQCG</td>
</tr>
</tbody>
</table>

*previously associated with cardiovascular disease or obesity

**rs10266732, which associated with PAI-1, is presented in Table 1.

***rs28429833, not listed, was in almost perfect linkage disequilibrium with rs28550932

Column abbreviations: Chr.=chromosome; MAF=minor allele frequency; BMI=body mass index; Bivariate= MultiPhen, which models genotype as a function of BMI and PAI-1.
Table 4. Associations ($p<10^{-4}$) with triglycerides and PAI-1 in 1032 Ghanaian participants. Using univariate and multivariate methods, 15,890 exonic SNPs (MAF≥0.20) were tested for association; all models were adjusted for age and sex.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Single Trait TG.</th>
<th>Single Trait PAI-1**</th>
<th>Bivariate</th>
<th>Homogeneity of Correlation</th>
<th>Gene</th>
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<td>8.95E-05</td>
<td>Loc100131135</td>
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<tr>
<td>rs896999</td>
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<td>A</td>
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<td>1.59E-05</td>
<td>0.02</td>
<td>1.66E-04</td>
<td>0.682</td>
<td>ANP32A*</td>
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<tr>
<td>rs13165786</td>
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<td>T</td>
<td>0.43</td>
<td>5.97E-05</td>
<td>0.653</td>
<td>1.31E-04</td>
<td>0.919</td>
<td>EDIL3*</td>
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<tr>
<td>rs3131875</td>
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<td>G</td>
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<td>9.81E-05</td>
<td>0.281</td>
<td>3.77E-04</td>
<td>0.627</td>
<td>ZFP57</td>
</tr>
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</table>

*previously associated with cardiovascular disease
** rs10266732, which associated with PAI-1, is presented in Table 1.
***rs29272, not listed, was in almost perfect linkage disequilibrium with rs29234

Column abbreviations: Chr.=chromosome; MAF=minor allele frequency; TG=triglycerides; Bivariate= MultiPhen, which models genotype as a function of TG and PAI-1.
Discussion

Endophenotypes such as PAI-1 have been considered promising targets for GWAS, in part because they exhibit higher heritability than the complex disease-related endpoints with which they associate\textsuperscript{6}. A key rationale for their proposed utility has been that the genotype-endophenotype map should be substantially simpler than the genotype-phenotype map, allowing for the efficient detection of variants of relatively large effect size. In the case of PAI-1, however, very few associations have been found, explaining little of the variance, and those that have been found have not been clinically relevant\textsuperscript{7}. To address this issue, we devised a novel way to study endophenotypes, such as PAI-1, that do not fit the above model.

Our underlying hypotheses are that (1) the intensity of association between PAI-1 and cardiovascular risk factors is, to some extent, under genetic control, and (2) that the mechanistic aspects of this control can be characterized as phenotypes, characterized by heritable variation. The genetic determinants of this control may be particularly relevant from a clinical perspective, because PAI-1 functions as a biochemical link between CVD risk factors and CVD endpoints. As such, variants that affect how PAI-1 concentration responds to CVD risk factors may have a greater impact on health than loci that influence PAI-1 independently of them. Indeed, a variant that raises PAI-1 a small amount regardless of any CVD risk factor may be trivial from a clinical perspective. In this regard, even the well-studied 4G-5G variant, which has been shown to affect PAI-1 independently, does not usually associate with CVD-related endpoints\textsuperscript{10}.

In our analyses, we found more significant heterogeneities of correlation by genotype than marginal effects, with no evidence of Type I inflation. This was surprising, because correlation relies on the second moment of two variables and therefore should (all else being equal) require larger sample sizes to provide similar power. Furthermore, p-values for marginal
effects were not corrected for multiple testing (i.e. doubled), as they would need to be to enable a fair comparison between tests per pair of risk factors. While our results would have to be validated by other studies before firm conclusions can be drawn, it may be the case that knowledge of the risk factors upstream of an endophenotype promotes the discovery of context-dependent genetic effects. Although such effects have frequently been found where sought, identifying the relevant “contexts” can be difficult. Genetic background, for example, though clearly a fundamental modifier of genetic effects, is difficult to define. However, the risk factors that precede an endophenotype on the etiological chain are natural candidates for context-dependent factors. Our focus on correlations may also have contributed to stronger-than-expected results, insofar as relationships between PAI-1 and cardiometabolic risk factors at the physiological level cannot be assumed to be causal, or unidirectionally causal, as regression analyses implicitly assume. There is evidence, for example, that PAI-1 is not only released by adipocytes, but can promote adipogenesis itself.\(^{32}\)

The most significant result in all univariate and multivariate tests in this study was the heterogeneity of correlation between MAP and PAI-1 for rs10738554, located near SLC24A2 (also known as NCKX2), a gene previously associated with high blood pressure in African Americans.\(^{33}\) SLC24A2 belongs to a family of proteins that transport sodium, potassium and calcium ions to regulate homeostasis, and can thus be plausibly implicated in the improper regulation of blood solutes that characterize hypertension. Within the context of the renin-angiotensin system, high blood pressure also promotes overexpression of PAI-1 levels.\(^{34}\) In addition to this biological plausibility, the high MAF at this locus (0.34), which increases the stability of its correlation estimate\(^{35}\), and our use of the conservative Spearman’s rank correlation for all tests, make the result particularly compelling.
While the biological relationship between PAI-1 and SLC24A2 has not been previously explored, a recent study found that the disruption of SLC24A2 (NCKX2) renders neurons more susceptible to ischemic insult. In particular, primary cortical neurons in SLC24A2 knockout models displayed a higher vulnerability and greater tendency to release Ca\(^{2+}\) ions under hypoxic conditions.\(^{36}\) Because hypoxia also stimulates PAI-1 expression,\(^{37}\) it is possible that in our study, PAI-1-level is serving as a proxy for hypoxic conditions, such that its increase corresponds with abnormal ion exchange in individuals with poorly functioning SLC24A2. If so, this context-dependence would explain why rs10738554 had no marginal effect on MAP. This interpretation is also consistent with the possibly recessive effect of rs10738554-C on the MAP-PAI-1 correlation, observable in Figure 5.

It is worth noting that rs10738554 would not have been detected by a gene-by-MAP interaction term in a regression analysis, because the correlation for the heterozygote genotype was lower than that for both homozygotes (Figure 5). Consistent with true overdominance is the fact that the minor allele frequency (MAF) of rs10738554 is close to 50% in all HapMap populations (in fact, its MAF of 36% in Yorubans is the lowest among continental populations), raising the possibility that balancing selection may be at play; barring further validation, however, it is perhaps more likely that an additive or recessive effect would emerge with greater sample size. Regardless of the true dominance deviation, this finding illustrates that complementing single variable tests for marginal effects with bivariate tests for homogeneity of correlation can offer unique insight.

When glucose was paired with PAI-1, rs404890 upstream of NOTCH4 on chromosome 6 was significant in tests for heterogeneity of correlation. Murine knockouts of NOTCH4 display severe angiogenic vascular remodeling defects, consistent with the well-known functional role of
Notch4 in promoting arterial endothelial cell specification. PAI-1 is also known to promote angiogenesis, although the exact mechanism has not been established. Plasma glucose has been shown to have an inverse relationship with vascular endothelial growth factor (VEGF) expression. Furthermore, severe, chronic hyperglycemia, as observed in cases of poorly controlled type 2 diabetes, damages vessels by non-enzymatic glycosylation, thereby increasing vessel permeability, atherogenesis and hyaline arteriolosclerosis. Proliferative diabetic retinopathy is another endpoint of poorly managed diabetes, the hallmark of which is aberrant angiogenesis leading to abnormal, fragile vessels in the eye. While threshold specific effects of Notch4 and PAI-1 are not well established, making it difficult to speculate on their physiological effects with respect to angiogenesis, the role of glucose is well known. The effects of clinical hyperglycemia, both through direct action on the vessels and indirect modulation of VEGF, fit the context-dependent model, where they become active and pathogenic beyond a certain level of vessel injury.

The single variable tests in this study generated surprisingly few associations at p<10^{-4}, e.g. none for fasting glucose and only one each PAI-1 and BMI (rs1026673 in MGAM, and rs1420101 in IL1RL1, respectively). MGAM has no clear connection to PAI-1 in the literature, but its role in starch digestion may be relevant. IL1RL1 is selectively expressed on Th2 cells and mast cells, and appears to be involved in inflammatory responses. Binding of IL1RL1 to its ligand, IL33, produces an IL4 mediated response in allergic airway inflammation of extrinsic asthma. A BMI-dependent increased risk of asthma has been reported for overweight and obese patients.

Interestingly, the bivariate tests in this study (performed using the MultiPhen platform) did not effectively complement the single variable tests for marginal effects, yielding only two
associations in total, and outperforming the single variable test only in identifying rs1048347 (p=2.03 x 10^{-05} vs. 1.98 x 10^{-04}), a locus with no apparent connection to either PAI-1, TG, or CVD. The poor performance of MultiPhen indicates that pleiotropy may play a limited role in shaping the phenotypes tested, perhaps because it is unlikely that the same genetic factors affect both an endophenotype and its correlated phenotype independently, i.e. when MultiPhen is designed to have the most power^{29}.

Our genome-wide scan for genetic effects on phenotypic correlation (which, to our knowledge, has not been previously performed), provides evidence in support of the hypothesis that context-dependent genetic variants play an important role in the genetic architecture of complex phenotypes. Our understanding of how genetic variation modulates the relationships between CVD risk factors and CVD endpoints is extremely limited. Yet, because marginal effects can be masked by context, as our results indicate, identifying context-dependent genetic effects will be necessary to inform genetic risk assessment and improve precision medicine in the future. Our method appears to be well suited for this task.
Figure 1. P-value distributions of univariate and multivariate tests assessing 15,890 exonic SNPs for association with PAI-1 and each of four cardiovascular risk factors in 1032 Ghanaian participants. The negative logarithms (base 10) of p-values are shown. Results for the univariate association tests of PAI-1 are depicted as green horizontal bars in each panel. Results for the univariate association tests of (a) MAP, (b) glucose (c) BMI, and (d) TG are depicted as purple plus-signs. P-values for the bivariate association tests (red circles) and the tests of homogeneity of correlation (black asterisks) for (a) MAP and PAI-1, (b) glucose and PAI-1, (c) BMI and PAI-1, and (d) TG and PAI-1 are also depicted.
Figure 5. Correlation between PAI-1 and MAP by genotype of rs10738554; (TT=0, CT=1, CC=2). The Spearman’s correlations for genotypes TT, CT, and CC are 0.33, 0.13, and 0.57, respectively. PAI-1 and MAP were adjusted for age and sex and standardized before stratification by genotype.
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References


substrate "Brake" on maltase-glucoamylase activity is located within the glucoamylase subunit. J Nutr 138, 685-692.
