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ORIGINAL ARTICLE

Differences in thrombin and plasmin generation potential between East African and Western European adults: The role of genetic and non-genetic factors

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Abstract

Background: Geographic variability in coagulation across populations and their determinants are poorly understood.

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Objective: To compare thrombin (TG) and plasmin (PG) generation parameters between healthy Tanzanian and Dutch individuals, and to study associations with inflammation and different genetic, host and environmental factors.

Methods: TG and PG parameters were measured in 313 Tanzanians of African descent living in Tanzania and 392 Dutch of European descent living in the Netherlands and related to results of a dietary questionnaire, circulating inflammatory markers, genotyping, and plasma metabolomics.

Results: Tanzanians exhibited an enhanced TG and PG capacity, compared to Dutch participants. A higher proportion of Tanzanians had a TG value in the upper quartile with a PG value in the lower/middle quartile, suggesting a relative pro-coagulant state. Tanzanians also displayed an increased normalized thrombomodulin sensitivity ratio, suggesting reduced sensitivity to protein C. In Tanzanians, PG parameters (lag time and TTP) were associated with seasonality and food-derived plasma metabolites. The Tanzanians had higher concentrations of pro-inflammatory cytokines, which correlated strongly with TG and PG parameters. There was limited overlap in genetic variation associated with TG and PG parameters between the two cohorts. Pathway analysis of genetic variants in the Tanzanian cohort revealed multiple immune pathways that were enriched with TG and PG traits, confirming the importance of co-regulation between coagulation and inflammation.

Conclusions: Tanzanians have an enhanced TG and PG potential compared to Dutch individuals, which may relate to differences in inflammation, genetics and diet. These observations highlight the importance of better understanding of the geographic variability in coagulation across populations.

KEYWORDS
ethnicity, genetic association studies, inflammation, metabolome, plasmin, thrombin

1 | INTRODUCTION

Thrombosis is the common cause of myocardial infarction, stroke, venous thrombosis and pulmonary embolism (venous thromboembolism, VTE). Low- and middle-income countries are disproportionally affected by cardiovascular diseases (CVD).[1,2] Contrary to other geographical regions, sub-Saharan Africa is witnessing a rapid increase in the burden of CVD, with stroke being a leading cause of cardiovascular death.[1,3] Population growth, aging, dietary and lifestyle changes are important contributory factors to this health transition. Data on the epidemiology of VTE in sub-Saharan Africa is very limited, but a recent systematic review suggested that the burden of VTE in sub-Saharan Africa is considerable.[4]

Coagulation pathways are influenced by genetic, environmental and host factors.[5–8] Variation in these factors contributes to differences in the epidemiology of thrombosis across populations. Studies outside the African continent have shown that individuals of African descent are disproportionally affected by cardiovascular diseases, including myocardial infarction, stroke and VTE.[9,10] African ancestry was also associated with a hypercoagulable state, including enhanced thrombin generation (TG).[11–13]

Essentials

- Geographic variability in coagulation across populations are poorly understood
- Thrombin (TG) and plasmin (PG) generation capacity were assessed in healthy Tanzanians and Dutch
- Tanzanians exhibit enhanced TG and PG with a decreased response to thrombomodulin
- Coagulation and inflammation were co-regulated with Tanzanians exhibiting a pro-inflammatory state

Simultaneous measurement of TG and plasmin generation (PG) provides a comprehensive evaluation of coagulation potential.[14] In addition, thrombomodulin (TM) acts as a cofactor for thrombin to activate protein C. TG in the presence of TM assesses the function of the protein C pathway.[15] Activated protein C resistance is a well-established risk factor for thrombosis. TG capacity has been associated with different clinical conditions, including the risk for VTE[16–19] and atherothrombosis.[20]

Previous studies have shown that TG is influenced by various factors, including genetic variation,[21,22] sex,[19,23] body mass index (BMI)[24–26] and inflammation.[27] Extensive cross-talk exists between inflammation and coagulation,[28,29] and the reciprocal activation of both systems is highly relevant in the setting of thrombosis-related diseases.[30,31] Nonetheless, when assessing these systems in different populations, it is hard to untangle which coagulation and plasminogen activation potential activities are related to inflammation beyond their genetic association. Therefore, we hypothesized that marked differences exist in TG and PG potential between people living in East Africa or Western Europe. Here, we compared TG, TM-modulated TG and PG parameters between healthy Tanzanians of African descent living in Tanzania and healthy Dutch of Western-European background living in the Netherlands, and studied associations with inflammation and different genetic, host and environmental factors.

2 | MATERIAL AND METHODS

2.1 | Study design and population

For this cross-sectional study, data from two cohorts embedded in the Human Functional Genomics Project were analyzed (https://www.humanfunctionalgenomics.org): the 300-Tanzania-FG (300TZFG) and the Dutch 500FG cohort. Characteristics of both cohorts of healthy subjects have been described earlier.[32,33] In summary, the 300TZFG cohort consists of 323 healthy Tanzanian individuals of African ancestry, aged between 18 and 65 years, living in the urban and rural areas in the Kilimanjaro region in Northern Tanzania. The urban participants were mostly from Moshi city, which is located at an elevation of around 880 m above sea level, whereas most of the rural participants resided on the slopes of Mount Kilimanjaro up to an altitude of approximately 2250 m. The cohort was enrolled between March and December 2017. Exclusion criteria were participants with any acute or chronic disease, use of antibiotics or anti-malaria medication in the 3 months before blood sampling, tuberculosis in the past year, a blood pressure ≤90/60 mmHg or ≥140/90 mmHg or a random blood glucose > 8.0 mmol/L. Pregnant, postpartum, or breastfeeding women were excluded. The 500FG cohort consists of 534 Dutch individuals of Western-European background, aged 18 years and older. Data were collected between August 2013 and December 2014 at the Radboud university medical center (Radboudumc) in the Netherlands. Exclusion criteria were the use of any medication in the past month and acute or chronic diseases at the time of blood sampling. Pregnant, postpartum, or breastfeeding women were excluded. TG and PG were measured simultaneously on stored samples of both cohorts. In addition, in order to assess the possible effects of storage time on the TG results, TG was also measured in samples of the so-called 50FG cohort, which consists of 56 participants of the initial 500FG cohort, who were resampled at 3-month intervals over one year in 2016.[34] TG was performed on two additional samples (collected in February and August 2016) of 51 participants of the 50FG cohort.

Data from both cohorts are available including metadata from questionnaires, circulating inflammatory markers and genotype data and are available upon request from the corresponding author. In the Tanzanian cohort, data from untargeted plasma metabolomics as well as dietary habits are also available.

2.2 | Sample collection and preparation

Sample collection and plasma preparation was harmonized as much as possible across the different HFGP cohorts reported here. Blood was obtained using a straight needle via antecubital venepuncture; the tourniquet was released immediately after needle insertion. Both EDTA tubes (Monoject^{TM}; Covidien) serum and citrate tubes (3.2% sodium citrate; Becton Dickinson) were used; the citrate tubes were filled last. Within 2–3 h after blood collection, platelet-poor plasma was generated by centrifugation (2000 g for 10 min) and stored at −80°C.

2.3 | Thrombin and plasmin generation assays

Thrombin generation and PG generation assays were performed on citrate anticoagulated platelet-poor plasma at Synapse Research Institute, Maastricht, the Netherlands. TG was performed using modified calibrated automated thrombography (MidiCAT; Synapse Research Institute, Maastricht, the Netherlands) as described before.[35] In short, 10 µl trigger (5 pM recombinant tissue factor and 4 µM phospholipids) and 10 µl α_2-macroglobulin-thrombin complex were added to the reaction and calibrator wells, respectively. Next, 40 µl plasma was added to each well and plates were heated for 10 min at 37°C. To initiate thrombin generation, 10 µl of a fluorescent substrate (Z-Gly-Gly-Arg-AMC) with calcium (FluCa) was added to the wells. TG was performed in the absence and presence of 7 nM thrombomodulin (TG−TM and TG+TM). The inter-assay variation was controlled by normalizing all TG parameters with normal pooled plasma (NPP). Details of the NPP preparation have been described previously.[36]

The plasmin generation (PG) assay was performed using a method similar to the CAT technique with the addition of a recombinant tissue plasminogen activator (rtPA).[37] To prevent the influence of plasma color on fluorescence intensity, each plasma was compared to its calibrator measurement. Reactions were monitored every 20 s with a fluorometer (Fluoroskan Ascent, Thrombinoscope, Maastricht, the Netherlands). The intra- and inter-assay coefficient of variation (CV) of TG parameters both in absence and presence of TM were all <7% and 13%, respectively. The intra- and inter-assay %CVs of PG parameters were all less than 8% and 12%, respectively. The biological variation of TG has been described before in 127 healthy donors.[38] Variation of TG triggered by 5pM TF in absence of TM was <19% for all parameters, whereas the variation of TG parameters in the presence of TM was <34%. Reference ranges for PG were determined in 112 healthy volunteers and the variation of PG parameters was <26%.

Thrombin and plasmin generation assay data were analyzed by Thrombinoscope software to generate the following parameters: lag-time (minutes at 6 nM thrombin/plasmin), time to peak (TTP; minutes), velocity index (VI, nM/min, Peak/[TTP-lag-time]), peak (nM), and endogenous thrombin/plasmin potential (ETP/EPP, nM × minute). The VI, Peak and ETP, both in the absence and presence of TM, of tested subjects were normalized as the percentage of that of a NPP tested without TM in the same run. Furthermore, a normalized thrombomodulin sensitivity ratio (n-TMsr) was determined for the ETP, peak and VI. This ratio is calculated by dividing the values of these parameters in the presence and absence of thrombomodulin, normalized against the same ratio determined in NPP. A higher ratio reflects a decreased anticoagulant response to TM in comparison to pooled normal plasma.

The analytical performance of the TG test was evaluated with NPP samples prior to the final testing. The distribution of the thrombin and plasmin parameters are shown in Figure S1A,B.

2.4 | Plasma metabolome

Plasma samples of the Tanzanian cohort were measured using the untargeted metabolomics workflow by General Metabolics with procedures as previously described.[39] In short, metabolites were measured by a high throughput mass spectrometry technique using the Agilent Series 1100 LC pump coupled to a Gerstel MPS2 autosampler and the Agilent 6520 Series Quadrupole Time-of-flight mass spectrometer (Agilent). The selection of food-derived metabolites was performed based on the ontology given in the HMDB (https://www.hmdb.ca/) as described previously.[32]

2.5 | Circulating inflammatory markers

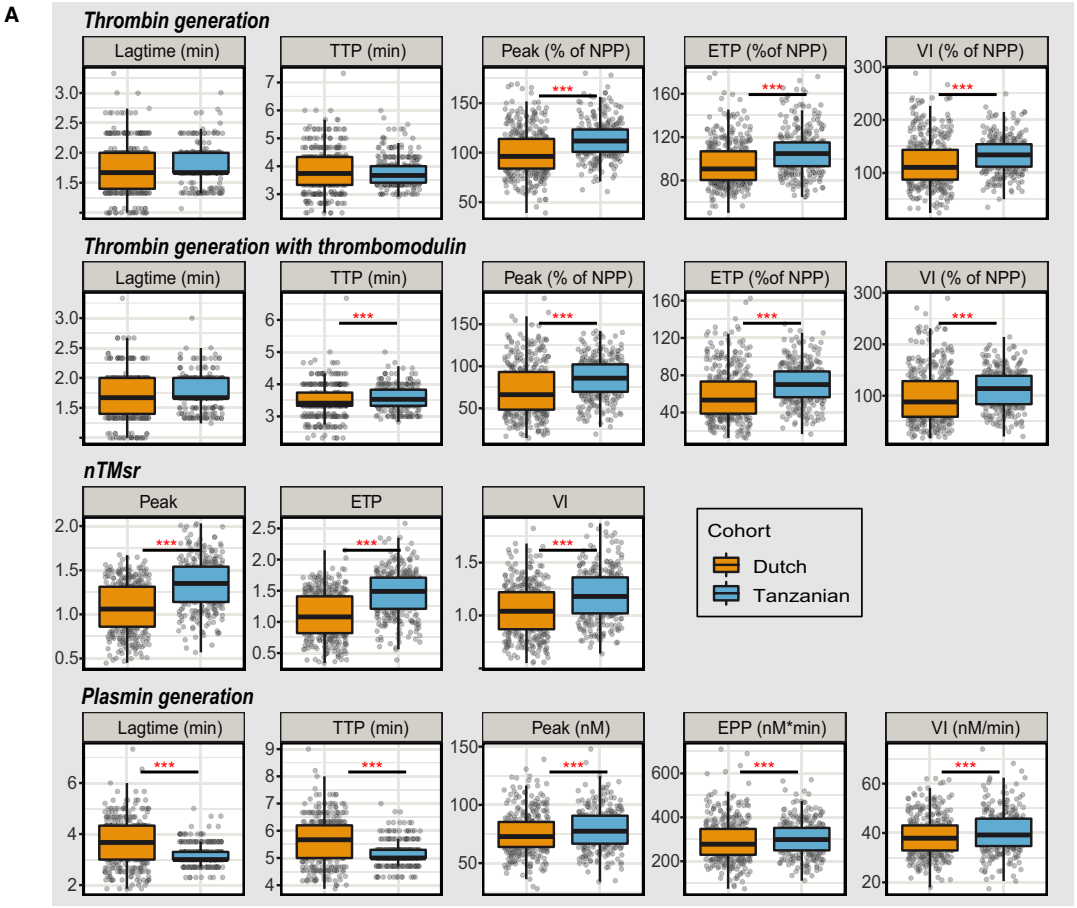
Concentrations of interleukin (IL)-6, IL-18, IL-1β, IL-1 receptor antagonist (IL-1Ra) were measured in EDTA plasma using the Simple

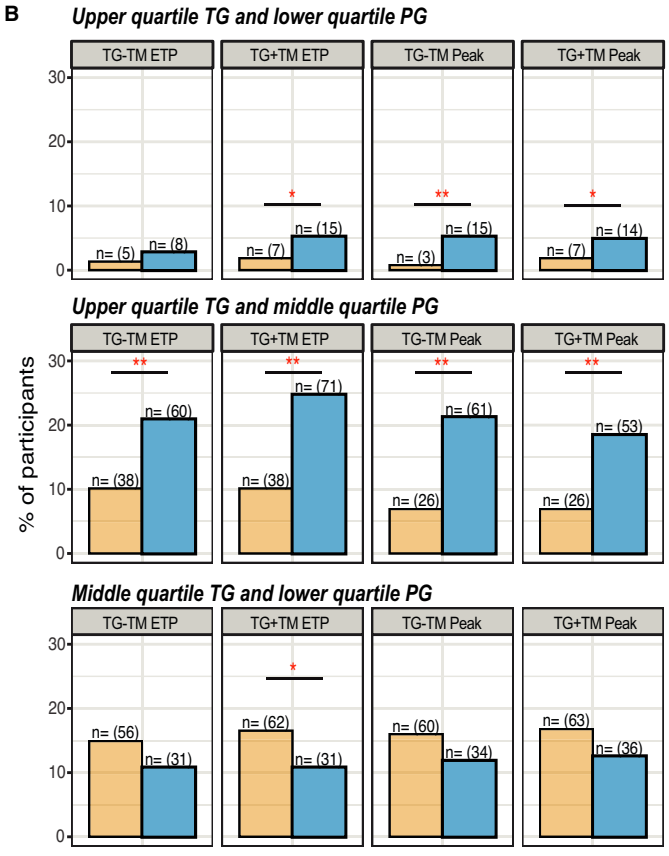
TABLE 1 Cohort characteristics

	Tanzanian (*N* = 313)	Dutch (*n* = 396)	*p*-value
Sex, women (*N*, %)	157 (50.2)	196 (49.5)	.880
Age, years (median, IQR)	30.3 (23.4–40.2)	23.5 (21.0–27.0)	<.001
Age category (*N*, %)			
18–30	153 (48.8)	316 (81.0)	<.001
31–40	80 (25.5)	19 (4.8)	
41–50	51 (16.2)	12 (3.0)	
50–60	25 (7.9)	18 (4.6)	
≥60	4 (1.2)	25 (6.4)	
BMI (median, IQR)	23.8 (21.5–27.3)	22.5 (20.8–24.4)	<.001
BMI category (*N*, %)			
≤24.9	190 (60.7)	320 (83.3)	<.001
≥25–29.9	76 (24.2)	57 (14.8)	
≥30	47 (15.0)	7 (1.82)	
BMI by sex (median, IQR)			
Men	22.8 (20.8–24.9)	22.9 (21.6–24.7)	.325
Women	25.7 (22.6–30.3)	21.7 (20.5–23.7)	<.001
Smoking (*N*, % of men)	45 (28.8)	37 (18.5)	.023
Smoking (*N*, % of women)	0 (0.0)	21 (10.7)	<.001
Hormonal contraceptives	24 (15.3)	75 (38.3)	<.001

Note: Data were compared using the χ^2-test, Fisher's exact test or Mann-Whitney-*U* test.

FIGURE 1 Differences in thrombin (TG) and plasmin generation parameters (PG) between the Tanzanian and Dutch cohort. (A) Boxplots depict TG parameters in the absence and presence of thrombomodulin (TM) and PG parameters in both cohorts. The analysis was performed using a linear regression model using age, sex, and hormonal contraceptive use as covariates. In the boxplots, the in-box line defines the median value, hinges depict 25th and 75th percentiles and whiskers extend to ±1.5 interquartile ranges; dots show data from individual participants. Significance level was set by FDR *p*-value <.05(*), <.005(**), and <.0001(***). (B) Figure show the comparison of the proportion of participants in the upper quartile of TG with either lower or middle quartile of PG, and participants in the middle quartile of TG with lower quartile of PG. Participants were grouped according to TG and PG quartiles in the Dutch cohort. Analysis was performed using chi-square test. *p*-value <.05(*); <.005(**). Abbreviations: TG-TM, thrombin generation in the absence of thrombomodulin; TG+TM, thrombin generation in the presence of thrombomodulin; nTMsr, normalized thrombomodulin sensitivity ratio; TTP, time to peak; VI, velocity index; ETP/EPP, endogenous thrombin/plasmin potential; NPP, normal pooled plasma


A
Thrombin generation
Lagtime (min)
TTP (min)
Peak (% of NPP)
ETP (%of NPP)
VI (% of NPP)
Thrombin generation with thrombomodulin
Lagtime (min)
TTP (min)
Peak (% of NPP)
ETP (%of NPP)
VI (% of NPP)
nTMsr
Peak
ETP
VI
Cohort
Dutch
Tanzanian
Plasmin generation
Lagtime (min)
TTP (min)
Peak (nM)
EPP (nM*min)
VI (nM/min)


B
Upper quartile TG and lower quartile PG
TG-TM ETP
TG+TM ETP
TG-TM Peak
TG+TM Peak
n= (5)
n= (8)
n= (7)
n= (15)
n= (3)
n= (15)
n= (7)
n= (14)
Upper quartile TG and middle quartile PG
% of participants
n= (38)
n= (60)
n= (38)
n= (71)
n= (26)
n= (61)
n= (26)
n= (53)
Middle quartile TG and lower quartile PG
n= (56)
n= (31)
n= (62)
n= (31)
n= (60)
n= (34)
n= (63)
n= (36)

Plex™ cartridges run on the Ella™ platform (Bio-Tech/R&D; SPCKC-PS-001559; Protein Simple) following the manufacturer's instructions as described previously.[33] The adipokines leptin, adiponectin, resistin, and the acute phase protein alpha-1-antitrypsin (AAT) were measured in EDTA plasma using R&D Systems DuoSet ELISA kits following the manufacturer's protocol. The distribution of all measured cytokines and adipokines is summarized by the histograms shown in Figure S2.

2.6 | Genotype and imputation

For the 300TZFG cohort, genotyping was performed using the Global Screening Arrays (GSA) SNP chip which is more suitable for African ancestry.[40] Optical 0.7.0 with default settings was used for genotype calling.[41] Filtering was performed by excluding variants with call rate >0.01, low minor allele frequencies (MAF < 0.001), and Hardy-Weinberg Equilibrium (HWE) with a p-value $<1 \times 10^{-4}$. The strands and variant identifiers were aligned to the 1000 Genome reference panel using Genotype Harmonizer.[42] Genotype imputation was performed using the Minimac4 software through the publicly available Michigan Imputation Server[43] with the Human Reference Consortium (HCR r.1.1 2016) being used as a reference panel. Data were phased using Eagle v2.3. Finally, we filtered out variants with imputation quality score (R^2) < .3. Genotyping and imputation generated a total of 5 271 779 variants.

Genotyping strategies of the 500FG cohort were already described previously.[44] In brief, genotyping was performed using the commercially available single-nucleotide polymorphism (SNP) chip, Illumina HumanOmniExpressExome-8 v1.0., and was imputed to obtain genotypes at approximately 7 million SNPs. The strands and variant identifiers were aligned to the reference Genome of The Netherlands (GoNL) dataset using Genotype Harmonizer.[42] Data were phased and imputed using the GoNL as a reference panel by SHAPEIT2 v2 and IMPUTE2, respectively.

2.7 | Statistical analysis

Values are displayed as median with interquartile range (IQR) or number with percentage for categorical variables. Outlier detection was performed using principal component analysis (PCA). Outliers were defined by a value >3 SD from the mean principal component (PC) 1 and 2 and were excluded from further analysis. Linear regression was used to adjust differences in TG and PG parameters for age, sex, BMI, and hormonal contraceptive use unless otherwise stated. Since a strong correlation was found between age and BMI in both cohorts, different adjustment for covariates was done in the analysis to prevent multicollinearity. Data were transformed using inverse ranked-based transformation before analysis. Unsupervised clustering (k-nearest neighbors with 100 repetitions) of metabolic profiles in the Tanzanian cohort was performed and visualized by the ComplexHeatmap package (v 2.7.1.1008). To address multiple testing, p-values were corrected according to the Benjamini-Hochberg procedure to decrease the false discovery rate (FDR).[45] Significance was defined by FDR p-values <.05.

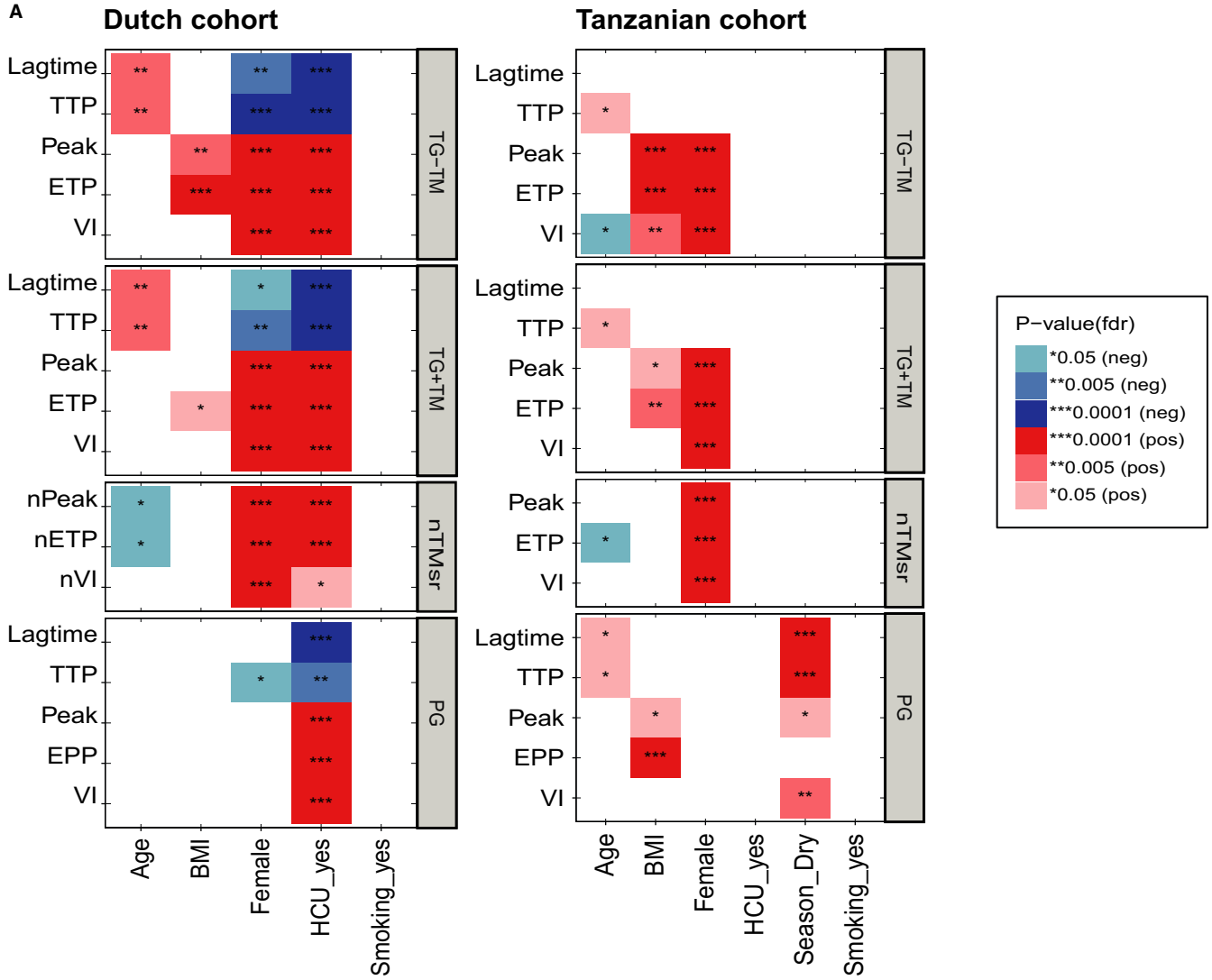
For both cohorts, we tested genetic variants for association with TG and PG traits with an additive linear regression model adjusted for age and sex. Before analysis, inverse ranked-based transformations were performed to normalize the distribution in each trait. Systematic inflation or deflation in test statistics over all loci was assessed through the quantile-quantile (QQ) plot for all TG and PG parameters. Genome-wide significance was defined by p-values $<5 \times 10^{-8}$, and suggestive significance was defined by p-values $<1 \times 10^{-6}$. A total number of 273 and 249 samples from Tanzania and 354 and 340 samples from Dutch were available for TG/n-TMsr and PG genetic analysis, respectively.

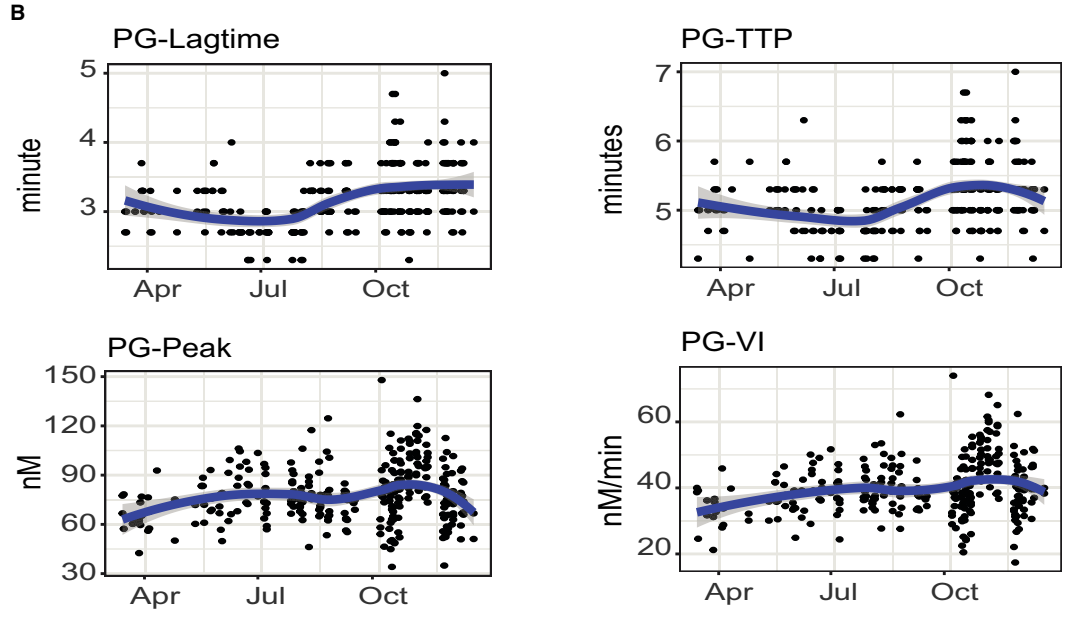
Furthermore, we performed a meta-analysis which is a standard approach to detect associations using the summary statistics of independent studies. Meta-analysis was performed using METAL using a sample size-weighted approach.[46] For this meta-analysis, we used variants that showed a p-value of association <.05 with each trait in both cohorts. To detect common signals for TG and PG profiles with the same effect, we selected SNPs that showed suggestive p-values and no significant heterogeneity in the meta-analysis (heterogeneity p-value >.05). For pathway analysis, gene set enrichment tests were performed using the Functional Mapping and Annotation (FUMA) bioinformatics tool.[47] A list of genes of interest was extracted within 250 kb of SNPs with p-value $<1 \times 10^{-4}$ before analysis. To test for the overrepresentation of biological functions, this list was tested against gene sets obtained from Reactome[48] using hypergeometric tests. We reported gene sets with FDR p-value <.01 and the number of genes that overlap with the gene set >1 by default.

2.8 | Ethical considerations

The 300TZFG study was approved by the Ethical Committees of the Kilimanjaro Christian Medical University College (CRERC) (No 2443)

FIGURE 2 Association of thrombin (TG) and plasmin generation (PG) parameters with selected host and environmental factors. (A) Heat map showing the associations between TG and PG parameters with a selection of host and environmental factors in the Tanzanian and Dutch cohort. The color-coding key depicts FDR corrected p-values. The analysis was performed by linear regression model with adjustment for other metadata. (B) Figures show the effect of seasonality on a selection of PG parameters in the Tanzanian cohort. The lines indicate the local regression (LOESS) fit; each dot represents an individual participant. Abbreviations: TG-TM, thrombin generation in the absence of thrombomodulin; TG+TM, thrombin generation in the presence of thrombomodulin; nTMsr, normalized thrombomodulin sensitivity ratio; TTP, time to peak; VI, velocity index; ETP/EPP, endogenous thrombin/plasmin potential. HCU, hormonal contraceptive use


A
Dutch cohort
Tanzanian cohort
Lagtime
TTP
Peak
ETP
VI
nPeak
nETP
nVI
EPP
TG−TM
TG+TM
nTMsr
PG
Age
BMI
Female
HCU_yes
Smoking_yes
Season_Dry
P−value(fdr)
*0.05 (neg)
**0.005 (neg)
***0.0001 (neg)
***0.0001 (pos)
**0.005 (pos)
*0.05 (pos)


B
PG-Lagtime
minute
PG-TTP
minutes
PG-Peak
nM
PG-VI
nM/min
Apr
Jul
Oct

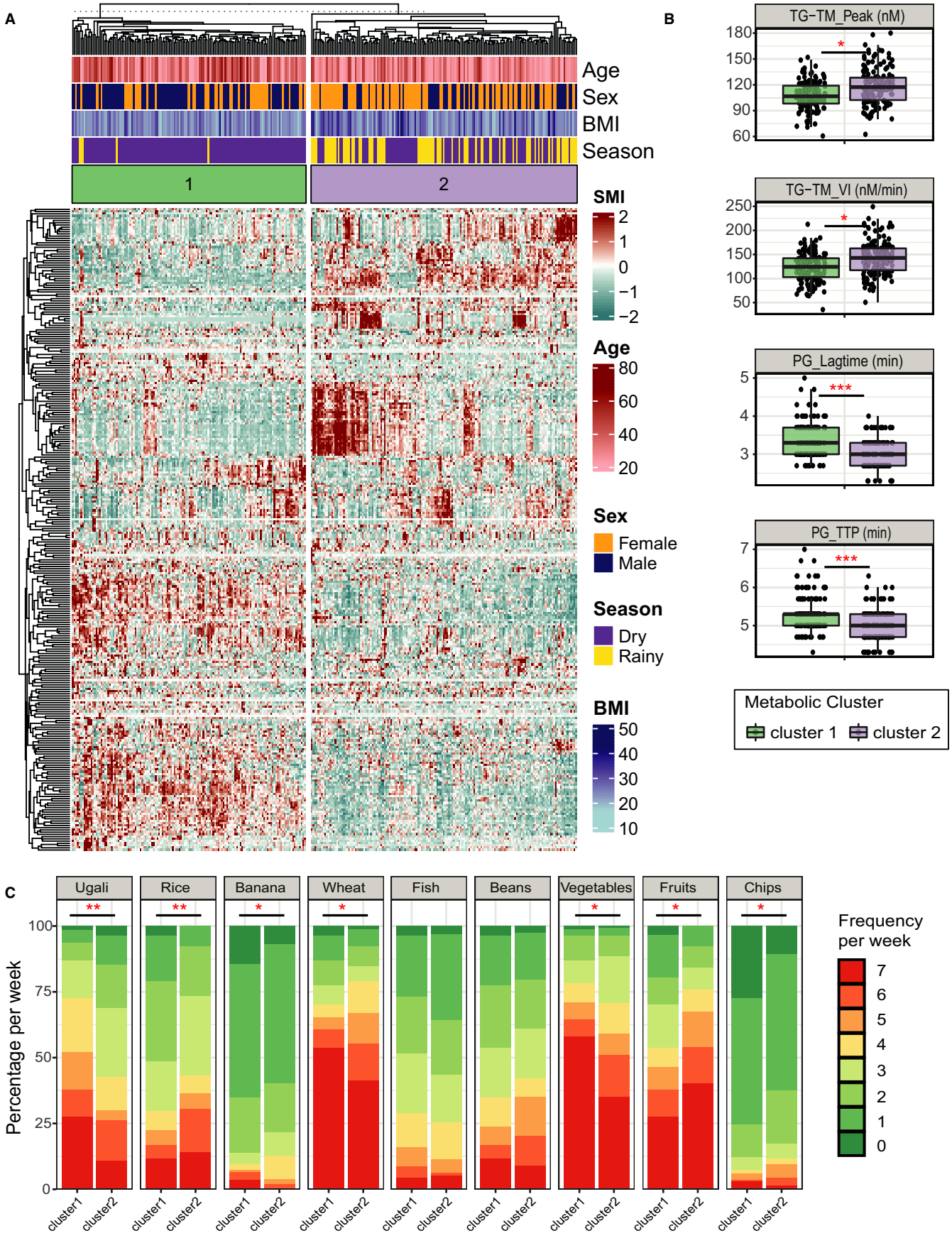

A
Age
Sex
BMI
Season
1
2
SMI
2
1
0
−1
−2
Age
80
60
40
20
Sex
Female
Male
Season
Dry
Rainy
BMI
50
40
30
20
10
B
TG−TM_Peak (nM)
180
150
120
90
60
*
TG−TM_VI (nM/min)
250
200
150
100
50
*
PG_Lagtime (min)
5
4
3
***
PG_TTP (min)
7
6
5
***
Metabolic Cluster
cluster 1
cluster 2
C
Ugali
Rice
Banana
Wheat
Fish
Beans
Vegetables
Fruits
Chips
**
**
*
*
*
*
*
Percentage per week
100
75
50
25
0
cluster1
cluster2
Frequency per week
7
6
5
4
3
2
1
0

FIGURE 3 Association of food-derived plasma metabolites with thrombin (TG) and plasmin generation (PG). (A) Unsupervised k-means clustering of individuals from the Tanzanian cohort ($n = 295$) according to the food-derived plasma metabolome (288 metabolites). Data are shown as scaled metabolites intensity (SMI). The color code indicates the relative abundance of metabolites across the samples of the two compared groups. Red and blue colors indicate higher and lower abundance, respectively. Presented are annotations for age, sex, BMI, seasonality and food-metabolome cluster. (B) Boxplot showing the comparison of TG and PG parameters between the two food-metabolome clusters. The analysis was performed by linear regression model using age, sex, and hormonal contraceptive use as covariates. In all box plots, the in-box line defines the median value, hinges depict 25th and 75th percentiles and whiskers extend to ±1.5 interquartile ranges; each dot indicates an individual participant. (C) Weekly food consumption of different products between the metabolic clusters. Differences in food consumption frequency categories were tested using the chi-squared test. The color-coding key of the food frequencies per week is depicted in the legend. Significance level was set by FDR p-value <.05(*), <.005(**), and <.0001(***). Abbreviations: TG-TM, thrombin generation in the absence of thrombomodulin; TTP, time to peak; VI, velocity index

and the National Institute for Medical Research (NIMR/HQ/R.8a/Vol. IX/2290 and NIMR/HQ/R.8a/Vol.IX/3318) in Tanzania. The 500FG cohort study was approved by the Ethical Committee of the Radboud University Medical Centre Nijmegen, the Netherlands (NL42561.091.12, 2012/550). Written informed consent was obtained from all subjects.

3 | RESULTS

3.1 | Characteristics of the study population

A total number of 323 Tanzanian and 534 Dutch healthy individuals were enrolled. TG and PG data were available for 313 and 287 of the Tanzanian participants, and for 392 and 375 of the Dutch participants, respectively. Study participants' characteristics are summarized in Table 1. The Tanzanian cohort was significantly older than the Dutch cohort with a median age of 30.3 years (IQR 23.4–40.2) vs. 23.5 years (IQR 21.0–27.0; p-value <.001). Tanzanian women had a higher BMI compared to Dutch women with a median of 25.7 kg/m^2 (IQR 22.6–30.3) vs. 21.7 kg/m^2 (IQR 20.5–23.7); p-value <.001, but there was no significant BMI difference between Dutch and Tanzanian men. 253 (78.3%) of the 323 Tanzanian participants lived in an urban area and 70 (21.7%) in a rural area.

3.2 | Increased TG, PG, and reduced protein C activation in Tanzanians

We first assessed the differences in TG and PG parameters between the Tanzanian and Dutch cohort. Compared with the Dutch cohort, Tanzanians had a significantly enhanced TG (higher ETP, peak, and VI; in the presence and absence of TM) and PG (higher EPP, peak, and reduced lag-time and TTP) (FDR p-value <.05; Figure 1A, Table S1). We next assessed the anticoagulant response to TM by calculating the normalized TM sensitivity ratio (n-TMsr) for ETP, peak, and VI. This ratio was increased for the Tanzanian cohort, i.e., TG in Tanzanians were more resistant to the anticoagulant effect of TM, suggesting an impaired anticoagulant effect of the protein C pathway or elevated FVIII levels. These differences persisted after adjustment for age, BMI, sex and hormonal contraceptive use.

To further assess the possible effects of the differences in age and in sample storage time between the cohorts, we used previously reported TG data derived from a sub-cohort of the Dutch 500FG cohort, namely the 50FG.[34] This cohort consists of 51 participants of the 500FG cohort, who were resampled in February and August 2016. Median age of these 51 participants was 30.0 years (IQR 25.8–53.0), which is comparable to that of the Tanzanian cohort. A principal component analysis that included the TG data of the three sampling time points (initial sampling in the 500FG in 2013/2014, and two times sampling in 2016) was performed, showing no significant separation across the time points (Figure S3A). Median values of the peak and ETP in the presence and absence of TM also did not differ across the three time points (Figure S3B). Moreover, the observed differences in TG parameters between the Tanzanians and the Dutch 500FG participants persisted when samples of the Tanzanians were compared with participants of the 50FG (Figure S3C). Together, these findings suggest that neither differences in sample storage time nor age explain the differences in TG between Tanzanians and Dutch in our study.

Next, to further assess differences in the balance between TG and PG across the Tanzanian and Dutch cohorts, we grouped participants according to TG and PG quartiles in the Dutch cohort. A significantly higher proportion of Tanzanian participants had a TG value in the upper quartile together with a PG value either in the lower or middle quartile (Figure 1B). This, together with the increased n-TMsr in the Tanzanians may signify that the enhanced TG activity in the Tanzanians is incompletely counterbalanced by an increased potential of activation of plasminogen and protein C.

3.3 | Influence of the host and environmental factors on TG and PG

To further understand the differences in TG and PG between the Tanzanians and Dutch, we first explored in both cohorts the associations of TG and PG parameters with host and environmental factors. Female sex,[23,49,50] aging,[23,49,50] and BMI[24–26] have previously been shown to be associated with enhanced TG in individuals living in high-income countries. Compared to men, Tanzanian and Dutch women both had a significantly enhanced TG (with and without TM,

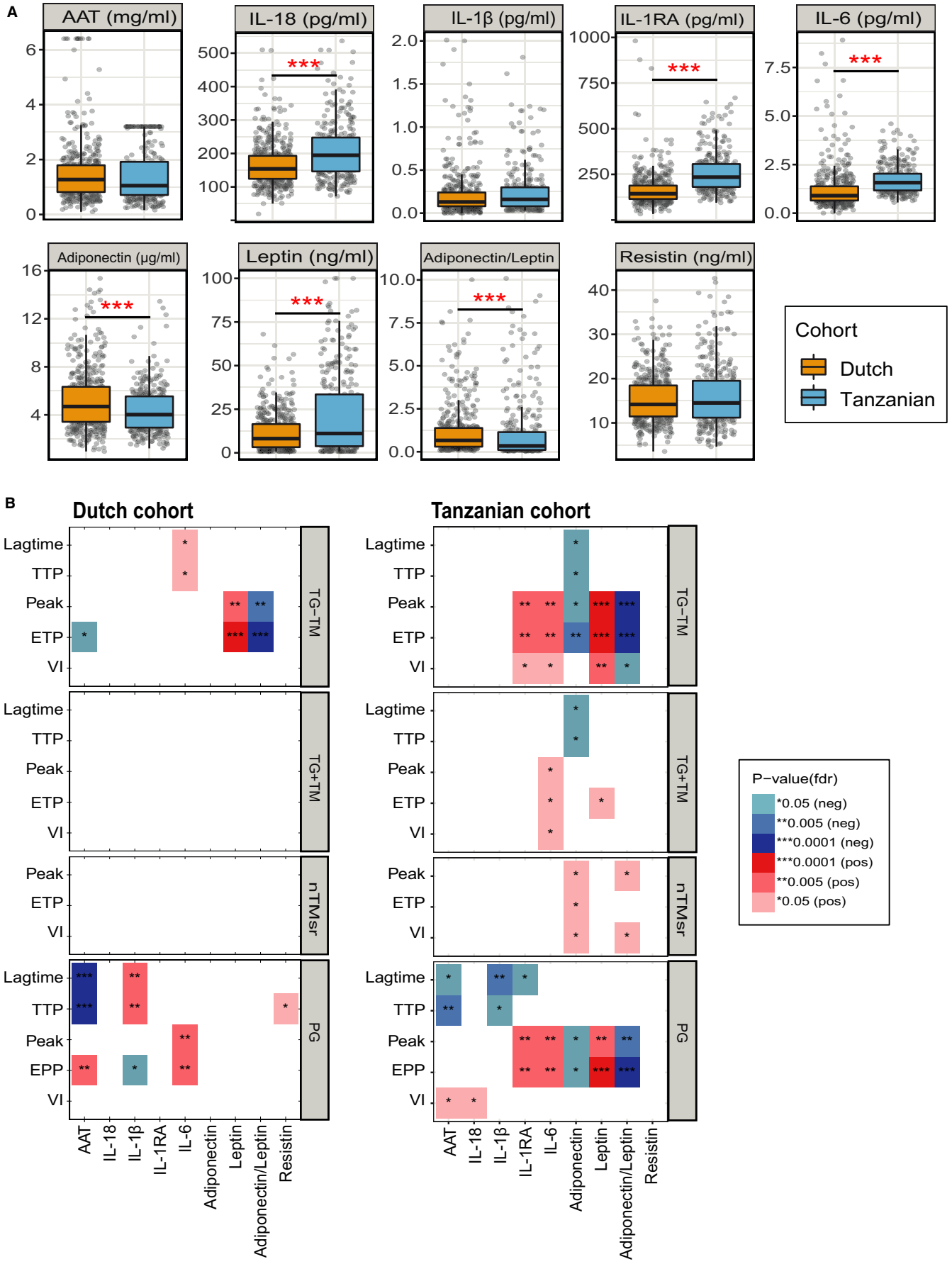

A
AAT (mg/ml)
IL-18 (pg/ml)
IL-1β (pg/ml)
IL-1RA (pg/ml)
IL-6 (pg/ml)
Adiponectin (μg/ml)
Leptin (ng/ml)
Adiponectin/Leptin
Resistin (ng/ml)
***
Cohort
Dutch
Tanzanian
B
Dutch cohort
Tanzanian cohort
Lagtime
TTP
Peak
ETP
EPP
VI
TG−TM
TG+TM
nTMsr
PG
AAT
IL-18
IL-1β
IL-1RA
IL-6
Adiponectin
Leptin
Adiponectin/Leptin
Resistin
P−value(fdr)
*0.05 (neg)
**0.005 (neg)
***0.0001 (neg)
***0.0001 (pos)
**0.005 (pos)
*0.05 (pos)

FIGURE 4 Associations of plasma inflammatory cytokines and adipokines with thrombin (TG) and plasmin generation (PG) parameters. (A) Comparison of plasma inflammatory mediators in the Tanzanian (blue; $n = 318$) and Dutch cohort (yellow; $n = 470$). The analysis was performed by linear regression model using age and sex as covariates. In all box plots, the in-box line defines the median value, hinges depict 25th and 75th percentiles and whiskers extend to ±1.5 interquartile ranges; each dot indicates an individual participant. Significance level was set by FDR p-value <.05(*), <.005(**), and <.0001(***). (B) Heat maps presenting FDR corrected p-values of a general linear regression model of plasma concentrations of cytokines and adipokines and TG and PG parameters (corrected for age, sex and, hormonal contraceptive use) in the Tanzanian and Dutch cohort. Abbreviations: AAT, alpha-1 antitrypsin; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; TG-TM, thrombin generation in the absence of thrombomodulin; TG+TM, thrombin generation in the presence of thrombomodulin; nTMsr, normalized thrombomodulin sensitivity ratio; TTP, time to peak; VI, velocity index; ETP/EPP, endogenous thrombin/plasmin potential

FDR p-value <.05) (Figure 2A). Women also had a higher n-TMsr. The use of hormonal contraceptives was associated with enhanced TG, PG and n-TMsr in Dutch women, as previously reported,[49,51,52] but not in Tanzanian women (Figure 2A) (FDR p-value <.05). Only a few women ($n = 24/157$; 15%) in the Tanzanian cohort used hormonal contraceptives of whom the majority ($n = 15/24$; 63%) used implant contraceptives. Correlations of TG and PG parameters with age were less outspoken, especially in the Tanzanians, and were mainly restricted to TTP and lag-time. A higher BMI was correlated with enhanced TG (ETP and peak) in both cohorts, and with EPP in the Tanzanian cohort (FDR p-value <.05). Smoking was not associated with TG or PG parameters. Moreover, there was no difference in TG and PG parameters between urban and rural dwellers in the Tanzanian cohort.

In the Tanzanians, PG parameters demonstrated a seasonal variation with a longer lag-time and a higher TTP, peak, and VI in participants recruited during the dry season (August–December; Figure 2A,B). Daily diet also varies across seasons with more availability of fresh staples during the dry season (the harvest period). We previously showed that food-derived metabolites in the plasma in this cohort are associated with diet.[32] We speculated that TG and PG potential is also associated with food-derived metabolites. We performed unsupervised clustering of the food-derived metabolome, which yielded two clusters. Participants in cluster one, who were mainly recruited during the dry season, had a lower TG (peak and VI) in the absence of TM and slower PG (higher lag-time and TTP) compared to cluster two (Figure 3A,B). Weekly food frequency consumption of ugali, rice, cooked banana (plantain), wheat and green vegetables differed significantly among metabolome clusters (Figure 3C). Individuals from the cluster one more frequently consumed ugali, cooked banana, and green vegetables and less frequently consumed rice and potato chips. We also related individual food metabolites to TG and PG and mainly observed associations with the lag time and time to peak of PG (FDR p-value <.05; Figure S4). A high-fat diet was recently shown to delay plasmin generation[37] and we found strong positive associations between food-derived metabolites that play a role in lipid metabolism with lag time and time to peak of PG in our cohort; for instance 1-(11 Z-eicosenoyl)-glycero-3-phosphate, lysoPE(0:0/20:0), and 1-(5Z,8 Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphate. Collectively, these results show that changes in food-derived metabolites in the Tanzanian cohort are associated with seasonality and TG and PG parameters.

3.4 | Higher inflammatory cytokines and adipokines associate with increased TG and PG potential in Tanzanians

Coagulation and inflammation share an intricate relation and previous studies have highlighted the bidirectional associations between inflammation and coagulation.[28,29] We postulated that differences in inflammatory markers between both cohorts contribute to the differences in coagulation profiles. We measured plasma concentrations of the inflammatory cytokines IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-6, and IL-18, as well as a selection of adipokines. Compared with Dutch participants, Tanzanians had significantly higher plasma concentrations of IL-1Ra, IL-6, IL-18, and leptin, and a lower concentration of adiponectin (Figure 4A; FDR p-value <.05; adjusted for age and sex). The latter is an anti-inflammatory adipokine and the low adiponectin-to-leptin ratio is a sign of adipose tissue dysfunction and insulin resistance.[53] A recent study also showed inverse correlation between plasma adiponectin and thrombin generation.[54] Concentrations of IL-6, IL-1RA, leptin and adiponectin and its ratio were significantly associated with TG and PG parameters in both cohorts, but the strength of the association was stronger in the Tanzanian cohort (Figure 4B; FDR p-value <.05; adjusted for age, sex, and hormonal contraceptive use). A strong relationship exists between obesity and circulating cytokine and adipokine concentrations,[55] and correcting the analysis for BMI resulted in attenuation of associations in the Tanzanian, but not in the Dutch cohort (Figure S5). Moreover, we found a significant association between α1-antitrypsin (AAT) with lower TG and higher PG, especially in the Dutch cohort. In a previous study, AAT was found to inhibit both TG and PG,[27] but a supraphysiological AAT concentration (500 μmol/L) was used to inhibit plasmin in this study.

3.5 | Limited overlap in the genetic regulation of TG and PG

Assessment of genetic variants associated with TG and PG parameters in the Tanzanians identified two genome-wide significant SNPs at chromosome 1 (rs111494301; MAF = 1.13% and p-value = 3.69×10^{-8}) and chromosome 19 (rs113038409; MAF = 2.49% and p-value = 2.45×10^{-8}). These SNPs were associated with the peak and ETP of TG, respectively (Figure 5A, Table 2). SNP rs111494301 is located close to the *AMPD2* gene (Figure 5B) and is in high linkage disequilibrium ($R^2 > .8$) with *GNAT2* and *GPR61* loci. In

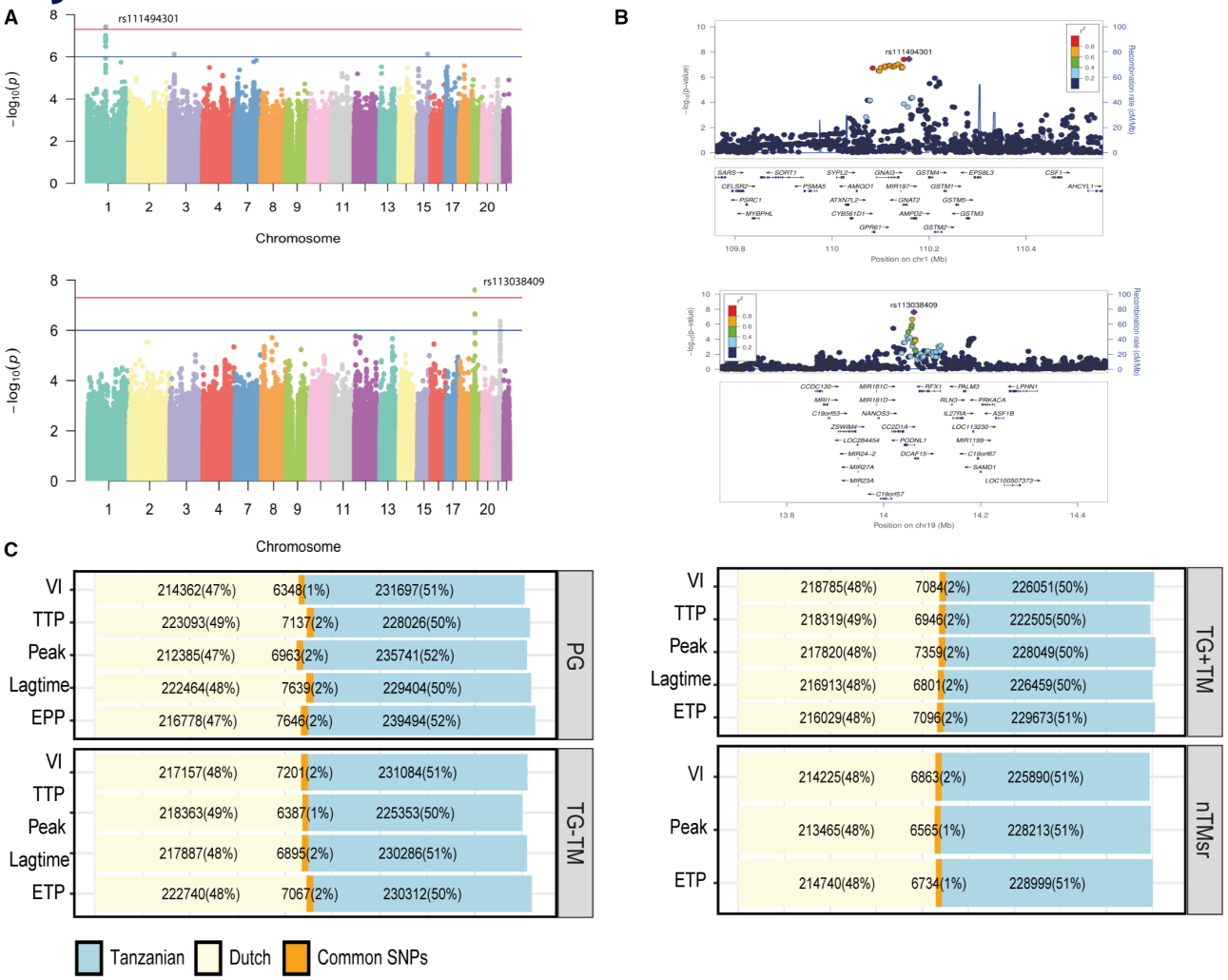

A
rs111494301
rs113038409
−log10(p)
Chromosome
B
rs111494301
rs113038409
Position on chr1 (Mb)
Position on chr19 (Mb)
Recombination rate (cM/Mb)
C
VI
TTP
Peak
Lagtime
EPP
ETP
214362(47%) 6348(1%) 231697(51%)
223093(49%) 7137(2%) 228026(50%)
212385(47%) 6963(2%) 235741(52%)
222464(48%) 7639(2%) 229404(50%)
216778(47%) 7646(2%) 239494(52%)
217157(48%) 7201(2%) 231084(51%)
218363(49%) 6387(1%) 225353(50%)
217887(48%) 6895(2%) 230286(51%)
222740(48%) 7067(2%) 230312(50%)
218785(48%) 7084(2%) 226051(50%)
218319(49%) 6946(2%) 222505(50%)
217820(48%) 7359(2%) 228049(50%)
216913(48%) 6801(2%) 226459(50%)
216029(48%) 7096(2%) 229673(51%)
214225(48%) 6863(2%) 225890(51%)
213465(48%) 6565(1%) 228213(51%)
214740(48%) 6734(1%) 228999(51%)
PG
TG-TM
TG+TM
nTMsr
Tanzanian
Dutch
Common SNPs
D
REGULATION OF IFNA SIGNALING
TRAF6 MEDIATED IRF7 ACTIVATION
INTERFERON ALPHA BETA SIGNALING
DDX58 IFIH1 MEDIATED INDUCTION OF INTERFERON ALPHA BETA
EXTRA NUCLEAR ESTROGEN SIGNALING
INTERLEUKIN 36 PATHWAY
CYTOKINE SIGNALING IN IMMUNE SYSTEM
FACTORS INVOLVED IN MEGAKARYOCYTE DEVELOPMENT AND PLATELET PRODUCTION
GLUCURONIDATION
ALPHA DEFENSINS
TNFS BIND THEIR PHYSIOLOGICAL RECEPTORS
METABOLISM OF RNA
PHASE II CONJUGATION OF COMPOUNDS
TNFR2 NON CANONICAL NF KB PATHWAY
SIGNALING BY INTERLEUKINS
SIGNALING BY NUCLEAR RECEPTORS
INTERLEUKIN 1 FAMILY SIGNALING
DEFENSINS
HEMOSTASIS
PLASMA LIPOPROTEIN ASSEMBLY REMODELING AND CLEARANCE
INNATE IMMUNE SYSTEM
PHASE 2 PLATEAU PHASE
RAB GEFS EXCHANGE GTP FOR GDP ON RABS
RAB REGULATION OF TRAFFICKING
DISEASE
COLLAGEN DEGRADATION
ACTIVATION OF MATRIX METALLOPROTEINASES
ION CHANNEL TRANSPORT
ESR MEDIATED SIGNALING
DEGRADATION OF THE EXTRACELLULAR MATRIX
POST TRANSLATIONAL PROTEIN MODIFICATION
GEN EXPRESSION TRANSCRIPTION
L1CAM INTERACTIONS
TRANSPORT OF SMALL MOLECULES
DEVELOPMENTAL BIOLOGY
ADAPTIVE IMMUNE SYSTEM
REGULATION_OF_INSULIN SECRETION
ESTROGEN DEPENDENT NUCLEAR EVENTS DOWNSTREAM OF ESR MEMBRANE SIGNALING
NEUTROPHIL DEGRANULATION
BIOLOGICAL OXIDATIONS
INTERFERON SIGNALING
SIGNALING BY GPCR
BETA CATENIN INDEPENDENT WNT SIGNALING
−log10(FDR p−value)
PG_Lagtime
PG_Peak
PG_TTP
PG_VI
TG−TM_ETP
TG−TM_Peak
TG−TM_VI
TG+TM_Lagtime
TG+TM_Peak
TG+TM_TTP
TG+TM_VI
TMsr_nETP
TMsr_nPeak
TMsr_nVI

FIGURE 5 Genetic regulation of thrombin (TG) and plasmin generation (PG) in the Tanzanian and Dutch cohort. (A) Manhattan plot of SNPs associated with lag-time and peak of TG in the absence of thrombomodulin in the Tanzanian cohort. The red line shows genome-wide significance (5×10^{-8}), while the blue line indicates the threshold for genetic variants that showed a suggestive significant association (1×10^{-6}) B. Regional association plots of genome-wide significant rs111494301 at chromosome 1 (upper) and rs113038409 at chromosome 19 (lower) (C) Frequency of common SNPs (*p*-value <.05) between the Tanzanian and Dutch cohorts significantly associated with TG and PG parameters (*p*-value <.05). (D) Gene set enrichment functional analysis for each TG and PG parameter in the Tanzanian cohort showing the significantly enriched pathways (FDR *p*-value <.01). Abbreviations: TG-TM, thrombin generation in the absence of thrombomodulin; TG+TM, thrombin generation in the presence of thrombomodulin; nTMsr, normalized thrombomodulin sensitivity ratio; TTP, time to peak; VI, velocity index; ETP/EPP, endogenous thrombin/plasmin potential

TABLE 2 Genome-wide loci (*p*-value $<5 \times 10^{-8}$) for thrombin generation and plasmin generation traits

SNPs	Trait	Chr	Position (bp)	Reference allele	Alternative allele	MAF	*p*-value	Beta	Gene(s)
rs111494301	TG-TM Peak	1	110158579	A	G	0.113	3.69×10^{-8}	−0.61	*AMPD2*[a], *GNAT2*[c], *GPR61*[c]
rs113038409	TG-TM Lag-time	19	14062443	G	A	0.249	2.45×10^{-8}	−0.62	*PODNL1*[a,b]

Abbreviation: TG-TM, thrombin generation in the absence of thrombomodulin.

[a]Genes in close proximity to thrombin generation associated SNPs.

[b]eQTL effect of thrombin generation associated SNPs based on publicly available database.

[c]Genes in close proximity to SNPs in LD with thrombin generation associated SNPs.

previous studies, the *AMPD2* locus has been associated with apolipoprotein B production.[56] This protein was recently shown to be more abundant in the plasma fibrin clot of patients with antiphospholipid syndrome compared to those with VTE.[57] In addition, variants located in the other two genes *GNAT2* and *GPR61* have been associated with BMI.[58,59] Furthermore, 26 independent SNPs showed a suggestive and significant association (*p*-value $<1 \times 10^{-6}$ and $>5 \times 10^{-8}$, respectively) with different TG and PG parameters (Table S2). Using publicly available eQTL databases from healthy blood donor samples,[60–62] we identified nine lead variants mapped to *cis*-eQTLs for 25 genes. The candidate gene list for lead SNPs of the genome-wide significant and suggestive loci is shown in Tables 2 and S2, respectively.

Next, we assessed the overlap in genetic variants association with TG and PG potential across the Tanzanian and Dutch cohorts. Approximately 200 000 SNPs were associated (*p*-value <.05) with TG or PG generation parameters in each cohort, but only approximately 7000 (3.5%) of them were overlapping (Figure 5C). A subsequent meta-analysis using the variants with *p*-value <.05, revealed SNP at chromosome 3 (rs2600154; *p*-value 1.16×10^{-8}) reaching the genome-wide significance threshold and associated with TTP of PG. Additionally, eight variants reached suggestive significant levels in the same direction (*p*-value $<1 \times 10^{-6}$; Table 3). Interestingly, the variant rs529565, which is located in the *ABO* gene, was associated with both the peak (*p*-value $= 5.23 \times 10^{-7}$) and ETP (*p*-value $= 2.18 \times 10^{-7}$) of TG in the presence of TM in both cohorts. This SNP was previously related to thrombosis in other populations,[63,64] and the ABO locus has been strongly associated with FVIII levels in African American individuals.[65]

Finally, we performed gene set enrichment functional analysis for each TG and PG parameter in the Tanzanian cohort using the FUMA platform.[47] This analysis revealed 43 pathways that were significantly enriched in different TG and PG parameters (FDR *p*-value <.01). The top significant pathways were related to immune function (Figure 5D), including 'regulation of IFN-α signaling', 'interleukin 36 pathway', 'signaling by interleukins', and 'interleukin 1 family signaling'. Interestingly, the type I interferon signaling was the top enriched pathway in the Tanzanian cohort.

4 | DISCUSSION

The current study assessed the differences in coagulation profiles between healthy adult individuals from Tanzania and the Netherlands and explored host, environmental, and genetic factors accounting for these differences. We show that Tanzanians had enhanced TG and PG capacity. Our observation that Tanzanians more often had a high TG together with a low or normal PG, and were more resistant to the anticoagulant effect of TM, suggest that Tanzanians are relatively hypercoagulable compared to individuals of Western-European ancestry. Considering the underlying mechanisms of this procoagulant potential, Tanzanians had higher circulating concentrations of cytokines and adipokines relative to Europeans. In addition, our genetic analysis suggested that there is a distinct genetic contribution of genetic variants to TG and PG between the two cohorts.

Extensive cross-talk exists between coagulation, inflammation and host defense.[28,29] Thrombo-inflammation is nowadays recognized to play a key role in the pathogenesis of cardiovascular diseases, including VTE,[30,31] and both thrombin and plasmin are effectors in infection and host responses.[66,67] Correlations of pro-inflammatory cytokines with TG and PG parameters were generally stronger in Tanzanian than in Dutch participants. In addition, we provide evidence

TABLE 3 Meta-analysis results of suggestive loci (p-value $<1 \times 10^{-6}$) for thrombin generation and plasmin generation traits

SNPs	Traits	Chr	A1[c]	Tanzanian cohort		Dutch cohort		Meta-analysis	Gene(s)
				p-value	MAF	p-value	MAF	p-value	
rs16831307	PG_Lag-time	2	G	3.08×10^{-4}	0.319	6.55×10^{-4}	0.327	7.64×10^{-7}	*FMNL2*[a,b], *NEB*[b]
rs2600154	PG_Lag-time	3	A	4.97×10^{-3}	0.474	4.30×10^{-6}	0.286	1.15×10^{-7}	*SRGAP3*[a], *TTLL3*[b]
rs2600154	PG_TTP	3	A	6.32×10^{-4}	0.474	4.31×10^{-6}	0.286	1.16×10^{-8}	*SRGAP3*[a], *TTLL3*[b]
rs368181	PG_VI	14	T	1.56×10^{-6}	0.394	7.68×10^{-3}	0.396	2.16×10^{-7}	*RP11-116N8.4*[a]
rs13414	TG-TM_ETP	17	A	6.83×10^{-4}	0.107	4.69×10^{-5}	0.191	1.18×10^{-7}	*RP5-1171I10.4*[a], *SUPT4H1*[b], *SEPT4*[b], *RAD51C*[b], *BZRAP1-AS1*[b], *PRR11*[b], *CTD-2510F5.4*[b], *BZRAP1*[b], *MSX2P1*[b], *AC099850.1*[b], *MTMR4*[b], *SKA2*[b], *TRIM37*[b], *TSPOAP1-AS1*[b]
rs529565[d]	TG-TM_Peak	9	T	4.38×10^{-4}	0.415	3.38×10^{-4}	0.342	5.23×10^{-7}	*ABO*[a,b], *GBGT1*[b], *SURF6*[b], *MED22*[b]
rs1914824	TG-TM_VI	8	A	1.08×10^{-4}	0.373	1.45×10^{-3}	0.143	7.04×10^{-7}	*PRAGMIN*[a], *RP11-62H7.2*[b], *MFHAS1*[b], *ALG1L13P*[b], *RP11-10A14.5*[b], *FAM86B3P*[b]
rs529565[d]	TG+TM_ETP	9	T	1.06×10^{-4}	0.415	5.01×10^{-4}	0.342	2.18×10^{-7}	*ABO*[a,b], *GBGT1*[b], *SURF6*[b], *MED22*[b]
rs529565[d]	TG+TM_Peak	9	T	8.04×10^{-5}	0.415	2.32×10^{-4}	0.342	7.55×10^{-8}	*ABO*[a,b], *GBGT1*[b], *SURF6*[b], *MED22*[b]
rs330061	nTMsr_VI	8	G	1.61×10^{-2}	0.472	4.25×10^{-6}	0.448	5.25×10^{-7}	*RP11-115J16.1*[a], *RP11-62H7.2*[b], *RP11-10A14.5*[b], *MSRA*[b], *MFHAS1*[b], *RP11-10A14.3*[b], *SGK223*[b]
rs2027169	nTMsr_VI	10	G	1.74×10^{-4}	0.104	3.49×10^{-4}	0.337	2.32×10^{-7}	*SEPHS1*[b], *BEND7*[b], *PHYH*[b], *RP11-295P9.3*[b]

Abbreviations: ETP, endogenous thrombin potential; nTMsr, normalized thrombomodulin sensitivity ratio; TG+TM, thrombin generation in the presence of thrombomodulin; TG-TM, thrombin generation in the absence of thrombomodulin; TTP, time to peak; VI, velocity index.

[a]Genes in close proximity to thrombin generation associated SNPs.

[b]eQTL effect of thrombin generation associated SNPs based on publicly available databases.

[c]Effect allele meta-analysis.

[d]SNPs that have been previously associated with thrombosis phenotype.

for coregulation of coagulation and plasminogen activation potential with the host immune system at the genetic level: different immune pathways were enriched for TG and PG traits in the Tanzanians, with 'type 1 interferon signaling' being the top enriched pathway. A recent study in mice reported the importance of type I interferon in linking innate immunity and coagulation.[68] Recently, we demonstrated in the same cohort that urban-living dwellers had increased cytokine production capacity compared with rural dwellers.[32] However, concentrations of plasma pro-inflammatory cytokines were similar between urban and rural dwellers, which may explain why no differences in TG or PG parameters were found. Altitude may also affect coagulation potential. Hypobaric hypoxia has been reported to increase thrombin generation.[69] Most of the rural dwellers resided on the slopes of Mount Kilimanjaro and therefore at a higher altitude than the rural dwellers. However, the absence of a difference in TG and PG between urban and rural dwellers argues against an important effect of altitude on the coagulation parameters in our study.

An interesting observation was the limited overlap of the genetic factors that regulate coagulation and plasminogen activation potential between Tanzanians and individuals of Western-European ancestry. These findings are in line with data from previous studies from the United Kingdom and the USA that showed that African ancestry was associated with a hypercoagulable state compared with non-African ancestry.[13] African ancestry was also reported to be associated with higher plasma fibrinogen[11,70] and lower plasminogen activator inhibitor-1 (PAI-1) concentrations.[11,71] Plasma fibrinogen and PAI-1 concentrations are known to influence TG and PG measurements. Unfortunately, fibrinogen and PAI-1 plasma concentrations were not available in our cohorts.

Another interesting observation of our study was the association of seasonality with PG parameters, which were related to differences in food-derived metabolites. We recently showed a similar seasonal trend for inflammation and cytokine production capacity in the same cohort, whereby individuals enrolled in the dry season exhibited less inflammation.[32] The incidence of VTE in Europe has a seasonal variation with a higher incidence in the winter months.[72–75] However, data on seasonal variation in coagulation profiles in African populations are not available. The region of Tanzania where the study was performed is located at 3° south of the Equator, but has a clear seasonal variation in precipitation with a wet and dry season. The dry season coincides with the main harvest time when people eat more fresh foods. In contrast, participants enrolled in the wet season consume a more Western-style diet and exhibit higher cytokine production and a plasma metabolome enriched in metabolic pathways, such as cholesterol metabolism.[32] Our present findings are also consistent with the results of a large community-based cohort study in the USA, which showed that a Western pattern diet was associated with a higher VTE risk,[76] and a recent study that showed that a high-fat diet delays PG in mice.[37]

A limitation of our study was the fact that the cohorts differed in some of the characteristics, e.g., Tanzanians had a higher median age and Tanzanian women had a higher BMI and less frequently used hormonal contraceptives than Dutch women. Due to these differences, all analyses were corrected for age or BMI, sex, and hormonal contraceptive use. Second, even though both cohorts are part of the Human Functional Genomics Project and study protocols were aligned as much as possible, differences in storage time and freeze-thawing cycles cannot be completely excluded and may have influenced the results.[77] We, therefore, validated our results by comparing TG parameters with existing data from another Dutch cohort (50FG) with comparable mean age, sample storage time, and freeze-thaw cycles as the Tanzanian cohort, and the increased TG potential in the Tanzanian cohort persisted.

In conclusion, our study shows pronounced differences in coagulation and plasminogen activation potential between healthy individuals from East Africa and Western Europe. These differences can be partly explained by differences in the host, environmental, and genetic factors that regulate coagulation and plasminogen activation potential. Our findings support the importance of a better understanding of geographic variability in coagulation across populations.

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CONFLICT OF INTEREST

J.W., D.H., B.d.L., and M.R., are employed by Synapse Research Institute, which is a member of the Stago group that markets the Calibrated Automated Thrombography. The other authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Q.d.M., A.V., M.G.N., L.A.B.J. and B.T.M. contributed to the conceptualization, study design and data interpretation and led the project; G.S.T., V.K., and M.J. contributed to participant recruitment, data collection and laboratory analyses; J.W., contributed to thrombin and plasmin generation measurements; N.V., G.S.T., and T.P., contributed to the formal analysis and analytical integration with metabolome data and interpretation; N.V., V.M., C.K.B. and V.K., contributed to genetics analysis and interpretation; G.S.T., N.V., and Q.d.M. wrote the original draft of the manuscript; and G.S.T., N.V., W.B., J.W., V.K., T.P., V.K., D.H., T.P., M.J., C.K.B., V.M., L.A.B.J., S.M.H.F., P.G.G., J.L.S., A.V., V.K., B.T.M., B.L., M.G.N., M.R., and Q.d.M. contributed to writing and editing the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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