

## ASSOCIATION STUDIES ARTICLE

# A genetic association study of activated partial thromboplastin time in European Americans and African Americans: the ARIC Study

Lu-Chen Weng<sup>1</sup>, Mary Cushman<sup>2</sup>, James S. Pankow<sup>1</sup>, Saonli Basu<sup>3</sup>, Eric Boerwinkle<sup>4</sup>, Aaron R. Folsom<sup>1</sup> and Weihong Tang<sup>1,\*</sup>

<sup>1</sup>Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN 55454, USA, <sup>2</sup>Departments of Medicine and Pathology, University of Vermont, Burlington, VT 05405, USA, <sup>3</sup>Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN 55455, USA and <sup>4</sup>Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA

\*To whom correspondence should be addressed at: Division of Epidemiology and Community Health, University of Minnesota, 1300 South Second Street, WBOB 300, Minneapolis, MN 55454, USA. Tel: +1 6126269140; Fax: +1 6126240315; Email: tang0097@umn.edu

## Abstract

Reduced activated partial thromboplastin time (aPTT) is a risk marker for incident and recurrent venous thromboembolism (VTE). Genetic factors influencing aPTT are not well understood, especially in populations of non-European ancestry. The present study aimed to identify aPTT-related gene variants in both European Americans (EAs) and African Americans (AAs). We conducted a genetic association study for aPTT in 9719 EAs and 2799 AAs from the Atherosclerosis Risk in Communities (ARIC) study. Using the Candidate Gene Association Resource (CARE) consortium candidate gene array, the analyses were based on ~50 000 SNPs in ~2000 candidate genes. In EAs, the analyses identified a new independent association for aPTT in F5 (rs2239852,  $P$ -value =  $1.9 \times 10^{-8}$ ), which clusters with a coding variant rs6030 ( $P$ -value =  $7.8 \times 10^{-7}$ ). The remaining significant signals were located on F5, HRG, KNG1, F11, F12 and ABO and have been previously reported in EA populations. In AAs, significant signals were identified in KNG1, HRG, F12, ABO and VWF, with the leading variants in KNG1, HRG and F12 being the same as in the EAs; the significant variant in VWF (rs2229446,  $P$ -value =  $1.2 \times 10^{-6}$ ) was specific to the AA sample (minor allele frequency = 19% in AAs and 0.2% in EAs) and has not been previously reported. This is the first study to report aPTT-related genetic variants in AAs. Our findings in AAs demonstrate transferability of previously reported associations with KNG1, HRG and F12 in EAs. We also identified new associations at F5 in EAs and VWF in AAs that have not been previously reported for aPTT.

## Introduction

The activated partial thromboplastin time (aPTT) is a measurement of clotting time between the activation of factor XII and the formation of fibrin clot, and is an indicator of the integrity of intrinsic and common coagulation pathways (1). The aPTT is a commonly used clinical test to screen for coagulation factor deficiencies (2). A prolonged aPTT is usually due to deficiencies

of coagulation factors or acquired inhibitors, such as lupus anticoagulants (1). In contrast, subjects with a shorter aPTT often have significant higher levels of coagulation factors V, VIII, XI, XII and von Willebrand factor than subjects with normal aPTT (3). Moreover, a shorter aPTT is a risk marker for first and recurrent venous thromboembolism (VTE), reflecting that the aPTT is associated with hypercoagulability (4–7).

Received: July 22, 2014. Revised: December 17, 2014. Accepted: December 22, 2014

© The Author 2014. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

Although the heritability of aPTT from family-based studies is 0.43–0.83 (8,9), genetic factors influencing aPTT are not well understood. Two genome-wide association studies (GWAS), both including only individuals of European ancestry, have been published (10,11). They both identified variants in the histidine-rich glycoprotein (*HRG*), kininogen 1 (*KNG1*), and coagulation factor XII (*F12*) genes related to aPTT (10,11). The larger study, which pooled GWAS data from several cohorts, reported additional associations between aPTT and variants in the coagulation factor V (*F5*), coagulation factor XI (*F11*), ABO blood group (*ABO*) and ATP/GTP binding protein-like 1 (*AGBL1*) genes (11). The six lead identified loci (*KNG1*, *HRG*, *F11*, *F12* and *ABO*) altogether contributed to an 8 s difference in the mean aPTT between those with the lowest and highest number of aPTT-increasing alleles and explained up to 29% of the variance of aPTT in EAs (11). More recently, a gene-centric analysis with ~36 000 single nucleotide polymorphisms (SNPs) in 2510 women of European origin also replicated the associations of aPTT with genetic variants in *HRG*, *KNG1*, kallikrein B, plasma (Fletcher factor) 1 (*KLKB1*)/*F11*, *F12* and *ABO* gene regions (12). Among the identified genetic signals for aPTT, associations with VTE risk have recently been reported for the variants in *F11* and *KNG1* in populations of European ancestry (13,14), implying a potentially important contribution of genetic determinants of aPTT to the risk of VTE.

Although several genetic loci associated with aPTT have been reported in participants of European ancestry, to our knowledge, there are no large-scale genetic association studies to date for aPTT focusing on other ethnic populations. We aimed to identify the associations of genetic variants with aPTT in both European Americans (EAs) and African Americans (AAs) in the Atherosclerosis Risk in Communities (ARIC) study, using ~50 000 SNPs genotyped by a cardiovascular-focused candidate gene array from the National Heart, Lung, and Blood Institute (NHLBI) Candidate Gene Association Resource (CARE) consortium.

## Results

Basic characteristics of the study participants are provided in Table 1. The distributions of age and aPTT were similar between EA and AA participants.

### Genetic associations in EAs

The aPTT was significantly associated with seventy-three SNPs on the array, including those in the *F5* region on chromosome 1q23 (top independent variants: rs9332701 and rs2239852), *HRG*

and *KNG1* on chromosome 3q27 (rs9898 in *HRG* and rs710446 in *KNG1*), *F11* on chromosome 4q35 (rs4253399 and rs1593), *F12* on chromosome 5q35.3 (rs1801020) and *ABO* on chromosome 9q34.2 (rs657152 and rs8176704) (Supplementary Material, Table S1). Table 2 shows the top independent signals with aPTT in EAs. Detailed descriptions of the primary and conditional results are presented in Supplementary Material, Results, as the main findings here largely overlap with previously published GWAS reports (10–12). Regional association plots are shown in Supplementary Material, Figs. S1–S6. All of the top independent variants except rs2239852 (*F5*, intron,  $\beta = 0.24$ ,  $P$ -value =  $1.9 \times 10^{-8}$ ) were previously reported in GWAS or candidate gene studies in populations of European ancestry (10–12).

### Genetic associations in AAs

A total of 27 SNPs exceeded the significance threshold of  $P$ -value  $< 2 \times 10^{-6}$  (Supplementary Material, Table S2), including 8 SNPs on chromosome 3 (3q27), 10 on chromosome 5 (5q35.3), 8 on chromosome 9 (9q34.2), and one on chromosome 12 (12p13.3). On chromosome 3q27, two independent signals emerged based on the primary and conditional analyses: rs710446 (*KNG1*, missense, Ile581Thr,  $P$ -value =  $8.4 \times 10^{-42}$ ) and rs9898 (*HRG*, missense, Pro204Ser,  $r^2$  with rs710446 = 0,  $P$ -value =  $4.6 \times 10^{-26}$  in conditional analysis) (Table 2). Each copy of the rs710446 C allele and rs9898 T allele was associated with 1.09 and 0.90 s shorter aPTT, respectively.

On chromosome 5, the top independent variant was rs1801020 (*F12*, mRNA-utr,  $P$ -value =  $1.0 \times 10^{-84}$ , Table 2). Each copy of the rs1801020 A allele was associated with 1.55 s longer aPTT.

On chromosome 9, two independent signals emerged: rs8176722 (*ABO*, intron,  $P$ -value =  $1.6 \times 10^{-29}$ ) and rs651007 (intergenic,  $P$ -value =  $2.2 \times 10^{-10}$  in conditional analysis) (Table 2). Each copy of rs8176722 A allele and rs651007 T allele was associated with 1.37 and 0.57 s shorter aPTT, respectively.

On chromosome 12, there was one significant signal from a missense variant in the von Willebrand factor gene (*VWF*) (rs2229446, missense, Arg2185Gln,  $P$ -value =  $1.2 \times 10^{-6}$ , Table 2). Each copy of the T allele was associated with a 0.51 s longer aPTT. Regional association plots for chromosomes 3, 5, 9 are shown in Supplementary Material, Fig. S7–S10.

### Prediction of functional changes

Using the Sorting Intolerant From Tolerant (SIFT) (15) and Polymorphism Phenotyping v2 (PolyPhen-2) (16) programs, we assessed the potential influence of the identified missense variants in our study on protein function. In PolyPhen-2, Met2148Thr (rs9332701; score = 0.999) in *F5* and Arg2185Gln (rs2229446; score = 1) in *VWF* were predicted to be damaging, whereas the other missense variants were predicted to be benign. The results from SIFT were consistent with those from PolyPhen-2.

The search for all identified, independent signals in HaploReg v2 (17) showed that each identified SNP may have a role in altering regulatory motifs (rs2239852, rs710446, rs9898, rs1593, rs8176704, rs8176722, rs651007 and rs2229446), altering regulatory protein binding (rs9332701 and rs1801020), or both (rs4253399 and rs657152) (Supplementary Material, Table S3). Additionally, a few signals were predicted to be located in regions of DNase hypersensitivity sites (rs4253399, rs1801020, rs8176722 and rs651007) or regions of multiple histone markers (rs710446, rs4253399, rs1801020, rs657152, rs8176722, rs651007 and rs2229446), suggesting the potential impact of these signals on

**Table 1.** Description of study participant characteristics in ARIC

	EAs	AAs
N	9719	2799
Age (years)	54.2 ± 5.7	53.3 ± 5.8
Age range (years)	44–66	44–66
Female (%)	53.5	62.4
BMI (kg/m <sup>2</sup> )	27.0 ± 4.8	29.6 ± 6.0
aPTT (s)	29.0 ± 2.8	29.0 ± 3.1
Median aPTT (s)	28.8	28.8
Q1–Q3 aPTT (s)	27.1–30.7	27.0–30.9

Data shown are means ± standard deviations unless otherwise indicated. EAs, European Americans; AAs, African Americans; N, sample size; BMI, body mass index; s, seconds; Q1–Q3, the first to the third quartiles.

**Table 2.** Top independent signals of aPTT in ARIC by race (in bold) and the corresponding results in the other race group<sup>a</sup>

Region	SNP	Position	Gene	Function	A1/A2	EAs				AAs			
						AFA1	$\beta$ (SE)	P-value	Var%	AFA1	$\beta$ (SE)	P-value	Var%
1q24	rs2239852	167779148	F5	Intron	T/C	<b>0.34</b>	<b>0.24 (0.04)</b>	<b><math>1.9 \times 10^{-8}</math></b>	<b>0.3</b>	0.38	0.03 (0.08)	0.74	0.004
1q24	rs9332701 <sup>b,c</sup>	167751391	F5	Missense (Met2148Thr)	G/A	<b>0.05</b>	<b>0.51 (0.09)</b>	<b><math>3.2 \times 10^{-8}</math></b>	<b>0.3</b>	0.01	-0.31 (0.45)	0.50	0.02
							0.61 (0.09)	$5.4 \times 10^{-11}$	0.4				
3q27	rs710446	187942621	KNG1	Missense (Ile581Thr)	C/T	<b>0.41</b>	<b>-1.18 (0.04)</b>	<b><math>2.3 \times 10^{-197}</math></b>	<b>8.6</b>	<b>0.50</b>	<b>-1.09 (0.08)</b>	<b><math>8.4 \times 10^{-42}</math></b>	<b>6.2</b>
3q27	rs9898 <sup>c</sup>	187873321	HRG	Missense (Pro204Ser)	T/C	<b>0.34</b>	<b>-0.98 (0.04)</b>	<b><math>1.2 \times 10^{-123}</math></b>	<b>5.5</b>	<b>0.60</b>	<b>-0.90 (0.08)</b>	<b><math>1.2 \times 10^{-27}</math></b>	<b>4.1</b>
							-0.86 (0.04)	$1.1 \times 10^{-103}$	4.2		-0.85 (0.08)	$4.6 \times 10^{-26}$	3.6
4q35	rs4253399	187425088	F11	Intron	G/T	<b>0.39</b>	<b>-0.43 (0.04)</b>	<b><math>1.4 \times 10^{-25}</math></b>	<b>1.1</b>	0.11	-0.39 (0.13)	0.003	0.3
4q35	rs1593 <sup>c</sup>	187432545	F11	Intron	T/A	<b>0.12</b>	<b>0.55 (0.06)</b>	<b><math>1.2 \times 10^{-19}</math></b>	<b>0.8</b>	0.17	-0.05 (0.11)	0.64	0.01
							0.41 (0.06)	$3.0 \times 10^{-11}$	0.4				
5q35.3	rs1801020	176769138	F12	mrna-utr	A/G	<b>0.24</b>	<b>1.55 (0.04)</b>	<b><math>8.3 \times 10^{-260}</math></b>	<b>11.2</b>	<b>0.45</b>	<b>1.55 (0.08)</b>	<b><math>1.0 \times 10^{-84}</math></b>	<b>12.5</b>
9q34.2	rs657152	135129086	ABO	Intron	A/C	<b>0.37</b>	<b>-0.75 (0.04)</b>	<b><math>5.0 \times 10^{-75}</math></b>	<b>3.3</b>	0.43	-0.76 (0.08)	$2.0 \times 10^{-20}$	3.0
9q34.2	rs8176704 <sup>c</sup>	135125373	ABO	Intron	A/G	<b>0.07</b>	<b>0.20 (0.08)</b>	<b>0.01</b>	<b>0.1</b>	0.05	0.20 (0.19)	0.29	0.04
							0.81 (0.08)	$1.7 \times 10^{-22}$	0.9				
9q34.2	rs8176722	135122575	ABO	Intron	A/C	0.09	-0.45 (0.07)	$7.6 \times 10^{-11}$	0.4	<b>0.12</b>	<b>-1.37 (0.12)</b>	<b><math>1.6 \times 10^{-29}</math></b>	<b>4.4</b>
9q34.2	rs651007 <sup>c</sup>	135143696	None	Intergenic	T/C	0.23	-0.83 (0.05)	$5.0 \times 10^{-68}$	3.0	<b>0.14</b>	<b>-0.57 (0.12)</b>	<b><math>1.1 \times 10^{-6}</math></b>	<b>0.8</b>
											-0.74 (0.12)	$2.2 \times 10^{-10}$	1.4
12p13.3	rs2229446	5973333	VWF	Missense (Arg2185Gln)	T/C	0.002	0.74 (0.49)	0.13	0.02	<b>0.19</b>	<b>0.51 (0.11)</b>	<b><math>1.2 \times 10^{-6}</math></b>	<b>0.8</b>

EAs, European Americans; AAs, African Americans; A1, coded allele; A2, non-coded allele; AFA1, allele frequency of A1;  $\beta$ , mean change in aPTT in seconds per each A1 allele increment; top independent SNPs are in bold for each specific race group; Var%, variance% explained by each SNP.

<sup>a</sup>Sample size:  $n = 9660-9717$  for EAs,  $n = 2742-2799$  for AAs.

<sup>b</sup> $r^2 = 0.016$  with rs2239852 for EAs.

<sup>c</sup>The second row refers to the conditional analysis result after adjustment for the top signal in the same region.

gene regulation. In expression quantitative trait loci analysis of data from lymphoblastoid cell lines using SNPexp v1.2 (18), rs2239852 and rs9332701 were associated with expression of F5 transcription with  $P$ -value  $< 0.01$ , while rs1801020 and rs651007 were nominally associated with F12 and ABO transcription levels, respectively ( $P$ -value  $< 0.05$ ) (Supplementary Material, Table S4). No other significant associations were observed between the identified signals and expression levels of their corresponding genes.

## Discussion

Using the CARE candidate gene array data, we conducted a large-scale genetic association study for aPTT in EA and AA individuals. In EAs, we replicated previously published association studies of aPTT with signals on the F5, HRG, KNG1, F11, F12 and ABO genes (10–12). Our study further identified an independent signal on F5 (rs2239852) that has not previously been reported. In AAs, we demonstrated transferability of the associations of aPTT with variants on the HRG, KNG1 and F12 genes previously identified in EA populations (10–12); the associations of aPTT with variants on the ABO gene were also replicated at the gene level. Thus, our study confirmed the contribution of the HRG, KNG1, F12 and ABO genes to the inter-individual variation of aPTT across different ethnic populations. In addition, a new signal on VWF (rs2229446) was identified only in AAs. Furthermore, our search of functional annotation suggested that these identified loci may play a role in protein function and/or gene expression/regulation. To the best of our knowledge, this is the first study to report genetic variants related to aPTT in an AA population.

Factor V deficiency is caused by mutations in the F5 gene and will prolong the aPTT (19,20). For EAs, our study replicated the F5 signal from the previous GWAS (rs9332701) (11) and identified a new, independent signal, rs2239852. This new signal is in high linkage disequilibrium (LD) with a coding non-synonymous F5 SNP rs6030 (Met1764Val,  $r^2 = 0.96$ ). We obtained genotype data for rs6030 in ARIC from another project (genotyped by Sequenom) and evaluated the association of aPTT with rs6030. The association of rs6030 with aPTT was highly significant ( $P$ -value =  $7.8 \times 10^{-7}$ ), and the effect sizes for rs6030 and rs2239852 were similar ( $\beta = 0.21$  and  $\beta = 0.24$ , respectively). In addition, the signal for rs2239852 was abolished by the adjustment for rs6030, while that for rs9332701 was not. Together, these results suggest that the signal for rs2239852 is driven by the signal for rs6030. In contrast, we did not observe significant associations between the F5 variants and aPTT in AAs, which may result from the rare allele frequency [minor allele frequency (MAF) = 0.01 for rs9332701] and smaller effect sizes in AAs ( $\beta = 0.03$  and  $\beta = 0.14$  s for rs2239852 and rs6030, respectively).

von Willebrand disease, caused by vWF deficiency, is a common genetic disease and can prolong the aPTT (21). rs2229446 is a non-synonymous variant of VWF (Arg2185Gln), and its minor allele (T) was associated with lower vWF and factor VIII activity levels in the AA cohort from the NHLBI Exome Sequencing Project (22). The positive association between rs2229446 (T) and aPTT observed in AAs in our study is in the direction expected from the association between vWF and aPTT. This variant is rare in EAs (MAF = 0.002) and therefore failed to show association with aPTT, indicating that this signal is specific to the AA population.

In this study, the strongest association was observed between aPTT and a F12 variant (rs1801020) in both EAs and AAs, explaining 11%–12% of total variance in the two ethnic groups. The F12

variant rs1801020 is located on the 5' untranslated exonic coding region of the F12 gene and has been associated with factor XII concentration (23), ischemic stroke (24), acute coronary artery disease (25) and venous thrombosis (26). This SNP was associated with aPTT in a UK candidate gene study (12) and is in high LD with the top variants in published GWASs (rs2731672:  $r^2 = 0.87$ ; rs2545801:  $r^2 = 0.97$ ) (10,11), indicating the same underlying locus in the F12 region. The direction of association between F12 rs1801020 T allele and VTE risk as reported in the literature (26) is not consistent with what is expected based on the association of this SNP with factor XII (23) and aPTT.

Two strong signals related to the aPTT in both EAs and AAs were identified on chromosome 3 (KNG1 and HRG), replicating previous results in populations of European ancestry (10–12). KNG1 encodes high molecular weight kininogen (HMWK), a cofactor in the intrinsic pathway involving the activation of coagulation factor XI and factor XII, while HRG encodes histidine-rich glycoprotein in plasma and platelets. Although the function of HRG is unclear, interactions between histidine-rich glycoprotein and heparin, thrombospondin, fibrinogen, and plasminogen were observed (27). Identification of the independent contribution of the HRG genetic signal to the aPTT in both ethnic populations provides further evidence to support the role of HRG in coagulation pathways, and indicates the need for further studies to investigate the clinical relevance of HRG in thrombotic diseases.

At the ABO region, we identified two different, independent signals for aPTT between EAs and AAs. The top signal in our EAs, rs657152, replicated or was in high LD with the previously reported ABO top SNP in candidate gene or GWAS studies ( $r^2 > 0.9$  with rs687621) (10,11); and both are tag SNPs for the ABO O blood type ( $r^2 = 0.67$ ). The second independent signal, rs8176704, which also replicated the previously reported GWAS finding in EAs (11), is a perfect tag for the ABO A2 variant ( $r^2 = 1$ ). In contrast, the top SNP in AAs, rs8176722, was in low LD with the top ABO SNP in EAs (rs687621:  $r^2 = 0.26$  and rs657152:  $r^2 = 0.18$ ). Instead, rs8176722 is a better tag for the B group in AAs ( $r^2 = 0.79$ – $0.86$  with rs8176743, rs8176746, rs8176747 and rs8176749) than the O group ( $r^2 = 0.48$ ). ABO blood group is also associated with plasma levels of factor VIII and vWF (28,29), and it has been hypothesized that the ABO blood group influences plasma vWF and FVIII levels via vWF clearance rate (i.e. vWF has a shorter half-life in persons with O blood type compared with non-O) (30,31). Previous clinical, epidemiological and GWAS reports have established an association between ABO genetic variants and VTE risk, with non-O blood groups having a higher risk of VTE than the O blood group (32–34). Other studies reported the association of blood type A and AB genetic groups with a higher risk of coronary artery disease and stroke, respectively (35,36). The different associations of ABO variants with aPTT between EAs and AAs in our study suggest that specific ABO blood groups may have different contributions to the inter-individual variation of aPTT and activity of the coagulation pathways between the two ethnic populations.

In this study, the top F11 variant rs4253399 (intronic) in EAs replicated or was in high LD with previously reported GWAS findings in EAs ( $r^2 = 0.70$  with rs2289252) (10,11). This SNP was associated with VTE in a large GWAS (13) and also closely correlated with the other known SNPs for FXI level and aPTT ratio (measured aPTT/reference aPTT) in women with thrombosis (37) (rs2289252; rs2036914:  $r^2 = 0.57$ ). The aPTT association with the top F11 variant rs4253399 in EAs was replicated in AAs at nominal significance ( $P$ -value = 0.003). The effect size for this variant was similar between the two ethnic groups ( $-0.39$  s per G allele in AAs versus  $-0.43$  in EAs), while its allele frequency

was lower in AAs (0.11 in AAs versus 0.39 in EAs). This lower prevalence and slightly smaller effect size in the AA group contributed to the lower statistical significance in AAs.

Using a gene-centric array, the nine SNPs identified in our EA sample (rs2239852, rs9332701, rs710446, rs9898, rs4253399, rs1593, rs1801020, rs657152 and rs8176704) explained 30.4% of the variance in aPTT, similar to the findings from the previous GWAS (10,11). In the AAs in our study, the six identified SNPs (rs710446, rs9898, rs1801020, rs8176722, rs651007 and rs2229446) explained 28.9% of the variance in aPTT. The consistently large percentage of variance explained by the identified loci for aPTT in both ethnic populations supports the important role that genetic factors play in the inter-individual variation of aPTT at population level, and also demonstrates a good selection of SNPs in the customized candidate gene array for the aPTT analysis.

Reduced aPTT is associated with increased risk of both incident and recurrent VTE (8,9), and prolonged aPTT is used clinically to detect coagulation disorders (38). The top signals in F11 (rs4253399) (13) and KNG1 (rs710446) in our study have recently been associated with VTE risk in populations of European ancestry (14). The associations of these variants with aPTT in AAs in this study support pursuit of further studies to investigate the genetic architecture of VTE in AAs, which is a poorly understood area.

There are some limitations in our analysis. Firstly, our main analysis only focused on SNPs from the CARE candidate gene array, and therefore may miss signals from genetic variants that were not included. However, the analyses of both AAs and EAs replicated the previous GWAS findings and also identified additional signals, which increased the explained variation of aPTT in both ethnic groups. Secondly, we did not evaluate interactions between genetic variants. The previous GWAS identified an interaction of KNG1 variants with HRG, F11 or F12 variants on aPTT (11), which supports the hypothesis that genetic interactions exist in the coagulation system. Further studies of gene effects and gene-gene interactions on aPTT, as it is a global measure, may help improve the understanding of genetic influences in the coagulation pathways. Finally, our study only evaluated the associations of aPTT with genetic variants in EAs and AAs. Since the allele frequencies of identified variants may vary across different ethnicities, additional analysis is required to investigate the generalizability of these identified genetic determinants of aPTT to other ethnic groups.

## Summary

This is the first study we are aware of to identify the genetic variants related to aPTT in AAs. Our study confirmed previously reported associations of aPTT in EAs and also observed most of these associations in AAs. One signal identified on F5 in EAs and one on VWF in AAs represent new genetic associations for aPTT. Future studies are needed to confirm these newly identified signals in other populations and to investigate the biological mechanisms by which the confirmed genetic loci influence the activity of coagulation pathways.

## Materials and Methods

### Study population

The ARIC Study is a prospective cohort study designed to investigate the etiology and natural history of atherosclerosis in four US communities (39). A total of 11 478 EA and 4266 AA participants aged 45–64 years were enrolled at baseline between 1987

to 1989 in the four ARIC field centers: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Follow-up exams were conducted periodically to draw fasting blood samples and obtain information on demographic and clinical characteristics. Blood samples collected at baseline or exam 2 were used for genotyping. Each participating field center received approval from its institutional review board, and all participants gave informed consent.

### Phenotype measurement

aPTT was tested at baseline in thawed plasma from the whole ARIC cohort within few weeks after blood collection (40). aPTT was measured using an automated coagulometer with a rabbit brain phospholipid reagent (Coag-A-Mate X-2, General Diagnostics, Morris Plains, NJ) following a standard protocol. The overall reliability coefficient of repeated measurements from a sample of 39 subjects over several weeks was 0.92 for aPTT (40), and the laboratory coefficient of variation estimated from 816 duplicate participant samples was 3% (41). The total numbers of individuals who have aPTT measurements were 11 353 and 3966 in EAs and AAs, respectively. Outliers that were larger or smaller than six standard deviations from the mean were excluded from analyses.

### Genotyping

In both EAs and AAs in ARIC, a total of 49 320 SNPs were genotyped on the CARE ITMAT-Broad-CARE (IBC) genotyping array at the Broad Institute of Harvard and the Massachusetts Institute of Technology. This array was customized to capture a selected set of ~50 000 SNPs in ~2000 candidate gene loci related to cardiovascular, sleep, lung, metabolic and inflammatory pathways and syndromes (42), which provides additional information than standard GWAS chip. Functional SNPs or tag SNPs that captured LD in corresponding loci were given a high priority in selection into the array. Details on SNP selection were described in previous publication (42). A set of 24 markers that were also on the IBC array, including a gender confirmation assay, were genotyped by another platform to serve as a genetic fingerprint. Exclusion criteria for samples included sex mismatch, duplication, >3 discordant fingerprint assays, genotyping rate < 95%, missing ethnicity, haploid heterozygous calls, extreme heterozygosity values, low-level identical by descent (IBD)/identical by state (IBS) sharing by many samples, and genetic outliers. Filters for SNPs included genotyping success rate < 95%, SNP missingness that can be predicted using surrounding haplotypes, and SNPs associated with chemistry plate or that caused heterozygous haploid calls. In addition, SNPs with Hardy-Weinberg Equilibrium (HWE)  $P$ -value <  $10^{-4}$  were removed. A total of 46 058 SNPs in EAs and 46 787 SNPs in AAs passed the quality control criteria. SNPs that have MAF < 0.01 were excluded from our analysis.

Participants were excluded if they were missing for genetic information ( $n = 1624$  for EAs and  $n = 571$  for AAs) or principal components for population stratification ( $n = 346$  AAs), failed to pass the QC filters ( $n = 247$  for AAs), or were outliers ( $n = 10$  for EAs and  $n = 3$  for AAs), resulting in a total of 9719 EAs and 2799 AAs for the genetic analyses.

### Statistical analysis

Linear regression of aPTT on the dosage of each SNP in an additive genetic model was conducted in each ethnic group, adjusting

for age, sex and field centers. Additionally, in AAs the first 10 principal components were estimated by EIGENSTRAT and added into the regression model as covariates to control for potential influence of population stratification (43). These 10 principal components were calculated with default parameters in the program smartpca using the cleaned IBC genotyped data from the CARE samples together with the HapMap populations (CEU, YRI, CHB+JPT) who were all genotyped on IBC. The association analyses were performed using PLINK version 1.0.7 (<http://pngu.mgh.harvard.edu/~purcell/plink/>) (44). To account for multiple testing and correlation among the SNPs, the array-wide significance threshold were set at  $P$ -value  $< 2 \times 10^{-6}$  (45). When multiple signals were observed in the same region, conditional analysis was performed to examine the independence of the rest of the signals from the top one. The top SNP with smallest  $P$ -value in that region was added as covariate into the regression model sequentially until all remaining significant signals were abolished. Correlation between SNPs was represented by the LD statistic  $r^2$ . The  $r^2$  for EAs was calculated based on the HapMap Phase 3 CEU data, and that for AAs was based on a combined African and African Americans samples from HapMap Phase 3.

### Functional annotation

Two web-based tools, SIFT (15) and PolyPhen-2 (16), were queried to evaluate the impact of amino acid changes on protein function for the functional SNPs identified in our analysis. A SIFT score  $< 0.05$  was suggested to affect protein function; the PolyPhen-2 score ranged from 0 to 1 and was classified as benign (low), possibly damaging and probably damaging (high). Only rs6030, rs710446 and rs9898 were included in SIFT. In addition, HaploReg v2 (17) was queried to investigate the functional annotation of all identified signals, including predicted chromatin state, conservation across mammals and effect of the SNPs on regulatory motifs. Moreover, using gene expression data of lymphoblastoid cell lines from 210 unrelated individuals in the HapMap phase II (release 23) project, we evaluated the association between gene expression level and each of the identified top SNPs in additive genetic model by the web tool SNPexp v1.2 (18).

### Supplementary Material

Supplementary Material is available at HMG online.

### Acknowledgements

The authors would like to thank the University of Minnesota Supercomputing Institute for use of the blade and Calhoun supercomputers and thank the staff and participants of the ARIC study for their important contributions. Additionally, the authors wish to acknowledge the support of the National Heart, Lung, and Blood Institute and the contributions of the research institutions, study investigators, field staff and study participants in creating this resource for biomedical research.

*Conflict of Interest statement.* None declared.

### Funding

This work was supported by National Heart, Lung, and Blood Institute contracts for the Atherosclerosis Risk in Communities (ARIC) Study (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, HHSN26820

1100012C). Part of this study was supported by National Heart, Lung, and Blood Institute grants (R01-HL095603, R01-HL59367). ARIC as part of CARE: CARE is supported by National Institutes of Health/National Heart, Lung, and Blood Institute (N01-HC-65226) through the Broad Institute of Harvard University and the Massachusetts Institute of Technology. A full listing of the grants and contracts that have supported individual cohorts of the CARE is provided at <http://public.nhlbi.nih.gov/GeneticsGenomics/home/care.aspx>.

### References

- Kamal, A.H., Tefferi, A. and Pruthi, R.K. (2007) How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. *Mayo Clin. Proc.*, **82**, 864–873.
- Schmaier, A.H. (2013) Laboratory Evaluation of Hemostatic and Thrombotic Disorders. In Hoffman, R., Benz, E.J. Jr, Silberstein, L.E., Heslop, H., Weitz, J. and Anastasi, J. (eds.), *Hematology: Basic Principles and Practice*. Churchill Livingstone Elsevier, Philadelphia, PA, pp. 1857–1866.
- Mina, A., Favalaro, E.J., Mohammed, S. and Koutts, J. (2010) A laboratory evaluation into the short activated partial thromboplastin time. *Blood Coagul. Fibrinolysis*, **21**, 152–157.
- Hron, G., Eichinger, S., Weltermann, A., Quehenberger, P., Halbmayer, W.M. and Kyrle, P.A. (2006) Prediction of recurrent venous thromboembolism by the activated partial thromboplastin time. *J. Thromb. Haemost.*, **4**, 752–756.
- Zakai, N.A., Ohira, T., White, R., Folsom, A.R. and Cushman, M. (2008) Activated partial thromboplastin time and risk of future venous thromboembolism. *Am. J. Med.*, **121**, 231–238.
- Legnani, C., Mattarozzi, S., Cini, M., Cosmi, B., Favaretto, E. and Palareti, G. (2006) Abnormally short activated partial thromboplastin time values are associated with increased risk of recurrence of venous thromboembolism after oral anticoagulation withdrawal. *Br. J. Haematol.*, **134**, 227–232.
- Tripodi, A., Chantarangkul, V., Martinelli, I., Bucciarelli, P. and Mannucci, P.M. (2004) A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. *Blood*, **104**, 3631–3634.
- Souto, J.C., Almasy, L., Borrell, M., Gari, M., Martinez, E., Mateo, J., Stone, W.H., Blangero, J. and Fontcuberta, J. (2000) Genetic determinants of hemostasis phenotypes in Spanish families. *Circulation*, **101**, 1546–1551.
- Warren, D.M., Soria, J.M., Souto, J.C., Comuzzie, A., Fontcuberta, J., Blangero, J., MacCluer, J.W. and Almasy, L. (2005) Heritability of hemostasis phenotypes and their correlation with type 2 diabetes status in Mexican Americans. *Hum. Biol.*, **77**, 1–15.
- Houlihan, L.M., Davies, G., Tenesa, A., Harris, S.E., Luciano, M., Gow, A.J., McGhee, K.A., Liewald, D.C., Porteous, D.J., Starr, J.M. et al. (2010) Common variants of large effect in F12, KNG1, and HRG are associated with activated partial thromboplastin time. *Am. J. Hum. Genet.*, **86**, 626–631.
- Tang, W., Schwienbacher, C., Lopez, L.M., Ben-Shlomo, Y., Oudot-Mellakh, T., Johnson, A.D., Samani, N.J., Basu, S., Gogele, M., Davies, G. et al. (2012) Genetic associations for activated partial thromboplastin time and prothrombin time, their gene expression profiles, and risk of coronary artery disease. *Am. J. Hum. Genet.*, **91**, 152–162.
- Gaunt, T.R., Lowe, G.D., Lawlor, D.A., Casas, J.P. and Day, I.N. (2013) A gene-centric analysis of activated partial thromboplastin time and activated protein C resistance using the

- HumanCVD focused genotyping array. *Eur. J. Hum. Genet.*, **21**, 779–783.
13. Tang, W., Teichert, M., Chasman, D.I., Heit, J.A., Morange, P.E., Li, G., Pankratz, N., Leebeek, F.W., Pare, G., de Andrade, M. et al. (2013) A genome-wide association study for venous thromboembolism: the extended cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium. *Genet. Epidemiol.*, **37**, 512–521.
  14. Morange, P.E., Oudot-Mellakh, T., Cohen, W., Germain, M., Saut, N., Antoni, G., Alessi, M.C., Bertrand, M., Dupuy, A.M., Letenneur, L. et al. (2011) KNG1 Ile581Thr and susceptibility to venous thrombosis. *Blood*, **117**, 3692–3694.
  15. Sim, N.L., Kumar, P., Hu, J., Henikoff, S., Schneider, G. and Ng, P.C. (2012) SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res.*, **40**, W452–W457.
  16. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S. and Sunyaev, S.R. (2010) A method and server for predicting damaging missense mutations. *Nat. Methods*, **7**, 248–249.
  17. Ward, L.D. and Kellis, M. (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.*, **40**, D930–D934.
  18. Holm, K., Melum, E., Franke, A. and Karlsen, T.H. (2010) SNPexp—a web tool for calculating and visualizing correlation between HapMap genotypes and gene expression levels. *BMC Bioinformatics*, **11**, 600.
  19. Asselta, R. and Peyvandi, F. (2009) Factor V deficiency. *Semin. Thromb. Hemost.*, **35**, 382–389.
  20. Nizzi, F.A. Jr, Sapatnekars, S. and Bick, R.L. (2002) Hereditary coagulation protein defects. In Bick, R.L. (Ed.), *Disorders of thrombosis and hemostasis: clinical and laboratory practice*. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 117–138.
  21. Lippi, G., Franchini, M., Poli, G., Salvagno, G.L., Montagnana, M. and Guidi, G.C. (2007) Is the activated partial thromboplastin time suitable to screen for von Willebrand factor deficiencies? *Blood Coagul. Fibrinolysis*, **18**, 361–364.
  22. Johnsen, J.M., Auer, P.L., Morrison, A.C., Jiao, S., Wei, P., Haessler, J., Fox, K., McGee, S.R., Smith, J.D., Carlson, C.S. et al. (2013) Common and rare von Willebrand factor (VWF) coding variants, VWF levels, and factor VIII levels in African Americans: the NHLBI Exome Sequencing Project. *Blood*, **122**, 590–597.
  23. Calafell, F., Almasy, L., Sabater-Lleal, M., Buil, A., Mordillo, C., Ramirez-Soriano, A., Sikora, M., Souto, J.C., Blangero, J., Fontcuberta, J. et al. (2010) Sequence variation and genetic evolution at the human F12 locus: mapping quantitative trait nucleotides that influence FXII plasma levels. *Hum. Mol. Genet.*, **19**, 517–525.
  24. Santamaria, A., Mateo, J., Tirado, I., Oliver, A., Belvis, R., Marti-Fabregas, J., Felices, R., Soria, J.M., Souto, J.C. and Fontcuberta, J. (2004) Homozygosity of the T allele of the 46 C→T polymorphism in the F12 gene is a risk factor for ischemic stroke in the Spanish population. *Stroke*, **35**, 1795–1799.
  25. Santamaria, A., Martinez-Rubio, A., Mateo, J., Tirado, I., Soria, J.M. and Fontcuberta, J. (2004) Homozygosity of the T allele of the 46 C→T polymorphism in the F12 gene is a risk factor for acute coronary artery disease in the Spanish population. *Haematologica*, **89**, 878–879.
  26. Tirado, I., Soria, J.M., Mateo, J., Oliver, A., Souto, J.C., Santamaria, A., Felices, R., Borrell, M. and Fontcuberta, J. (2004) Association after linkage analysis indicates that homozygosity for the 46C→T polymorphism in the F12 gene is a genetic risk factor for venous thrombosis. *Thromb. Haemost.*, **91**, 899–904.
  27. Jones, A.L., Hulett, M.D. and Parish, C.R. (2005) Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. *Immunol. Cell. Biol.*, **83**, 106–118.
  28. Gill, J.C., Endres-Brooks, J., Bauer, P.J., Marks, W.J. Jr and Montgomery, R.R. (1987) The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*, **69**, 1691–1695.
  29. Souto, J.C., Almasy, L., Muniz-Diaz, E., Soria, J.M., Borrell, M., Bayen, L., Mateo, J., Madoz, P., Stone, W., Blangero, J. et al. (2000) Functional effects of the ABO locus polymorphism on plasma levels of von Willebrand factor, factor VIII, and activated partial thromboplastin time. *Arterioscler. Thromb. Vasc. Biol.*, **20**, 2024–2028.
  30. Nossent, A.Y., van Marion, V., van Tilburg, N., Rosendaal, F.R., Bertina, R.M., van Mourik, J.A. and Eiklenboom, H.C. (2006) von Willebrand factor and its propeptide: the influence of secretion and clearance on protein levels and the risk of venous thrombosis. *J. Thromb. Haemost.*, **4**, 2556–2562.
  31. O'Donnell, J. and Laffan, M.A. (2001) The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. *Transfus. Med.*, **11**, 343–351.
  32. Germain, M., Saut, N., Greliche, N., Dina, C., Lambert, J.C., Perret, C., Cohen, W., Oudot-Mellakh, T., Antoni, G., Alessi, M.C. et al. (2011) Genetics of venous thrombosis: insights from a new genome wide association study. *PLoS One*, **6**, e25581.
  33. Heit, J.A., Armasu, S.M., Asmann, Y.W., Cunningham, J.M., Matsumoto, M.E., Petterson, T.M. and De Andrade, M. (2012) A genome-wide association study of venous thromboembolism identifies risk variants in chromosomes 1q24.2 and 9q. *J. Thromb. Haemost.*, **10**, 1521–1531.
  34. Tregouet, D.A., Heath, S., Saut, N., Biron-Andreani, C., Schved, J.F., Pernod, G., Galan, P., Drouet, L., Zelenika, D., Juhan-Vague, I. et al. (2009) Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. *Blood*, **113**, 5298–5303.
  35. Schunkert, H., Konig, I.R., Kathiresan, S., Reilly, M.P., Assimes, T.L., Holm, H., Preuss, M., Stewart, A.F., Barbalic, M., Gieger, C. et al. (2011) Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat. Genet.*, **43**, 333–338.
  36. Zakai, N.A., Judd, S.E., Alexander, K., McClure, L.A., Kissela, B.M., Howard, G. and Cushman, M. (2014) ABO blood type and stroke risk: the REasons for Geographic And Racial Differences in Stroke Study. *J. Thromb. Haemost.*, **12**, 564–570.
  37. Lunghi, B., Cini, M., Legnani, C., Bernardi, F. and Marchetti, G. (2012) The F11 rs2289252 polymorphism is associated with FXI activity levels and APTT ratio in women with thrombosis. *Thromb. Res.*, **130**, 563–564.
  38. Lusher, J.M. (1996) Screening and diagnosis of coagulation disorders. *Am. J. Obstet. Gynecol.*, **175**, 778–783.
  39. The ARIC investigators. (1989) The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. *Am. J. Epidemiol.*, **129**, 687–702.
  40. Chambless, L.E., McMahon, R., Wu, K., Folsom, A., Finch, A. and Shen, Y.L. (1992) Short-term intraindividual variability in hemostasis factors. The ARIC Study. Atherosclerosis Risk in Communities Intraindividual Variability Study. *Ann. Epidemiol.*, **2**, 723–733.
  41. Chambless, L.E., McMahon, R., Finch, A., Sorlie, P., Heiss, G., Lyles, R. and Wu, K.K. (1993) ARIC hemostasis study—III. Quality control. Atherosclerosis Risk in Communities. *Thromb. Haemost.*, **70**, 588–594.
  42. Keating, B.J., Tischfield, S., Murray, S.S., Bhangale, T., Price, T.S., Glessner, J.T., Galver, L., Barrett, J.C., Grant, S.F., Farlow, D.N.

- et al. (2008) Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. *PLoS One*, **3**, e3583.
43. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. and Reich, D. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.*, **38**, 904–909.
44. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.*, **81**, 559–575.
45. Wassel, C.L., Lange, L.A., Keating, B.J., Taylor, K.C., Johnson, A.D., Palmer, C., Ho, L.A., Smith, N.L., Lange, E.M., Li, Y. et al. (2011) Association of genomic loci from a cardiovascular gene SNP array with fibrinogen levels in European Americans and African-Americans from six cohort studies: the Candidate Gene Association Resource (CARE). *Blood*, **117**, 268–275.