

Genetic Associations for Activated Partial Thromboplastin Time and Prothrombin Time, their Gene Expression Profiles, and Risk of Coronary Artery Disease

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Activated partial thromboplastin time (aPTT) and prothrombin time (PT) are clinical tests commonly used to screen for coagulation-factor deficiencies. One genome-wide association study (GWAS) has been reported previously for aPTT, but no GWAS has been reported for PT. We conducted a GWAS and meta-analysis to identify genetic loci for aPTT and PT. The GWAS for aPTT was conducted in 9,240 individuals of European ancestry from the Atherosclerosis Risk in Communities (ARIC) study, and the GWAS for PT was conducted in 2,583 participants from the Genetic Study of Three Population Microisolates in South Tyrol (MICROS) and the Lothian Birth Cohorts (LBC) of 1921 and 1936. Replication was assessed in 1,041 to 3,467 individuals. For aPTT, previously reported associations with *KNGL1*, *HRG*, *F11*, *F12*, and *ABO* were confirmed. A second independent association in *ABO* was identified and replicated (rs8176704, $p = 4.26 \times 10^{-24}$). Pooling the ARIC and replication data yielded two additional loci in *F5* (rs6028, $p = 3.22 \times 10^{-9}$) and *AGBL1* (rs2469184, $p = 3.61 \times 10^{-8}$). For PT, significant associations were identified and confirmed in *F7* (rs561241, $p = 3.71 \times 10^{-56}$) and *PROCR/EDEM2* (rs2295888, $p = 5.25 \times 10^{-13}$). Assessment of existing gene expression and coronary artery disease (CAD) databases identified associations of five of the GWAS loci with altered gene expression and two with CAD. In summary, eight genetic loci that account for ~29% of the variance in aPTT and two loci that account for ~14% of the variance in PT were detected and supported by functional data.

Activated partial thromboplastin time (aPTT) and prothrombin time (PT) are clinical tests commonly used to screen for coagulation-factor deficiencies.^{1–3} aPTT measures the clotting time between the activation of factor XII (FXII) and the formation of a fibrin clot; thus, aPTT reflects the integrity of the intrinsic and common coagulation pathways.^{2,3} PT measures the clotting time from the activation

of FVII and reflects the integrity of the extrinsic and common coagulation pathways.^{2,3} Shorter aPTT is a risk marker for incident and recurrent venous thromboembolism (VTE) in the general population,^{4,5} and activated coagulation is implicated in arterial thrombosis.⁶ An abnormal PT may reflect liver damage, deficiencies in clotting factors and vitamin K, or the use of blood thinners.³

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Table 1. Description of Basic Study Participant Characteristics: Mean ± SD or Percentage unless Otherwise Mentioned

	ARIC	MICROS	LBC1936	LBC1921	CaPS	MARTHA08	MARTHA10
Total n ^a	9,240	1,334	925	471	891	649	392
Age, in years	54.3 ± 5.7	44.7 ± 16.7	69.6 ± 0.84	79.1 ± 0.57	56.9 ± 4.3	44.4 ± 14.3	48.3 ± 16.1
Age range	45–64	9–94	68–71	78–81	48–67	1–90	7–85
% Female	52.9	57.2	49.4	59.6	0	75.8	63.3
BMI, in kg/m ²	27.0 ± 4.8	25.4 ± 4.8	27.8 ± 4.4	26.1 ± 4.2	26.7 ± 3.6	24.3 ± 4.4	25.8 ± 4.8
aPTT n	9,240	1,215	925	471	891	649	392
aPTT, in s	29.0 ± 2.9	26.6 ± 2.4	28.3 ± 2.6	0.90 ± 0.12 ^b	33.0 ± 2.8	31.3 ± 3.4	31.6 ± 3.2
Median aPTT	28.8	26.0	28.0	0.90 ^b	32.7	31.0	31.4
IQR aPTT	27.1–30.7	25.0–28.0	26.0–30.0	0.82–0.99 ^b	31.2–34.7	29.0–33.4	29.3–33.3
PT (INR) n	NA	1,218	922	443	NA	612	374
PT (INR)	NA	1.03 ± 0.12	0.96 ± 0.06	0.98 ± 0.08	NA	99.8 ± 13.5 ^c	101.4 ± 10.5 ^c
Median PT (INR)	NA	1.02	1.00	0.98	NA	99.0 ^c	101.0 ^c
IQR PT (INR)	NA	0.98–1.06	0.90–1.00	0.93–1.03	NA	91.0–107.0 ^c	94.2–108.0 ^c

BMI, body mass index; aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; IQR, interquartile range; NA, not available.

^aOverall n in the genome-wide association study of aPTT or INR after phenotype exclusions.

^baPTT ratio.

^cThe Quick value in %, which is in inverse relationship to INR.

Family-based studies have yielded heritability estimates of 0.43 to 0.83 for aPTT and 0.50 to 0.53 for PT, respectively,^{7,8} and shared genetic correlations have been observed between coagulation and thrombosis.⁹ To date, one genome-wide association study (GWAS) of aPTT, based on 542,050 genotyped SNPs in 1,477 participants of the Lothian Birth Cohorts (LBC) of 1921 and 1936 (LBC1921, LBC1936),¹⁰ has been reported. In that study, genome-wide significant associations were identified for variants at *F12* (MIM 610619), *KNG1* (MIM 612358), and *HRG* (MIM 142640).¹⁰ No GWAS has been reported to date for PT. We report a community-based GWAS and meta-analysis for aPTT and PT among participants of European ancestry and assess the functional and clinical relevance of identified loci.

The study population for aPTT comprised five community-based cohorts and two samples of individuals with VTE (Table 1). The community-based cohorts included the Atherosclerosis Risk in Communities (ARIC) study (final sample size n = 9,240; USA),¹¹ the Genetic Study of Three Population Microisolates in South Tyrol (MICROS) (n = 1,215; Italy),¹² LBC1921 (n = 471; UK), LBC1936 (n = 925; UK),¹³ and the Caerphilly Prospective Study (CaPS) (n = 891; UK).¹⁴ We studied individuals with VTE from two independent samples of the *Marseille Thrombosis Association* (MARTHA; France) study: MARTHA08 (n = 649) and MARTHA10 (n = 392). Given that the sample size for ARIC is much larger than that of the other cohorts, the ARIC cohort was analyzed as the discovery cohort and the others as replication cohorts.

PT was represented with the international normalized ratio (INR), which standardizes the PT measure across

studies. Data for INR were available from MICROS (n = 1,218), LBC1921 (n = 443), and LBC1936 (n = 922) (Table 1). All the three samples were analyzed as discovery cohorts. Data from MARTHA08 (n = 612) and MARTHA10 (n = 374), which recorded only the Quick value (a PT ratio of the coagulation time as a percentage of normal), were analyzed as replications. Each study was approved by the institutional review board of the corresponding institutes, and participants gave informed consent.

aPTT and PT were measured in plasma with the use of automated coagulometers and on the basis of standard protocols specified in the individual studies. Tests were conducted on the same day as blood collection or within a few weeks (after freezing). aPTT in seconds (s) was available in all the cohorts except LBC1921, in which only the aPTT ratio was available. The aPTT ratio was calculated as the ratio of the subject's aPTT to the reference value of the laboratory and correlates nearly perfectly ($R^2 = 0.95$) with aPTT values in LBC1936. INR was calculated as (subject's PT / mean normal PT)^{ISI}, where ISI (international sensitivity index) is a measure of the sensitivity of a particular PT reagent and is determined by the manufacturers. Additional details on the coagulation measurements are provided in Table S1 available online.

Each study independently performed assays for genome-wide SNPs, quality control, and imputation on ~2.5 million autosomal SNPs identified in the HapMap II sample with European ancestry (CEU [Utah residents with ancestry from northern and western Europe]). DNA samples were obtained from blood. The SNP arrays used were Affymetrix 6.0 in ARIC, Illumina HumHap300v2 Infinium in MICROS, Illumina Human 610_Quadv1 in

both LBC cohorts, Illumina Human 610_Quad in MARTHA08, and Illumina Human 660W_Quad in MARTHA10. Before imputation, SNPs were filtered on the basis of sample and SNP call rates, Hardy-Weinberg equilibrium p values, and minor allele frequencies. Imputation was performed with the use of MACH software^{15,16} in all cohorts for estimation of the “allele dosage.” The accuracy of imputation was reflected by the ratio of observed to expected variance (OEV) of the dosage statistic for each SNP. In CaPS, de novo replication genotyping was performed with a fluorescence-based competitive allele-specific PCR system by KBioscience (Hoddesdon, UK). Genotyping was successful in 22 out of 24 SNPs analyzed. Details are provided in Table S1. In addition, the FV Leiden variant (rs6025, MIMs 188055 and 612309) and the top SNP for aPTT on chromosome 5 (rs2545801) were genotyped in ARIC with an Illumina assay as part of the Candidate gene Association Resource (CARE) project. The CARE data for rs2545801 were analyzed for confirmation of the GWAS results for this variant, which was imputed with borderline precision in ARIC (OEV = 0.3).

The association of aPTT or INR with genotyped or imputed SNPs was tested with the use of an additive genetic model in accordance with a unified analysis plan. Outliers or users of anticoagulants were excluded. Analysis was conducted with linear regression models for studies of unrelated individuals. A score test¹⁷ was used for testing the genetic associations while accounting for familial correlations in MICROS, which is a population study that contains related individuals in extended families. All analyses were adjusted for age, sex, and other cohort-specific covariates and were performed with ProbABEL-GenABEL,¹⁸ Mach2QTL,^{15,16} or PLINK (Table S1).¹⁹

For aPTT, we performed an effective-sample-size-weighted meta-analysis to combine p values and the direction of genetic effects from the replication (MICROS, LBC1921, LBC1936, and CaPS) and discovery (ARIC) cohorts. The p value-based approach was chosen because of mixed aPTT and aPTT-ratio measurements across cohorts. For INR, a fixed-effect, inverse-variance-weighted meta-analysis approach was used to pool p values and β coefficients from MICROS, LBC1921, and LBC1936. The genomic-control correction was applied to each study before the meta-analysis. The genomic-control inflation factor λ was around 1 (0.98–1.03) for both aPTT and INR in all cohorts and in the meta-analyses. The meta-analyses were conducted with the METAL package.²⁰ The MARTHA data were not pooled with the community-based cohorts because of the possibility of different allele frequencies,²¹ linkage disequilibria (LD), and effect sizes in individuals with VTE compared to the general population.

To examine the evidence for additional independent signals in the presence of several significant associations at a given locus, we performed additional conditional analyses by adjusting for the top SNP observed in the region until there were no significant residual signals detected. LD statistics (r^2) were estimated from combined data of

the HapMap CEU and SeattleSNPs Programs for Genomic Applications (PGA) European panel. Interaction was tested between top SNPs of identified genes that have apparent biological effects (*KNG1*, *HRG*, *F11* [MIM 264900], and *F12*) and was represented by a cross-product term between dosages of two tested SNPs. Multiple testing was accounted for by Bonferroni correction.

The aPTT GWAS findings are described in this and the next six paragraphs. As shown in Figures S1 and S2 and Table S2, 331 SNPs in ARIC from six regions exceeded the genome-wide significance threshold of $p < 5 \times 10^{-8}$. These included one SNP within *F5* (MIM 612309), 107 SNPs in *KNG1/HRG*, 28 SNPs in *CYP4V2* (MIM 608614) / *KLKB1* (MIM 229000) / *F11*, 110 SNPs in *F12*, four SNPs in *C6orf10*, and 81 SNPs in *ABO* (MIM 110300). In the meta-analysis pooling data from ARIC, MICROS, LBC1936, and LBC1921 (Figures S3 and S4), a locus at chromosome 15q25.3 (three SNPs, within ATP/GTP binding protein-like 1, *AGBL1*) and another group of 29 SNPs within the *F5* region emerged as statistically significant. Associations of aPTT with lead SNPs at the seven regions are presented in Table 2, Tables S3 and S4, and Figures S5–S14.

More specifically, we confirmed independent associations with aPTT for rs710446 in *KNG1*, rs9898 in *HRG*, and rs2731672 in *F12*, previously reported in the LBC cohorts ($r^2 = 0.93$ between rs2731672 and our top SNP, rs2545801).¹⁰ We also provided confirmation with genome-wide significance for the previously reported association of the ABO O blood group with aPTT in candidate-gene studies (top SNP: rs687621, $r^2 = 0.67$ with the O functional variant).^{22,23}

At the *CYP4V2/KLKB1/F11* region, conditional analysis in ARIC identified two sets of independent signals led by two intronic variants in *F11*: rs2289252 (top SNP, $p = 4.30 \times 10^{-30}$) and rs1593 ($p = 1.25 \times 10^{-17}$). These two SNPs were in LD ($r^2 = 0.09$). After adjusting for rs2289252, rs1593 was still associated with aPTT ($p = 2.01 \times 10^{-8}$). The first locus tagged by rs2289252 was identified as suggestive by a pathway-based approach in the previously published GWAS for aPTT in LBC (top SNP: rs4253399, $p = 1.8 \times 10^{-5}$, $r^2 = 0.79$ with rs2289252).¹⁰ The second locus led by rs1593 has not previously been associated with aPTT, and it explains an additional 0.33% of the variance in aPTT in ARIC beyond what was explained by rs2289252 (Table S3).

The associations we identified for aPTT with *F5*, *C6orf10*, *AGBL1*, and a second independent locus in *ABO* have not been previously reported for aPTT. The two loci in *F5*, tagged by rs9332701 and rs6028, reside in coding regions of *F5* and are independent ($r^2 = 0.015$). In ARIC, adjustment for rs9332701 only minimally influenced the signal for rs6028 ($p = 3.84 \times 10^{-7}$ and 4.27×10^{-6} before and after adjustment, respectively). Heterozygotes for rs9332701 have 25% lower plasma FV levels.²⁴ The associations between aPTT and the two *F5* variants in this study were not due to their LD with the FV Leiden variant

Table 2. Association Parameters for Top SNP Associations in Activated Partial Thromboplastin Time Genome-wide Association Study

Region	Top SNP	Position	Gene/Var	A1/A2/AF1	Imput	Beta/SE/Var%	p Value/Direction			Comments
						ARIC	ARIC	Replication	MARTHA ^b	
						n = 9,240	n = 9,240	n ^a = 869–3,467	n = 1,041	
Achieving Genome-wide Significance in ARIC Alone										
1q24.2	rs9332701 ^c	167751391	<i>F5</i> /cns	A/G/0.95	-	0.54/0.10/0.30	$3.70 \times 10^{-8}/+$	0.62/+	NA	present study
3q27	rs710446 ^c	187942621	<i>KNG1</i> /cns	T/C/0.59	0.98–1.00	-1.19/0.04/7.92	$1.06 \times 10^{-185}/-$	$2.72 \times 10^{-34}/-$	$7.25 \times 10^{-22}/-$	significant in PGWA
3q27	rs9898 ^d	187873321	<i>HRG</i> /cns	C/T/0.65	0.87–1.00	-1.01/0.04/5.41	$1.65 \times 10^{-116}/-$	$5.03 \times 10^{-6}/-$	$2.58 \times 10^{-14}/-$	significant in PGWA
4q35	rs2289252 ^c	187444375	<i>F11</i> /intr	C/T/0.59	0.86–0.97	-0.48/0.04/1.31	$4.30 \times 10^{-30}/-$	$3.10 \times 10^{-16}/-$	$3.83 \times 10^{-5}/-$ ^e	suggestive in PGWA
4q35	rs1593 ^d	187432545	<i>F11</i> /intr	A/T/0.87	0.86–0.90	0.54/0.06/0.77	$1.25 \times 10^{-17}/+$	0.005/+	0.08/+	present study
5q33-qter	rs2545801 ^c	176773945	<i>F12</i> /pro	C/T/0.84	0.30–0.89 ^f	1.55/0.05/10.64 ^g	$4.13 \times 10^{-60}/+^h$	$3.49 \times 10^{-63}/+$	$6.20 \times 10^{-11}/+$	significant in PGWA
6p21.3	rs2050190	32447054	<i>C6orf10</i> /intr	A/G/0.66	0.94–0.99	-0.25/0.04/0.33	$1.29 \times 10^{-8}/-$	0.47/-	0.77/+	present study
9q34.1–2	rs687621 ^c	135126886	<i>ABO</i> /intr	A/G/0.65	0.99–1.00	-0.82/0.04/3.52	$3.64 \times 10^{-81}/-$	$7.25 \times 10^{-30}/-$	$5.18 \times 10^{-10}/-$	significant in PCGS
9q34.1–2	rs8176704 ^{d,i}	135125373	<i>ABO</i> /intr	G/A/0.93	0.90–1.00	0.89/0.09/1.04	$4.26 \times 10^{-24}/+$	$1.19 \times 10^{-5}/+$	0.002/+	present study
Achieving Genome-wide Significance in Meta-analysis of ARIC, MICROS, LBC1936, and LBC1921										
1q24.2	rs6028 ^d	167818306	<i>F5</i> /cs	T/C/0.69	0.97–0.99	0.23/0.04/0.25	$3.84 \times 10^{-7}/+$	0.001/+	0.59/+	present study
15q25.3	rs2469184	84992981	<i>AGBL1</i> /intr	G/A/0.55	0.99–1.00	0.16/0.04/0.15	$8.19 \times 10^{-5}/+$	$8.87 \times 10^{-6}/+$	0.35/+	present study

Beta, change in activated partial thromboplastin time (in s) per copy of the A2 allele (minor allele); Var%, percentage of variance explained; Direction (+ or -), direction of association associated with the minor allele; Var, variant of the top SNP; A1, allele 1 (major allele); A2, allele 2 (minor allele); Imput, ratio of observed to expected variance as a measure of imputation quality (range presented here, 1 for genotyped SNPs); AF1, allele frequency for A1; cns, coding nonsynonymous; NA, not available; PGWA, previously published genome-wide association study (GWAS) in Lothian Birth Cohorts (LBC); intr, intron; pro, promoter region; PCGS, previously published candidate-gene studies; cs, coding synonymous.

^an for replication: 869 is the n for rs9332701 that was only available in CaPS; n range for the other SNPs is 2,252–3,467.

^bBased on meta-analysis of MARTHA08 and MARTHA10.

^cTop SNP in that region if there is more than one independent SNP.

^dSecond independent SNP, p value after adjusting for the top SNP of the region in ARIC: 9.95×10^{-92} for rs9898, 2.01×10^{-8} for rs1593, and 4.27×10^{-6} for rs6028.

^eResult for proxy rs925451 ($r^2 = 0.81$ with rs2289252).

^f0.30 is the imputation quality score (OEI) in ARIC GWAS, OEI > 0.84 in the other cohorts.

^gBased on the results of ARIC genotyped data from the Candidate gene Association Resource (CARE) project.

^hp value < 10^{-150} based on the ARIC genotype data from the CARE project.

ⁱResults are after additionally adjusting for rs687621 in all cohorts.

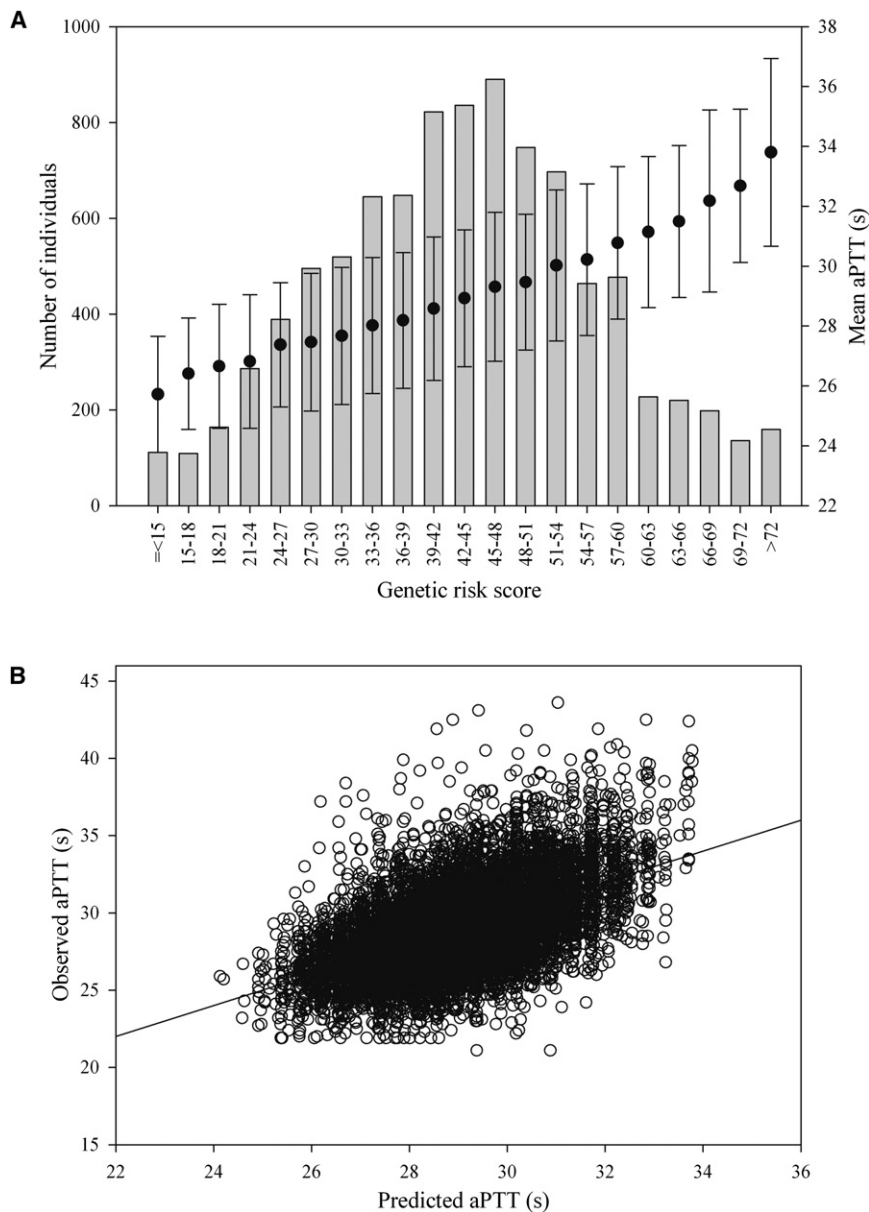


Figure 1. Cumulative Effect of the Lead SNPs in *KNG1*, *HRG*, *F11*, *F12*, and *ABO* on aPTT in ARIC

(A) Distribution of the genetic risk score (range: 0.24–85.1), and relation of risk-score categories to the mean aPTT (in s). The histogram, with reference to the y axis on the left, represents the number of individuals in each risk-score category. The black circles and error bars, with reference to the y axis on the right, indicate the mean aPTT \pm SD (in s).

(B) Predicted versus observed aPTT values. The predicted aPTT values were calculated on the basis of the regression of the observed aPTT on the genetic risk score.

in the replication samples (Table 2). The associations for rs9332701 in *F5*, rs1593 in *F11*, adjusted for rs2289252, and rs2050190 in *C6orf10* were not significantly replicated (Table 2), possibly due to limited replication data. The two associations that emerged only in the meta-analysis (rs6028 in *F5* and rs2469184 in *AGBL1*) were consistent across individual cohorts but require additional replication (Table 2 and Tables S2–S4). Of note, *AGBL1* and *C6orf10* were not previously known to affect coagulation.

In ARIC, we evaluated ten interactions for aPTT among the top SNPs in *KNG1* (rs710446), *HRG* (rs9898), *F11* (rs2289252 and rs1593), and *F12* (rs2545801). On the basis of a Bonferroni-adjusted alpha of 0.005, the interactions for rs710446 \times rs9898, rs710446 \times rs1593, and rs710446 \times rs2545801 were statistically significant (Table S5).

The interactions of rs710446 with rs9898 or with rs2545801 offset the marginal effects of the aPTT-decreasing alleles (Figure S15).

In ARIC, the six loci in *KNG1*, *HRG*, *F11*, *F12*, and *ABO*, which were replicated, together explained 28.7% of the variation in aPTT, with 6.0% attributable to the genome-wide significant associations in *F11* (rs2289252) and *ABO* (rs687621 and rs8176704) that were not reported in the previous GWAS. A genetic risk score was created by summing the product of the number of aPTT-increasing alleles and the β coefficient from the meta-analysis for the lead SNPs in the six loci, with the genetic risk score rescaled to 100%. The genetic risk score showed an accurate prediction of aPTT values (Figure 1). The Pearson correlation was 0.53 ($p < 0.0001$), and the linear-regression coefficient \pm SE was 1.00 ± 0.016 between the observed and predicted aPTT values. The mean aPTT ranged from

(rs6025), because rs6025 was neither associated with aPTT ($p = 0.54$ for rs6025 in ARIC) nor correlated with the two *F5* variants ($r^2 < 0.005$). The second independent locus at the *ABO* region for aPTT included 20 significant SNPs and emerged after additionally adjusting for the top SNP, rs687621. The associations were led by rs4363269 and rs8176704. Of the 20 significant signals, only rs8176704 is located within a gene (intronic to *ABO*, $p = 0.02$ and 4.26×10^{-24} before and after adjustment for rs68762, respectively); it tags the *ABO* A2 variant ($r^2 = 1$), is in weak LD with rs687621 ($r^2 = 0.20$), and explains an additional 1.0% of the variance in aPTT beyond what is explained by rs687621.

Among the six genome-wide significant associations for aPTT, identified in ARIC or the meta-analysis, the top signal in *F11* (rs2289252) and two independent ones in the *ABO* region (rs687621 and rs8176704) were confirmed

Table 3. p Values and Association Parameters for Top SNPs in Genome-wide Association Study for Prothrombin Time by INR

Region	Top SNP	Position	Gene/Var	A1/A2/AF1	MICROS (n = 1,218)			LBC1936 (n = 922)			LBC1921 (n = 443)			MARTHA (n = 986) ^a		
					T/C/0.71	Beta/SE/Var%	p	Beta/SE/Var%	p	Beta/SE/Var%	p	Beta/SE/Var%	p	Beta/SE/Var%	p	Beta/SE/Var%
13q34	rs561241 ^c	112808035	F7/Inear 5'		0.035/0.003/11.76	7.80 × 10 ⁻²⁷	0.040/0.004/8.78	2.75 × 10 ⁻¹⁹	0.059/0.007/12.12	1.56 × 10 ⁻¹⁵	7.05 × 10 ⁻²⁰	-7.60				
13q34	rs6046 ^c	112821160	F7/cns	G/A/0.71	0.035/0.003/11.74	7.55 × 10 ⁻²⁷	0.040/0.005/8.61	4.89 × 10 ⁻¹⁹	0.060/0.007/12.16	1.24 × 10 ⁻¹⁵	3.77 × 10 ⁻²¹	-7.99				
20q11.2	rs2295888 ^d	33186524	EDEM2/intr	A/G/0.85	-0.020/0.004/2.53	5.87 × 10 ⁻⁸	-0.018/0.005/1.60	7.77 × 10 ⁻⁵	-0.027/0.009/1.64	0.005	3.29 × 10 ⁻⁴	3.02				
20q11.2	rs867186 ^d	33228215	PROCR/cns	A/G/0.84	-0.020/0.004/2.50	9.98 × 10 ⁻⁸	-0.017/0.005/1.38	2.44 × 10 ⁻⁴	-0.024/0.009/1.37	9.6 × 10 ⁻³	3.46 × 10 ⁻⁴	2.97				

Var, variant of the top SNP; A1, allele 1 (major allele); A2, allele 2 (minor allele); AF1, allele frequency for A1; Beta, change in international normalized ratio (INR) per copy of the A2 allele (minor allele); Var%, percentage of variance explained; cns, coding nonsynonymous; intr, intron.

^aBased on meta-analysis of MARTHA08 and MARTHA10.

^bThe Quick value, which is in inverse relationship to INR, was used in MARTHA.

^{c-d}SNPs labeled with similar symbols are in high linkage disequilibrium with each other. Imputation quality scores ≥ 0.97 for all SNPs.

25.7 s in individuals in the lowest risk-score category (risk score ≤ 15, n = 111) to 33.8 s in those in the highest risk-score category (>72, n = 159). Each 11-unit increase in the genetic risk score corresponds to 1.21 s higher aPTT, which corresponds to the mean aPTT difference between VTE cases and controls.⁵

For the PT-related analyses, the INR GWAS results are presented in Table 3, Table S6, and Figures S16–S19. Two loci exceeded the genome-wide significance threshold of $p < 5 \times 10^{-8}$: 24 SNPs near or within *F7* (MIM 613878) (top SNP: rs561241, near *F7* 5', $p = 3.71 \times 10^{-56}$) and 40 SNPs near or within *EDEM2* (MIM 610302) and *PROCR* (MIM 600646) (top SNP: rs2295888, intronic to *EDEM2*, $p = 5.25 \times 10^{-13}$). Of note, rs867186, another top variant ($p = 4.65 \times 10^{-12}$), is a coding nonsynonymous variant in the endothelial protein C receptor gene (*PROCR* or *EPCR*). The lead SNPs at the two loci together account for 10%–14% of the variation in INR. The associations were significantly replicated in MARTHA (Table 3).

We next investigated pathways and networks by entering the genome-wide-significant hits in the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database.²⁵ Two networks were identified, characterizing mainly the intrinsic and extrinsic pathways for aPTT and PT, respectively. Other members of the identified networks were tested further at the gene level with the program VEGAS (Versatile Gene-based Association Study), which performs gene-based tests after taking into account of LD among SNPs.²⁶ In this pathway- and gene-based analysis (Figure S20), another gene, *PLG* (plasminogen, MIM 173350), emerged as a suggestive locus for aPTT, and *F5* emerged as a suggestive locus for INR ($p = 0.0048$ for *PLG* and 0.017 for *F5*, significant threshold = 0.0033 for aPTT genes and 0.006 for INR genes after Bonferroni correction). Plasmin, the activated form of plasminogen, not only dissolves fibrin in blood clots, but also mediates the activation of the contact coagulation system.²⁷

We next conducted gene expression association analysis to focus on SNPs with $p < 10^{-4}$ for aPTT (n = 1,124 SNPs) or INR (n = 340) from the meta-analysis. These SNPs were searched against a collected database of expression quantitative trait locus (eQTL) results on the basis of blood-derived and related organ tissues, including CD4+ lymphocytes,²⁸ monocytes,²⁹ adipose and blood samples,^{30,31} liver,^{31–33} and brain tissues (V.E., unpublished data). eQTL results were judged significant based on $p < 4 \times 10^{-5}$ and 1×10^{-4} for aPTT and INR, respectively, after accounting for multiple testing. Table 4 presents the smallest gene expression association p values for each relevant tissue at the corresponding GWAS regions. When the top GWAS SNPs were not available in the expression databases, expression SNPs (eSNPs) that correlated with the top GWAS SNPs at $r^2 \geq 0.8$ were considered as strongly supporting eSNPs. As shown in Table 4 and Tables S7 and S8, five of the GWAS loci (*F5*, *F12*, *ABO*, *F7*, and *EDEM2/PROCR*) contained at least one strongly supporting

Table 4. Selected Top Gene Expression Associations Related to Loci for aPTT or INR

eSNP	Region	Tissue	Genes Nearby ^a	p Value Expression/n	Transcript	p Value GWAS	r ² to Top GWAS SNP	Top GWAS SNP
aPTT								
rs1894702	1q24.2	CD4+ lymphocytes ²⁸	F5 ;SELP	7.59 × 10 ⁻¹⁷ /200	F5	8.11 × 10 ⁻⁹	0.92	rs6028
rs7542088		monocytes ²⁹	F5 ;SELP	4.74 × 10 ⁻¹⁴ /1,490	F5	9.37 × 10 ⁻⁹	0.96	rs6028
rs3917854		whole-blood ³⁰	F5 ;SELP	3.90 × 10 ⁻⁵⁰ /1,469	F5	3.05 × 10 ⁻⁸	0.96	rs6028
rs2731672	5q33	liver ³¹	<i>F12</i> ;GRK6; SLC34A1; PRR7; RGS14;DBN1	6.13 × 10 ⁻⁵³ /607	F12	2.43 × 10 ⁻³⁴	0.93	rs2545801
		liver ³²		1.16 × 10 ⁻²³ /472	F12			
		liver ³³		<1.00 × 10 ⁻¹⁶ /206	F12			
		liver ³³		1.52 × 10 ⁻⁶ /60	F12			
rs505922	9q34.2	CD4+ lymphocytes ²⁸	ABO ;SURF6;MED22	2.69 × 10 ⁻⁵ /200	ABO	1.04 × 10 ⁻⁹⁹	0.97	rs687621
rs2437792	15q25.3	CB ^b	AGBL1	5.34 × 10 ⁻⁵ /496	AA620749 ^c	2.66 × 10 ⁻⁷	0.87	rs2469184
INR								
rs7981123 ^d	13q34	liver ³³	F7 ; MCF2L; F10	2.78 × 10 ⁻¹⁰ /266	F7	7.24 × 10 ⁻⁵⁶	0.82	rs561241
		liver ³³		4.88 × 10 ⁻¹⁰ /206	F7			
		liver ³³		9.62 × 10 ⁻³ /60	F7			
rs488703		omental adipose ³¹	F7 ; MCF2L; F10	4.77 × 10 ⁻¹⁰ /848	F7	1.23 × 10 ⁻⁵⁵	0.88	rs561241
rs10665		subcutaneous adipose ³¹	<i>F7</i> ; MCF2L; F10	2.07 × 10 ⁻⁶ /701	F7	6.45 × 10 ⁻⁵³	0.92	rs561241
		DLPFC ^b		1.82 × 10 ⁻⁷ /583	F7			
		VC ^b		1.33 × 10 ⁻⁷ /409	F7			
rs11906318	20q11.2	liver ³¹	EDEM2 ;PROCR	3.20 × 10 ⁻²⁰ /607	PROCR	7.27 × 10 ⁻¹³	1.00	rs2295888
rs11907010		omental adipose ³¹	<i>EDEM2</i> ;PROCR	5.62 × 10 ⁻¹⁸ /848	PROCR	1.03 × 10 ⁻¹²	1.00	rs2295888
rs8119351		whole-blood ³⁰	<i>EDEM2</i> ;PROCR	2.60 × 10 ⁻⁴⁶ /1,469	EIF6	1.19 × 10 ⁻¹²	1.00	rs2295888
rs6060244		monocytes ²⁹	<i>EDEM2</i> ;TRPC4AP	5.26 × 10 ⁻²⁴ /1,490	EIF6	7.02 × 10 ⁻¹²	0.82	rs2295888

eSNP, expression SNP; GWAS, genome-wide association study; aPTT, activated partial thromboplastin time; INR, international normalized ratio; CB, cerebellum; DLPFC, dorsolateral prefrontal cortex; VC, visual cortex; EIF6, ITGB4BP.

^a"Nearby" is defined by 60,000 bp from gene boundaries; the genes are ordered by distance to the eSNP; the gene in which the top eSNP is located is in bold font ^bV.E., unpublished data.

^cAA620749 maps to unspliced and spliced expressed sequence tags that are -25–60 kb 5' of AGBL1.

^drs7981123 is positioned at -350 nt from the F7 transcription start site and is within regulatory elements based on alignment of ENCODE histone, DNase I hypersensitivity, and ChIP-sequencing data; in dbSNP, rs7981123 has merged into rs510335.

eSNP in at least one tissue, with strong support for *F5*, *F12*, *F7*, *PROCR*, and *EIF6* (eukaryotic initiation translation factor 6, MIM 602912) as important genes.

We next assessed the clinical relevance of the top ten independent SNPs associated with aPTT or INR using the CAD GWAS data from the Coronary Artery Disease Genome-wide Replication and Meta-analysis (CARDIOGRAM) study, which comprises 22,233 cases of CAD and 64,762 controls of European ancestry.^{34,35} The threshold for statistical significance was set at $p < 0.005$ for aPTT loci and $p < 0.025$ for INR after adjustment for multiple testing. As shown in Table S9, CAD was strongly and significantly associated with the proxy SNP for the O group (rs687621: odds ratio [OR] [95% confidence interval (CI)] = 1.06 [1.03–1.10] per one copy increase in the minor

allele, $p = 4.0 \times 10^{-5}$) and with the top variants at the *PROCR/EDEM2* locus (rs867186: OR [95% CI] = 0.93 [0.89–0.97], $p = 0.002$). The ABO-O association was consistent with previously published results in CARDIOGRAM.³⁴ These results highlight that the coagulation pathways represent major molecular mechanisms through which some of the recently identified CAD risk alleles³⁴ may be operating.

In this and the next four paragraphs, we offer a discussion of our results. This study provides evidence confirming the contributions of genetic factors in the *CYP4V2/CLKB1/F11* region to normal variation of aPTT. Multiple SNPs in *CYP4V2*, *CLKB1*, and *F11* have been independently associated with deep vein thrombosis (DVT)^{36,37}, and the first top SNP for aPTT, rs2289252, was confirmed

as a strong DVT variant.^{37,38} Another SNP, rs2036914, was associated with DVT independent of rs2289252.³⁷ We noted that rs1593, the second top independent variant for aPTT, associated more strongly with rs2036914 than with rs2289252 ($r^2 = 0.17$ versus 0.09). These observations, combined with the GWAS data presented in this study, suggest that more than one locus at this region influences coagulation function and the risk of DVT.

We not only confirmed the previously reported positive association between the ABO-O blood group and aPTT,^{22,23} but also identified a second independent locus represented by rs8176704, a perfect tag for the A2 group. The genome-wide significant associations for the second locus emerged after adjustment for the top SNP, rs687621, a tag for the O group. The adjustment substantially improved the signal for rs8176704, due to the existence of negative confounding between the two variants. There is a modest LD between the two variants ($r^2 = 0.2$), with the minor allele of rs8176704 residing on the same ancestral haplotype block as the minor allele of rs687621 (Figure S21). These two alleles showed opposite directions of association with aPTT and, therefore, counteracted the marginal association with each other when the adjustment was not considered. ABO encodes a glycosyltransferase with three main alleles: A, B, and O. The A and B glycosyltransferases transfer N-acetyl-D-galactosamine and D-galactose to H antigen, respectively, whereas the O glycosyltransferase does not possess any enzymatic function.³⁹ The A2 subgroup is characterized by a single-base deletion near the carboxyl terminal, resulting in a protein with little or no A-type transferase activity.⁴⁰ The influence of the ABO blood group on coagulation function has been postulated to be mediated through plasma clearance of von Willebrand factor (vWF), i.e., quicker clearance of vWF in O and A2 groups.⁴¹ Both the O and A2 groups are associated with reduced risk of VTE,⁴² indicating that both loci for aPTT are clinically relevant.

The significant genetic interactions of *KNG1* with *HRG* and *F12* have not been previously reported. In the intrinsic or contact coagulation pathway, high-molecular-weight kininogen (HMWK), encoded by *KNG1*, acts as a cofactor for activation of kallikrein and FXI and FXII.⁴³ *HRG* encodes a histidine-rich glycoprotein, which is located in the plasma and platelets. The physiological function of HRG is not well understood, but it is known to interact with heparin, thrombospondin, and plasminogen.⁴⁴ Human cases with congenital deficiency of HRG manifested thrombophilia (MIM 613116).^{45,46} Mice deficient in HRG showed enhanced blood coagulation and fibrinolysis.⁴⁷ *KNG1* and *HRG* are located immediately adjacent to each other on chromosome 3. There is a high degree of structural similarity between them,⁴⁸ indicating the possibility of shared function.⁴⁸ The interaction detected in our study suggests that HRG may interact with the same receptors as HMWK in the intrinsic or contact coagulation pathway.

SNPs in *F7* were a major contributor to variation of the functional index of the extrinsic pathway, explaining

8.6%–12.2% of variance in PT (INR). One of the top variants at this region, rs6046, results in an arginine-glutamine (R353Q) substitution. It was associated with FVII antigen and activity in a published GWAS.⁴⁹ Interestingly, this variant explains more variance in INR than in FVII antigen and activity,⁴⁹ suggesting that additional mechanisms, such as the interaction of FVII with other coagulation factors in the extrinsic pathway, may underlie the observed genetic associations.

Positive associations of the minor allele of rs867186 (*PROCR* S219G) with circulating levels of protein C and soluble EPCR (sEPCR) have been reported consistently in candidate-gene^{50–52} and GWAS studies.⁵³ This variant explained 10%–13% of variation in protein C and 75% in sEPCR.^{51,53} The association between PT and the variants at this locus may be mediated through the interaction of EPCR with FVII, as the binding of activated FVII to EPCR has been observed.^{54,55} Our study has extended the previously reported genetic associations with FVII antigen and activity^{49,52} to the global functional assay of the extrinsic pathway. The association of rs867186 with CAD has been investigated in two small-sized candidate-gene studies, which observed null or opposite directions of association compared to ours.^{50,51} Our study, based on data from a larger CAD consortium, has greater statistical power. The minor allele that was associated with decreased risk of CAD in our study was also associated with increased protein C levels,^{51,53} consistent with the hypothesis of a protective effect of the protein C-activated protein C pathway on CAD risk.⁵⁶ Interestingly, the rs867186/rs2295888 locus also showed strong associations with the expression of the *ITGB4BP/EIF6*, which is involved in regulation of translation in response to extracellular signals.⁵⁷ As such, the gene expression data may point to a novel mechanism by which this locus influences coagulation and CAD risk.

In summary, we identified and replicated genome-wide significant associations at *KNG1*, *HRG*, *F11*, *F12*, and *ABO* for aPTT and at the *F7* and *PROCR/EDEM2* regions for PT. The loci identified in previous studies and in this study together explained a substantial proportion of the variance in aPTT and PT, which are important clinical tests. The underlying causal variants in *KNG1*, *HRG*, and *F12* may interact with each other in influencing coagulation and aPTT levels. This GWAS, together with gene expression and clinical CAD data, improves our understanding of the role of biological mechanisms in the regulation of hemostasis and may offer new approaches to the prevention and treatment of arterial and venous thrombosis.

Supplemental Data

Supplemental Data include 21 figures, nine tables, acknowledgments and funding information for each individual study, disclosures, and a complete list of the CARDIoGRAM members and can be found with this article online at <http://www.cell.com/AJHG/>.

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Web Resources

The URLs for data presented herein are as follows:

dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>

GeneCards, <http://www.genecards.org/>

International HapMap Project, <http://www.hapmap.org>

KBioscience genotyping services, http://www.kbioscience.co.uk/MACH_1.0_and_Mach2QTL, <http://www.sph.umich.edu/csg/abecasis/MACH/>

METAL, <http://www.sph.umich.edu/csg/abecasis/metal>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

PLINK, <http://pngu.mgh.harvard.edu/~purcell/plink>

ProbABEL and GenABEL, <http://www.genabel.org/packages>

SeattleSNPs, <http://pga.gs.washington.edu/>

STRING database, <http://string-db.org/>

UCSC Human Genome Browser Gateway, <http://genome.ucsc.edu/cgi-bin/hgGateway>

VEGAS, <http://gump.qimr.edu.au/VEGAS/>

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