

Genetic Predictors of Fibrin D-Dimer Levels in Healthy Adults

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Received November 24, 2010; accepted February 18, 2011.

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Guest editor for this article was Donna K. Arnett, PhD.

The online-only Data Supplement is available with this article at <http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.110.009480/DC1>.

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Circulation is available at <http://circ.ahajournals.org>

DOI: 10.1161/CIRCULATIONAHA.110.009480

Background—Fibrin fragment D-dimer, one of several peptides produced when crosslinked fibrin is degraded by plasmin, is the most widely used clinical marker of activated blood coagulation. To identify genetic loci influencing D-dimer levels, we performed the first large-scale, genome-wide association search.

Methods and Results—A genome-wide investigation of the genomic correlates of plasma D-dimer levels was conducted among 21 052 European-ancestry adults. Plasma levels of D-dimer were measured independently in each of 13 cohorts. Each study analyzed the association between ≈ 2.6 million genotyped and imputed variants across the 22 autosomal chromosomes and natural-log-transformed D-dimer levels using linear regression in additive genetic models adjusted for age and sex. Among all variants, 74 exceeded the genome-wide significance threshold and marked 3 regions. At 1p22, rs12029080 ($P=6.4\times 10^{-52}$) was 46.0 kb upstream from *F3*, coagulation factor III (tissue factor). At 1q24, rs6687813 ($P=2.4\times 10^{-14}$) was 79.7 kb downstream of *F5*, coagulation factor V. At 4q32, rs13109457 ($P=2.9\times 10^{-18}$) was located between 2 fibrinogen genes: 10.4 kb downstream from *FGG* and 3.0 kb upstream from *FGA*. Variants were associated with a 0.099-, 0.096-, and 0.061-unit difference, respectively, in natural-log-transformed D-dimer and together accounted for 1.8% of the total variance. When adjusted for nonsynonymous substitutions in *F5* and *FGA* loci known to be associated with D-dimer levels, there was no evidence of an additional association at either locus.

Conclusions—Three genes were associated with fibrin D-dimer levels. Of these 3, the *F3* association was the strongest, and has not been previously reported. (*Circulation*. 2011;123:1864-1872.)

Key Words: epidemiology ■ fibrin fragment D ■ genome-wide association study ■ hemostasis ■ meta-analysis ■ thrombosis

Fibrin fragment D-dimer is one of several peptides produced when crosslinked fibrin is degraded by plasmin. The amount of D-dimer in plasma is of scientific interest. Several assays of D-dimer have demonstrated their clinical usefulness in determining noninvasively the likelihood of clinically suspected venous thrombosis and pulmonary embolism.^{1,2} D-dimer levels have also been associated with the risks of subsequent arterial and venous thrombotic events in middle-aged and older adults.^{3–10}

Clinical Perspective on p 1872

Estimates of the heritability of plasma D-dimer levels range from 23% to 65% in Northern Europeans.^{11–13} Several genetic predictors of D-dimer levels have been previously reported. A 2008 publication clearly identified the association of variation within the fibrinogen gene cluster, particularly *FGA* and *FGG*, with D-dimer levels.¹⁴ There was also evidence of an association with genetic variation in 2 other fibrinolysis-related genes, urokinase plasminogen activator (*PLAU*) and plasminogen activator inhibitor 1 (*SERPINE1*). In other studies, the factor V Leiden variant (rs6025, 1691 G>A) and the factor II 20210A variant (rs1799963, G>A) have both been associated with D-dimer levels.⁸ Although genetic influences beyond genes in the clotting cascade have been proposed, none have been identified.¹⁵

The aim of this study was to identify areas of the genome that are statistically associated with plasma levels of fibrin D-dimer. To date, no large-scale genome-wide investigation of the genomic correlates of D-dimer plasma levels has been published. Using data from 21 052 European-ancestry adults, we investigated genome-wide associations between common genetic variation and plasma levels of D-dimer.

Methods

Setting

The meta-analysis was conducted in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, which includes data from several prospective, population-based cohorts of

adults in the United States and Europe.¹⁶ D-dimer measures were available from 3 of the cohorts: the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Rotterdam Study (RS). The meta-analysis also included data from 10 other studies with D-dimer measures and genome-wide markers: the British 1958 Birth Cohort (B58C) from which nonoverlapping subsets were used as controls for the Wellcome Trust Case-Control Consortium (WTCCC); the Type 1 Diabetes Genetics Consortium (T1DGC); a European asthma genetics consortium (GABRIEL); the Cooperative Research in the Region of Augsburg (KORA) F3 study; the Lothian Birth Cohorts of 1921 and 1936 (LBC1921 and LBC1936); the Orkney Complex Disease Study (ORCADES); the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER); TwinsUK; and the CROATIA-Vis study. These studies have been described elsewhere.^{17–33}

Subjects

Eligible participants for these analyses had a D-dimer measure and high-quality data from the genome-wide scans (see below), and were not using a coumarin-based anticoagulant at the time of the phenotype measurement. Participants were of European ancestry by self-report. Each study received institutional review board or ethics approval, and all participants provided written informed consent for the use of their DNA in research.

Measures

D-Dimer

Plasma measures of D-dimer were obtained at the time of cohort entry for CHS, CROATIA-Vis, KORA-F3, ORCADES, PROSPER, RS, and TwinsUK and at a follow-up visit for B58C (2002 and 2003 examinations), FHS (examination cycle 5, 1991 to 1995), LBC1921 (wave 3 in 2007 to 2008), and LBC1936 (wave 2 from 2007 to 2010). The D-dimer phenotype was measured by ELISA using monoclonal antibodies in a kit or prepared in the laboratory: ELISA assay (Hyphen, Paris, France) in B58C, CROATIA-Vis, and ORCADES¹⁷; VIDAS D-dimer (bioMérieux, Marcy Etoile, France) in LBC1921 and LBC1936 ($n=391$)¹⁸; ELISA HemosIL D-dimer (Instrumentation Laboratory, Italy) in LBC1936 ($n=162$)¹⁹; Biopool AB in PROSPER²⁰; Enzygnost D-dimer micro (Behringwerke Diagnostica, Marburg, Germany) in RS²¹; and Biopool TintElize ELISA D-dimer (Trinity Biotech, Bary, Ireland) in TwinsUK.²² Two monoclonal antibodies directed against nonoverlapping antigenic determinants were used to detect D-dimer from crosslinked fibrin but not D-monomer in CHS, FHS, and KORA-F3.^{4,23,24}

Table 1. Characteristics of the Study Participants at the Time of D-Dimer Measurement

Characteristic	B58C-WTCCC	B58C-T1DGC	B58C-GABRIEL	CHS	CROATIA-Vis	FHS
Counts	1460	2475	657	1667	884	3094
Mean age (SD), y	44.9 (0.4)	45.3 (0.3)	45.2 (0.4)	72.2 (7.5)	56.4 (15.5)	58.5 (9.7)
Men, %	50.4	48.2	47.2	41.3	42.0	46.9
European ancestry, %	100	100	100	100	100	100
Mean BMI (SD), kg/m ²	27.4 (4.9)	27.5 (4.9)	27.2 (5.0)	26.4 (6.3)	27.32 (4.3)	27.9 (5.1)
Arterial disease hx, %	UNK	UNK	UNK	0.0	11.4	10.8
Venous disease hx, %	UNK	UNK	UNK	4.4	UNK	UNK
Diabetes mellitus, %	1.9	1.3	2.0	27.5	14.4	11.4
Median D-dimer (IQR), ng/dL	154 (114–215)	157 (114–218)	166 (115–235)	133 (89–202)	201 (134–317)	317 (205–473)
Median In D-dimer (IQR), ng/dL	5.04 (4.74–5.37)	5.06 (4.74–5.38)	5.11 (4.74–5.46)	4.97 (4.49–3.10)	5.33 (4.89–5.76)	5.76 (5.32–6.16)

B58C indicates British 1958 Birth Cohort; WTCCC, Wellcome Trust Case-Control Consortium; T1DGC, Type 1 Diabetes Genetics Consortium; GABRIEL, European asthma genetics consortium; CHS, Cardiovascular Health Study; FHS, Framingham Heart Study; KORA, Cooperative Research in the Region of Augsburg; LBC, Lothian Birth Cohort; ORCADES, Orkney Complex Disease Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; BMI, body mass index; Arterial disease hx, history of myocardial infarction, angina, stroke, or transient ischemic attack; Venous disease hx, history of deep vein thrombosis or pulmonary embolism; UNK, unknown; and IQR, interquartile range. KORA-F3: arterial disease is the only myocardial infarction; venous thrombosis is the only inpatient treatment. RS: arterial disease is the only myocardial infarction. LBC1921 and LBC1936: arterial disease is cardiovascular history and history of stroke.

*In LBC1921 and LBC1936, the Vidas method measurements were available only in increments of 100 ng/dL.

Baseline measures of clinical and demographic characteristics were obtained at the time of cohort entry for CHS, CROATIA-Vis, KORA-F3, ORCADES, PROSPER, RS, and TwinsUK and at the time of phenotype measurement for B58C, FHS, LBC1921, and LBC1936. Measures, taken with standardized methods as specified by each study, included in-person measures of height and weight and self-reported treatment of diabetes mellitus and prevalent cardiovascular disease (history of myocardial infarction, angina, coronary revascularization, stroke, transient ischemic attack) or venous thrombotic disease (history of deep vein thrombosis or pulmonary embolism).

Genotyping and Imputation

Genotyping was performed with DNA collected from phlebotomy from all cohorts except B58C, which used cell lines. Genome-wide assays of single-nucleotide polymorphisms (SNPs) were conducted independently in each cohort with various Affymetrix and Illumina panels (Table 1 in the online-only Data Supplement). Genotype quality control and data cleaning, which included assessing Hardy-Weinberg equilibrium and variant call rates, were conducted independently by each study; details have been published elsewhere and are provided in Table 1 in the online-only Data Supplement.^{16,25,26}

We investigated genetic variation in the 22 autosomal chromosomes. Genotypes were coded as 0, 1, and 2, representing the number of copies of the coded alleles. Each study independently applied quality-control criteria (Table 2 in the online-only Data Supplement) and imputed their genotype data to the ≈ 2.6 million SNPs derived from the HapMap white sample from the Centre d'Etude du Polymorphisme Humain using imputation software that included MACH, Bayesian Imputation Based Association Mapping, or IMPUTE.^{27–29} Imputation results were summarized as an “allele dosage” defined as the expected number of copies of the minor allele at that SNP (a continuous value between 0 and 2) for each genotype. Each cohort calculated a ratio of observed to expected variance of the dosage statistic for each SNP. This value, which generally ranges from 0 to 1 (poor to excellent), reflects imputation quality.

Statistical Analyses

Investigators from all cohorts developed the prespecified analytic plan described below. Each study independently analyzed their genotype-phenotype data. All studies used linear regression to conduct association analyses between measured and imputed SNPs and natural-log-transformed D-dimer measures except for CROATIA-Vis, FHS, and ORCADES, which used a linear mixed-effects model to account for family relationships, and TwinsUK,

which used a score test and variance components method as implemented in Merlin.^{30–33} An additive genetic model with 1 *df* was adjusted for age and sex. In addition, CHS and PROSPER adjusted for field site or center; CROATIA-Vis, FHS, and ORCADES adjusted for generation and ancestry using principal components; and B58C adjusted for nuisance blood-draw variables.^{17,34} For each analysis, a genomic control coefficient, which estimated the extent of underlying population structure on the basis of test-statistic inflation, was used to adjust standard errors.³⁵

Within-study findings were combined across studies to produce summary results using standard meta-analytic approaches. A fixed-effects, inverse-variance weighted meta-analysis was performed, and summary *P* values and β coefficients were calculated. Parameter coefficient represents natural-log-transformed plasma D-dimer differences associated with a 1-unit change in allele dosage. All meta-analyses were conducted with MetABEL (<http://mga.bionet.nsc.ru/~yurii/ABEL>). For loci containing genes already known to be associated with D-dimer, we conducted secondary analyses, adjusting for ≥ 1 SNPs within the gene in addition to the variant with an association with D-dimer that has already been established. This allowed us to assess possible novel associations independently of previously known strong signals.

The a priori threshold of genome-wide significance was set at a value of $P=5.0 \times 10^{-8}$. When >1 SNP clustered at a locus, we chose the SNP with the smallest *P* value to represent the locus. The amount of variation explained by the top SNPs was the difference in the r^2 value when comparing a model containing only the adjustment variables, age, sex, and study-design variables with a model also containing top genome-wide significant SNPs. Each study calculated the amount of variation explained, and these estimates were combined across cohorts using sample-size weighted averages. We also identified subthreshold loci, those marked by variants with a value of $P < 1.0 \times 10^{-5}$ but greater than the level of genome-wide significance. We limited our report to those subthreshold loci at a distance of ≥ 200 kb from other reported loci.

Results

A total of 21 052 participants of European ancestry were eligible from the 13 studies. Counts of participants and their characteristics are provided in Table 1. The average age in each cohort ranged from 44.9 to 86.6 years, and 43% of the participants were men. Summary statistics for the untrans-

Table 1. Continued

KORA-F3	LBC 1921 (Vidas)	LBC 1936 (Vidas)	LBC 1936 (HemosIL)	ORCADES	PROSPER	RS	TwinsUK
1588	152	391	162	883	5076	552	2166
62.3 (10.1)	86.6 (0.4)	72.00 (0.5)	72.9 (0.3)	53.5 (15.7)	75.3 (3.4)	71.7 (8.9)	54.2 (12.4)
49.4	46.1	52.7	54.9	46.1	47.6	42.2	8.4
100	100	100	100	100	100	100	100
28.1 (4.5)	26.2 (4.2)	27.7 (4.2)	27.2 (3.4)	27.66 (4.8)	26.8 (4.2)	26.3 (3.6)	26.4 (5.0)
3.3	29.6	29.4	36.1	9.7	39.3	25.1	1.4
2.4	UNK	UNK	UNK	UNK	UNK	UNK	UNK
10.8	5.9	10.6	11.4	3.0	10.4	15.6	2.5
22.5 (15.1–43.7)	200 (100–300)*	100 (100–200)*	144 (102–222)	79 (47–124)	265 (191–383)	39.0 (22.3–74.1)	77 (56–114)
3.24 (2.71–3.78)	5.30 (4.61–5.70)	4.61 (4.61–5.30)	4.98 (4.63–5.40)	4.37 (3.85–4.82)	5.59 (5.27–5.95)	3.66 (3.10–4.30)	4.3 (4.03–4.74)

formed and natural-log-transformed D-dimer phenotype are also listed in Table 1.

Within the 13 cohorts, the genomic control coefficients were small (<1.036), suggesting negligible test statistic inflation. The Figure presents all 2 522 393 meta-analysis P

values organized by chromosome and genomic position. Among these variants, 74 exceeded the genome-wide significance threshold and marked 3 regions on 2 chromosomes: 1p22, 1q24, and 4q32. Table 2 lists the top variant for each chromosomal region. The amount of variance in the natural-log-transformed

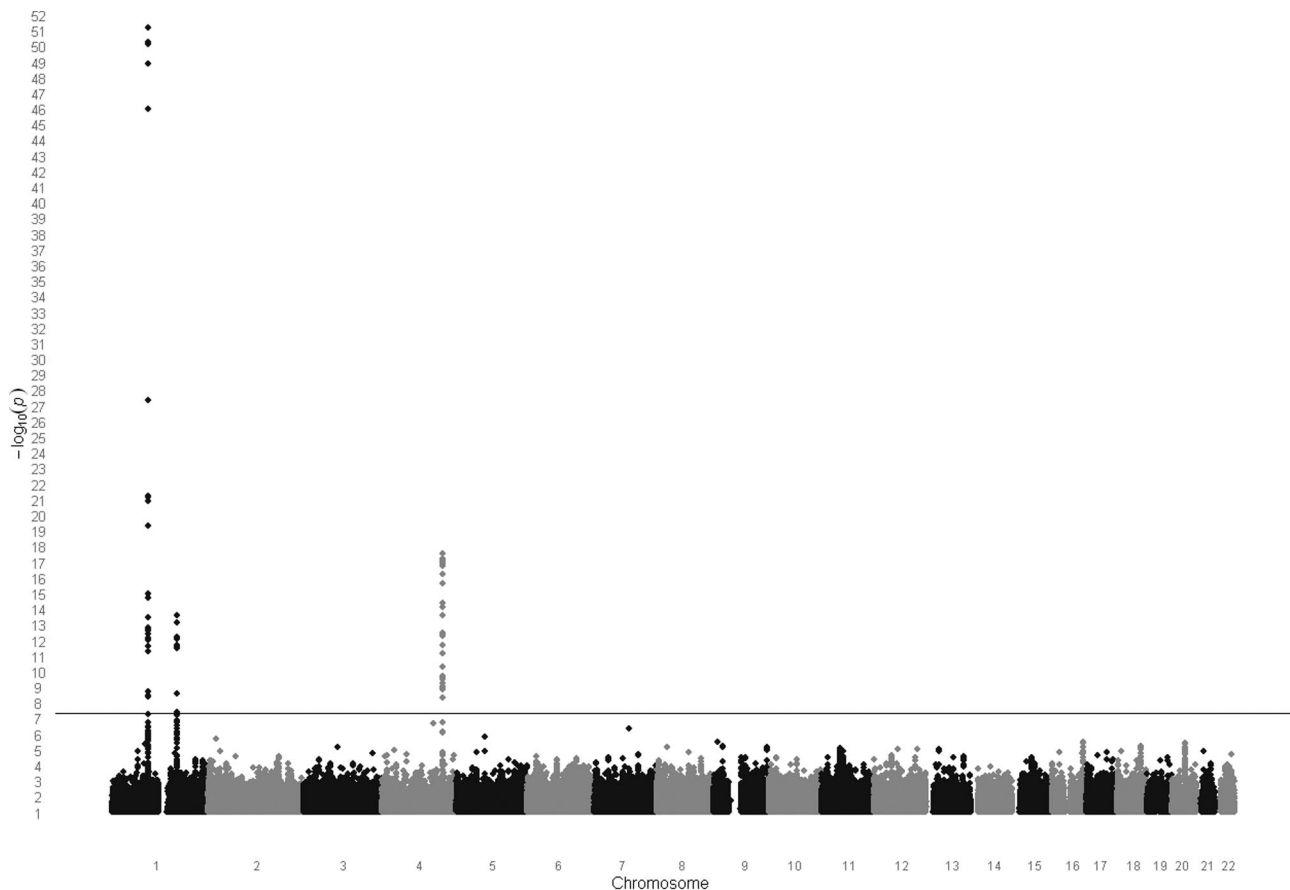


Figure. The genome-wide $\log_{10} P$ value plots of D-dimer for the 2 522 393 single-nucleotide polymorphisms meta-analyzed. The horizontal line marks the 5.0×10^{-8} P value threshold of genome-wide significance.

Table 2. Description of Single-Nucleotide Polymorphisms Associations With P Values $<5.0 \times 10^{-8}$

Region	SNP		Position	Variant	MAF	P	Parameter Coefficient,* ln (CI)	Change,† % (CI)	Closest Gene
	Significant, n	Most Significant							
1p22	29	rs12029080‡	94825941	T to G	0.305	6.4×10^{-52}	0.099 (0.064–0.134)	10.4 (6.6–14.3)	<i>F3</i> (46.0 kb upstream)
1q24	14	rs6687813§	167744198	C to A	0.059	2.4×10^{-14}	0.096 (0.027–0.165)	10.1 (2.7–17.9)	<i>F5</i> (79.7 kb downstream)
4q32	31	rs13109457‡	155734329	G to A	0.250	2.9×10^{-18}	0.061 (0.023–0.099)	6.3 (2.3–10.4)	<i>FGA</i> (3.0 kb upstream), <i>FGG</i> (10.4 kb downstream)

SNP indicates single-nucleotide polymorphism; MAF, weighted minor allele frequency; and CI, confidence interval based on a 2-sided $\alpha=0.00000005$.

*Parameter coefficient represents the change associated with a 1-unit change in allele dosage.

†Estimated percentage the change in median D-dimer associated with a 1-unit change in allele dosage.

‡ R^2 value for quality of measurement >0.90 .

§ R^2 value for quality of measurement >0.85 .

D-dimer phenotype explained by the top 3 genetic variants was 1.8% across the 13 cohorts (range, 0% to 4.2%).

We identified genome-wide significant signals at chromosomal position 1p22 (Figure I in the online-only Data Supplement). Rs12029080 was associated with the smallest P value in this region ($P=6.4 \times 10^{-52}$; minor allele frequency=0.305) and was 46.0 kb upstream from *F3*, coagulation factor III, also known as tissue factor. Each copy of the G allele was associated with a 0.099-unit (genome-wide significance confidence interval [CI], 0.064 to 0.134) increase in the natural-log-transformed D-dimer (ng/dL) or a 10.4% (CI, 6.6 to 14.3) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Figure II in the online-only Data Supplement.

A second genome-wide significant locus on chromosome 1 was found at 1q24 (Figure III in the online-only Data Supplement). Rs6687813 was associated with the smallest P value at this locus ($P=2.4 \times 10^{-14}$; minor allele frequency=0.059). Each copy of the A allele was associated with a 0.096-unit (CI, 0.027 to 0.165) increase in the natural log of D-dimer or a 10.1% (CI, 2.7 to 17.9) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Figure IV in the online-only Data Supplement. This SNP was located 79.7 kb downstream of *F5*, coagulation factor V. The rs6687813 variant is one of several weak proxies for the *F5* Leiden R506Q (rs6025) variant ($r^2=0.12$, $D'=1.0$). In a subset of 5 cohorts in which the *F5* Leiden variant was genotyped (B58C-WTCCC, CHS, CROATIA-Vis, ORCADES, and RS), we reanalyzed the data from chromosome 1 and adjusted for rs6687813 and rs6025 to estimate the amount of variation explained independently by each SNP. The P value for rs6687813 decreased from 2.5×10^{-5} to 4.4×10^{-2} when also adjusted for rs6025, and was no longer significantly associated with the phenotype. In the subset of cohorts, the *F5* R506Q (rs6025) variant was associated with a 0.22-unit (CI, 0.013 to 0.419) increase in natural-log-transformed D-dimer ($P=6.4 \times 10^{-9}$) or a 24% (CI, 1.3 to 52.0) increase in median D-dimer level. A forest plot of rs6025 is provided in Figure V in the online-only Data Supplement.

A third genome-wide significant locus was identified at chromosomal position 4q32 (Figure VI in the online-only Data Supplement). Rs13109457 had the smallest P value ($P=2.9 \times 10^{-18}$; minor allele frequency=0.250) and was

located between 2 fibrinogen genes: 10.4 kb downstream from *FGG*, fibrinogen γ chain, and 3.0 kb upstream from *FGA*, fibrinogen α chain. Each copy of the A allele was associated with a 0.061-unit (CI, 0.023 to 0.099) increase in the natural log of D-dimer or a 6.3% (CI, 2.3 to 10.4) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Figure VII in the online-only Data Supplement. The rs13109457 variant is in strong linkage disequilibrium with rs6050 ($r^2=0.96$, $D'=1.0$), which codes a missense substitution at amino acid site 331 (Thr to Ala) in *FGA*. The rs6050 SNP was associated with a value of $P=1.8 \times 10^{-17}$, and each additional risk allele of rs6050 was associated with a 0.06-unit (CI, 0.022 to 0.098) increase in natural-log-transformed plasma D-dimer or a 6.3% (CI, 2.3 to 10.4) increase in median D-dimer level. When we adjusted for age, sex, and the Thr331Ala variant, D-dimer levels were no longer associated with rs13109457 ($P=8.1 \times 10^{-1}$).

An additional 13 loci across 8 chromosomes had ≥ 1 variants with a P value that did not exceed the threshold of genome-wide significance ($P=5.0 \times 10^{-8}$), but was smaller than 1.0×10^{-5} . Details for these 13 loci, including closest reference gene, are presented in Table II in the online-only Data Supplement.

Discussion

A genome-wide investigation of the plasma fibrin D-dimer phenotype in $>20\,000$ adults of European ancestry yielded 3 loci that exceeded the threshold of significance. All 3 loci were associated with genes previously known to be involved in the coagulation cascade: *F3*, *F5*, and *FGA*. The association of genetic variation in *F3* with D-dimer is novel and has not been previously reported. The associations with *F5* and *FGA* replicate previous findings.

Genome-Wide Significant Associations

The *F3* locus, also known as tissue factor or thromboplastin, was associated with the smallest P values, and was located in the presumed regulatory region of *F3*, ≈ 46.0 kb upstream from the start of transcription. The variant with the smallest P value, rs12029080, was common, and was among 29 SNPs that reached genome-wide significance within a region that spanned 48 kb. Although very highly significant, its association with an increase in plasma levels of D-dimer was modest. The activated serine protease, factor VIIa, bound to

its cofactor, tissue factor produced by *F3*, initiates the activation of the extrinsic coagulation pathway. There has been no prior report of genetic variation in or upstream from *F3* affecting levels of D-dimer, and there are no known direct roles of tissue factor, the protein product of *F3*, on fibrin degradation. It is presumed that *F3* modulates D-dimer through the initiation of the extrinsic pathway, which leads to changes in coagulation and a subsequent modification of fibrinolysis and a change in the amount of D-dimer fragments shed. There is increasing interest in the roles of tissue factor in the initiation of hemostasis, arterial and venous thrombosis, inflammation, and tumor growth and metastasis.³⁶

The variants with the smallest *P* values tagging the *F5* and *FGA* loci were immediately downstream and upstream, respectively, from the start sites of transcription. When adjusted for 2 nonsynonymous variants known to be associated with D-dimer levels, the *F5* R506Q variant (rs6025) and the *FGA* Thr331Ala variant (rs6050), there was no longer strong evidence of an association of D-dimer levels with top SNPs at either locus; it was concluded that the rs6025 and rs6050 variants, which produce a prothrombotic environment by increasing resistance to activated protein C and by producing stiffer, more intensively crosslinked clots, respectively, likely accounted for the observed genetic signal.^{37–39}

Relevance for Cardiovascular Disease and Cardiovascular Outcomes

Activation of blood coagulation plays a key role in hemostasis and in arterial and venous thrombosis (see Figure IV in the online-only Data Supplement). Fibrin D-dimer is the most widely used clinical marker of activated blood coagulation. Epidemiological and clinical studies over the last 20 years have established its associations with risks of arterial and venous thromboembolic events and with other pathologies, including disseminated intravascular coagulation, cancer progression, and cognitive decline.^{1–10,20,40} The present study confirms a previous report from a smaller study that D-dimer levels are associated with the factor V Leiden *F5* R506Q variant (rs6025), which is associated with risks of arterial and venous thrombosis.^{8,41} Also confirmed is a previous report that D-dimer levels are associated with the *FGA* Thr331Ala variant (rs6050) variant, which is also associated with risks of arterial and venous thrombosis.^{14,42,43} However, the most important finding in this report is the novel association of D-dimer levels with variation upstream from *F3*, which produces tissue factor. Although there is considerable experimental evidence that tissue factor plays an important role in the initiation of hemostasis and arterial thrombosis, there has been very limited epidemiological evidence to date that tissue factor initiates activation of blood coagulation in generally healthy individuals.³⁶ Our demonstration that a genetic variant upstream from *F3* is associated with variation in D-dimer levels in generally healthy populations supports the concept that *F3* might be a potential therapeutic target to reduce thrombotic risk.³⁶ The measurement of plasma D-dimer has clinical utility when ruling out a pulmonary embolism diagnosis in the acute phase for symptomatic patients, but is otherwise a weak predictor of the risk of arterial and venous thrombotic events in healthy persons.^{1–10} The overall genetic

contribution of the 3 loci to D-dimer variation identified in this report was modest, <2% of variation in the phenotype, and is unlikely to have immediate applications to clinical prediction models for arterial or venous thrombosis in the general population.

Subthreshold Associations

Among the 13 subthreshold loci identified, 7 had high-signal markers in genes and another 3 had markers within 50 kb of genes. Among the 7 were 2 related to coagulation phenotypes, the ABO blood group (*ABO*) and the endothelial protein C receptor (*PROCR*). Larger sample sizes are necessary to identify whether there are genome-wide significant associations with these 13 loci.

Strengths and Limitations

This is the first genome-wide association study to attempt to discover novel genetic associations with plasma fibrin D-dimer levels. The meta-analysis included >20 000 individuals of European ancestry and examined >2.5 million markers spread throughout the genome. The novel association identified was for a variant with a *P* value that was >40 orders of magnitude smaller than the significance threshold of 5.0×10^{-8} set as significant. Genetic associations of such strength have consistently been replicated in other settings; for this reason, we did not seek replication.^{44–46} With a sample size of $\approx 21\,000$, we had 80% power (2-sided $\alpha = 5.0 \times 10^{-8}$) to detect a 0.067-unit difference in log D-dimer (mean=5, SD=1) for a variant with a minor allele frequency of 0.3. Most of the subthreshold findings had smaller differences in log D-dimer levels than the 0.067 difference. The D-dimer phenotype was measured in a standardized fashion within each cohort, but measurement methods differed between cohorts and likely introduced between-group variability.⁴⁰ This variability may decrease statistical power to find associations of smaller magnitudes. Not all SNPs tested were directly genotyped, and the imputation quality varied across SNPs and cohorts. For poorly imputed SNPs, there was reduced statistical power to detect an association. For each identified locus, we chose the SNP with the smallest *P* value, but the causal variant—if one exists—need not be the one with the smallest *P* value or may not have been measured or imputed. There was variability in the cohort-specific point estimates around the meta-analysis-estimated mean effect of each SNP. This type of variation is expected and most likely attributable to random variation in the genotype-phenotype association but may also be influenced by other unmeasured factors.

Conclusions

Using data from 13 cohorts that included 21 052 participants, we identified 3 genes associated with fibrin D-dimer levels (*F3*, *F5*, and *FGA*), of which the *F3* association has not been previously reported. The proportion of variation in D-dimer explained by these variants was modest and, although unlikely to be useful for clinical prediction, should provide further insights into the molecular pathways underlying activation of blood coagulation and possibly subsequent fibrinolysis.

Acknowledgments

We acknowledge the essential role of the CHARGE Consortium in the development and support of this manuscript. CHARGE members include National Heart, Lung, and Blood Institute's (NHLBI) Atherosclerosis Risk in Communities Study, National Institute on Aging Iceland Age, Gene/Environment Susceptibility Study, the NHLBI's CHS and the FHS, and the Netherlands RS. We also acknowledge the thousands of study participants who volunteered their time to help advance science and the scores of research staff and scientists who have made this research possible. TwinsUK would to acknowledge the essential contributions from Peter Grant and Angela Carter from the Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK, for measurements of clotting factors phenotypes. The CROATIA-Vis study would like to acknowledge the invaluable contributions of the recruitment team (including those from the Institute of Anthropological Research in Zagreb) in Vis, the administrative teams in Croatia and Edinburgh, and the people of Vis. Genotyping was performed at the Wellcome Trust Clinical Research Facility in Edinburgh. The LBC studies thank Janie Corley, Caroline Brett, Caroline Cameron, Michelle Taylor, Ross Henderson, Catherine Murray, and Alison Pattie for data collection and data entry; study secretary Paula Davies; the nurses and staff at the Wellcome Trust Clinical Research Facility, where subjects were tested and genotyping was performed; Paul Redmond for technical assistance; the staff at the Department of Hematology, Western General Hospital for the D-dimer measurements; and the staff at the Lothian Health Board and Scottish Council for Research in Education Centre, University of Glasgow. The Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/KORA Augsburg studies thank all members of the Institute of Epidemiology at the Helmholtz Zentrum München and the field staff in Augsburg who were involved in the planning and conduct of the MONICA/KORA Augsburg studies. We also thank Gerlinde Trischler (University of Ulm) for excellent technical assistance. ORCADES acknowledges the invaluable contributions of Lorraine Anderson, the research nurses in Orkney, and the administrative team in Edinburgh; DNA extractions were performed at the Wellcome Trust Clinical Research Facility in Edinburgh. The RS thanks Pascal Arp, Mila Jhamai, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the genome-wide association study database.

Sources of Funding

We acknowledge the use of phenotype and genotype data from the B58C DNA collection, funded by Medical Research Council (MRC) grant G0000934 and Wellcome Trust grant 068545/Z/02 (<http://www.b58cgene.sgul.ac.uk/>). Genotyping for the B58C-WTCCC subset was funded by Wellcome Trust grant 076113/B/04/Z. The B58C-TIDGC genotyping used resources provided by the TIDGC, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Allergy and Infectious Diseases, National Human Genome Research Institute, National Institute of Child Health and Human Development, and Juvenile Diabetes Research Foundation International and supported by U01 DK062418. B58C-TIDGC genome-wide association study data were deposited by the Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research, University of Cambridge, which is funded by Juvenile Diabetes Research Foundation International, the Wellcome Trust, and the National Institute for Health Research Cambridge Biomedical Research Centre; the Cambridge Institute for Medical Research has received a Wellcome Trust Strategic Award (079895). The B58C-GABRIEL genotyping was supported by a contract from the European Commission Framework Programme 6 (018996) and grants from the French Ministry of Research. The CHS is supported by contracts N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01-HC-15103, N01-HC-55222, N01-HC-75150, and N01-HC-45133 and grants U01 HL080295 and R01 HL 087652 from the NHLBI, with additional contribution from the National Institute of Neurological Disorders and Stroke. A full list of principal CHS investigators and institutions

can be found at <http://www.chs-nhlbi.org/pi.htm>. Support was also provided by NHLBI grants HL073410 and HL095080 and the Leducq Foundation, Paris, France, for the development of Transatlantic Networks of Excellence in Cardiovascular Research. DNA handling and genotyping were supported in part by National Center for Research Resources grant M01-RR00425 to the Cedars-Sinai General Clinical Research Center Genotyping core and National Institute of Diabetes and Digestive and Kidney Diseases grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The CROATIA-Vis study in the Croatian island of Vis was supported by grants from the Medical Research Council UK and Ministry of Science, Education and Sport of the Republic of Croatia (No. 108-1080315-0302) and the European Union framework program 6 European Special Populations Research Network project (contract LSHG-CT-2006-018947). This research was conducted in part using data and resources from the FHS of the NHLBI of the NIH and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the FHS investigators participating in the SNP Health Association Resource project. Partial investigator support was provided by National Institute of Diabetes and Digestive and Kidney Diseases K24 DK080140 (J.B. Meigs). This work was partially supported by the NHLBI's FHS (contract N01-HC-25195) and its contract with Affymetrix, Inc, for genotyping services (contract N02-HL-6-4278). A portion of this research used the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. The MONICA/KORA Augsburg studies were financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, and supported by grants from the German Federal Ministry of Education and Research. Part of this work was financed by the German National Genome Research Network (project 01GS0834) and through additional funds from the University of Ulm. The research was supported within the Munich Center of Health Sciences as part of the Ludwig Maximilians University innovative. The LBC whole-genome association study was funded by the Biotechnology and Biological Sciences Research Council (BBSRC; Reference BB/F019394/1). The LBC1936 research was supported by a program grant from Research Into Ageing (Reference 251) and continues with program grants from Help the Aged/Research Into Ageing (Disconnected Mind). The LBC1921 data collection was funded by the Chief Scientists Office of the Scottish Executive (CZB/4/505). The study was conducted within the University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, supported by the BBSRC, Engineering and Physical Sciences Research Council, Economic and Social Research Council, and MRC, as part of the cross-council Lifelong Health and Wellbeing Initiative (G0700704/84698). Dr Luciano is a Royal Society of Edinburgh/Lloyds TSB Foundation for Scotland Personal Research Fellow. Dr Visscher acknowledges support from the Australian National Health and MRC. ORCADES was supported by the Chief Scientist Office of the Scottish Government, the Royal Society, and the European Union framework program 6 European Special Populations Research Network project (contract LSHG-CT-2006-018947). The PROSPER study was supported by an investigator-initiated grant obtained from Bristol-Myers Squibb. Dr Jukema is an established clinical investigator of the Netherlands Heart Foundation (grant 2001 D 032). Support for genotyping was provided by the seventh framework program of the European commission (grant 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging grant 050-060-810). The RS is supported by the Erasmus Medical Center and Erasmus University Rotterdam; the Netherlands Organization for Scientific Research (NWO); the Netherlands Organization for Health Research and Development; the Research Institute for Diseases in the Elderly (RIDE); the Netherlands Heart Foundation; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission; and the Municipality of Rotterdam. Dr Dehghan is supported by the Netherlands Organization for Scientific Research (NWO, VICI No. 918-76-619). Support for genotyping was provided

by the Netherlands Organization of Scientific Research NWO Investments (No. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative/Netherlands Consortium for Healthy Aging project No. 050-060-810. Dr Nicole Soranzo is supported by the Wellcome Trust (Core Grant Number 091746/Z/10/Z). The TwinsUK study was funded by the Wellcome Trust; European Community's Sixth and Seventh Framework Programmes (FP-6/2005-2008) Life Sciences and Health (Reference 005268, genetic regulation of the end-stage clotting process that leads to thrombotic stroke: The EuroClot Consortium and (FP7/2007 to 2013), ENGAGE project HEALTH-F4-2007-201413, and the FP-5 GenomeUtwinn Project (QLG2-CT-2002-01254). The study also receives support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London. Dr Spector is an NIHR senior investigator. The project also received support from a Biotechnology and Biological Sciences Research Council project grant (G20234). The authors acknowledge the funding and support of the National Eye Institute via an NIH/CIDR genotyping project (principal investigator, Terri Young).

Disclosures

Dr Cushman reports receiving NIH grants. Dr Deary has received Age UK, MRC, BBSRC, and CIMEF Research Office grants. Dr Gow has received Scottish Executive Chief Scientific Office grants. Dr Porteous has received various government and research grants and Wellcome Trust honoraria. Dr Starr reports receiving LBC1921 and LBC1936 grants. Dr Tenesa has received a genome-wide association study of nonpathological cognitive decline grant. Dr Grant has received BHF Programme and FBG EuroClot grants. Dr Wright reports receiving an MRC Programme grant. Dr Campbell has received MRC and EU FBG grants. Dr Rudan has received MRC and Croatia government grants and Glaxo-Smith-Kline honoraria, and has been a Child Health and Nutrition Research Initiative board member. Dr Hastie has received an MRC Programme grant. Dr Polasek reports receiving MRC UK and Croatian Ministry of Science grants. The other authors report no conflicts.

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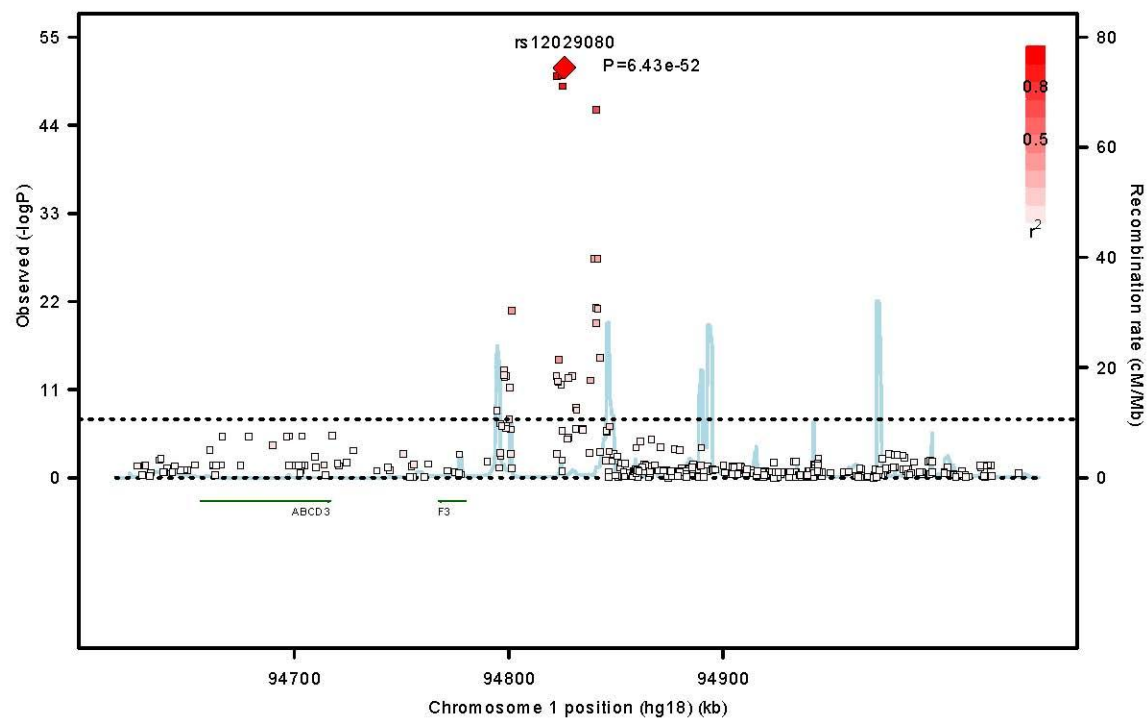
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CLINICAL PERSPECTIVE

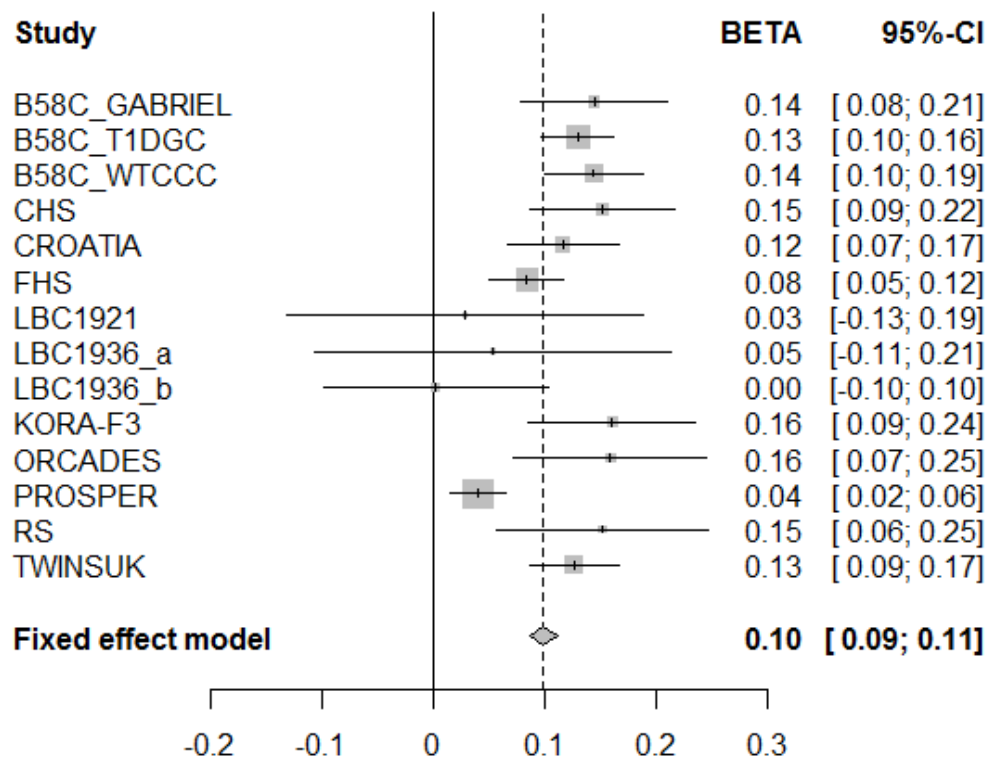
Activation of blood coagulation plays a key role in hemostasis and in arterial and venous thrombosis. Fibrin D-dimer is the most widely used clinical marker of activated blood coagulation. Epidemiological and clinical studies over the last 20 years have established its associations with risk of arterial and venous thromboembolic events and with other pathologies. This article reports a novel association of plasma D-dimer levels with variation upstream from *F3*, the gene for tissue factor. The manuscript also confirms a previous report that D-dimer levels are associated with the factor V Leiden *F5* R506Q variant (rs6025) and the *FGA* Ala331Thr variant (rs6050) variant, which are also associated with risk of arterial and venous thrombosis. Our demonstration that a genetic variant upstream from *F3* is associated with variation in D-dimer levels in generally healthy populations supports the concept that *F3* might be a potential therapeutic target to reduce thrombotic risk.

Supplemental Materials

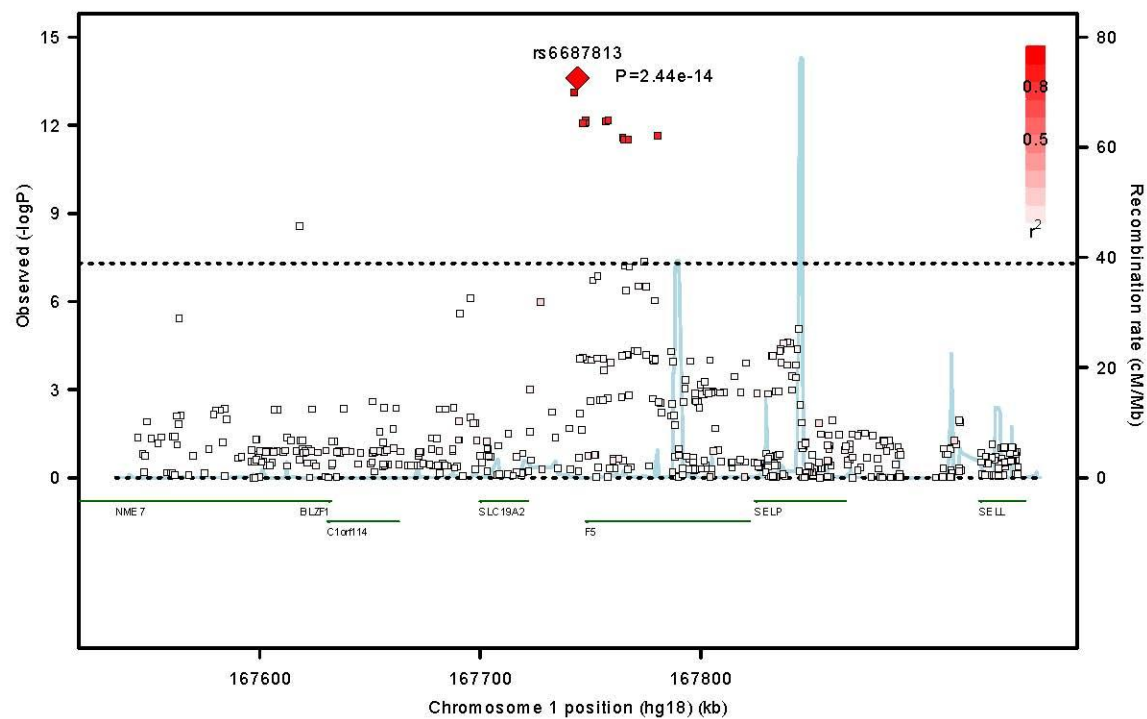
Supplemental Figure S1. Regional plot of *F3*



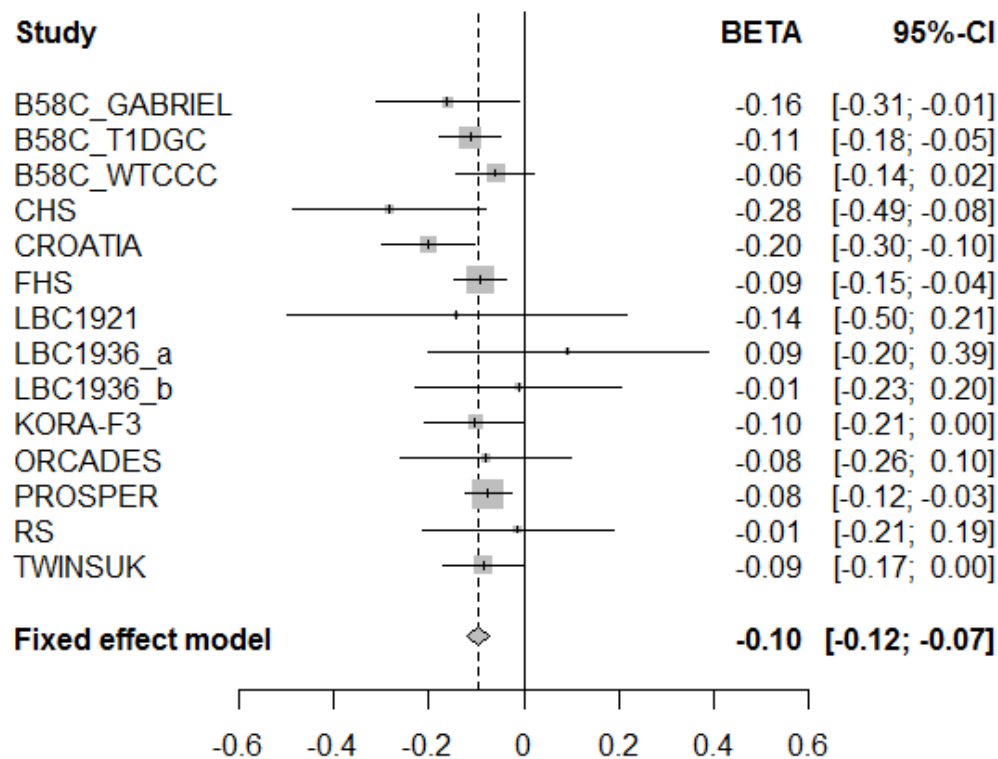
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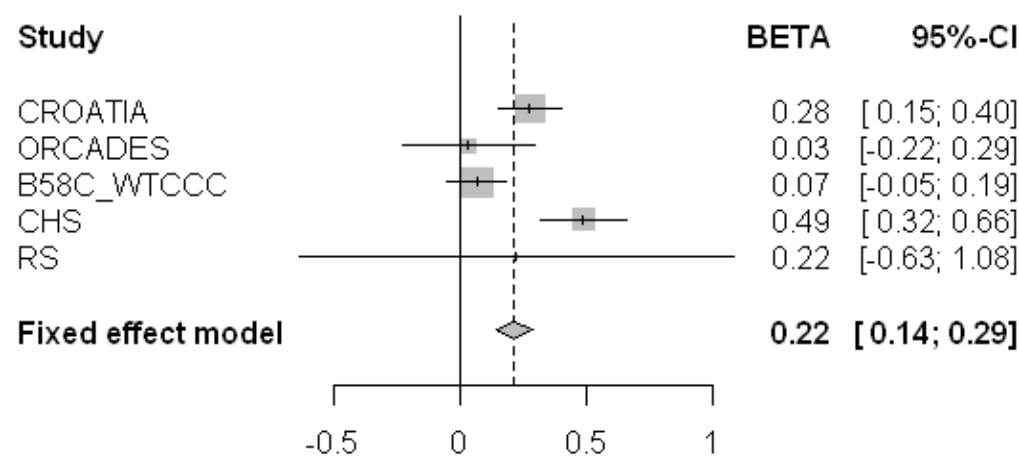
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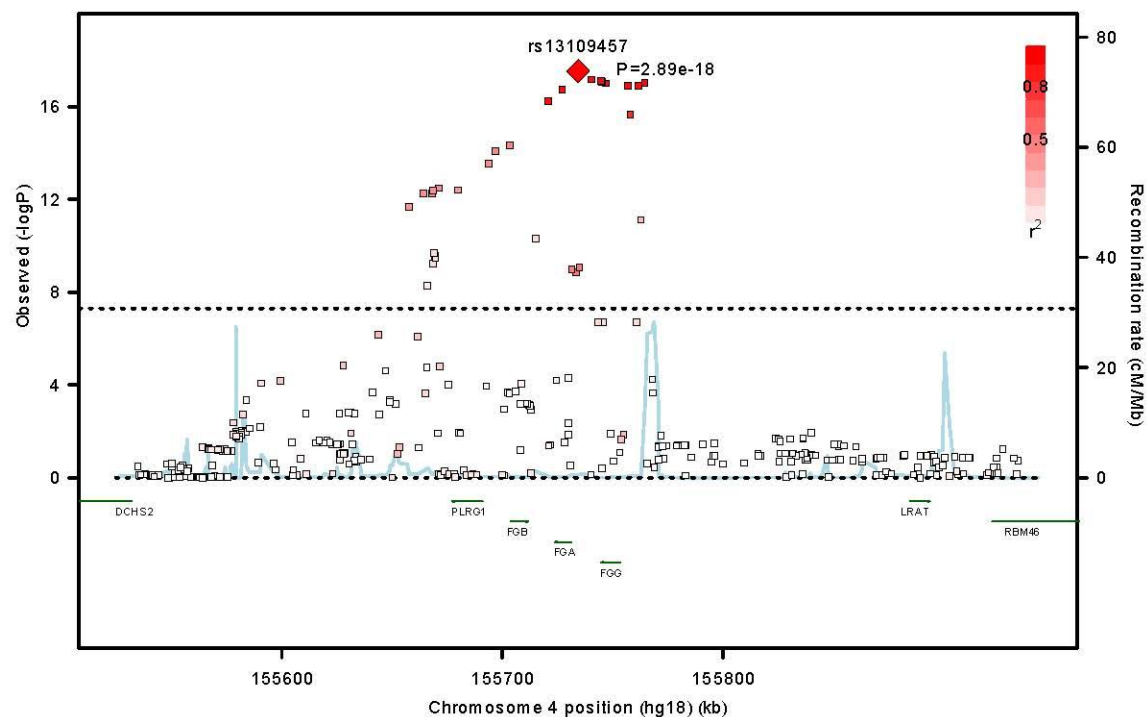
Supplemental Figure S4. Forest plot of rs6687813



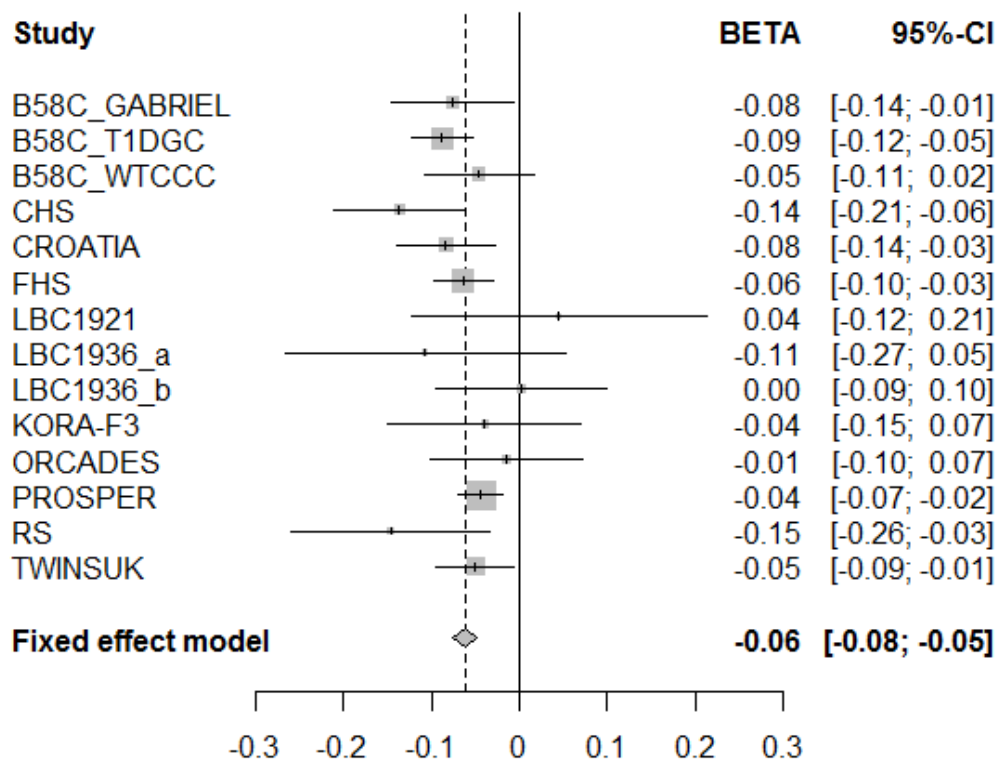
Supplemental Figure S5. Forest plot of rs6025



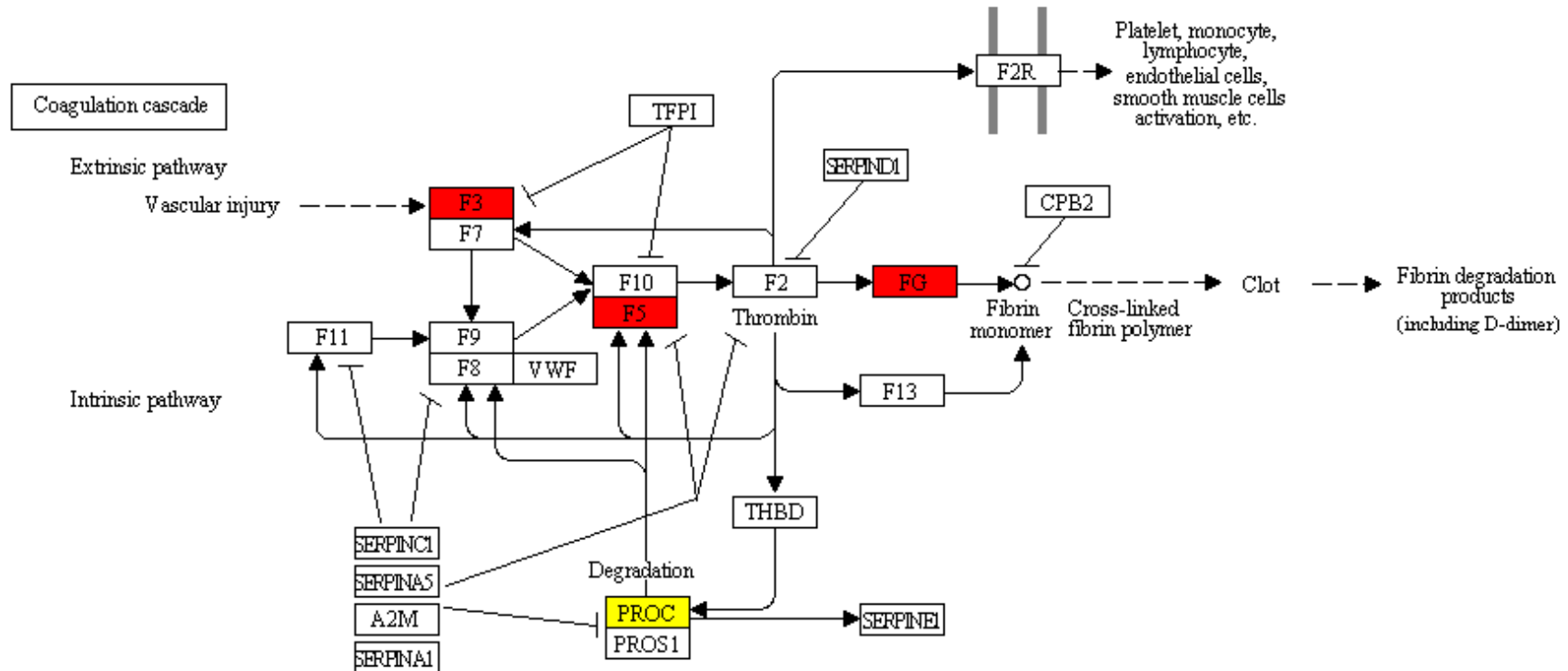
Supplemental Figure S6. Regional plot of *FGA*



Supplemental Figure S7. Forest plot of rs13109457



Supplement Figure S7. Depiction of major components of the coagulation cascade.



From Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg-bin/show_pathway?map04610)

A2M = alpha-2-macroglobulin; **CPB2** = plasma carboxypeptidase B2 (thrombin-activatable fibrinolysis inhibitor); **F2** = coagulation factor II (prothrombin); **F2R** = coagulation factor 2 receptor; **F3** = coagulation factor III (tissue factor); **F5** = coagulation factor V; **F7** = coagulation factor VII; **F8** = coagulation factor VIII; **F9** = coagulation factor IX; **F10** = coagulation factor X; **F11** = coagulation factor XI; **F13** = coagulation factor XIII; **FG** (includes *FGA*, *FGB*, *FGG*) = alpha, beta, and gamma fibrinogen; **PROC** = protein C; **PROS1** = protein S; **SERPINA1** = alpha-1-antitrypsin; **SERPINA5** = protein C inhibitor; **SERPINC1** = antithrombin; **SERPIND1** = heparin cofactor II; **SERPINE1** = plasminogen activator inhibitor-1; **TFPI** = tissue-factor pathway inhibitor; **THBD** = thrombomodulin; **VWF** = von Willebrand factor.

Supplemental Table S1: Genotyping and imputation methods for autosomal chromosomes by study

Methods	B58C-WTCCC	B58C-T1DGC	B58C-GABRIEL	CHS	Croatia-Vis	FHS
Platform	Affymetrix	Illumina	Illumina	Illumina	Illumina	Affymetrix
Chip	500K	550K	610K	370 CNV	300v1	500K + 50K
SNPs investigated	490,032	539,438	582,892	306,655	317,500	490,700 (500K) 48,195 (50K)
SNP exclusion criteria*	Sample-wise	Call rates <0.98	excluded			
Call rate	none	none	none	$\hat{0}.97$	$\hat{0}.98$	$\hat{0}.97$
HWE p-value	None	None	None	$<1.0 \times 10^{-5}$	$<1.0 \times 10^{-6}$	$<1 \times 10^{-6}$
Variants included for imputation	490,032	539,438	582,892	291,322	299,829	343,361 (500K) 34,841 (50K)
Percent of variants included	100%	100%	100%	95%	94.5%	70% (500k) 72% (50K)
Imputation software	IMPUTE	MACH	MACH	BIMBAM	MACH	MACH
Imputation software version	0.1.3	1.0.13	1.0.16	0.99	1.0.16	1.0.15
Genome build	35	35	36	36	36	36.2
Total number of SNPs	2,236,936	2,557,252	2,543,887	2,543,887	2,453,887	2,543,887

Methods	KORA-F3	LBC (all)	ORCADES	PROSPER	RS	Twins UK
Platform	Affymetrix	Illumina	Illumina	Illumina	Illumina	Illumina
Chip	500K	610-Quad v1	300 v2	660K	550 v3	300, 610Q, 1M, 1M-Duo, 1.2MDuo
SNPs I	490,032	542,050	373397	561.490	561,466	307,739 (300) 598,207 (610Q) 892,943 (1M)
SNP E						
Call	1.00	$\hat{0}.98$	$\hat{0}.98$	$\hat{0}.95$	$\hat{0}.98$	$\hat{0}.97$ (MAF $\hat{0}.05$) $\hat{0}.99$ (0.01MAF $\hat{0}.05$)
HWE	-	<0.001	$<1.0 \times 10^{-6}$	$<1.0 \times 10^{-6}$	$<1.0 \times 10^{-6}$	$<1.0 \times 10^{-6}$
Variants	490,032	535,709	293,700	557,192	512,349	889,685 (Merged)
Percent	100%	99%	92%	99.2%	91.3%	99.6% (1M)
Software	MACH	MACH	MACH	MACH	MACH	Impute
Version	1.0.9	1.0.16	1.0.16	1.0.15	1.0.15	2
Build	35.21	36	36	36	36	36
Total SNPs	2,557,252	2,543,887	2,453,887	2,543,887	2,543,887	2,657,661

Supplemental Table S2. Description of subthreshold single-nucleotide-polymorphism marker associations with p-values less than 1.0×10^{-5} but more than 5.0×10^{-8}

Chromosome	SNP		Position	Variant	MAF	P-value	Parameter coefficient*	Closest Gene (location/distance)
	Number of SNPs	Most significant						
1	4	rs2774920	94383888	G	0.085	1.1×10^{-6}	0.0513	<i>ABCA4</i> (25kb 5'); <i>ARHGAP29</i> (23kb 3')
1	2	rs16861990	167401751	C	0.061	1.9×10^{-6}	0.0614	<i>NME7</i> (intronic)
5	1	rs16871023	73250993	G	0.046	1.4×10^{-6}	-0.0761	<i>RGNEF</i> (intronic)
9	6	rs4246856	24082072	C	0.201	6.2×10^{-6}	0.0333	**
9	6	rs687621	135126886	G	0.317	7.0×10^{-6}	0.0284	<i>ABO</i> (intronic)
11	1	rs2306029	46849684	C	0.448	7.8×10^{-6}	-0.0269	<i>LRP4</i> (exonic: Ser1554Gly)
11	1	rs7117404	47083729	G	0.153	8.5×10^{-6}	-0.0398	<i>C11orf49</i> (intronic)
11	1	rs11039571	48163835	G	0.131	9.4×10^{-6}	-0.0390	<i>PTPRJ</i> (15kb 3')
11	5	rs1351696	48339047	G	0.135	7.9×10^{-6}	-0.0382	<i>OR4C45</i> (9kb 5')
12	1	rs7314285	110006409	G	0.065	9.5×10^{-6}	0.0542	<i>CUX2</i> (intronic)
16	4	rs1991867	81076729	C	0.111	3.5×10^{-6}	-0.0440	<i>CDH13</i> (141kb 5')
18	34	rs8083346	61149755	G	0.346	6.2×10^{-6}	-0.0278	**
20	6	rs867186	33228215	G	0.091	3.7×10^{-6}	0.0484	<i>PROCR</i> (exonic: Ser219Gly)

CI = confidence interval based on a 2-sided $\chi^2 = 0.00000005$; MAF = weighted minor allele frequency; SNP = single nucleotide polymorphism.

*Parameter coefficient represents change (% of activity or antigen) associated with 1-unit change in allele dosage. ** No genes within 200kb on either side of SNP.

Genetic Predictors of Fibrin D-Dimer Levels in Healthy Adults

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Circulation. 2011;123:1864-1872; originally published online April 18, 2011;
doi: 10.1161/CIRCULATIONAHA.110.009480

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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