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Genetic Predictors of Fibrin D-Dimer Levels in Healthy Adults

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From the Division of Community Health Sciences, St. George's, University of London, London, UK (D.P.S., A.R.R.); Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK (G.D.O.L., A.R.); ALSPAC Laboratory, University of Bristol, Bristol, UK (W.L.M.); Departments of Epidemiology (N.L.S., B.M.P.), Medicine (J.C.B., B.M.P.), Biostatistics (T.L., B.M.), and Health Services (B.M.P.), University of Washington, Seattle; Group Health Research Institute, Group Health Cooperative, Seattle, WA (N.L.S., B.M.P.); Seattle Epidemiologic Research and Information Center, Veteran Affairs Office of Research and Development, Seattle, WA (N.L.S.); Departments of Pathology (M.C., E.G.B.) and Medicine (M.C.), University of Vermont, Burlington; Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA (X.G.); Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK (V.V., S.C., N.D.H., C.H., A.T., J.E.H., A.F.W.); Centre for Population Health Sciences, University of Edinburgh Medical School, Edinburgh, UK (I.R., S.H.W., J.F.W., H.C.); Gen Info Ltd, Zagreb, Croatia (O.P., I.R.); Medical School, University of Zagreb, Zagreb, Croatia (O.P.); Division of Haematology, Department of Internal Medicine, Clinical Hospital Center Zagreb, Zagreb, and Medical School, University of Osijek, Osijek, Croatia (D.P.); Croatian Centre for Global Health, University of Split Medical School, Split, Croatia (I.R.); National Heart, Lung, and Blood Institute's Framingham Heart Study (J.H., C.J.O.) and Division of Intramural Research (C.J.O.), National Heart, Lung, and Blood Institute, Bethesda, MD; Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA (C.J.O.); Departments of Biostatistics (Q.Y.) and Neurology (M.H.C.), Boston University, Boston, MA; Royal North Shore Hospital, University of Sydney, Sydney, Australia (G.T.); Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Genetic Epidemiology (C.G.), Unit of Molecular Epidemiology (T.I.), and Institute of Epidemiology II (J.B., B.T., A.P.), Neuherberg, Germany; Department of Internal Medicine II–Cardiology, University of Ulm Medical Center, Ulm, Germany (M.K., W.K.); Centre for Cognitive Ageing and Cognitive Epidemiology (L.M.L., S.E.H., D.C.L., A.J.G., M.L., J.M.S., D.J.P., P.M.V., I.J.D.), Department of Psychology (L.M.L., G.D., D.C.L., A.J.G., M.L., I.J.D.), Geriatric Medicine Unit (J.M.S.), and Medical Genetics Section (S.E.H., D.J.P.), University of Edinburgh, Edinburgh, UK; Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK (A.T.); Queensland Statistical Genetics, Queensland Institute of Medical Research, Brisbane, Australia (P.M.V.); Departments of Cardiology (S.T., W.J.), Gerontology and Geriatrics (S.T., A.J.M.d.C., R.G.J.W.), and Molecular Epidemiology (P.E.S.), Leiden University Medical Center, Leiden, the Netherlands; Netherlands Consortium for Healthy Ageing, Rotterdam, the Netherlands (P.E.S., R.G.J.W., A.D., A.G.U., A.H., J.C.M.W.); Division of Cardiovascular and Medical Sciences (D.J.S., G.D.O.L., A.R.) and Robertson Center for Biostatistics (I.F.), University of Glasgow, Glasgow, UK; BHF Glasgow Cardiovascular Research Centre, Faculty of Medicine, Glasgow, UK (N.S.); Department of Pharmacology and Therapeutics, University College Cork, Cork, Ireland (B.M.B.); Durrer Center for Cardiogenetic Research, Amsterdam, the Netherlands (W.J.); Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands (W.J.); Departments of Epidemiology (A.D., A.G.U., A.H., J.C.M.W.) and Internal Medicine (A.G.U.), Erasmus University Medical Center, Rotterdam, the Netherlands; Human Genetics Department, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK (S.Y.S., N.S.); Department of Twin Research and Genetic Epidemiology Unit, King's College London, London, UK (T.D.S., N.S.); and Division of Cardiovascular and Diabetes Research, Leeds University, Leeds, UK (A.M.C., P.J.G.).

*These authors contributed equally as first authors.

†These authors contributed equally as last authors.

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Correspondence to Nicholas L. Smith, PhD, Cardiovascular Health Research Unit, University of Washington, 1730 Minor Ave, Ste 1360, Seattle, WA 98101 (E-mail nlsmith@u.washington.edu); or Caroline Hayward, PhD, Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK (E-mail C.Hayward@hgu.mrc.ac.uk).

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***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 identity 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 ($P=6.4\times10^{-52}$) was 46.0 kb upstream from *F3*, coagulation factor III (tissue factor). At 1q24, rs6687813 ($P=2.4\times10^{-14}$) was 79.7 kb downstream of *F5*, coagulation factor V. At 4q32, rs13109457 ($P=2.9\times10^{-18}$) 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 ORCADES[17]; 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 PROSPER[20]; Enzygnost D-dimer micro (Behringwerke Diagnostica, Marburg, Germany) in RS[21]; 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]

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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 ln 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 I 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 I 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 II 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 $P=5.0\times10^{-8}$. 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 $P<1.0\times10^{-5}$ 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-

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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

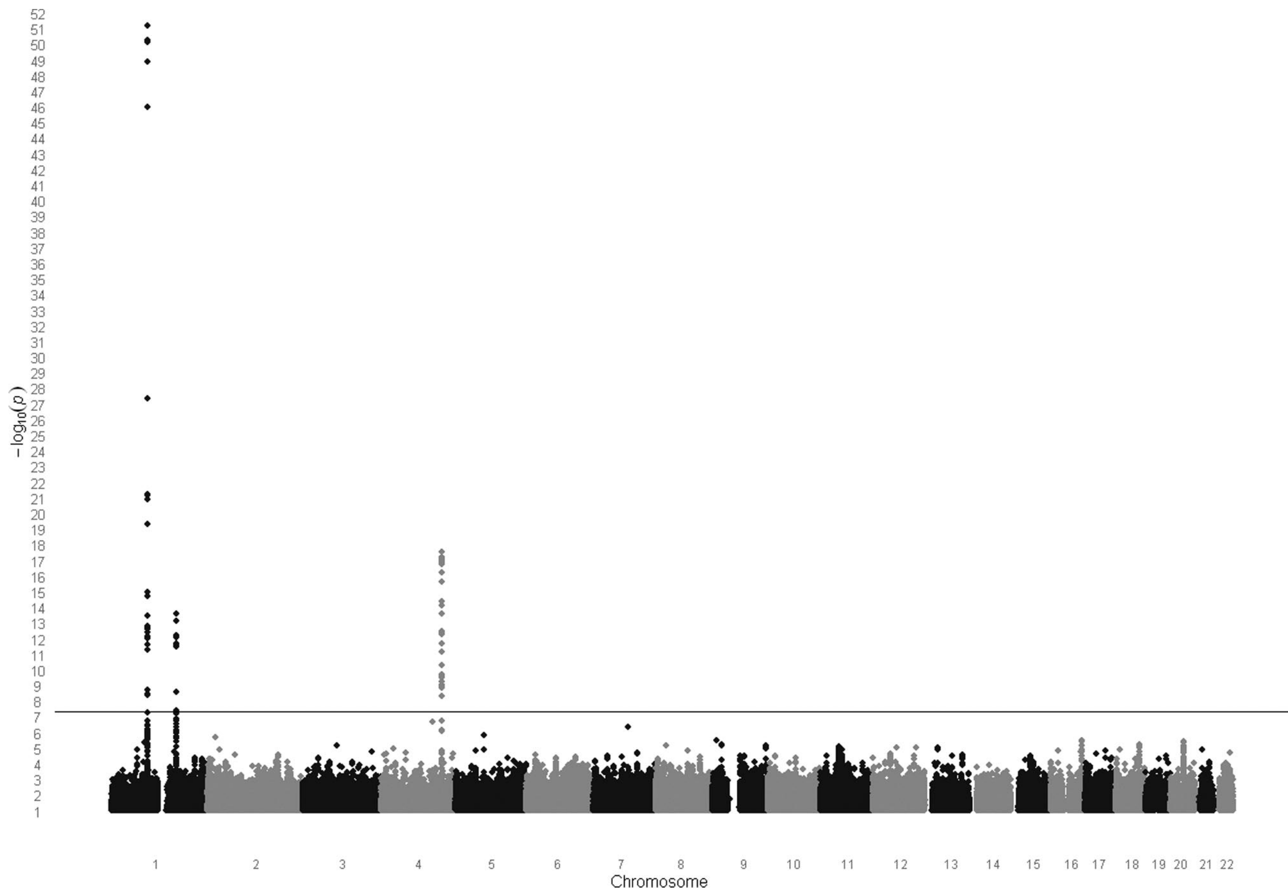

Figure. The genome-wide $\log_{10}$ *P* value plots of D-dimer for the 2 522 393 single-nucleotide polymorphisms meta-analyzed. The horizontal line marks the 5.0×10^{-8} *P* value threshold of genome-wide significance.

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

Region	SNP Significant, n	SNP Most Significant	Position	Variant	MAF	P	Parameter Coefficient,* ln (CI)	Change,† % (CI)	Closest Gene
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)	*F3* (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)	*F5* (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)	*FGA* (3.0 kb upstream), *FGG* (10.4 kb downstream)

SNP indicates single-nucleotide polymorphism; MAF, weighted minor allele frequency; and CI, confidence interval based on a 2-sided α=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 ($P=6.4\times10^{-52}$; 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 ($P=2.4\times10^{-14}$; 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 ($P=6.4\times10^{-9}$) 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 ($P=2.9\times10^{-18}$; 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 $P=1.8\times10^{-17}$, 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 ($P=8.1\times10^{-1}$).

An additional 13 loci across 8 chromosomes had ≥1 variants with a P value that did not exceed the threshold of genome-wide significance ($P=5.0\times10^{-8}$), 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

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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 ≈21 000, we had 80% power (2-sided $\alpha=5.0\times10^{-8}$) 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.

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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.

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Supplemental Materials

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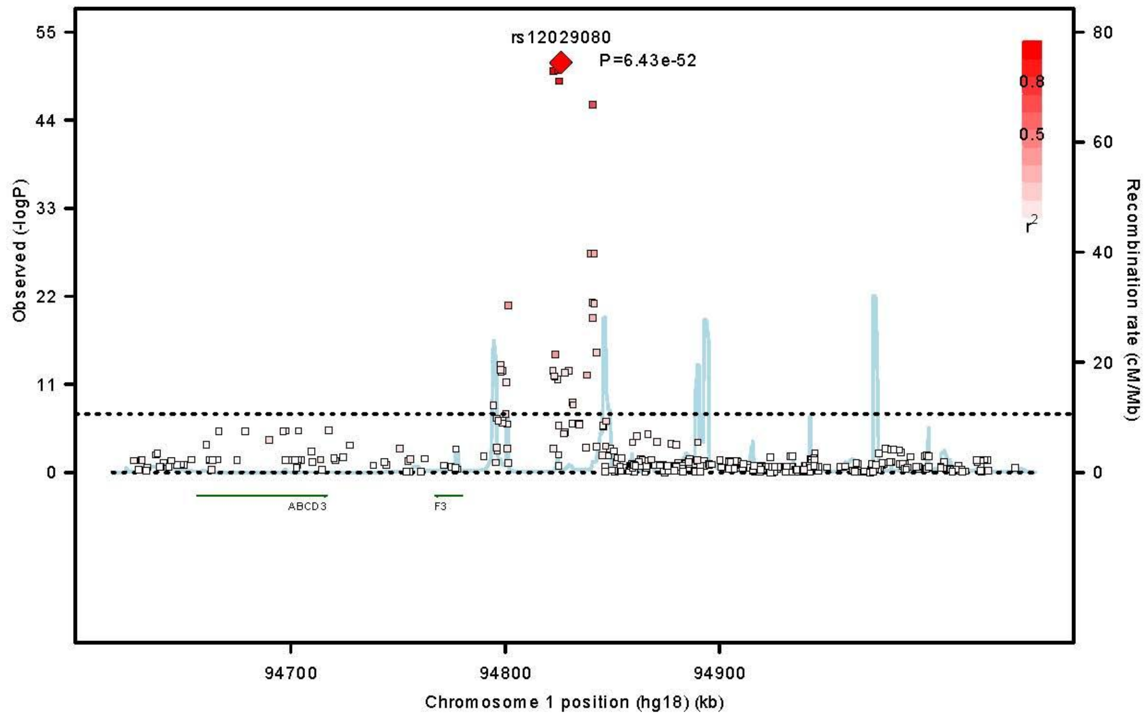
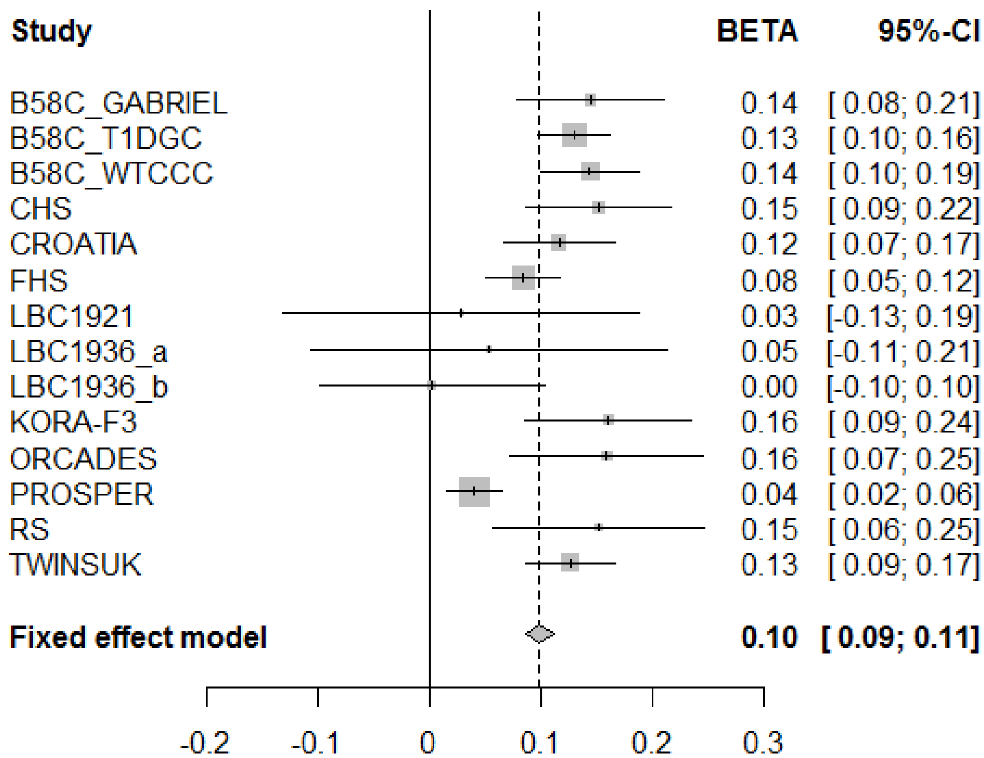
Supplemental Figure S1. Regional plot of *F3*



Supplemental Figure S2. Forest plot of rs12029080



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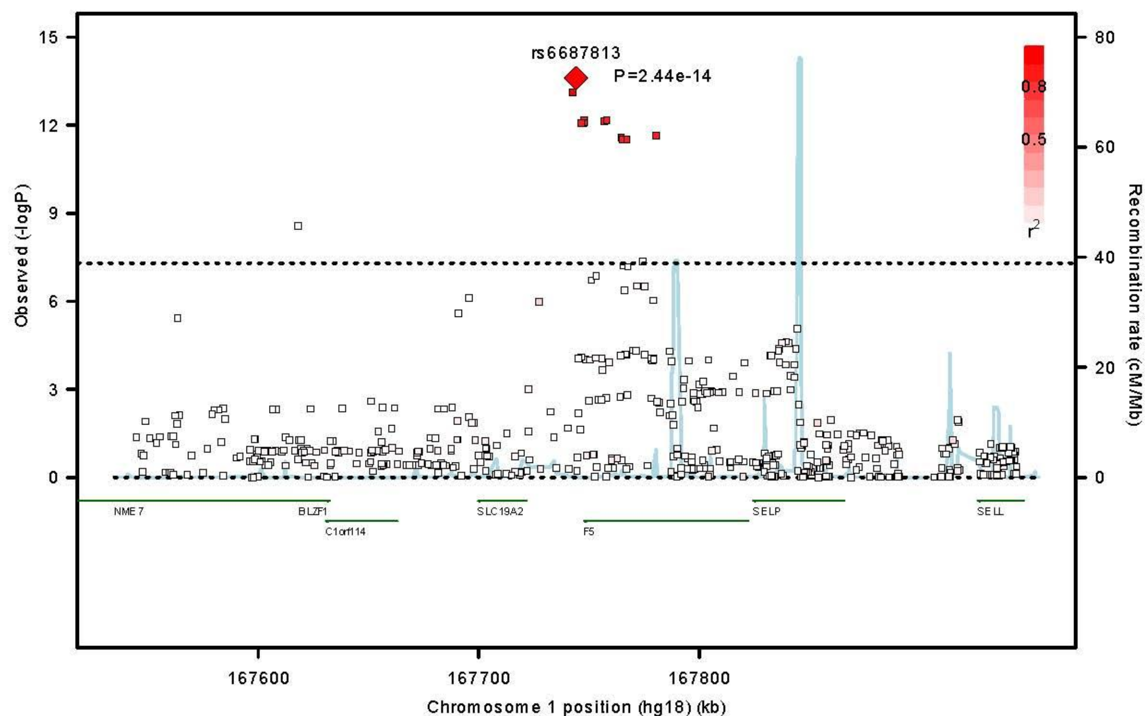
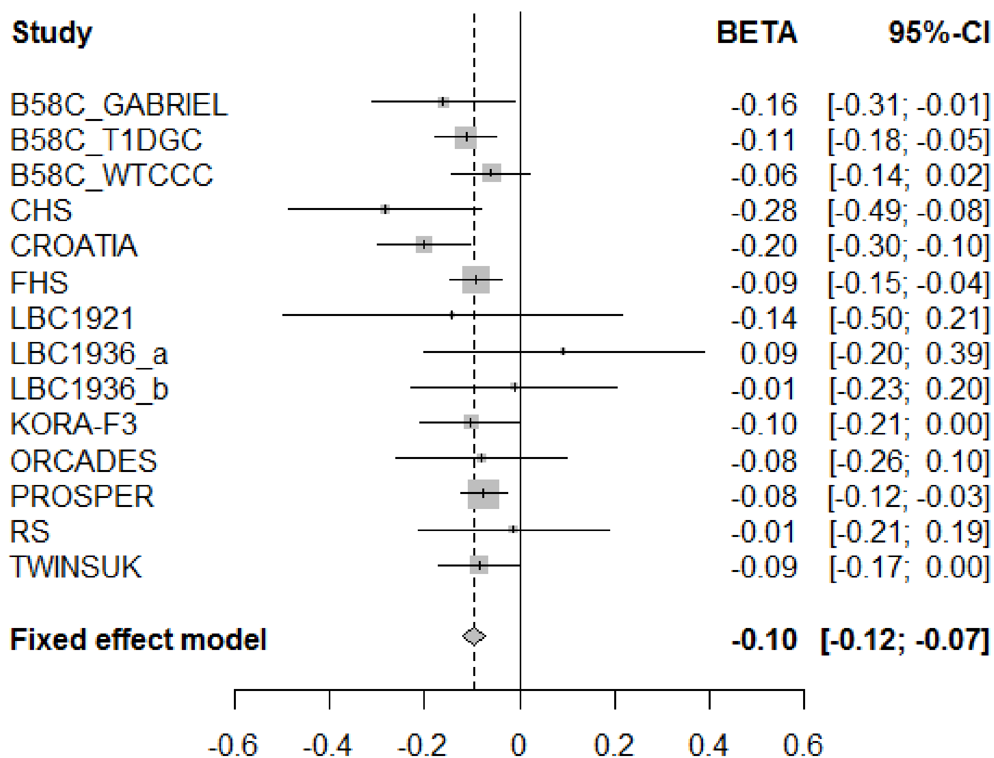
Supplemental Figure S3. Regional plot of *F5*



Supplemental Figure S4. Forest plot of rs6687813



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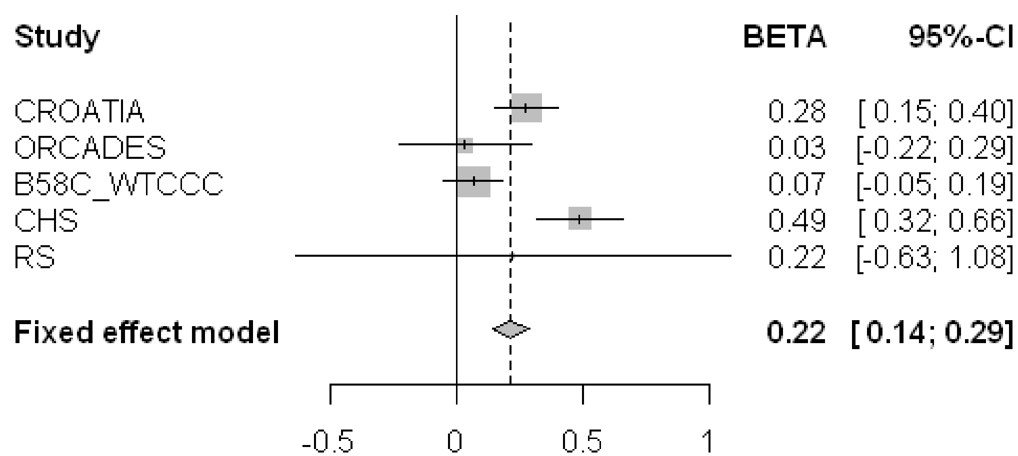
Supplemental Figure S5. Forest plot of rs6025



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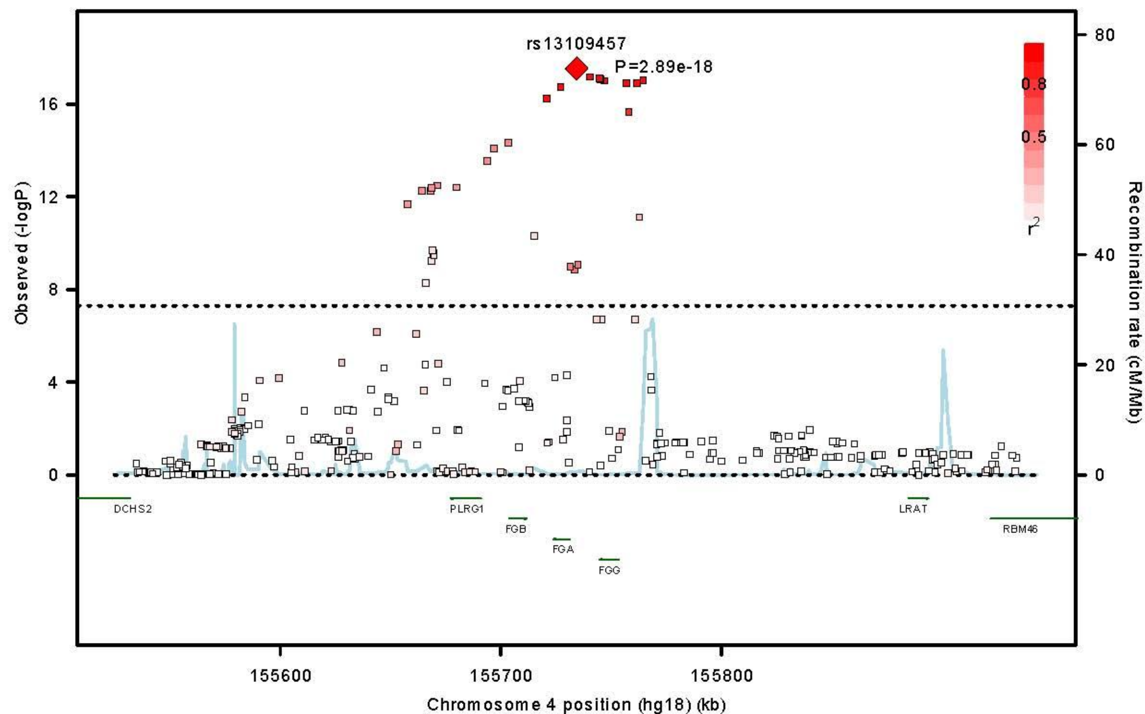
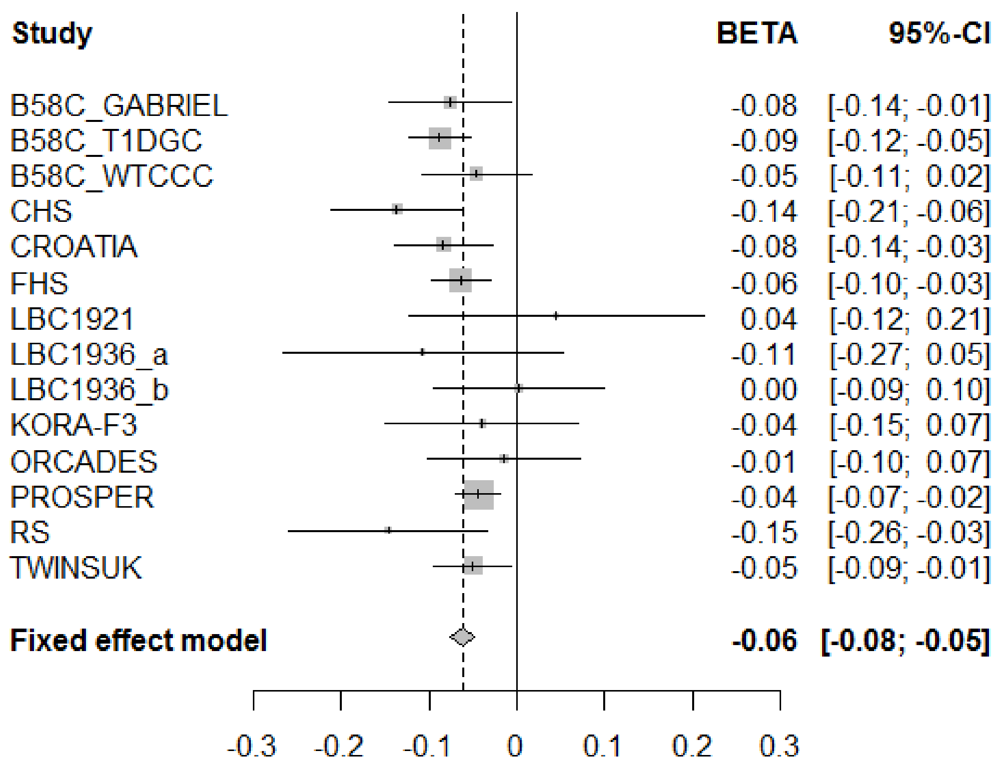
Supplemental Figure S6. Regional plot of *FGA*



Supplemental Figure S7. Forest plot of rs13109457



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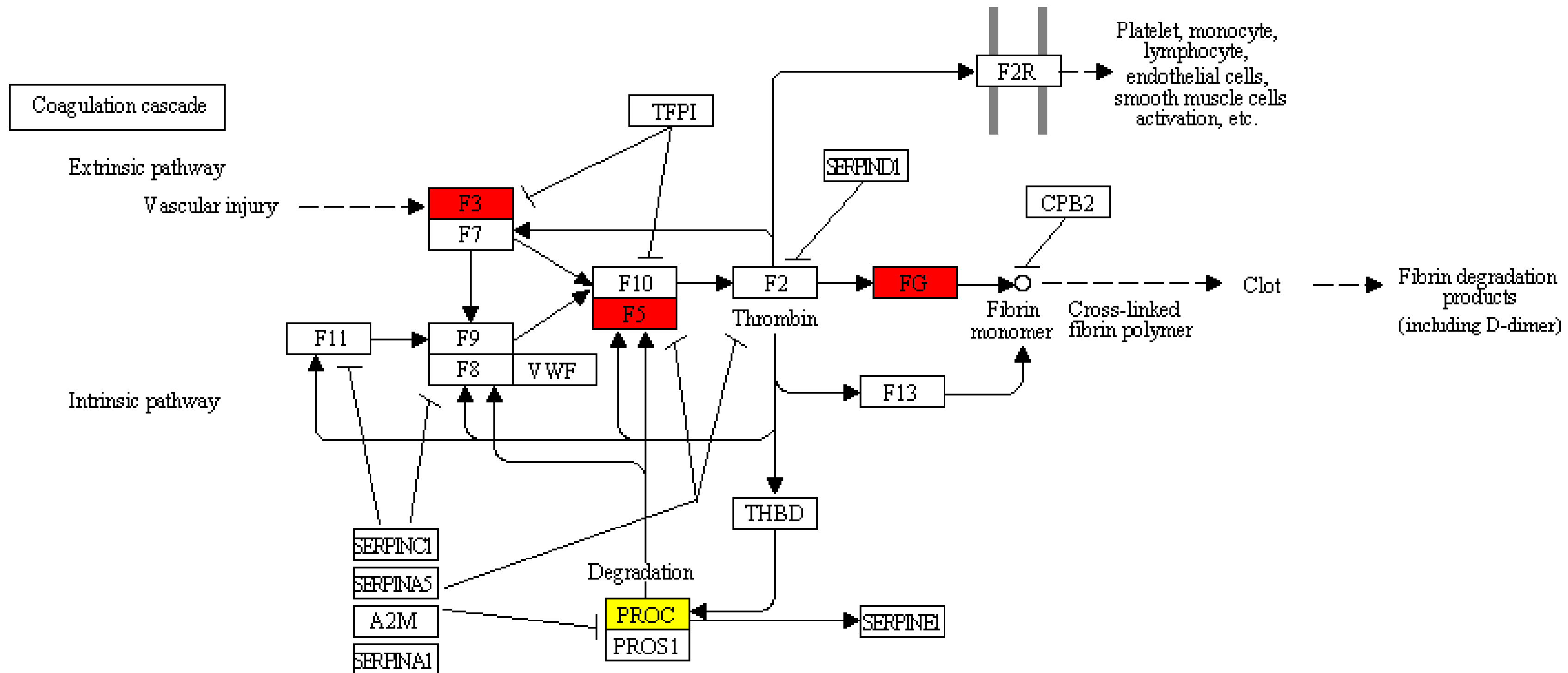
Supplement Figure S7. Depiction of major components of the coagulation cascade.



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 gama 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.

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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	m0.97	m0.98	m0.97
HWE p-value	None	None	None	$<1.0x10^{-5}$	$<1.0x10^{-6}$	<1 x 10E-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	m0.98	m0.98	m0.95	m0.98	m0.97 (MAF⁻ 0.05) m0.99 (0.01mMAFm0.05)
HWE	-	<0.001	$<1.0x10^{-6}$	$<1.0x10^{-6}$	$<1.0x10^{-6}$	$<1.0x10^{-6}$
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

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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	*ABCA4* (25kb 5′); *ARHGAP29* (23kb 3′)
1	2	rs16861990	167401751	C	0.061	1.9×10^{-6}	0.0614	*NME7* (intronic)
5	1	rs16871023	73250993	G	0.046	1.4×10^{-6}	-0.0761	*RGNEF* (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	*ABO* (intronic)
11	1	rs2306029	46849684	C	0.448	7.8×10^{-6}	-0.0269	*LRP4* (exonic: Ser1554Gly)
11	1	rs7117404	47083729	G	0.153	8.5×10^{-6}	-0.0398	*C11orf49* (intronic)
11	1	rs11039571	48163835	G	0.131	9.4×10^{-6}	-0.0390	*PTPRJ* (15kb 3′)
11	5	rs1351696	48339047	G	0.135	7.9×10^{-6}	-0.0382	*OR4C45* (9kb 5′)
12	1	rs7314285	110006409	G	0.065	9.5×10^{-6}	0.0542	*CUX2* (intronic)
16	4	rs1991867	81076729	C	0.111	3.5×10^{-6}	-0.0440	*CDH13* (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	*PROCR* (exonic: Ser219Gly)

CI = confidence interval based on a 2-sided = 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.

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