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Genetic predictors of fibrin D-dimer levels in healthy adults

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Abstract

Background—Fibrin fragment D-dimer is one of several peptides produced when cross-linked fibrin is degraded by plasmin, and 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-value 6.4×10^{-52}) was 46.0 kb upstream from *F3*, coagulation factor III (tissue factor). At 1q24, rs6687813 (p-value 2.4×10^{-14}) was 79.7 kb downstream of *F5*, coagulation factor V. At 4q32, rs13109457 (p-value 2.9×10^{-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 non-synonymous 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 which the *F3* association was the strongest and has not been previously reported.

Keywords

genome-wide variation; D-dimer; epidemiology; meta-analysis; thrombosis; hemostasis

Fibrin fragment D-dimer is one of several peptides produced when cross-linked 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 non-invasively 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

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 While genetic influences beyond genes in the clotting cascade have been proposed, none has 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 US 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 non-overlapping subsets were used as controls for the Wellcome Trust Case-Control Consortium (WTCCC), the Type 1 Diabetes Genetics Consortium (T1DGC), and a European asthma genetics consortium (GABRIEL); 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); Twins UK; and the CROATIA-Vis study. These studies have been described elsewhere.^{17,33}

Subjects

Eligible participants for these analyses had a D-dimer measure, 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 Twins UK, and at a follow-up visit for B58C (examination 2002 and 2003), FHS (examination cycle 5, 1991-1995), LBC1921 (wave 3 in 2007-2008), and LBC1936 (wave 2 from 2007-2010). The D-dimer phenotype was measured by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies in kit or prepared in the lab: 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 Biopool TintElize ELISA D-dimer (Trinity Biotech, Bary, Ireland) in UK Twins;²² and using 2 monoclonal antibodies directed against nonoverlapping antigenic determinants to detect D-dimer from cross-linked fibrin but not D-monomer in CHS, FHS, and KORA-F3.^{4, 23, 24}

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 Twins UK, and at the time of phenotype measurement for B58C, FHS, LBC1921, and LBC1936. Measures, taken using standardized methods as specified by each study, included in-person measures of height and weight, and self-reported treatment of diabetes, 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 using 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 using various Affymetrix and Illumina panels (Supplemental Table S1). Genotype quality control and data cleaning that included assessing Hardy-Weinberg equilibrium and variant call rates, were conducted independently by each study; details have been published elsewhere and are also provided in Supplemental Table S1.^{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 (Supplemental S2) and imputed their genotype data to the ~2.6 million SNPs derived from the HapMap Caucasian (CEU) sample from the Centre d'Etude du Polymorphisme Humain using imputation software that included MACH, BIMBAM 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 (OEV) 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 pre-specified 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 for Twins UK, which used a score test and variance components method as implemented in Merlin.³⁰⁻³³ An additive genetic model with 1 degree of freedom 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, that estimated the extent of underlying population structure based on 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 1-unit change in allele dosage. All meta-analyses were conducted using 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 or more SNPs within the gene in addition to the variant whose association with D-dimer has already been established. This allowed us to assess possible novel associations independent of previously-known strong signals.

The *a priori* threshold of genome-wide significance was set at a p-value of 5.0×10^{-8} . When more than 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, to a model that also included 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 sub-threshold loci, those marked by variants with a p-value less than 1.0×10^{-5} but greater than level of genome-wide significance. We limited our report to those sub-threshold loci at a distance of 200kb or more 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 untransformed 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 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 (Supplemental Figure S1). Rs12029080 was associated with the smallest p-value in this region (6.4×10^{-52} , minor allele frequency [MAF] = 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]: 0.064-0.134) increase in the natural-log transformed of D-dimer (ng/dl), or a 10.4% (6.6-14.3%) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Supplemental Figure S2.

A second genome-wide significant locus on chromosome 1 was found at 1q24 (Supplemental Figure S3). Rs6687813 was associated with the smallest p-value at this locus (2.4×10^{-14} , MAF = 0.059). Each copy of the A allele was associated with a 0.096 unit (CI: 0.027-0.165) increase in the natural log of D-dimer, or a 10.1% (CI: 2.7-17.9%) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Supplemental Figure S4. 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 where the *F5* Leiden variant was genotyped (B58C-WTCCC, CHS, CROATIA-Vis, ORCADES, and RS), we re-analyzed the data from chromosome 1 and adjusted for rs6687813 and rs6025 in order 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 additionally adjusting 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: 0.013-0.419) increase in natural-log transformed D-dimer (p-value = 6.4×10^{-9}), or a 24% (CI 1.3-52.0%) increase in median D-dimer level. A forest plot of rs6025 is provided in Supplemental Figure S5.

A third genome-wide significant locus was identified at chromosomal position 4q32 (Supplemental Figure S6). Rs13109457 had the smallest p-value (2.9×10^{-18} , MAF = 0.250) and was located between 2 fibrinogen genes: 10.4 kb downstream from *FGG*, fibrinogen gamma chain, and 3.0 kb upstream from *FGA*, fibrinogen alpha chain. Each copy of the A allele was associated with a 0.061 unit (CI: 0.023-0.099) increase in the natural log of D-dimer, or a 6.3% (CI: 2.3-10.4%) increase in median D-dimer level. A forest plot of the meta-analyzed studies is depicted in Supplemental Figure S7. The rs13109457 variant is in strong LD 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 p-value of 1.8×10^{-17} and each additional risk allele of rs6050 was associated with a 0.06 unit (CI: 0.022-0.098) increase in natural-log transformed plasma D-dimer, or a 6.3% (2.3-10.4%) 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 (8.1×10^{-1}).

An additional 13 loci across 8 chromosomes had 1 or more variants with a p-value that did not exceed the threshold of genome-wide significance, 5.0×10^{-8} , but was smaller than

1.0×10^{-5} . Details about these 13 loci including closest reference gene are presented in Supplemental Table S2.

Discussion

A genome-wide investigation of the plasma fibrin D-dimer phenotype in over 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*, approximately 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 D-dimer fragments shed. There is increasing interest in the roles of tissue factor in initiation of hemostasis and 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 non-synonymous 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 cross-linked 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 Supplemental Figure S4). 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 as well as with other pathologies including disseminated intravascular coagulation, cancer progression, and cognitive decline.^{1-10, 20, 40} The current 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 also 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. While there is considerable experimental evidence that tissue factor plays an important role in initiation of hemostasis

and arterial thrombosis, to date there has been very limited epidemiological evidence 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, less than 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.

Sub-Threshold Associations

Among the 13 sub-threshold loci identified, 7 had high-signal markers in genes and another 3 had markers within 50kb 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 will be 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 over 20,000 individuals of European ancestry and examined over 2.5 million markers spread throughout the genome. The novel association identified was for a variant whose p-value was over 40 orders of magnitude smaller than the significance threshold of 5.0×10^{-8} set as significant. Genetic associations of such strength have consistently replicated in other settings, and for this reason, we did not seek replication.^{44,46} With a sample-size of ~21,000, we had 80% power (2-sided alpha = 5.0×10^{-8}) to detect a 0.067 unit difference in log D-dimer (mean = 5, standard deviation = 1) for a variant with an MAF of 0.3. Most of the sub-threshold 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-analytic estimated mean effect of each SNP. This is 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.

Summary

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.

Clinical Commentary

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 as well as with other pathologies. This manuscript 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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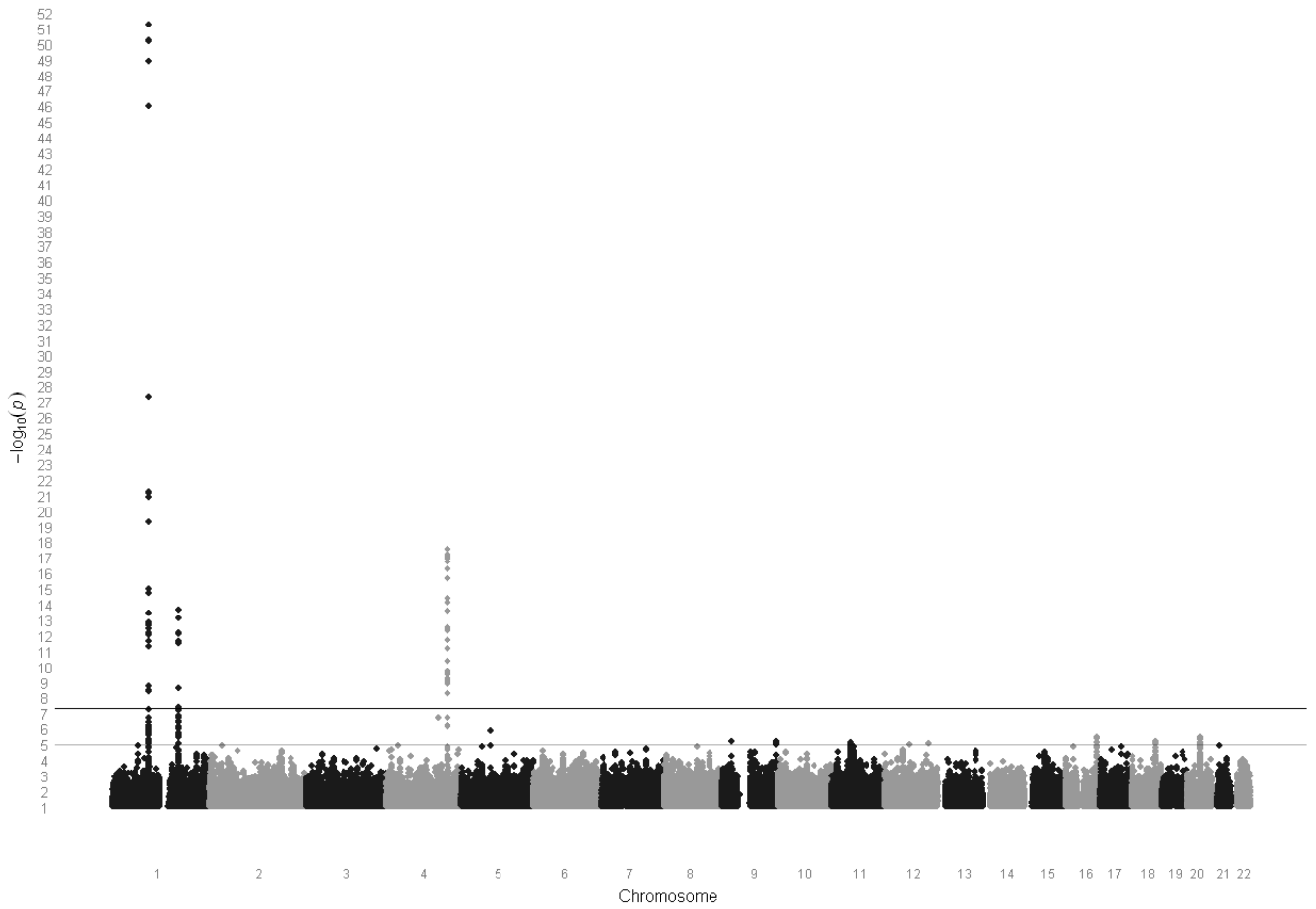


Figure. The genome-wide \log_{10} p-value plots of D-dimer for the 2,522,393 single-nucleotide polymorphisms meta-analyzed. The horizontal line marks the 5.0×10^{-8} p-value threshold of genome-wide significance.

Table 1
Characteristics of the study participants at the time of D-dimer measurement

Characteristic	B58C-WTCCC	B58C-TIDGC	B58C-GABRIEL	CHS	CROATIA-Vis	FHS
Counts	1,460	2,475	657	1,667	884	3,094
Mean age, years (SD)	44.9 (0.35)	45.3 (0.34)	45.2 (0.37)	72.2 (7.5)	56.4(15.54)	58.5 (9.7)
Male, %	50.4	48.2	47.2	41.3	42.0	46.9
European ancestry, %	100	100	100	100	100	100
BMI, kg/m ² (SD)	27.4 (4.9)	27.5 (4.9)	27.2 (5.0)	26.4 (6.3)	27.32(4.27)	27.9 (5.1)
Arterial disease hx, %	unk	unk	Unk	0.0	11.36	10.8
Venous disease hx, %	unk	unk	Unk	4.4	unk	unk
Diabetes, %	1.9	1.3	2.0	27.5	14.4	11.4
Median D-dimer, ng/dL (IQR)	154 (114-215)	157 (114-218)	166 (115-235)	133 (89-202)	201 (134-317)	317 (205-473)
Median In D-dimer, ng/dL (IQR)	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)

KORA-F3	LBC 1921 (Vidas)	LBC 1936 (Vidas)	LBC 1936 (HemoSii)	ORCADES	PROSPER	RS	Twins UK
1,588	152	391	162	883	5,076	552	2,166
62.3 (10.1)	86.6 (0.39)	72.00 (0.48)	72.9(0.33)	53.5(15.73)	75.3 (3.4)	71.7 (8.9)	54.2 (12.42)
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 (4.21)	27.7 (4.15)	27.2 (3.43)	27.66(4.84)	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)

IQR: interquartile range; SD = standard deviation; 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. KORA-F3: arterial disease is only MI; venous thrombosis is only inpatient treatment. RS: arterial disease is only MI. LBC1921 and LBC1936: Arterial disease is cardiovascular history and history of stroke.

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

Table 2Description of single-nucleotide polymorphisms associations with p-values less than 5.0×10^{-8}

Region	SNP		Position	Variant	MAF	P-value	Parameter coefficient, ln* (CI)	Change**, % (CI)	Closest Gene
	Number significant	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)

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

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

** Estimated percentage change in median D-dimer associated with 1-unit change in allele dosage.

[†] R²-value for quality of measurement >0.90.[‡] R²-value for quality of measurement >0.85.