Genetic predictors of fibrin D-dimer levels in healthy adults

Nicholas L. Smith, PhD, Jennifer E. Huffman, MSc, David P. Strachan, MD, Jie Huang, MD, MPH, Abbas Dehghan, MD PhD, Stella Trompet, PhD, So-Youin Shin, PhD, Jens Baumert, PhD, Veronique Vitart, PhD, Joshua C. Bis, PhD, Sarah H. Wild, MD, PhD, Ann Rumley, PhD, Qiong Yang, PhD, Andre G Uitterlinden, PhD, David J. Stott, MD, PhD, Gail Davies, PhD, Angela M. Carter, PhD, Barbara Thorand, PhD, MPH, Ozren Polašek, MD, PhD, Barbara McKnight, PhD, Harry Campbell, MD, Alicja R. Rudnicka, PhD, Ming-Huei Chen, PhD, Brendan M. Buckley, PhD, Sarah E. Harris, PhD, Frances M. K. Williams, PhD, Annette Peters, PhD, Drazen Pulaic, MD, PhD, Thomas Lumley, PhD, Anton J.M. de Craen, PhD, David C. Liewald, Christian Gieger, PhD, Susan Campbell, HND, Ian Ford, PhD, Alan J. Gow, PhD, Michelle Luciano, PhD, David J. Porteous, PhD, Xiuling Guo, PhD, Naveed Sattar, MD, PhD, Albert Tenesa, PhD, Mary Cushman, MD, P. Eline Slagboom, PhD, Peter M. Visscher, PhD, Tim D. Spector, MD, Thomas Illig, PhD, Igor Rudan, MD, PhD, Edwin G. Bovill, MD, Alan F. Wright, PhD, Wendy L. Mc Ardle, PhD, Geoffrey Tolfer, PhD, Albert Hofman, MD, PhD, Rudi G.J. Westendorp, MD, PhD, John M. Starr, MD, Peter J. Grant, MD, Mahir Karakas, MD, Nicholas D. Hastie, PhD, Bruce M. Psaty, MD, PhD, James F. Wilson, DPhil, Gordon D. O. Lowe, MD, Christopher J O’Donnell, MD, MPH, Jacqueline CM Witteman, PhD, J. Wouter Jukema, MD, PhD, Ian J. Deary, PhD, Nicole Soranzo, PhD, Wolfgang Koenig, MD, and Caroline Hayward, PhD

: Division of Community Health Sciences, St George’s, University of London, UK (DPS, ARR); Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK (GDOL, AR); ALSPAC Laboratory, University of Bristol, UK (WLM).

: Departments of Epidemiology (NLS, BMP), Medicine (JCB, BMP), Biostatistics (TL, BM), Health Services (BMP), University of Washington; and Group Health Research Institute (NLS, BMP), Group Health Cooperative, Seattle, Washington, USA; Seattle Epidemiologic Research and Information Center (NLS), Office of Research & Development, Seattle, WA, USA; Departments of Pathology (MC, EGB), Medicine (MC), University of Vermont, Burlington, Vermont USA; Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA (XG).

: MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK (VV, SC, NDH, CH); Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK (C.Hayward@hgu.mrc.ac.uk).

*These authors contributed equally as first authors.
**These authors contributed equally as last authors.

Corresponding authors: Nicholas L. Smith, PhD Cardiovascular Health Research Unit, University of Washington 1730 Minor Ave, Suite 1360, Seattle WA 98101 Phone: 206-287-2777; ns smith@u.washington.edu Caroline Hayward, PhD MRC Human Genetics Unit; Institute of Genetics and Molecular Medicine, Western General Hospital; Edinburgh, UK Phone: (44) 131 332 2471, C.Hayward@hgu.mrc.ac.uk.

Disclosures No author has a real or perceived conflict of interest to report for this manuscript except the following: Mary Cushman (NIH grants); Ian J. Deary (Age UK, MRC, BBSRC, and CIMEF Research Office grants); Alan Gow (Scottish Executive Chief Scientific Office grants); David J. Porteous (various government and research grants; Wellcome Trust honoraria); John Starr (LBC 1921 and 1936 grants); Albert Tenesa (genome-wide association study of non-pathological cognitive decline grant); P. J. Grant (BHF Programme and FBG Euroclot grants); Alan Wright (MRC Programme grant); Harry Campbell (MRC and EU FBG grants); Igor Rudan (MRC and Croatia government grants; Glaxo-Smith-Kline honoraria; Child Health and Nutrition Research Initiative board member); Nicole Hastie (MRC Programme grant); and Ozren Polasek (Medical Research Council UK and Croatian Ministry of Science grants).

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Published in final edited form as:
Edinburgh Medical School, Edinburgh, UK (IR); Gen Info Ltd, Zagreb, Croatia (OP, IR); Medical School, University of Zagreb, Zagreb, Croatia (OP); Division of Haematology, Department of Internal Medicine, Clinical Hospital Center Zagreb, Zagreb, Croatia, Medical School, University of Osijek, Osijek, Croatia (DP); Croatian Centre for Global Health, University of Split Medical School, Split, Croatia (IR)

: National Heart, Lung, and Blood Institute’s Framingham Heart Study (JH, CJO) and Division of Intramural Research (C.J.O.), National Heart, Lung, and Blood Institute, Bethesda, MD; Cardiology Division (C.J.O.), Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts USA; Departments of Biostatistics (QY) and Neurology (MHC), Boston University, Boston, Massachusetts USA; Royal North Shore Hospital, University of Sydney, Australia (GT)

: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Genetic Epidemiology (CG), Unit of Molecular Epidemiology (TI), Institute of Epidemiology II (JB, BT, AP), Neuherberg, Germany, Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany (MK, WK)

: Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, 7 George Square, Edinburgh, EH8 9JZ, UK (LML, SEH, DCL, AJG, ML, JMS, DJP, PMV, IJD); Department of Psychology, The University of Edinburgh, 7 George Square, Edinburgh, EH8 9JZ, UK (LML, GD, DCL, AJG, ML, IJD); Geriatric Medicine Unit, The University of Edinburgh, Royal Victoria Craighielth Road, Edinburgh, EH2 2DN, UK (JMS); Medical Genetics Section, The University of Edinburgh, Edinburgh, EH4 2XU, UK (SEH, DJP); MRC Human Genetics Unit, The Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, EH4 2XU, UK (AT); The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, UK (AT); Queensland Statistical Genetics, Queensland Institute of Medical Research, Brisbane, Australia (PMV)

: MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, Western General Hospital, Edinburgh, UK (JEH, AFW); Centre for Population Health Sciences, University of Edinburgh Medical School, Edinburgh, UK (SHW, JFW, HC)

: Departments of Cardiology (ST, WJ), Gerontology and Geriatrics (ST, AJMdC, RGJW) and Molecular Epidemiology (PES) Leiden University Medical Center, The Netherlands; Netherlands Consortium of Healthy Ageing (PES, RGJW), The Netherlands; Division of Cardiovascular and Medical Sciences (DJS, GDOL, AR) and Robertson Center for Biostatistics (IF) University of Glasgow, Glasgow, UK; BHF Glasgow Cardiovascular Research Centre (NS), Faculty of Medicine, Glasgow, UK; Department of Pharmacology and Therapeutics, University College Cork, Ireland (BMB); Durrer Center for Cardiogenetic Research, Amsterdam, The Netherlands (WJ); Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands (WJ).

: Department of Epidemiology (AD, AGU, AH, JCMW) and Internal Medicine (AGU), Erasmus University Medical Center, Rotterdam, The Netherlands; Netherlands Consortium for Healthy Aging (AD, AGU, AH, JCMW)

: Human Genetics Department (SYS, NS), Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; Department of Twin Research & Genetic Epidemiology Unit (FMKW, TDS, NS), King’s College London (AMC, PJG), London, UK; Division of Cardiovascular and Diabetes Research, Leeds University, Leeds, UK.

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. D-dimer levels have also been associated with the risks of subsequent arterial and venous thrombotic events in middle-aged and older adults. Estimates of the heritability of plasma D-dimer levels range from 23% to 65% in Northern Europeans. 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. 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. While genetic influences beyond genes in the clotting cascade have been proposed, none has been identified. 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. 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 Enzygnost D-Dimer micro (Behringwerke Diagnostica, Marburg, Germany) in RS;20 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

Circulation. Author manuscript; available in PMC 2011 November 01.
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. 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. 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. 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.

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 r² 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 ($6.4 \times 10^{-52}$, 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 ($2.4 \times 10^{-14}$, 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 reanalyzed 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 $2.5 \times 10^{-5}$ to $4.4 \times 10^{-2}$ 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 = $6.4 \times 10^{-9}$), 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 ($2.9 \times 10^{-18}$, 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 $1.8 \times 10^{-17}$ 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 ($8.1 \times 10^{-1}$).

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, $5.0 \times 10^{-8}$, 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 tops 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. 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. 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. 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. 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. 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

The authors acknowledge the essential role of the CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium in development and support of this manuscript. CHARGE members include National Heart, Lung, and Blood Institute’s (NHLBI) Atherosclerosis Risk in Communities (ARIC) Study, NIA’s Iceland Age, Gene/Environment Susceptibility (AGES) Study, NHLBI’s Cardiovascular Health Study and Framingham Heart Study, and the Netherlands’ Rotterdam Study. The authors 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.

Further, Twins UK would to acknowledge essential contribution of Peter Grant and Angela Carter from the Leeds Institute of Genetics, Health and Therapeutics, University of 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 Lothian Birth Cohort studies also thank Janie Corley, Caroline Brett, Caroline Cameron, Michelle Taylor, Ross Henderson, Catherine Murray and Alison Pattie for data collection and data entry; the study secretary Paula Davies; the nurses and staff at the Wellcome Trust Clinical Research Facility, where subjects were tested and the genotyping was performed; Paul Redmond for technical assistance; the staff at the Department of Haematology, Western General Hospital for the D-Dimer measurements; and the staff at the Lothian Health Board and at the SCRE Centre, University of Glasgow.

The 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. Furthermore, we 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 Rotterdam study thanks Pascal Arp, Mila Jhamai, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the GWAS database.

Funding Sources
We acknowledge use of phenotype and genotype data from the British 1958 Birth Cohort DNA collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. The B58C-WTCC subset was funded by the Wellcome Trust grant 076113/B/04/Z. The B58C-T1DGC genotyping utilized resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. B58C-T1DGC GWAS data were deposited by the Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research (CIMR), 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 CIMR is in receipt of 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 Cardiovascular Health Study (CHS) is supported by contract numbers N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, grant numbers U01 HL080295 and R01 HL 087652 from NHLBI, with additional contribution from the National Institute of Neurological Disorders and Stroke. A full list of principal 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 was supported in part by National Center for Research Resources grant M01RR00425 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 through the grants from the Medical Research Council UK and Ministry of Science, Education and Sport of the Republic of Croatia (number 108-100315-0302) and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).

This research was conducted in part using data and resources from the Framingham Heart Study of the NHLBI of the NIH and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHArE) project. Partial investigator support was provided by National Institute of Diabetes and Digestive and Kidney Diseases K24 DK080140 (JB Meigs). This work was partially supported by NHLBI’s Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc., for genotyping services (Contract No. N02-HL-6-4278). A portion of this research utilized 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 (BMBF). Part of this work was financed by the German National Genome Research Network (NGFNPlus, project number 01GS0834) and through additional funds from the University of Ulm. Furthermore, the research was supported within the Munich Center of Health Sciences (MC Health) as part of the Ludwig Maximilians University (LMU) innovative.

The Lothian Birth Cohorts whole genome association study was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) (Ref. BB/F019394/1). The LBC1936 research was supported by a programme grant from Research Into Ageing (Ref. 251) and continues with programme 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 (CZ/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 (EPSRC), Economic and Social Research Council (ESRC), and Medical Research Council (MRC), as part of the cross-council Lifelong Health and Wellbeing Initiative (G0700704/84698). ML is a Royal Society of Edinburgh/Lloyds TSB Foundation for Scotland Personal Research Fellow. PMV acknowledges support from the Medical Research Council, Medical Research Council (UK) and Ministry of Science, Education and Sport of the Republic of Croatia (number 108-100315-0302) and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).

ORCADES was supported by the Chief Scientist Office of the Scottish Government, the Royal Society and the European Union framework program 6 EUROSPAN project (contract no. LSHG-CT-2006-018947).

The PROSPER study was supported by an investigator initiated grant obtained from Bristol-Myers Squibb. Prof. Dr. J. W. 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...
The Rotterdam Study 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 (ZonMw); 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. Abbas 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 (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Consortium for Healthy Aging (NCHA) project nr. 050-060-810.

The Twins UK study was funded by the Wellcome Trust; European Community’s Sixth and Seventh Framework Programmes (FP-6/2005-2008) LIFE SCIENCES & HEALTH (Ref 005268 Genetic regulation of the end stage clotting process that leads to thrombotic stroke: The EuroClot Consortium and (FP7/2007-2013), ENGAGE project HEALTH-F4-2007-201413 and the FP-5 GenomEUtwin Project (QLG2-CT-2002-01254). The study also receives support from the Dept 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. TD is an NIHR Senior Investigator. The project also received support from a Biotechnology and Biological Sciences Research Council (BBSRC) project grant. (G20234). The authors acknowledge the funding and support of the National Eye Institute via an NIH/CIDR genotyping project (PI: Terri Young).

References


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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>B58C-WTCCC</th>
<th>B58C-T1DGC</th>
<th>B58C-GABRIEL</th>
<th>CHS</th>
<th>CROATIA-Vis</th>
<th>FHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts</td>
<td>1,460</td>
<td>2,475</td>
<td>657</td>
<td>1,667</td>
<td>884</td>
<td>3,094</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>44.9 (0.35)</td>
<td>45.3 (0.34)</td>
<td>45.2 (0.37)</td>
<td>72.2 (7.5)</td>
<td>56.4 (15.54)</td>
<td>58.5 (9.7)</td>
</tr>
<tr>
<td>Male, %</td>
<td>50.4</td>
<td>48.2</td>
<td>47.2</td>
<td>41.3</td>
<td>42.0</td>
<td>46.9</td>
</tr>
<tr>
<td>European ancestry, %</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BMI, kg/m² (SD)</td>
<td>27.4 (4.9)</td>
<td>27.5 (4.9)</td>
<td>27.2 (5.0)</td>
<td>26.4 (6.3)</td>
<td>27.32 (4.27)</td>
<td>27.9 (5.1)</td>
</tr>
<tr>
<td>Arterial disease hx, %</td>
<td>unk</td>
<td>unk</td>
<td>Unk</td>
<td>0.0</td>
<td>11.36</td>
<td>10.8</td>
</tr>
<tr>
<td>Venous disease hx, %</td>
<td>unk</td>
<td>unk</td>
<td>Unk</td>
<td>4.4</td>
<td>unk</td>
<td>unk</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>1.9</td>
<td>1.3</td>
<td>2.0</td>
<td>27.5</td>
<td>14.4</td>
<td>11.4</td>
</tr>
<tr>
<td>Median D-dimer, ng/dL (IQR)</td>
<td>154 (114-215)</td>
<td>157 (114-218)</td>
<td>166 (115-235)</td>
<td>133 (89-202)</td>
<td>201 (134-317)</td>
<td>317 (205-473)</td>
</tr>
<tr>
<td>Median ln D-dimer, ng/dL (IQR)</td>
<td>5.04 (4.74-5.37)</td>
<td>5.06 (4.74-5.38)</td>
<td>5.11 (4.74-5.46)</td>
<td>4.97 (4.49-3.10)</td>
<td>5.33 (4.89-5.76)</td>
<td>5.76 (5.32-6.16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KORA-F3</th>
<th>LBC 1921 (Vidas)</th>
<th>LBC 1936 (Vidas)</th>
<th>LBC (HemoSil)</th>
<th>ORCADES</th>
<th>PROSPER</th>
<th>RS</th>
<th>Twins UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,588</td>
<td>152</td>
<td>391</td>
<td>162</td>
<td>883</td>
<td>5,076</td>
<td>552</td>
<td>2,166</td>
</tr>
<tr>
<td>62.3 (10.1)</td>
<td>86.6 (0.39)</td>
<td>72.00 (0.48)</td>
<td>72.90 (0.33)</td>
<td>53.5 (15.73)</td>
<td>75.3 (3.4)</td>
<td>71.7 (8.9)</td>
<td>54.2 (12.42)</td>
</tr>
<tr>
<td>49.4</td>
<td>46.1</td>
<td>52.7</td>
<td>54.9</td>
<td>46.1</td>
<td>47.6</td>
<td>42.2</td>
<td>8.4</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>28.1 (4.5)</td>
<td>26.2 (4.21)</td>
<td>27.7 (4.15)</td>
<td>27.2 (3.43)</td>
<td>27.66 (4.84)</td>
<td>26.8 (4.2)</td>
<td>26.3 (3.6)</td>
<td>26.4 (5.0)</td>
</tr>
<tr>
<td>3.3</td>
<td>29.6</td>
<td>29.4</td>
<td>36.1</td>
<td>9.7</td>
<td>39.3</td>
<td>25.1</td>
<td>1.4</td>
</tr>
<tr>
<td>2.4</td>
<td>unk</td>
<td>unk</td>
<td>Unk</td>
<td>Unk</td>
<td>unk</td>
<td>unk</td>
<td>unk</td>
</tr>
<tr>
<td>10.8</td>
<td>5.9</td>
<td>10.6</td>
<td>11.4</td>
<td>3.0</td>
<td>10.4</td>
<td>15.6</td>
<td>2.5</td>
</tr>
<tr>
<td>22.5 (15.1-43.7)</td>
<td>200 (100-300) $^a$</td>
<td>144 (102-222)</td>
<td>79 (47-124)</td>
<td>265 (191-383)</td>
<td>39.0 (22.3-74.1)</td>
<td>77 (56-114)</td>
<td></td>
</tr>
<tr>
<td>3.24 (2.71-3.78)</td>
<td>5.30 (4.61-5.70)</td>
<td>4.61 (4.61-5.30)</td>
<td>4.98 (4.63-5.40)</td>
<td>4.37 (3.85-4.82)</td>
<td>5.59 (5.27-5.95)</td>
<td>3.66 (3.10-4.30)</td>
<td>4.3 (4.03-4.74)</td>
</tr>
</tbody>
</table>

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.

$^a$In LBC1921 and LBC1936, the Vidas method measurements were only available in increments of 100 ng/dL.
Table 2

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

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

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