Published in final edited form as:
Thromb Res. 2014 August ; 134(2): 462–467. doi:10.1016/j.thromres.2014.05.018.

A genetic association study of D-dimer levels with 50K SNPs from a candidate gene chip in four ethnic groups

Lu-Chen Weng[1], **Weihong Tang**[*,1], **Stephen S. Rich**[2], **Nicholas L. Smith**[3], **Susan Redline**[4], **Christopher J. O'Donnell**[5], **Saonli Basu**[6], **Alexander P. Reiner**[3], **Joseph A. Delaney**[3], **Russell P. Tracy**[7], **Cameron D. Palmer**[8], **Taylor Young**[8], **Qiong Yang**[9], **Aaron R. Folsom**[1], and **Mary Cushman**[10]

[1]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA

[2]Department of Public Health Sciences and the Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA

[3]Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA, USA; also: Group Health Research Institute, Group Health Cooperative, Seattle WA; Seattle Epidemiologic Research and Information Center, Department of Veterans Affairs Office of Research and Development, Seattle WA

[4]Division of Sleep Medicine, Department of Medicine, Brigham and Women's Hospital and Beth Israel Deaconess Medical Center, Harvard Medical School. Boston, MA, USA

[5]National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

[6]Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN

[7]Departments of Pathology and Biochemistry, University of Vermont, Burlington, VT, USA

[8]Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, MA, USA

[9]The NHLBI's Framingham Heart Study, Framingham, MA, USA; Department of Biostatistics, School of Public Health, Boston University, Boston, MA,USA

[10]Departments of Medicine and Pathology, University of Vermont, Burlington, VT, USA

Abstract

[*]Corresponding author: Division of Epidemiology and Community Health University of Minnesota 1300 South Second Street, WBOB 300 Minneapolis, MN 55454 tang0097@umn.edu Phone (612) 626-9140 Fax (612) 624-0315.

Authorship Contributions and Disclosure of Conflict-of-interest

Contribution: L.-C. W. analyzed the data and wrote the manuscript; W.T. designed the research, supervised the analysis, and edited the manuscript; C.D.P. and T.Y. analyzed the data; and all the others edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Introduction—D-dimer, a fibrin degradation product, is related to risk of cardiovascular disease and venous thromboembolism. Genetic determinants of D-dimer are not well characterized; notably, few data have been reported for African American (AA), Asian, and Hispanic populations.

Materials and Methods—We conducted a large-scale candidate gene association study to identify variants in genes associated with D-dimer levels in multi-ethnic populations. Four cohorts, comprising 6,848 European Americans (EAs), 2,192 AAs, 670 Asians, and 1,286 Hispanics in the NHLBI Candidate Gene Association Resource (CARe) consortium, were assembled. Approximately 50,000 genotyped SNPs in 2,000 cardiovascular disease gene loci were analyzed by linear regression, adjusting for age, sex, study site, and principal components in each cohort and ethnic group. Results across studies were combined within each ethnic group by meta-analysis.

Results—Twelve SNPs in coagulation factor V (*F5*) and 3 SNPs in the fibrinogen alpha chain (*FGA*) were significantly associated with D-dimer level in EAs with $p < 2.0\times10^{-6}$. The signal for the most associated SNP in *F5* (rs6025, *F5* Leiden) was replicated in Hispanics ($p = 0.023$), while that for the top functional SNP in *FGA* (rs6050) was replicated in AAs ($p = 0.006$). No additional SNPs were significantly associated with D-dimer.

Conclusions—Our study replicated previously reported associations of D-dimer with SNPs in *F5* (*F5*-Leiden) and *FGA* in EAs; we demonstrated replication of the association of D-dimer with *FGA* rs6050 in AAs and the *F5*-Leiden variant in Hispanics.

Keywords

D-dimer; genetic association study; CARe consortium; single nucleotide polymorphisms

D-dimer is a plasma fibrin degradation product and a biomarker of thrombosis. It is generated through fibrinolysis during which fibrin polymers are cleaved by circulating enzyme plasmin. Therefore, D-dimer level may be influenced by coagulation, fibrinolysis, or regulatory factors of these processes. In previous studies, higher plasma D-dimer concentration correlated with higher levels of coagulation and inflammatory markers, such as fibrinogen, factor VIII coagulant activity, C-reactive protein, interleukin-6, and with carriage of the factor V (*F5*) Leiden polymorphism [1-3]. Also, D-dimer has been shown to be associated with the risk of several diseases in prospective studies, including cardiovascular disease and first/recurrent venous thromboembolism [3-5].

A study focusing on an elderly population showed that single nucleotide polymorphisms (SNPs) in the fibrinogen gamma chain (*FGG*), fibrinogen alpha chain (*FGA*), urokinase-type plasminogen activator (*PLAU*), and serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (*SERPINE1*) genes were significantly associated with D-dimer levels in European Americans (EAs), but no SNPs were associated in African Americans (AAs) [6]. A genome-wide association study (GWAS) of 13 European ancestry cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium identified significant evidences of associations with D-dimer concentration near genes coding for coagulation factor III (*F3*), *F5*, *FGG*, and *FGA* [7]. Populations of other ethnicities were not included in the GWAS. Mean D-dimer levels

differed between EAs and other ethnic populations (*e.g.*, AAs and Asians) [8], and the percentage of European ancestry was associated with lower D-dimer in AAs [6]. However, genetic determinants of D-dimer in AAs, Hispanics, and Asians have not been well understood.

The ITMAT-Broad-Candidate Gene Association Resource (CARe) (IBC) genotyping array is a customized SNP genotyping platform [9]. This platform includes about 50,000 SNPs covering ~2,000 cardiovascular disease (CVD) gene loci. We analyzed the associations between D-dimer levels and SNPs on the IBC candidate gene array in multi-ethnic populations from the NHLBI CARe consortium; EA and AA samples were obtained from the Multi-Ethnic Study of Atherosclerosis (MESA), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), and Cleveland Family Study (CFS); Hispanic and Asian participants in the MESA study were analyzed for replication of significant SNP associations detected in the EA and AA populations.

Materials and Methods

Study population

The NHLBI CARe consortium was initiated to study genetic variations in cardiovascular, pulmonary, hematologic, and sleep-related traits in over 40,000 participants from 9 community-based cohorts [10] of four ethnic groups: EAs, AAs, Hispanic-, and Asian-Americans. Four cohorts (CFS, CHS, FHS, and MESA) measured D-dimer concentrations and thus contributed to the analyses. D-dimer levels were measured for the entire population at baseline in MESA [8] and for the FHS Offspring cohort without prior cardiovascular disease at exam 5 [11]. In CFS, D-dimer levels were measured in 735 participants who attended the visit 5 exam [12]. In CHS, plasma D-dimer levels were measured in a sub-population of ~1,800 EAs and ~300 AAs as part of a nested case-control study for cardiovascular disease [13]. All participating institutions in this study received approval from their institutional review boards, and informed consents were obtained from all participants. Details of each participating cohort are described in Supplemental Methods.

Phenotype measurement

In CFS and MESA, fasting blood samples were centrifuged and then stored at −80 °C until assayed at the University of Vermont using the STa-R analyzer, an immunoturbidimetric assay (Liatest D-DI; Diagnostica Stago) [8, 12]. The analytic coefficients of variation (CV) were 1% for CFS and 8% for MESA [12, 14]. D-dimer concentration in CHS and FHS were measured with an enzyme-linked immunosorbent assay (ELISA, Biopool AB) [6, 15-17]. The CVs for D-dimer measurements were 7% for CHS and 11.7% for FHS. Participants who were taking warfarin or had D-dimer concentrations more than 6 standard deviations from the mean were excluded from analysis.

Genotyping and imputation

A total of 49,320 SNPs in ~2,000 candidate gene loci are included on the IBC genotyping array, including at least 17% of SNPs that were not in HapMap at the time of construction [18]. The genes on the array were selected to represent candidates for a range of

cardiovascular, sleep, lung, metabolic and inflammatory pathways and syndromes [9]. The SNPs in the selected genes were chosen to capture the linkage disequilibrium (LD) for European- and African-ancestry populations using a cosmopolitan tagging approach, based on data in HapMap, SeattleSNPs, and Environmental Genome Projects. Additional SNPs were included if they had putative functional significance in genes. Details on SNP selection strategy can be found in Supplemental Methods.

Imputation of non-genotyped SNPs in the ~2,000 gene loci was performed separately in each race group using MACH 1.0.16 [19] based on the HapMap 2 reference panels. The CEU panel was used as the reference for EAs and the JPT+CHB panel was used for Asians; a combined CEU and YRI panel with 1:1 ratio was created as the reference for imputation in AAs. In Hispanics, a combination of CEU, YRI, and JPT+CHB with 1:1:1 ratio was created as reference panel for imputation. All imputed SNPs were represented as “dosage” based on the expected number of copies of the reference allele. Details of genotyping, imputation, and quality control are provided in Supplemental Methods.

Statistical Analysis

D-dimer level was regressed on age, sex, and study site (if applicable) in a general linear model to calculate the inverse normal transformed residuals in SAS (SAS version 9.2). In CHS, the regression included additional adjustment for case-control status as well as subgroup status of controls, based on the nested case-control design of the original study [13]. The residuals were calculated by study and ethnic group and used in the genetic analysis. Linear associations between genetic variants and the inverse normal transformed residuals of D-dimer were analyzed in each study and ethnic group. Ten principal components calculated by EIGENSTRAT were included as covariates in all genetic analyses (within each ethnic group) to control for population stratification [20]. For cohorts of unrelated individuals, linear regression analyses were performed using PLINK version 1.0.7 [21-22]. For family cohorts, a linear mixed effects model (LME) was used to adjust for correlations between individuals of the same families [23]. METAL was used to meta-analyze the genetic associations across studies within the same ethnic groups using the inverse-variance weighted approach [24]. Linear associations between the top SNPs and raw D-dimer value (i.e., untransformed) were also performed to obtain clinically meaningful estimate of SNP effect. SNPs with minor allele count (minor allele frequency × sample size) less than 10 or imputed SNPs with imputation quality score (RSQ) (Squared correlation between imputed and true genotypes) ≤ 0.6 were excluded from the meta-analysis. The threshold for statistical significance was set at $p < 2\times10^{-6}$ to account for multiple testing and correlation among SNPs.

When multiple significant SNPs occurred in the same region, conditional analyses were performed to examine the independence of those SNPs from the most associated SNP. The LD statistics, r^2, was used to represent the correlation between SNPs within a region. The r^2 calculated from the HapMap 3 CEU was used to represent the LD for CARe EAs, and the r^2 calculated based on the HapMap 3 African and African American data were used for the CARe AAs. For Asians, the r^2 was calculated based on CHB+JPT from the HapMap 3.

Results

A total of 6,848 EA, 2,192 AA, 670 Asian, and 1,286 Hispanic participants were included in these analyses. As shown in Table 1, the mean age for these 4 studies ranged from 38 to 73 years, and 39-50% of participants were male. Within each study, AA participants had higher D-dimer levels than EAs; Asians had the lowest levels. The quantile-quantile (Q-Q) plots of genetic associations in EAs and AAs are shown in Supplemental Figure S1-S2. The inflation factors for the analyses of EAs and AAs were 0.98 and 1.0, respectively, suggesting minimal influence of population admixture.

In EAs, 15 SNPs were significantly ($p < 2\times10^{-6}$) associated with D-dimer (Supplemental Table 1), located in the genes *F5* on chromosome 1q23 (12 SNPs) and *FGA* on chromosome 4q28 (3 SNPs). Table 2 presents the most significantly associated SNPs for D-dimer in EAs and the corresponding results in AAs, Asian and Hispanic Americans. The most associated SNP in *F5* was rs6025 (the Factor V Leiden, Arg506Gln, $p = 7.41\times10^{-16}$). Each one copy of the rs6025 A allele was associated with 0.46 higher level of inverse normal transformed residual of D-dimer. After conditioning on rs6025, none of the remaining 11 SNPs at the F5 region attained significance. Notably, the association between rs6025 and D-dimer was replicated in Hispanic Americans (Table 2).

Three SNPs in or close to the *FGA* were significantly associated with D-dimer in EAs - rs13109457 (3 kb from *FGA*, $p = 7.52\times10^{-7}$), rs6050 (coding-nonsynonymous, Thr331Ala, $p = 1.24\times10^{-6}$), and rs6825454 (3.1 kb from FGA, $p = 1.67\times10^{-6}$) (Table 2 and Supplemental Table 1). Each one copy of the rs6050 A allele was associated with 0.1 lower level of inverse normal transformed residual of D-dimer. These three SNPs were highly correlated with each other in EAs (r^2=0.91-0.96), so that in the conditional analysis adjusting for rs6050, the association of the remaining two SNPs no longer attained statistical significance. The association of D-dimer level with rs6050 was replicated in AAs (Table 2).

Regional association plots for the *F5* locus on chromosome 1 and *FGA* locus on chromosome 4 are presented in Supplemental Figures S3 and S4. No other SNPs were significantly associated with D-dimer levels in AA, Asian, or Hispanic populations.

We also conducted a look-up of seven variants in *F3*, *F5*, *FGG*, *FGA*, *PLAU*, and *SERPINE1* that were previously reported for association with D-dimer from the literature [6-7]. Of the seven variants, rs12029080 in *F3* was not available in this study and the following variants reached nominal significance: rs6687813 (*F5*) in EAs ($p=1.71\times10^{-05}$) and Hispanics (p=0.007), rs2066865 (*FGG)* in EAs ($p=8.81\times10^{-06}$) and AAs (p=0.002), rs2070006 (*FGA)* in EAs (p=0.001) and AAs (p=0.018), rs2070011 (*FGA)* in EAs (p=0.004) (Supplemental Table 2). In addition, we did not observe any significant signals at chromosome 2q, 5p, 5q and 14q loci reported in the previous linkage studies [25-26] (data not shown).

Discussion

This study, based on 49,320 tagging and functional SNPs in ~2,000 CVD-related gene loci in the cohorts of the NHLBI CARe consortium, identified significant associations between

D-dimer levels and SNPs in the *F5* and *FGA* genes in 6,848 EAs. The association of D-dimer with the *F5*-Leiden variant and the *FGA* rs6050 was replicated at nominal significance in Hispanic Americans and AAs, respectively. No significant genetic associations for D-dimer level were observed in Asian Americans. To the best of our knowledge, this is the first report of candidate gene variants for D-dimer level in Hispanic-Americans and also the second but the largest study of this association in AAs.

The SNP with the smallest p-value in this analysis was rs6025, which is located in the *F5* gene and is recognized as *F5*-Leiden. The *F5*-Leiden variant is present in 4-6% of the U.S. population, delays the inactivation of Factor Va by activated protein C [27], and increases risk of venous thrombosis [27]. A previous GWAS in EAs reported that rs6687813, a weak proxy for rs6025 (r^2=0.32), was significantly associated with D-dimer [7]. Our finding directly demonstrated a significant association between D-dimer level and rs6025 (*F5* Leiden). Our study is the first we aware of that demonstrates an association between D-dimer and rs6025 in Hispanic Americans.

The top two signals in *FGA*, rs6050 and rs13109457, were in strong LD (r^2=0.91 in EA), with rs6050 being a coding-nonsynonymous variant. The Ala allele of rs6050 increases FXIIIa-dependent fibrinogen alpha chain cross-linking and formation of thicker fibrin fibers, resulting in stiffer clots [28]. Several case-control studies have found a positive association between the Ala allele of rs6050 and risk of venous thromboembolism [29-31]. A GWAS and a small candidate gene association study in populations of European ancestry further demonstrated a positive association between the Ala allele of rs6050 and D-dimer levels [6-7]. This small candidate gene study also included 327 elderly AAs, in which no significant associations were detected between the *FGA* SNPs and D-dimer level [6]. Our study extended the significant associations for *FGA* SNPs to AAs. Notably, minor alleles for all of the 3 significant *FGA* SNPs were positively associated with D-dimer level in both EAs and AAs; however, the strength of associations between EAs and AAs was less comparable for rs13109457 (beta=0.1 for EAs and beta=0.035 for AAs) than for rs6050 (beta=-0.1 for EAs and beta=-0.09 for AAs), and this larger discrepancy for rs13109457 was likely due to different LD at the *FGA* gene between EAs and AAs (r^2 between rs13109457 and rs6050 = 0.91 for EAs and 0.47 for AAs). Taken together, our data suggest that the functional variant rs6050 is likely the causal variant for the clustering of signals at the *FGA* region.

D-dimer level is 23-65% heritable [25, 32]. Genetic linkage studies for D-dimer level have reported putative quantitative trait loci on chromosome 5p [25], 2q [25], 5q [26], and 14q [26], while no loci were detected in either association or linkage analyses in the FHS cohort [11]. A candidate gene study [6] and a GWAS in populations of European ancestry [7] both showed that identified gene variants together explained ~2% of the total variance in D-dimer. Agreeing with these studies, across our EA populations, the *F5*-Leiden variant and *FGA* SNPs explained 0.6%-2% and 0.01%-1% of the total variance in D-dimer levels, respectively. Further studies using other methods, such as next-generation sequencing, are required to search for the missing heritability.

In our look-up of the previously reported variants (Supplemental Table 2), we did not identify any variants in *PLAU*, *SERPINE1*, or *F3* genes associated with plasma D-dimer

level in any ethnic group with p-value exceeding nominal significant threshold of $p<0.05$. Small p-values were observed for rs2066865 in *FGG* in both EAs and AAs ($p=8.81\times10^{-06}$ for EAs and p=0.002 for AAs); however, they did not exceed our array-wide p-value threshold. Interestingly, the effect size of rs2066865 was similar to that of rs6050 (rs2066865: beta=0.09 for EAs and 0.1 for AAs; rs6050: beta=-0.1 for EAs and -0.09 in AAs), and these two SNPs are in high LD in EAs ($r^2=0.88$) and moderate LD in AAs ($r^2=0.40$). The signal of rs2066865 was attenuated after adjusting for rs6050 in EAs (beta=-0.032, p=0.62).

Identification of genetic determinants for D-dimer levels might aid risk prediction and stratification in clinical practice, and the minor alleles in *F5* and *FGA* are associated with increased risk of venous thromboembolism [33-34]. However, based on our current understanding of D-dimer genetics, it is not practical to simply measure SNPs identified for D-dimer levels to predict new and recurrent venous thromboembolism, because all of the SNPs identified in this and the other studies explained at most 2% of total variance in D-dimer levels. Improved utility of D-dimer genetic variants in venous thromboembolism risk prediction might await identification of more variants that can explain a substantial percentage of variance in D-dimer levels.

According to this and previous [8] studies, D-dimer levels were higher in AAs and lower in Asians. Further different patterns of genetic associations for D-dimer were observed between race groups in linkage studies [11, 25-26] and in the present study. However, only 2% of the total variance in plasma D-dimer level can be explained by genetic variants, suggesting that cultural or environmental factors may contribute to D-dimer variation in different ethnic groups. Lower alcohol, including wine, consumption [35], poor sleep [36], smoking [37], and leisure inactivity [37] were associated with higher D-dimer levels in other studies. Systolic blood pressure was also associated with higher level of D-dimer [37]. Future studies are needed to evaluate whether these factors contribute to the race-related differences in D-dimer levels.

Limitations

First, our study focused on SNPs of candidate genes. This approach may miss genes/SNPs that were not selected or regulatory elements that are not located in the selected genes. Also, these analyses focused on the main effect of SNPs in the candidate genes, and it is possible that evaluating gene-gene or gene-environment interactions may be able to identify additional genetic factors for D-dimer levels. Second, additional effects of less common and rare variants on D-dimer levels may be missed because this study focused on common variants. Third, while it is of concern that many of the EA participants in our study were also included in the CHARGE GWAS study [7], only EA participants in CHS and FHS were included in that study. CFS (270 EAs and 340 AAs) and MESA (2264 EAs, 1583 AAs, 670 Asians, and 1286 Hispanics) were added in this study. Furthermore, our study aimed to identify the genetic associations for D-dimer in multi-ethnic populations, in contrast to the CHARGE GWAS study which only focused on populations of European ancestry. The CARe IBC chip is comprised of functional SNPs in the candidate genes, which enables this study to gain additional information than standard GWAS. Therefore, our study

complements the CHARGE GWAS report by including additional information for different populations, ethnicities, and genetic variants. Fourth, two laboratory assays were used in the study populations, which might reduce the comparability of D-dimer measurements from different studies. However, D-dimer measurements in each study were carefully quality controlled to reduce measurement error and the assays correlate quite well (correlation coefficient = 0.69 provided by Dr. Mary Cushman). In addition, we ran a sensitivity analysis of the top variants by using a sample size-weighted meta-analysis, which used p-value from individual studies. This approach converts the individual p-value and the direction of effect for each cohort to a cohort-specific Z-score and then meta-analyzes them to a summarized Z-score. This method allows us to combine effect sizes in different units as p-value is determined by relative rank of an individual by genotype and phenotype, not the absolute unit of phenotype measurement. We obtained similar results in this sensitivity analysis (data not shown). Therefore, different laboratory assays across studies likely had little influence in our association analysis. Lastly, the sample sizes for the AA, Asian- and Hispanic-American populations studied here were relatively small. This, in combination with smaller effect sizes for rs13109457 and rs6050 in Hispanics and Asians, and the rarer frequencies of rs6025 in AAs and Asians, contributed to the lack of replication for these variants in the non-EA populations. For example, the minimum sample sizes to have 80% power for detecting the associations of corresponding magnitudes between rs6050 and D-dimer at $p<0.05$ were 5,625 for Asians and 6,141 for Hispanics, and an even larger sample would be required for rs13109457 (n=16,749 for AAs, 5,646 for Asians, and 12,357 for Hispanics). Therefore, larger studies are needed to clarify these un-replicated genetic associations for D-dimer in non-EA populations.

Summary

Among 6,848 EAs from four population-based studies, SNPs in the *F5* and *FGA* genes were significantly associated with D-dimer levels, replicating results from a previously published GWAS in EA. The genetic signals in *F5* (rs6025, *F5*-Leiden) and *FGA* (rs6050) were replicated in Hispanic-American and AA populations, respectively. A lack of replications for these signals in the other non-EA populations may be due to rarer allele frequencies, smaller effect sizes, and/or limited sample sizes. Given the important relationships of D-dimer with risk of venous and arterial thrombosis, and the small fraction of D-dimer variance explained by the genetic variants in our (and others') results, future work is needed to further understand the genetic underpinnings of D-dimer variability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

CARe Acknowledgement: The authors wish to acknowledge the support of the National Heart, Lung, and Blood Institute and the contributions of the research institutions, study investigators, field staff and study participants in creating this resource for biomedical research. The following four parent studies have contributed parent study data, ancillary study data, and DNA samples through the Broad Institute of Harvard University and the Massachusetts Institute of Technology (N01-HC-65226) to create this genotype/phenotype data base for this project:

Cardiovascular Health Study (CHS): University of Washington (N01-HC-85079), Wake Forest University (N01-HC-85080), Johns Hopkins University (N01-HC-85081), University of Pittsburgh (N01-HC-85082), University of California, Davis (N01-HC-85083), University of California, Irvine (N01-HC-85084), New England Medical Center (N01-HC-85085), University of Vermont (N01-HC-85086), Georgetown University (N01-HC-35129), Johns Hopkins University (N01 HC-15103), University of Wisconsin (N01-HC-75150), Geisinger Clinic (N01-HC-45133), University of Washington (N01 HC-55222, U01 HL080295);

Cleveland Family Study (CFS): Case Western Reserve University (RO1 HL46380-01-16);

Framingham Heart Study (FHS): Boston University (N01-HC-25195);

Multi-Ethnic Study of Atherosclerosis (MESA): University of Washington (N01-HC-95159), Regents of the University of California (N01-HC-95160), Columbia University (N01-HC-95161), Johns Hopkins University (N01-HC-95162), University of Minnesota (N01-HC-95163), Northwestern University (N01-HC-95164), Wake Forest University (N01-HC-95165), University of Vermont (N01-HC-95166), New England Medical Center (N01-HC-95167), Johns Hopkins University (N01-HC-95168),Harbor-UCLA Research and Education Institute (N01-HC-95169);

Part of the work was supported by grant R01-HL095603. We would like to thank the University of Minnesota Supercomputing Institute for use of the Blade and Calhoun supercomputers. Acknowledgements for each individual parent study of the CARe Consortium are listed in the Supplemental Acknowledgements.

Abbreviations

AAs	African Americans
CARe	the Candidate Gene Association Resource
CFS	Cleveland Family Study
CHARGE	the Cohorts for Heart and Aging Research in Genomic Epidemiology
CHS	Cardiovascular Health Study
CV	coefficients of variation
CVD	cardiovascular disease
EAs	European Americans
ELISA	enzyme-linked immunosorbent assay
FGA	fibrinogen alpha chain gene
FGG	fibrinogen gamma chain gene
FHS	Framingham Heart Study
F3	factor III gene
F5	factor V gene
FXIIIa	activated factor XIII
GWAS	genome-wide association study
IBC	the ITMAT-Broad-CARe
LD	linkage disequilibrium
LME	linear mixed effects model
MESA	the Multi-Ethnic Study of Atherosclerosis study

PLAU	urokinase-type plasminogen activator gene
Q-Q plot	quantile-quantile plot
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 gene
SNP	single nucleotide polymorphism

References

1. Lowe GD, Sweetnam PM, Yarnell JW, Rumley A, Rumley C, Bainton D, Ben-Shlomo Y. C-reactive protein, fibrin D-dimer, and risk of ischemic heart disease: the Caerphilly and Speedwell studies. Arterioscler Thromb Vasc Biol. 2004; 24:1957–1962. [PubMed: 15308549]
2. Koenig W, Rothenbacher D, Hoffmeister A, Griesshammer M, Brenner H. Plasma fibrin D-dimer levels and risk of stable coronary artery disease: results of a large case-control study. Arterioscler Thromb Vasc Biol. 2001; 21:1701–1705. [PubMed: 11597948]
3. Cushman M, Folsom AR, Wang L, Aleksic N, Rosamond WD, Tracy RP, Heckbert SR. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003; 101:1243–1248. [PubMed: 12393393]
4. Eichinger S, Minar E, Bialonczyk C, Hirschl M, Quehenberger P, Schneider B, Weltermann A, Wagner O, Kyrle PA. D-dimer levels and risk of recurrent venous thromboembolism. JAMA. 2003; 290:1071–1074. [PubMed: 12941680]
5. Zakai NA, Katz R, Jenny NS, Psaty BM, Reiner AP, Schwartz SM, Cushman M. Inflammation and hemostasis biomarkers and cardiovascular risk in the elderly: the Cardiovascular Health Study. J Thromb Haemost. 2007; 5:1128–1135. [PubMed: 17388967]
6. Lange LA, Reiner AP, Carty CL, Jenny NS, Cushman M, Lange EM. Common genetic variants associated with plasma fibrin D-dimer concentration in older European- and African-American adults. J Thromb Haemost. 2008; 6:654–659. [PubMed: 18208536]
7. Smith NL, Huffman JE, Strachan DP, Huang J, Dehghan A, Trompet S, Lopez LM, Shin SY, Baumert J, Vitart V, Bis JC, Wild SH, Rumley A, Yang Q, Uitterlinden AG, Stott DJ, Davies G, Carter AM, Thorand B, Polasek O, McKnight B, Campbell H, Rudnicka AR, Chen MH, Buckley BM, Harris SE, Peters A, Pulanic D, Lumley T, de Craen AJ, Liewald DC, Gieger C, Campbell S, Ford I, Gow AJ, Luciano M, Porteous DJ, Guo X, Sattar N, Tenesa A, Cushman M, Slagboom PE, Visscher PM, Spector TD, Illig T, Rudan I, Bovill EG, Wright AF, McArdle WL, Tofler G, Hofman A, Westendorp RG, Starr JM, Grant PJ, Karakas M, Hastie ND, Psaty BM, Wilson JF, Lowe GD, O'Donnell CJ, Witteman JC, Jukema JW, Deary IJ, Soranzo N, Koenig W, Hayward C. Genetic predictors of fibrin D-dimer levels in healthy adults. Circulation. 2011; 123:1864–1872. [PubMed: 21502573]
8. Lutsey PL, Cushman M, Steffen LM, Green D, Barr RG, Herrington D, Ouyang P, Folsom AR. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. J Thromb Haemost. 2006; 4:2629–2635. [PubMed: 17002663]
9. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, Galver L, Barrett JC, Grant SF, Farlow DN, Chandrupatla HR, Hansen M, Ajmal S, Papanicolaou GJ, Guo Y, Li M, Derohannessian S, de Bakker PI, Bailey SD, Montpetit A, Edmondson AC, Taylor K, Gai X, Wang SS, Fornage M, Shaikh T, Groop L, Boehnke M, Hall AS, Hattersley AT, Frackelton E, Patterson N, Chiang CW, Kim CE, Fabsitz RR, Ouwehand W, Price AL, Munroe P, Caulfield M, Drake T, Boerwinkle E, Reich D, Whitehead AS, Cappola TP, Samani NJ, Lusis AJ, Schadt E, Wilson JG, Koenig W, McCarthy MI, Kathiresan S, Gabriel SB, Hakonarson H, Anand SS, Reilly M, Engert JC, Nickerson DA, Rader DJ, Hirschhorn JN, Fitzgerald GA. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One. 2008; 3:e3583. [PubMed: 18974833]
10. Musunuru K, Lettre G, Young T, Farlow DN, Pirruccello JP, Ejebe KG, Keating BJ, Yang Q, Chen MH, Lapchyk N, Crenshaw A, Ziaugra L, Rachupka A, Benjamin EJ, Cupples LA, Fornage M,

Fox ER, Heckbert SR, Hirschhorn JN, Newton-Cheh C, Nizzari MM, Paltoo DN, Papanicolaou GJ, Patel SR, Psaty BM, Rader DJ, Redline S, Rich SS, Rotter JI, Taylor HA Jr. Tracy RP, Vasan RS, Wilson JG, Kathiresan S, Fabsitz RR, Boerwinkle E, Gabriel SB. Candidate gene association resource (CARe): design, methods, and proof of concept. Circ Cardiovasc Genet. 2010; 3:267–275. [PubMed: 20400780]

11. Yang Q, Kathiresan S, Lin JP, Tofler GH, O'Donnell CJ. Genome-wide association and linkage analyses of hemostatic factors and hematological phenotypes in the Framingham Heart Study. BMC Med Genet. 2007; 8(Suppl 1):S12. [PubMed: 17903294]
12. Mehra R, Xu F, Babineau DC, Tracy RP, Jenny NS, Patel SR, Redline S. Sleep-disordered breathing and prothrombotic biomarkers: cross-sectional results of the Cleveland Family Study. Am J Respir Crit Care Med. 2010; 182:826–833. [PubMed: 20508215]
13. Crainich P, Jenny NS, Tang Z, Arnold AM, Kuller LH, Manolio T, Sharrett AR, Tracy RP. Lack of association of the plasminogen activator inhibitor-1 4G/5G promoter polymorphism with cardiovascular disease in the elderly. J Thromb Haemost. 2003; 1:1799–1804. [PubMed: 12911596]
14. Arnett DK, McClelland RL, Bank A, Bluemke DA, Cushman M, Szalai AJ, Jain N, Gomes AS, Heckbert SR, Hundley WG, Lima JA. Biomarkers of inflammation and hemostasis associated with left ventricular mass: The Multiethnic Study of Atherosclerosis (MESA). Int J Mol Epidemiol Genet. 2011; 2:391–400. [PubMed: 22200000]
15. Cushman M, Lemaitre RN, Kuller LH, Psaty BM, Macy EM, Sharrett AR, Tracy RP. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 1999; 19:493–498.
16. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006; 355:2631–2639. [PubMed: 17182988]
17. Schnabel RB, Larson MG, Yamamoto JF, Sullivan LM, Pencina MJ, Meigs JB, Tofler GH, Selhub J, Jacques PF, Wolf PA, Magnani JW, Ellinor PT, Wang TJ, Levy D, Vasan RS, Benjamin EJ. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. Circulation. 2010; 121:200–207. [PubMed: 20048208]
18. Wassel CL, Lange LA, Keating BJ, Taylor KC, Johnson AD, Palmer C, Ho LA, Smith NL, Lange EM, Li Y, Yang Q, Delaney JA, Tang W, Tofler G, Redline S, Taylor HA Jr. Wilson JG, Tracy RP, Jacobs DR Jr. Folsom AR, Green D, O'Donnell CJ, Reiner AP. Association of genomic loci from a cardiovascular gene SNP array with fibrinogen levels in European Americans and African-Americans from six cohort studies: the Candidate Gene Association Resource (CARe). Blood. 2011; 117:268–275. [PubMed: 20978265]
19. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]
20. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]
21. [Accessed June 1, 2010] Purcell S PLINK (1.0.7). http://pngu.mgh.harvard.edu/purcell/plink/
22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]
23. Chen MH, Yang Q. GWAF: an R package for genome-wide association analyses with family data. Bioinformatics. 2010; 26:580–581. [PubMed: 20040588]
24. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–2191. [PubMed: 20616382]
25. Diego VP, Almasy L, Rainwater DL, Mahaney MC, Comuzzie AG, Cole SA, Tracy RP, Stern MP, Maccluer JW, Blangero J. A quantitative trait locus on chromosome 5p influences d dimer levels in the san antonio family heart study. Int J Vasc Med. 2010; 2010:490241. [PubMed: 21151504]

26. Williams FM, Carter AM, Kato B, Falchi M, Bathum L, Surdulescu G, Kyvik KO, Palotie A, Spector TD, Grant PJ. Identification of quantitative trait loci for fibrin clot phenotypes: the EuroCLOT study. Arterioscler Thromb Vasc Biol. 2009; 29:600–605. [PubMed: 19150881]
27. Price DT, Ridker PM. Factor V Leiden mutation and the risks for thromboembolic disease: a clinical perspective. Ann Intern Med. 1997; 127:895–903. [PubMed: 9382368]
28. Standeven KF, Grant PJ, Carter AM, Scheiner T, Weisel JW, Ariens RA. Functional analysis of the fibrinogen Aalpha Thr312Ala polymorphism: effects on fibrin structure and function. Circulation. 2003; 107:2326–2330. [PubMed: 12707238]
29. Carter AM, Catto AJ, Kohler HP, Ariens RA, Stickland MH, Grant PJ. alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism. Blood. 2000; 96:1177–1179. [PubMed: 10910940]
30. Rasmussen-Torvik LJ, Cushman M, Tsai MY, Zhang Y, Heckbert SR, Rosamond WD, Folsom AR. The association of alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism in the LITE study. Thromb Res. 2007; 121:1–7. [PubMed: 17433418]
31. Le Gal G, Delahousse B, Lacut K, Malaviolle V, Regina S, Blouch MT, Couturaud F, Mottier D, Oger E, Gruel Y. Fibrinogen Aalpha-Thr312Ala and factor XIII-A Val34Leu polymorphisms in idiopathic venous thromboembolism. Thromb Res. 2007; 121:333–338. [PubMed: 17568659]
32. Ariens RA, de Lange M, Snieder H, Boothby M, Spector TD, Grant PJ. Activation markers of coagulation and fibrinolysis in twins: heritability of the prethrombotic state. Lancet. 2002; 359:667–671. [PubMed: 11879863]
33. Voorberg J, Roelse J, Koopman R, Buller H, Berends F, ten Cate JW, Mertens K, van Mourik JA. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet. 1994; 343:1535–1536. [PubMed: 7911872]
34. Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. A meta-analysis involving approximately 120,000 cases and 180,000 controls. Thromb Haemost. 2009; 102:360–370. [PubMed: 19652888]
35. Wannamethee SG, Lowe GD, Shaper G, Whincup PH, Rumley A, Walker M, Lennon L. The effects of different alcoholic drinks on lipids, insulin and haemostatic and inflammatory markers in older men. Thromb Haemost. 2003; 90:1080–1087. [PubMed: 14652640]
36. von Kanel R, Dimsdale JE, Ancoli-Israel S, Mills PJ, Patterson TL, McKibbin CL, Archuleta C, Grant I. Poor sleep is associated with higher plasma proinflammatory cytokine interleukin-6 and procoagulant marker fibrin D-dimer in older caregivers of people with Alzheimer's disease. J Am Geriatr Soc. 2006; 54:431–437. [PubMed: 16551309]
37. Lee AJ, Fowkes GR, Lowe GD, Rumley A. Determinants of fibrin D-dimer in the Edinburgh Artery Study. Arterioscler Thromb Vasc Biol. 1995; 15:1094–1097. [PubMed: 7627701]

Thromb Res. Author manuscript; available in PMC 2015 August 01.

Table 1

Characteristics of the study participants

Characteristic	CHS	CFS	FHS	MESA
European Americans				
N	1673	223	2688	2264
Mean age, years	73.1 ± 5.9	41.3 ± 19.3	58.3 ± 9.6	62.7 ± 10.3
Male, %	43.6	41.7	46	47.8
BMI, kg/m^2	26.3 ± 4.55	29.8 ± 7.77	26.1 ± 4.58	27.7 ± 5.04
Current Smoker, %	11.7	18.4	33.1	11.6
Median D-dimer (IQR), ng/mL	141 (90-218)	270 (170-470)	315 (202-462)	200 (130-350)
African Americans				
N	299	310		1583
Mean age, years	72.9 ± 5.6	37.7 ± 18.7		62.2 ± 10.1
Male, %	38.5	40.3		45.8
BMI, kg/m^2	28.4 ± 5.37	31.5 ± 8.58		30.2 ± 5.86
Current Smoker, %	15.1	14.5		18.1
Median D-dimer (IQR), ng/mL	215 (126-384)	340 (220-570)		250 (150-440)
Asians				
N				670
Mean age, years				62.6 ± 10.5
Male, %				49.7
BMI, kg/m^2				23.9 ± 3.28
Current Smoker, %				5.5
Median D-dimer (IQR), ng/mL				180 (100-300)
Hispanics				
N				1286
Mean age, years				61.2 ± 10.3
Male, %				47.9
BMI, kg/m^2				29.5 ± 5.15
Current Smoker, %				13.9
Median D-dimer (IQR), ng/mL				230 (130-390)

Data are stated as mean ± SD or percentage, unless otherwise stated.

CHS indicates Cardiovascular Health Study, CFS, Cleveland Family Study; FHS, Framingham Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; IQR, interquartile range.

Table 2

Top SNPs associated with D-dimer in European Americans and replication in the other ethnic groups

Counts	rs6025 (FV Leiden)	rs13109457*	rs6050
Region	1q23	4q28	4q28
Genes/Function	FV/coding-nonsynonymous (Arg506Gln)	~3kb from FGA/intergenic	FGA/coding-nonsynonymous (Thr331Ala)
A1/A2†	A/G	A/G	A/G
European Americans			
N	6625	6710	6848
Allele A1 Frequency	0.026	0.254	0.761
Beta (Beta_raw)‡	0.459 (114.25)	0.102 (11.31)	−0.100 (−7.89)
P-value	7.41×10^{-16}	7.52×10^{-7}	1.24×10^{-6}
African Americans			
N	– §	2192	2194
Allele A1 Frequency	-	0.256	0.641
Beta (Beta_raw)	-	0.035 (17.56)	−0.093 (−34.13)
P-value	-	0.329	0.006
Asians			
N	-	670	670
Allele A1 Frequency	-	0.439	0.553
Beta (Beta_raw)		0.053 (29.23)	−0.053 (−29.01)
P-value	-	0.361	0.367
Hispanics			
N	1286	1286	1286
Allele A1 Frequency	0.013	0.252	0.732
Beta (Beta_raw)	0.401 (43.65)	0.041 (1.40)	−0.057 (−7.87)
P-value	0.023	0.377	0.221

CHS indicates Cardiovascular Health Study, CFS, Cleveland Family Study; FHS, Framingham Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis.

*r^2 between rs13109457 and rs6050 in EA, AA, and Asian are 0.91, 0.47, and 1.00, respectively, r^2 is not available in Hispanics;

†A1: coded allele, A2=non-coded allele;

[‡]Beta: regression coefficient refers to the change in inverse normal transformed residuals of D-dimer level per 1-allele increase in the A1 allele for each race group; Beta_raw: regression coefficient refers to the change in raw D-dimer level (i.e., untransformed, ng/mL) per 1-allele increase in the A1 allele for each race group

[§]Very rare or monomorphic.