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A Multi-Ethnic Meta-Analysis of Genome-Wide Association Studies in Over 100,000 Subjects Identifies 23 Fibrinogen-Associated Loci but no Strong Evidence of a Causal Association between Circulating Fibrinogen and Cardiovascular Disease

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Abstract

Background—Estimates of the heritability of plasma fibrinogen concentration, an established predictor of cardiovascular disease (CVD), range from 34 to 50%. Genetic variants so far identified by genome-wide association (GWA) studies only explain a small proportion (< 2%) of its variation.

Methods and Results—We conducted a meta-analysis of 28 GWA studies, including more than 90,000 subjects of European ancestry, the first GWA meta-analysis of fibrinogen levels in 7 African Americans studies totaling 8,289 samples, and a GWA study in Hispanic-Americans totaling 1,366 samples. Evaluation for association of SNPs with clinical outcomes included a total of 40,695 cases and 85,582 controls for coronary artery disease (CAD), 4,752 cases and 24,030 controls for stroke, and 3,208 cases and 46,167 controls for venous thromboembolism (VTE). Overall, we identified 24 genome-wide significant ($P<5\times10^{-8}$) independent signals in 23 loci, including 15 novel associations, together accounting for 3.7% of plasma fibrinogen variation. Gene-set enrichment analysis highlighted key roles in fibrinogen regulation for the three structural fibrinogen genes and pathways related to inflammation, adipocytokines and thyrotrophin-releasing hormone signaling. Whereas lead SNPs in a few loci were significantly associated with CAD, the combined effect of all 24 fibrinogen-associated lead SNPs was not significant for CAD, stroke or VTE.

Conclusion—We identify 23 robustly associated fibrinogen loci, 15 of which are new. Clinical outcome analysis of these loci does not support a causal relationship between circulating levels of fibrinogen and CAD, stroke or VTE.

Keywords

Fibrinogen; cardiovascular disease; genome-wide association study

Introduction

Fibrinogen plays a major role in wound healing and thrombosis. Circulating levels of fibrinogen are upregulated in inflammatory conditions, consequently serving as an important

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marker of inflammation. Fibrinogen is a well-established predictor of cardiovascular disease (CVD) outcomes, such as myocardial infarction,[1, 2] stroke[3] and venous thromboembolism (VTE) .[4, 5]

It is estimated that 34 (extended pedigrees study) to 44% (twins study) of the inter-individual variation in fibrinogen levels is heritable,[6, 7] indicating a substantial influence of genetics. Two recent meta-analyses of genome-wide association (GWA) studies, conducted in cohorts of European ancestry, identified several genetic variants affecting fibrinogen levels.[8, 9] These variants account only for a small proportion (< 2%) of plasma fibrinogen variation, suggesting that additional genetic variants with more modest effects may remain to be detected.

There is now increasing evidence that a substantial proportion of consequential genetic variation for many phenotypes is tagged by common SNPs[10], although most of these SNPs cannot pass the restrictive genome-wide significance level of $p<5\times10^{-8}$ in a typical association study. To overcome this limitation, increased sample sizes are needed. We conducted a large meta-analysis of 28 GWA studies including more than 90,000 individuals of European ancestry, a 4-fold increase in sample size compared to prior meta-analyses.[8, 9] We included data from an additional 8,423 samples from the first GWA studies of African Americans and 1,447 Hispanic individuals to also explore whether ethnic differences exist in the genetic regulation of plasma fibrinogen concentration. To further elucidate possible biological mechanisms underlying fibrinogen regulation, we examined genome-wide significant loci in relation to expression levels of nearby genes, and in gene pathway analyses. Finally, we examined whether fibrinogen related genes affect risk of coronary artery disease (CAD), stroke and VTE.[11–16]

Methods

Cohorts and Plasma Fibrinogen Measurements

Twenty-eight studies contributed to the discovery GWA study meta-analysis of European-ancestry individuals. Characteristics of all participating studies are provided in Supplementary Methods and Supplementary Table S1. In 7 cohorts, with 33,745 individuals, plasma fibrinogen concentration was measured by an immunonephelometric method.[17] For the other 21 European-ancestry cohorts (57,578 individuals), plasma fibrinogen levels were determined by a functional method (based on the Clauss method).[18] Seven African-American cohorts with GWA data, including a total of 8,423 individuals (5,937 with Clauss and 2,486 with immunonephelometric measures)and one cohort of 1,447 Hispanics with immunonephelometric fibrinogen measures was also analyzed (Supplementary Methods and Supplementary Table S2). Exclusion criteria applied in individual cohorts are provided in Supplementary Methods. All studies were approved by the relevant research ethics committees.

Genotyping, Quality Control of Genotype Data and Imputation

Commercial arrays were used for genome-wide genotyping in all cohorts, and quality control (QC) filtering of SNP genotype data was generally performed in individual cohorts by call rate, minor allele frequency (MAF) and deviation from Hardy-Weinberg equilibrium (HWE) (Supplementary Methods, Supplementary Tables S3 and S4). Approximately 2.5 million autosomal SNPs were imputed cohorts using the HapMap II Caucasian (CEU, Centre d'Etude du Polymorphisme Humain) sample as reference panel for the European-ancestry cohorts, a combined CEU+YRI reference panel for the African-American cohorts, and a combined CEU+YRI+CHB+JPT reference panel for the Hispanic sample. MACH or IMPUTE software[19–21] were used in the imputation (Supplementary Tables S3 and S4).

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Meta-Analysis of GWA Studies

Values of plasma fibrinogen concentration were natural logarithm-transformed prior to analysis. Association analyses were conducted in each cohort of measured and imputed autosomal SNP allele dosage with fibrinogen values, using a linear regression model assuming additive genetic effects adjusted for age and sex. Additional adjustments for principal components or multi-dimensional scaling, country, or center were made, when necessary, by individual cohorts to account for population stratification (see Supplementary Methods). Relatedness was accounted for in family studies by applying linear mixed-effect models. Genotype-phenotype association results from the 28 cohorts were then meta-analyzed by using an inverse-variance model with fixed effects in METAL (http://www.sph.umich.edu/csg/abecasis/Metal/index.html).[22] In order to identify additional independent association signals in the genome-wide significant loci, conditional GWA analysis was performed as described in Supplementary Methods. Overall, we selected for further analysis only SNPs from genome-wide significantly associated loci, including the lead SNP for each locus in the initial meta-analysis along with one additional lead SNP representing a new clear signal identified in the conditional analysis.

In order to identify genes that regulate fibrinogen levels in other ethnic groups, we conducted a separate GWA meta-analysis using 7 separate GWA scans in African Americans totaling 8,289 samples, and a single GWA analysis in a cohort of Hispanic-Americans totaling 1,366 samples

The threshold of genome-wide significance was set at $P=5.0\times10^{-8}$ for the primary analyses of GWA with plasma fibrinogen levels and their heterogeneity measures, as well as for the conditional meta-analysis. We used Bonferroni correction for the exploration of the 24 lead-SNPs in African-American and Hispanic samples, and for the lookups in clinical outcomes ($P<0.002$).

Genetic risk score

A genetic risk score (GRS) was computed using data from 88,251 European-ancestry individuals to model the increase in fibrinogen levels according to number of fibrinogen-raising alleles for each of the lead SNPs. Methods are further described in Supplementary Methods.

Multivariable adjusted model

We re-analyzed the association with plasma fibrinogen concentration of the lead SNPs, using a linear model with further adjustment for BMI and smoking, in addition to sex and age and the extra covariates used in each cohort in the discovery analyses. Association results from all cohorts were then meta-analyzed using inverse-variance weighted fixed-effects meta-analysis implemented in METAL.

Pathway Analyses

MAGENTA and GRAIL[23, 24] were used to assess putative relationships between the lead SNPs and to infer genes and pathways underlying SNP associations with plasma fibrinogen levels. MAGENTA v. 2 analysis was performed as described,[24] including gene sets from Gene Ontology (GO), KEGG, PANTHER, and Ingenuity downloaded in June 2011 (http://www.broadinstitute.org/mpg/magenta/). Gene set statistics were determined for an empirically derived 95th percentile threshold of gene-wide adjusted *P* values. Only gene sets meeting a false discovery rate (FDR) < 0.05 were considered for further inspection. Candidate SNPs were identified in the MAGENTA analysis as SNPs with nominal locus-wide corrected *P*-values (corrected $P<0.05$) mapping to genes in gene sets that met FDR<0.05. GRAIL analysis was performed as described (http://www.broadinstitute.org/

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mpg/grail/) using the pair-wise similarity metric compiled from the literature in December 2006 to limit bias, as recommended [25].

Association with Gene Expression in Human Liver

The lead SNPs and their perfect proxies (r^2=1) were further analyzed with respect to association with expression levels of nearby genes (located within ±200 kilobases (kb) of the SNP). Global gene expression data from human liver were obtained from the Advanced Study of Aortic Pathology (ASAP).[26] Details of the ASAP biobank and the methods for gene expression analysis and genotyping are provided in the Supplementary Methods. Further queries were made against significant results from four other liver eQTL analyses whose methods were previously published.[27–30]

Associations with Clinical Outcomes

We examined associations of the 24 lead SNPs with prevalent CAD, stroke and VTE. Genotype-CAD association results for the selected SNPs were obtained from the Coronary ARtery DIsease Genome-wide Replication And Meta-analysis (CARDIoGRAM) and Europe South Asia Coronary Artery Disease Genetics (C4D) consortia, including a total of 40,695 CAD cases and 85,582 controls. Lead SNP associations with stroke were explored in data generated from four large cohorts composing the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, including 1544 incident strokes (1164 ischemic strokes) developed over an average follow-up of 11 years, and 18,058 controls, and in data generated from four cohorts comprising the Welcome Trust Case Control Consortium (WTCCC), including 3,548 cases with ischemic stroke and 5,972 controls. The SNP genotype-VTE association results were generated in 3,208 VTE cases and 46,167 controls from the French MARTHA and the CHARGE studies. Definitions of the disease phenotypes adopted in each individual study are detailed elsewhere.[11–15, 31] Each of the 24 fibrinogen-associated SNPs was tested for association with each of the clinical outcomes by logistic regression, adjusting for age and sex. The log-odds-ratios and their standard errors for each SNP were standardized for direction and magnitude to correspond to the change in allele dosage that accounted for a 3.1% relative increase in circulating fibrinogen level (fibrinogen-effect associated with the FGB variant rs1800789). These harmonised effect estimates were then pooled by fixed-effects (inverse-variance weighted) meta-analysis (stroke and VTE) or by random-effects meta-analysis (for CAD, due to significant heterogeneity in both direction and magnitude of the harmonised log-odds-ratios).

Results

Meta-analysis in European-Ancestry Samples

Meta-analysis was performed for 2,515,567 SNPs on individual GWA study results generated in 28 European-ancestry cohorts including a total of 91,323 individuals. A total of 985 SNPs, located in 23 chromosomal loci, passed the genome-wide significance threshold of P=5.0×10^{-8} (Figure 1). Among the 23 loci (designated according to nearest gene), 8 (*IL6R, NLRP3, IL1RN, CPS1, PCCB, FGB, IRF1* and *CD300LF*) represent replications of previously identified fibrinogen-associated loci and 15 are novel associations (*JMJD1C, LEPR, PSMG1, CHD9, SPPL2A, PLEC1, FARP2, MS4A6A, TOMM7/IL6, ACTN1, HGFAC, IL1R1, DIP2B* and *SHANK3/CPT1B*). More information about these genes is provided in Supplementary Table S5. Further information about the lead SNPs and their association with fibrinogen levels is listed in Table 1.

To search for further independent association signals within the 23 loci, we repeated the individual GWA analyses, conditioning on the 23 lead SNPs. This analysis revealed two genome-wide significant SNPs located, respectively, in the *FGA* gene (rs2070016,

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P=3.9×10^{-8}) and on chromosome 5 (rs11242111, P=1.60X10^{-21}) (Supplementary Figure S1). Accordingly, rs11242111 was added to the list of independent lead SNPs selected for further analyses (Table 1). The rs2070016, in *FGA*, showed evidence of correlation with the lead SNP rs1800789 in *FGB* (r^2 =0.364 according to 1000 Genomes Map Pilot 1); hence, we did not select this SNP for further analyses. After adjusting for number of tests, none of the 24 lead SNPs showed significant heterogeneity across European-ancestry cohorts. Regional association plots for the 24 loci are shown in Supplementary Figure S2.

Further adjustment for body mass index (BMI) and smoking, which together explained 5.3% of the variation in plasma fibrinogen level amongst 81,511 individuals from the European-ancestry meta-analysis, resulted in stronger associations for most of the lead SNPs but no new discoveries (Table 1).

Meta-analysis and Validation of European-ancestry Loci in African-American and Hispanic Samples

The Manhattan and QQ plots (λ=1.012) reporting the results for the African-American samples are shown in the Supplementary Figure S3. Only the *FGA/FGB/FGG* locus on chromosome 4 reached genome-wide significance in the African American meta-analysis, with the most strongly associated SNP being rs4463047, P=4.63×10^{-10}, at 12,790 bp from rs1800789 (P=4.02×10^{-7}). No single SNP attained genome-wide significance in the Hispanic samples (Supplementary Figure S3).

We tested the association of the 24 European-ancestry lead SNPs in the African American meta-analysis (Supplementary Table S6). After correcting for 24 statistical tests (P-value threshold < 0.002) only the two lead-SNPs, rs1800798 (*FGB*) and rs6734238 (*ILRN*) passed the significant threshold. However, 5 other lead-SNPs, located in the *IRF1*, *IL6R, CHD9, JMJD1C* and *MS4A6A* loci, were associated at P<0.05, with consistent directions of effect in both populations (Supplementary Table S7). Furthermore, at 20 of the 24 lead SNPs the direction of the beta estimate was the same in the European and African-American samples (P=0.00077, sign test). In the Hispanic samples, 3 European-ancestry lead SNPs, in *FGB* (rs1800798), *IL6R* (rs6734238) and *CHD9* (rs7204230), passed the significance threshold (24 SNPs / P < 0.002) for association, and 3 additional lead SNPs were associated at a nominally significant threshold of P<0.05, with consistent directions of effect in both populations (Supplementary Table S6). In addition, the direction of the beta estimate at 20 of the 24 lead SNPs was the same in the European and Hispanic samples (P=0.00077, sign test).

GRS and Proportion of Variance Explained

Figure 2 presents the average fibrinogen values (in g/l) across categories of the GRS. The mean percentage of residual variance (after adjustment for age and sex) explained by 24 lead-SNPs was 3.7% in all European-ancestry cohorts (range 1.4–7.6% in individual cohorts). The heritability of plasma fibrinogen concentration estimated from the family cohorts within this study (NTR, CROATIA-Vis, CROATIA-Korcula, ORCADES, FHS and SardiNIA) ranged from 15% to 51% (mean(SD)=31(15)%) (Supplementary Results). The proportion of variance in fibrinogen levels explained by common SNPs (MAF>0.01) was calculated in one of our participant cohorts (WGHS, n=21,336) using the method proposed by Yang and Visscher[10]. Results showed that 16% (SE=0.017) of the variance in fibrinogen levels was explained by common SNPs.

Finally, the GRS was strongly associated with levels of fibrinogen in the combined African-American cohorts (P= 1.5×10^{-8}) and the Hispanic cohort (P=3.8×10^{-15}).

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Pathway and Expression QTL Analyses

We performed additional *in silico* pathway analyses using GRAIL and MAGENTA (Supplementary Table S8). The GRAIL results identified 6 SNPs (rs6734238, rs12712127, rs8192284, rs10157379, rs1938492 and rs6831256) that were located within or near genes (*IL1RN, IL1R1, IL6R, NLRP3, LEPR* and *LRPAP1*) with significantly related function among all of the genes in the vicinity of the 24 lead SNPs, suggesting that these genes should be prioritized as the most plausible functional candidate genes within the associated loci. Gene-set enrichment analysis using MAGENTA (based on the whole genome-wide genetic dataset) identified several gene sets and pathways that were enriched in the analysis (Supplementary Table S9). Apart from the three structural genes, the most represented pathways were related to inflammation (acute-phase response, interleukin signaling), adipocytokine signaling and thyrotrophin-releasing hormone signaling. According to these results, several genes (*LEPR, IL6R, IL1R, IL1F10/IL1F5/IL1F8/IL1RN, FGA/FGB, ACTN1* and *CPT1B*) were prioritized as plausible candidate genes within our 23 genomic regions. A comprehensive SNP list, which includes both the 24 lead SNPs and the SNPs selected by either GRAIL or MAGENTA on the whole genome-wide genetic dataset, is reported in Supplementary Table S9.

We then interrogated the 24 lead SNPs and their perfect proxies with respect to their associations with expression levels of nearby genes (located within ±200 kb of the lead SNP) in 5 human liver databases. Expression levels of *LEPR*, *PCCB*, *MSL2L1*, *NGFRAP1*, *FGB* and *TOMM7* were significantly associated with allelic differences in one of the 24 lead SNPs (results are shown in Supplementary Table S8). Finally, to assess the functional role of SNPs in Fibrinogen genes we also studied the eQTL associations of all SNPs within 100Kb of the fibrinogen genes cluster. The highest association with expression of fibrinogen transcripts within the fibrinogen cluster was found for SNP rs4220 (P=1.38×10^{-20}), causing a missense mutation in the FGB gene. All positive associations with fibrinogen transcripts are shown in Supplementary Table S10.

Associations with Clinical Outcomes

After correction for multiple testing (P<0.002 threshold), rs4129267 located in the *IL6R* locus, rs6734238 in the *IL1F10/IL1RN* locus and rs1154988 in the *PCCB* locus were found to be significantly associated with CAD; however, the directions of the effects on CAD and fibrinogen levels were consistent only for rs4129267 in the *IL6R* locus . The pooled association for the 24 lead SNPs with CAD was not significant (OR(CI$_{95\%}$)= 1.00 (0.97,1.03)). None of the fibrinogen-associated lead SNPs was significantly associated with stroke or VTE after correction for multiple testing. The pooled results were suggestive for stroke (stroke OR(CI$_{95\%}$)= 1.03 (1.00,1.07); but not for VTE OR(CI$_{95\%}$)= 0.96 (0.92,1.01)) (Table 2). Additional results from the WTCCC stroke consortium, generated according to clinical subphenotypes, are shown in Supplementary Table S11. No significant associations with stroke subphenotypes were found after correction for multiple hypothesis testing.

Discussion

The present study represents the largest effort to identify novel gene loci regulating plasma fibrinogen levels. Overall, we identified 24 independent genome-wide significant SNPs in 23 loci, including 15 loci with newly discovered fibrinogen associations. Using our genetic findings, we found no evidence for a causal role of fibrinogen in CAD, stroke, and VTE.

The proportion of variance in plasma fibrinogen level accounted for by all 24 fibrinogen-associated lead SNPs increased to 3.7% (a detailed description of the novel nearby candidate genes is presented in Supplementary Table S5). These results support the notion that

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regulation of plasma fibrinogen levels is driven by multiple genes, each having a modest effect on the phenotype. It is likely that even more loci with smaller effects remain to be discovered.

Relevance of the Fibrinogen-Related Loci in Non-European Ancestry Individuals

We performed the first meta-analysis of GWA studies on African-American samples and we provide evidence for significant association of a weighted SNP score based on the 24 lead SNPs from the European-ancestry meta-analysis with levels of fibrinogen in both African-Americans (P=1.5×10^{-8}) and Hispanics (P=3.8×10^{-15}). Thus, despite differences in allele frequencies and/or differences in the relative impact of covariates associated with fibrinogen among populations, loci identified in European-ancestry samples collectively contribute to the regulation of plasma fibrinogen in African-American and Hispanic populations. 20 of 24 lead SNPs showed the same direction of effect when comparing the European sample with either the African-American or the Hispanic samples. The substantially smaller size of the African-American and Hispanic cohorts compared to the total sample with European ancestry restricted available power and may have limited the significance of the candidate SNP associations in these populations (see power calculations in Supplementary Methods).

Pathways Involved in Regulation of Plasma Fibrinogen Level

It is interesting to note that several of the genome-wide significant loci identified in the present study harbor inflammatory genes, a remarkable set of which relate to the IL1 pathway, indicating the importance of this pathway in the regulation of fibrinogen. Most of these inflammatory genes have been previously reported in relation to other inflammation-related phenotypes and diseases. For example, *IL6R, NLRP3, IL1RN/ILF10,* and *IRF1* were recently identified in a GWA study meta-analysis of C-reactive protein (CRP) conducted on European samples.[32] Both fibrinogen and CRP are acute-phase proteins whose levels are largely influenced by inflammatory triggers. It is thus not surprising that they are both partly regulated by a common group of genes that are implicated in the immune response. These results are also consistent with our *in silico* gene-set enrichment analyses, which showed that inflammation-related pathways, including acute-phase response and interleukin signaling, were most enriched for fibrinogen-associated genes. In this regard, interesting new plausible candidate genes could be discerned within the newly identified loci, including *IL6*, located in the *TOMM7-IL6* locus on chromosome 7, and *IL1R1*, located in the cytokine receptor gene cluster on chromosome 2.

Our gene-set enrichment analysis also highlighted genes regulating fat metabolism as important in the control of plasma fibrinogen concentration, as indicated by the strong representation of adipocytokine signaling genes. This is consistent with our observation that smoking and BMI contributed about 5.3% of the plasma fibrinogen variation and with data from *The Fibrinogen Studies Collaboration*, reporting that 7% of the variation in plasma fibrinogen concentration was accounted for by smoking, BMI and high-density lipoprotein (HDL) cholesterol[33]

Relations to Cardiovascular Disease

Although plasma fibrinogen concentration has been identified as a predictor of incident CAD events,[1, 34] it has been argued that increased plasma fibrinogen levels in population subgroups at increased CAD risk could be due to other mechanisms, including existing atherosclerosis, which might induce a pro-inflammatory state with a subsequent increase in acute-phase reactants such as fibrinogen or CRP. Given the associations of fibrinogen levels with other established CAD risk factors (such as smoking and BMI), it remains uncertain whether these other factors may confound the association of fibrinogen with disease risk. Prior studies that assessed the causality of the association between plasma fibrinogen

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concentration and risk of CAD by Mendelian randomization (MR), using 2 common SNPs located in the promoter region of the FGB gene, found no significant association of this locus with CAD, concluding that the relationship was non-causal.[35, 36] One limitation of these studies is that this single locus might have biologically unusual effects on measured fibrinogen levels. [35, 36] Our analysis of 23 other fibrinogen-associated SNPs offers a broader perspective, and thus a more robust and generalisable evaluation of the causal relationship between fibrinogen and cardiovascular events. A further strength of our study is that we present estimates of the effects on risk of clinical outcomes individually for each SNP as well as globally for all SNPs combined.

Our results do not support a causal relationship between plasma fibrinogen level and CAD. In fact, consistent with the negative results from previous MR, the lead SNP located in the *FGB* gene showed no association with CAD. Whereas SNPs rs4129267, rs6734238, and rs1154988, located in the *IL6R, IL1F10/IL1RN* and *PCCB* loci, respectively, were significantly associated with CAD in CARDIoGRAM and C4D, the direction of effect was consistent only for SNP located in the *IL6R* locus (i.e., the allele that lowered the plasma fibrinogen concentration also lowered CAD risk). Furthermore, the global effect of all 24 fibrinogen-associated SNPs was not associated with CAD risk ($OR(CI_{95\%})$= 1.00 (0.97,1.03).

Overall, our results suggest that systemic inflammation both causes raised fibrinogen level and (by a different mechanism) is associated with increased risk of CAD. The lack of overlap between the top CAD-associated SNPs from the literature and the fibrinogen-associated SNPs identified in our study further argues against a reverse causality hypothesis, where inflammation caused by the atherosclerosis process would raise the fibrinogen level.

Although not as consistent as for CAD or MI, some studies have also suggested that an elevated fibrinogen concentration is a risk factor for stroke.[3, 37–39] In the present study, none of the fibrinogen-associated SNPs were significantly associated with stroke. Our findings suggest that similar to what we observed for CAD, a raised fibrinogen concentration is not causally related to stroke, although a positive trend was observed that warrants further investigation. Similarly, our results show that none of the fibrinogen-associated SNPs was significantly associated with VTE after correction for multiple testing, although rs1800789G in the fibrinogen gene cluster, which is associated with higher fibrinogen level in our discovery study, showed a clear trend (P=0.004). However, given the small sample size of the VTE cases examined, the power for detection of VTE association in our data is substantially lower than for stroke and CAD (Supplementary Methods).

Conclusions

The present meta-analysis of fibrinogen GWA studies, based on a 4-fold greater sample size than previous meta-analyses (≈91,500 individuals), identified 24 independent signals in 23 loci (of which 15 are new) and increased the proportion of variance of plasma fibrinogen level accounted for by all lead SNPs in genome-wide significant loci from <2% to 3.7%. For some of these loci, our pathway and eQTL analyses provided supporting evidence regarding the most plausible candidate genes. Finally, our study does not support causal involvement of fibrinogen in CVD, particularly in clinically apparent CAD. Functional studies are needed to confirm and characterize candidate genes suggested by the *in silico* analyses presented here.

Future studies aimed at explaining the substantial missing heritability of plasma fibrinogen concentration should focus on exploring gene-gene and gene-environment interactions as well as on applying resequencing technologies to elucidate the role of rare variants.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Perspective

Plasma fibrinogen concentration is a predictor of cardiovascular disease independent of other traditional risk factors, and variation in fibrinogen concentration has a substantial heritable component. We conducted a meta-analysis of 28 genome-wide association studies, including more than 90,000 subjects of European ancestry and substantial numbers of African Americans and Hispanic-Americans. We identified 24 genome-wide significant ($P<5\times10-8$) independent single nucleotide polymorphisms (SNPs) in 23 genetic loci, including 15 novel associations, together accounting for 3.7% of plasma fibrinogen variation. Gene-set enrichment analysis highlighted potential key roles in fibrinogen regulation for the known structural fibrinogen genes as well as inflammation and other candidate pathways. However, in an evaluation for associations of the top fibrinogen SNPs with coronary artery disease, stroke and venous thromboembolism in very large case-control genomewide studies, there was no evidence for association with any of these clinical outcomes of either the single SNP most closely related to fibrinogen level (in the fibrinogen gene) or the combined effect of all 24 fibrinogen-associated SNPs (across 23 distinct loci). Our findings in a very large total study population provide comprehensive data for new and known genetic variants underlying fibrinogen concentration in human populations including multiple ethnic groups. Our findings highlight potential pathways for future study of the role of fibrinogen in the pathophysiology of atherosclerosis and cardiovascular disease. Clinical outcome analysis does not support a strong causal relationship between circulating levels of fibrinogen and coronary artery disease, stroke or venous thromboembolism.

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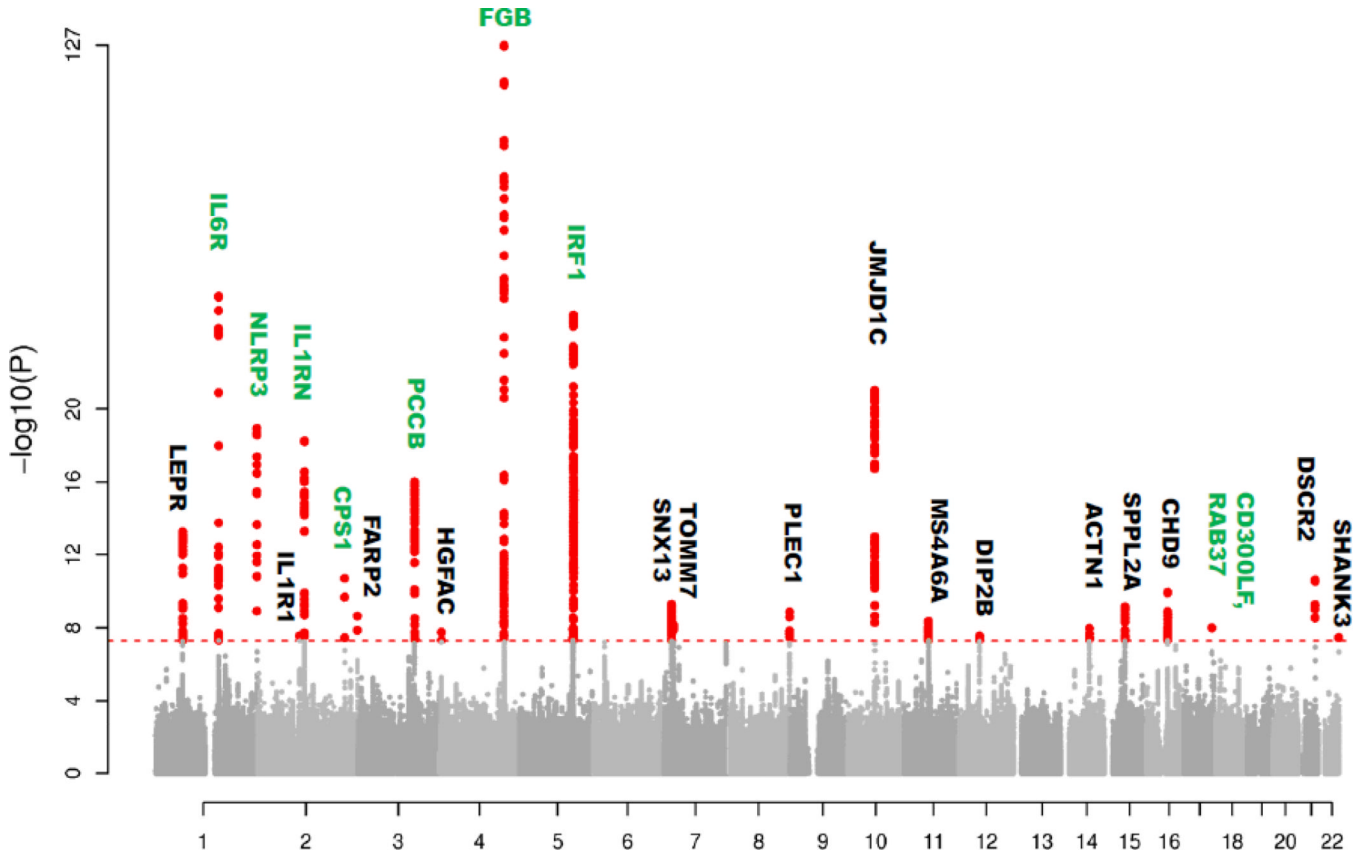

Figure 1.

Manhattan plot of the association *P*-values for plasma fibrinogen concentration in the meta-analysis performed on European-ancestry samples. Analyzed SNPs are plotted on the X-axis ordered by chromosomal position. Y-axis plots the logarithm of the *P*-values. Gene loci labeled in green were previously known; gene loci labeled in black are novel discoveries in this meta-analysis. The dotted line indicates the threshold for genome-wide significance ($P=5\times10^{-8}$).

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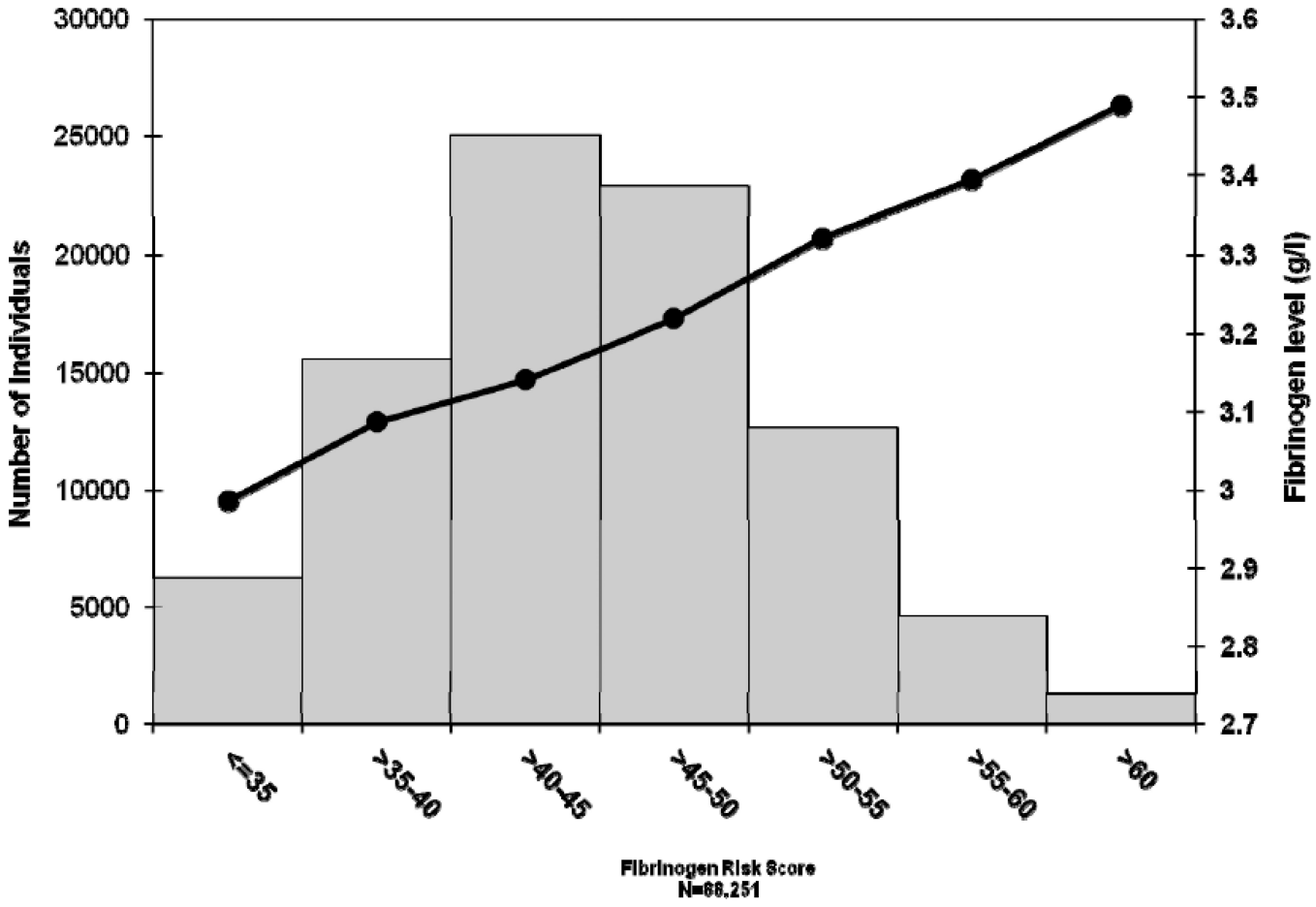

Figure 2.

Mean values for plasma fibrinogen concentration in g/l (right Y-axis) plotted by categories of fibrinogen-associated single nucleotide polymorphism (SNP) score (X-axis), represented by the black dots. Number of individuals in each category is represented by the grey bars (left Y-axis).

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Table 1

Details of the 24 lead SNPs and association β and *P*-values for the original meta-analysis performed with the European-ancestry cohorts (adjusted for age and sex) together with the corresponding values of the values obtained in the same cohorts with further adjustments for BMI and smoking.

								Original meta-analysis						Further adjustment for BMI and smoking		
SNP	**Band**	**Position**	**Closest gene**	**In gene**	**Distance (bp)**	**A1**	**A2**	**Freq**	**Beta**	**SE**	***P***	**Het P**	**N**	**Beta**	**SE**	***P***
rs1938492	1p31.3	65890417	*LEPR*		14653	A	C	0.62	0.008	0.001	5.28×10^{-14}	0.438	89330	0.008	0.001	1.12×10^{-15}
rs4129267	1q21.3	152692888	*IL6R*	intron		T	C	0.39	−0.011	0.001	5.97×10^{-27}	0.724	91419	−0.011	0.001	4.57×10^{-30}
rs10157379	1q44	245672222	*NLRP3*	intron		T	C	0.62	0.010	0.001	1.15×10^{-19}	0.416	86730	0.010	0.001	3.12×10^{-22}
rs12712127	2q11.2	102093093	***IL1R1****/IL1R2*		43740	A	G	0.41	0.006	0.001	2.72×10^{-08}	0.097	91406	0.006	0.001	3.66×10^{-10}
rs6734238	2q13	113557501	***IL1F10****/IL1RN*		7603	A	G	0.58	−0.009	0.001	5.77×10^{-19}	0.487	91426	−0.010	0.001	6.66×10^{-22}
rs715	2q34	211251300	*CPS1*	exon		T	C	0.68	0.009	0.001	1.98×10^{-11}	0.153	74715	0.011	0.001	3.95×10^{-19}
rs1476698	2q37.3	241945122	*FARP2*	intron		A	G	0.65	0.007	0.001	2.24×10^{-09}	0.420	91419	0.007	0.001	1.44×10^{-10}
rs1154988	3q22.3	137407881	***MSL2****/PCCB*		10503	A	T	0.78	−0.010	0.001	9.64×10^{-17}	0.154	91416	−0.012	0.001	2.98×10^{-24}
rs16844401	4p16.2	3419450	***HGFAC****/LRPAP1*	exon		A	G	0.08	0.015	0.003	1.74×10^{-08}	0.077	74680	0.014	0.002	7.07×10^{-09}
rs1800789	4q32.1	155702193	*FGB*		1388	A	G	0.21	0.031	0.001	1.68×10^{-127}	0.001	91301	0.031	0.001	1.94×10^{-140}
rs11242111	5q31.1	131783957	***C5orf56****/IRF1*	intron		A	G	0.05	0.023	0.002	1.60×10^{-21}	0.353	91423	0.024	0.002	1.14×10^{-23}
rs2106854	5q31.1	131797073	*C5orf56/IRF1*	intron		T	C	0.21	−0.019	0.001	1.72×10^{-48}	0.082	91406	−0.019	0.001	1.93×10^{-54}
rs10226084	7p21.1	17964137	***SNX13****/PRPS1L1*		17481	T	C	0.52	−0.007	0.001	5.05×10^{-10}	0.441	91403	−0.007	0.001	6.68×10^{-11}
rs2286503	7p15.3	22823131	*TOMM7*	intron		T	C	0.36	−0.006	0.001	6.88×10^{-09}	0.845	91413	−0.005	0.001	2.26×10^{-07}
rs7464572	8q24.3	145093155	*PLEC1*	intron		C	G	0.60	−0.007	0.001	1.33×10^{-09}	0.123	82730	−0.006	0.001	7.41×10^{-09}
rs7896783	10q21.3	64832159	*JMJD1C*	intron		A	G	0.48	−0.010	0.001	8.90×10^{-22}	0.754	91412	−0.009	0.001	4.43×10^{-20}
rs1019670	11q12.1	59697175	*MS4A6A*	EXON		A	T	0.36	−0.007	0.001	4.37×10^{-09}	0.696	9018	−0.006	0.001	8.09×10^{-08}
rs7968440	12q13.13	49421008	*DIP2B*	intron		A	G	0.64	0.006	0.001	2.74×10^{-08}	0.360	91405	0.006	0.001	1.37×10^{-09}
rs434943	14q24.1	68383812	*ACTN1*		26780	A	G	0.31	0.007	0.001	1.08×10^{-08}	0.014	86189	0.008	0.001	1.73×10^{-10}
rs12915708	15q21.2	48835894	*SPPL2A*	intron		C	G	0.30	−0.007	0.001	6.87×10^{-10}	0.625	91434	−0.007	0.001	3.45×10^{-11}
rs7204230	16q12.2	51749832	*CHD9*	intron		T	C	0.70	0.008	0.001	1.18×10^{-10}	0.493	82835	0.008	0.001	6.40×10^{-12}
rs10512597	17q25.1	70211428	*CD300LF*	intron		T	C	0.18	−0.008	0.001	9.92×10^{-09}	0.108	86737	−0.009	0.001	4.23×10^{-11}
rs4817986	21q22.2	39387382	*PSMG1*		81871	T	G	0.28	−0.008	0.001	2.46×10^{-11}	0.539	85293	−0.009	0.001	3.39×10^{-14}
rs6010044	22q13.33	49448804	***SHANK3****/ARSA*		11131	A	C	0.80	−0.008	0.001	3.41×10^{-08}	0.582	89138	−0.008	0.001	7.07×10^{-09}

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The closest gene is indicated in bold. Beta values and frequencies refer to allele 1 (A1).

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Table 2

Association results for the 24 lead SNPs with coronary artery disease (CAD), stroke and venous thromboembolism (VTE).

						CAD*			Stroke**			VTE***		
SNP	**Band**	**Allele1**	**Allele2**	**Freq1**	**Closest gene**	**OR**	**SE**	***P***	**OR**	**SE**	***P***	**OR**	**SE**	***P***
rs1938492	1p31.3	A	C	0.597	*LEPR*	0.98	0.011	0.038	0.98	0.025	0.405	1.00	0.032	0.892
rs4129267	1q21.3	T	C	0.378	*IL6R*	0.96	0.011	**1.73×10^{-05}**	0.97	0.024	0.212	1.01	0.032	0.838
rs10157379	1q44	T	C	0.603	*NLRP3*	1.00	0.011	0.883	1.02	0.025	0.329	1.04	0.032	0.204
rs12712127	2q11.2	A	G	0.451	*IL1R1/IL1R2*	1.00	0.011	0.985	0.98	0.025	0.423	1.00	0.032	0.909
rs6734238	2q13	A	G	0.589	*IL1F10/IL1RN*	1.04	0.011	**9.44×10^{-05}**	1.00	0.025	0.974	1.01	0.032	0.702
rs715	2q34	T	C	0.685	*CPS1*	1.03	0.013	0.011	1.01	0.029	0.822	0.91	0.054	0.081
rs1476698	2q37.3	A	G	0.615	*FARP2*	1.00	0.011	0.873	1.02	0.026	0.388	1.06	0.033	0.089
rs1154988	3q22.3	A	T	0.778	*MSL2/PCCB*	1.04	0.013	**0.002**	0.95	0.029	0.100	0.95	0.037	0.186
rs16844401	4p16.2	A	G	0.089	*HGFAC/LRPAP1*	1.03	0.024	0.263	1.01	0.052	0.848	0.92	0.082	0.285
rs1800789	4q32.1	A	G	0.2	*FGB*	1.00	0.014	0.939	0.99	0.031	0.828	0.89	0.04	0.004
rs11242111	5q31.1	A	G	0.101	*C5orf56/IRF1*	0.95	0.024	0.02	1.09	0.057	0.145	0.97	0.079	0.72
rs2106854	5q31.1	T	C	0.267	*C5orf56/IRF1*	0.98	0.012	0.068	0.99	0.030	0.671	1.05	0.039	0.191
rs2286503	7p15.3	T	C	0.397	*TOMM7*	0.97	0.011	0.005	0.97	0.025	0.173	0.99	0.033	0.641
rs10226084	7p21.1	T	C	0.543	*SNX13/PRPS1L1*	1.01	0.011	0.497	1.02	0.024	0.379	0.98	0.032	0.614
rs7464572	8q24.3	C	G	0.624	*PLEC1*	1.02	0.011	0.03	0.98	0.028	0.526	0.99	0.041	0.724
rs7896783	10q21.3	A	G	0.508	*JMJD1C*	1.02	0.01	0.14	0.98	0.024	0.449	0.98	0.032	0.512
rs1019670	11q12.1	A	T	0.381	*MS4A6A*	1.01	0.012	0.311	0.96	0.028	0.173	1.02	0.036	0.597
rs7968440	12q13.13	A	G	0.69	*DIP2B*	1.00	0.012	0.825	1.00	0.025	0.989	1.01	0.033	0.819
rs434943	14q24.1	A	G	0.305	*ACTN1*	1.01	0.013	0.314	1.03	0.027	0.256	0.97	0.035	0.366
rs12915708	15q21.2	C	G	0.3	*SPPL2A*	0.98	0.012	0.063	1.00	0.027	0.889	1.00	0.034	0.915
rs7204230	16q12.2	T	C	0.682	*CHD9*	0.99	0.012	0.419	1.01	0.029	0.721	0.96	0.044	0.401
rs10512597	17q25.1	T	C	0.202	*CD300LF*	1.02	0.014	0.218	1.00	0.032	0.909	0.99	0.041	0.781
rs4817986	21q22.2	T	G	0.268	*PSMG1*	1.02	0.013	0.182	1.00	0.027	0.928	1.03	0.035	0.486
rs6010044	22q13.33	A	C	0.777	*SHANK3/ARSA*	0.97	0.014	0.012	0.97	0.030	0.364	0.96	0.042	0.368

Abbreviations: Freq1= frequency of allele1; OR= Odds ratio; SE= Standard error;

*Joint meta-analysis of results from the Coronary ARtery DIsease Genome-wide Replication And Meta-analysis (CARDIoGRAM) and Europe South Asia Coronary Artery Disease Genetics (C4D) consortia.

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** Joint meta-analysis of results from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium and the Wellcome Trust Case-Control Consortium (WTCCC).

*** Meta-analysis result from the French MARseille THrombosis Association (MARTHA) Consortium and the CHARGE Consortium Studies on Venous Thrombosis.