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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)

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ABSTRACT

Several common genomic loci, involving various immunity-and metabolism-related genes, have been associated with plasma fibrinogen in European Americans (EA). The genetic determinants of fibrinogen in African Americans (AA) are poorly characterized. Using a vascular gene-centric array in 23,634 EA and 6,657 African-American (AA) participants from 6 studies comprising the Candidate gene Association Resource (CARe) project, we examined the association of 47,539 common and lower frequency variants with fibrinogen concentration. We identified a rare Pro265Leu variant in *FGB* (rs6054) associated with lower fibrinogen. Common fibrinogen gene SNPs (*FGB* rs1800787 and *FGG* rs2066861) significantly associated with fibrinogen in EAs were prevalent in AA and showed consistent associations. Several fibrinogen locus SNPs associated with lower fibrinogen were exclusive to AA; these include a newly reported association with *FGA* rs10050257. For *IL6R*, *IL1RN*, and *NLRP3* inflammatory gene loci, associations with fibrinogen were concordant between EA and AA, but not at other loci (*CPS1*, *PCCB*, and *SCL22A5-IRF1*). The association of *FGG* rs2066861 with fibrinogen differed according to assay type used to measure fibrinogen. Further characterization of common and lower-frequency genetic variants that contribute to inter-population differences in fibrinogen phenotype may help refine our understanding of the contribution of hemostasis and inflammation to athero-thrombotic risk.

INTRODUCTION

Fibrinogen plays a central role in hemostasis and is also a marker of inflammation. Increased plasma fibrinogen concentration is a well-established risk factor for cardiovascular disease (CVD)[1]. Recent genome-wide scans have provided strong evidence that common polymorphisms of the fibrinogen structural genes on chromosome 4 (*FGA*, *FGB*, and *FGG*) and several other genomic loci (*IL6R*, *CPS1*, *PCCB*, *NLRP3*, *IL1RN*, *CD300LF*, *IRF1-SCL22A5*) influence fibrinogen in European-American (EA) populations [2-6]. Nonetheless, common polymorphisms explain only a small portion of the heritable component of fibrinogen, which has estimates of ~20% to 50% in EAs[7-9]. Thus, additional genetic variants with more subtle effects, gene-environment interactions, or lower frequency variants with large effects might account for additional inter-individual variation in fibrinogen.

Fibrinogen levels differ by race/ethnicity, with higher levels among African Americans (AA) than EA[10-14]. Fibrinogen predicts CVD risk in AA as well as EA[15]. Heterogeneity between EA and AA in the allelic patterns of fibrinogen phenotype association has been noted for the fibrinogen structural genes on chromosome 4[3, 16]. Little is known about other genomic loci that might account for inter-individual differences in fibrinogen between EA and other U.S. minority populations.

The ITMAT-Broad-CARe (IBC) genotyping array is a custom, CVD gene-centric single nucleotide polymorphism (SNP) genotyping platform that contains greater SNP marker density and linkage disequilibrium coverage for over 2000 CVD candidate regions than current genome-wide arrays. The IBC array is particularly informative for individuals of African descent, enabling analyses of common and lower frequency variants in diverse populations[17].

We analyzed the association between the SNPs on the IBC candidate gene array and fibrinogen levels in a large number of EA and AA participants from 6 population-based cohorts from the Candidate gene Association Resource (CARe) project. Our objectives were (1) to perform a detailed characterization and comparative analysis of the allelic patterns of association for the fibrinogen gene locus and other fibrinogen-associated candidate gene regions in EA and AA; and (2) evaluate whether other common or less frequent variants at these or other candidate gene loci, or gene-environment interaction, explain any of the "missing heritability" of fibrinogen. Secondarily, we assessed the potential heterogeneity of fibrinogen SNP associations according to the type of assay used to measure fibrinogen (functional versus immunologic).

MATERIALS AND METHODS

The CARe Consortium is a NHLBI supported resource for analyses of the association of genotypes with heart, lung, and blood phenotypes. CARe cohort studies with fibrinogen measurements include the Atherosclerosis Risk in Communities (ARIC) study, the Coronary Artery Risk Development in Young Adults (CARDIA) study, the Cleveland Family Study (CFS), the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Multi-Ethnic Study of Atherosclerosis (MESA). All participating institutions and care sites gave institutional review board approval for this study. Further details of the participating CARe studies are given under **Supplemental Methods**.

Fibrinogen Measurement

In ARIC, CFS, and CHS, the Clauss clotting rate method[18] was used for measuring plasma fibrinogen. In MESA and CARDIA, fibrinogen was determined by an immunonephelometric method (Dade Behring Marburg GmbH, Marburg, Germany) on a Behring Nephelometer II analyzer. The FHS used the Clauss method in the offspring and generation 3 participants, and a modified method of Ratnoff and Menzie[19] in the original cohort subjects.

Genotyping

The ITMAT-Broad-CARe (IBC) genotyping array[17] enables efficient capture of genetic diversity across approximately 2,100 genes related to cardiovascular, inflammatory, hemostasis/coagulation, and metabolic phenotypes and pathways. There are a total of 49,320 SNPs on the IBC array, including approximately 15,000 candidate gene SNPs not present in the HapMap. The tagging approach utilized on the IBC array was designed to capture maximal

genetic information from the HapMap populations as well as European and African American individuals for both common and lower frequency SNPs[17]. Further details regarding the SNP selection and tagging approach are given under **Supplemental Methods**.

After excluding study participants who did not provide informed consent for genetic testing, subjects with a mismatch between genotypic and phenotypic sex, and other individuals who were genetic outliers there were a total of 23,634 EA and 6,657 AA CARe study participants available for analysis (see **Supplemental Methods**).

Statistical Analysis

Sex-specific fibrinogen phenotype residuals were constructed within cohort and race strata, after accounting for age and field site/center. A normal quantile transformation was performed on the covariate-adjusted residuals to improve normality of model residuals and to preserve the rank order of measurements assayed in different laboratories and in some instances, using different fibrinogen assays. For FHS, since fibrinogen was measured at different times using different assays for the parent, offspring, and generation 3 cohorts, phenotype residuals were constructed separately within each FHS cohort.

Genotype-phenotype data analyses were performed separately for EA and AA subjects within each study. To further adjust for population stratification, principal components were incorporated as covariates in analyses (see **Supplemental Methods)**. For studies involving unrelated individuals, linear regression was used on quantile transformed standardized fibrinogen residuals, assuming an additive genetic model. Association analyses were performed in PLINK V 1.0.7[20]. To allow for the presence of related individuals in CFS and FHS, linear mixed effects

models (LME) were used[21]. Results from each study were combined using fixed and random effects meta-analysis based on inverse-variance weighting. To assess heterogeneity of SNP-fibrinogen association results between cohort studies, we used the I^2 inconsistency metric.

To correct for multiple testing, we first determined the effective number of independent SNPs present on the IBC array is 26,482 for AA and 20,544 for EA. To maintain an overall type 1 error rate of 5%, a statistical threshold of $\alpha = 2 \times 10^{-6}$ was used to declare array-wide significance. To test for gene-environment interaction, we estimated the effect of the top SNPs within strata of sex, obesity (body mass index ≥ 30 kg/m^2 versus < 30), and current smoking status by introducing an interaction term into the linear model.

To assess the number of independent SNPs associated with fibrinogen phenotype within a candidate region, association signals were initially characterized by grouping significant SNPs according to linkage disequilibrium, using a pair-wise r^2 threshold of greater than 0.50 to define a smaller number of clusters of correlated SNPs. To further assess the presence of multiple independent SNPs in a candidate region, regression analyses were repeated, conditional on the mostly strongly associated SNP in the region. Finally, for fine-mapping of candidate regions on chromosomes 4q32 and 5q31, additional SNP genotypes were imputed using phase 2 HapMap genotype data. Further details regarding imputation and haplotype estimation can be found in the **Supplemental Methods**.

RESULTS

The sample size and participant characteristics from each study are shown in **Table 1**. Mean fibrinogen levels were higher in AA than EA. A total of 32 SNPs spanning 11 candidate gene regions were significantly associated with fibrinogen in EA (p-value range 10^{-6}-10^{-39}, **Supplemental Table 1** and **Supplemental Figure 1**). Results were similar when the analyses were restricted to the approximately 3,867 CARe EA participants from CARDIA, MESA, and CFS, who were not included as part of the CHARGE consortium fibrinogen GWAS (data not shown). In the smaller AA sample, a total of 6 SNPs were significantly associated with fibrinogen (p-value range 10^{-6}-10^{-11}, **Supplemental Table 2 and Supplemental Figure 1**), all located in the chromosome 4 fibrinogen structural gene region.

Common fibrinogen-associated SNPs in EA

The majority of SNPs significantly associated with fibrinogen in EA were common, with minor allele frequencies >20%. Of the 32 SNPs that reached the threshold of experiment-wide significance, 14 SNPs could be considered to represent potential independent association signals. SNPs previously associated with fibrinogen in GWAS of EA that are replicated in the current report, and those SNPs newly associated with fibrinogen in the current study, are indicated in **Table 2**. Based on assessment of regional linkage disequilibrium patterns and conditional regression analyses in the overall CARe EA sample (n=23,634), there was most likely a single association signal present within each of the *IL6R*, *CPS1*, *PCCB*, and *HDLBP* genes (tagged by rs7529229, rs7422339, rs3821445, and rs6752050, respectively). In 4 genes, we found evidence of more than one independent fibrinogen-associated SNP. Within *NLRP3*, the minor allele of rs4925659 associated with higher fibrinogen and the minor allele of rs12239046 associated with lower levels. The minor allele of *IL1RN* rs315921 was associated with lower fibrinogen levels.

At a slightly less restrictive significance threshold, (p-value $<10^{-5}$) *IL1RN* rs4251961 was associated with higher fibrinogen levels. In the fibrinogen structural gene region, 2 discrete clusters of SNPs associated with fibrinogen phenotype could be distinguished: 1 associated with higher fibrinogen levels derived from the *FGB* region (tagged by rs1800787) and the other associated with lower fibrinogen levels derived primarily from the *FGG* region (tagged by rs2066861). The strongest fibrinogen association signal in the cytokine gene cluster region on chromosome 5 was derived from a block of highly correlated SNPs, tagged by rs6874639, that span the genes encoding *SLC22A5* and *IRF1* (meta-analysis p-values in the range of 10^{-19} to 10^{-20}) (**Table 2 and Figure 2**). This LD bin includes rs1016988 and rs2522056, which were previously reported to be associated with circulating fibrinogen[5, 6]. In a conditional regression analysis of this region (adjusting for rs6874639), only rs6873426 remained associated with fibrinogen ($p=1.2 \times 10^{-5}$).

Low frequency fibrinogen-associated variants

Two SNPs with minor allele frequencies of <5% showed evidence of association with fibrinogen in EA (**Table 2**). A low frequency non-synonymous Pro265Leu variant of the fibrinogen beta-chain encoded by *FGB* rs6054 (MAF=0.4% in EA and 0.2% in AA) was significantly associated with fibrinogen concentration, even following adjustment for the top common *FGB* variant rs1800787 ($p=1 \times 10^{-18}$). Haplotype analysis across the *FGB* region confirmed that the rs6054 rare variant allele likely arose on a common ancestral haplotype (allele frequency ~30% in EA) that is distinct from the 'high-fibrinogen' *FGB* haplotype tagged by rs1800787. A non-synonymous coding variant of *HNF4A* with a MAF of 3% in EA (rs1800961 encoding an

Ile139Thr missense substitution) was associated with lower fibrinogen, though it did not reach the threshold of statistical significance ($p=5 \times 10^{-6}$).

Fibrinogen-associated loci in AA and comparison with EA results

The 6 SNPs significantly associated with fibrinogen in the AA sample (**Table 3**) had minor allele frequencies that ranged from ~2 to 12%; these SNPs represent 5 independent association signals within the *FGB-FGA-FGG* gene cluster. The association of fibrinogen with rs10050257, located 5 kb upstream of the FGA transcription start site, has not been previously reported. Two additional independent signals, tagged by rs10034922 and rs4463047, were present among the imputed HapMap SNPs in this region.

Table 4 compares the top SNP genotype effects (change in fibrinogen per minor allele, adjusted for age, sex, and clinic site) and minor allele frequencies for EA and AA, for each region significantly associated with fibrinogen phenotype in either population. At the *FGB-FGA-FGG* fibrinogen gene locus, *FGB* 1800787 and *FGG* 2066861 were less common in AA, but showed consistent direction and magnitude of phenotypic effects in AA. In contrast, several fibrinogen-associated SNPs were exclusive to AA (rs2066874, rs10050257, rs2070017, rs2066877, and rs6058); all had minor allele frequencies of < 0.1% in EA.

For several additional loci (*IL6R*, *IL1RN* rs4251961, *NLRP3* rs12239046, and *SLC22A4* rs270607), the EA fibrinogen phenotype association results were concordant (similar direction of effect and nominal p-values <0.05) in AA. In contrast, there was no evidence of fibrinogen phenotype association in AA for *NLRP3* rs4925659, *CPS1*, *PCCB*, and *SLC22A5-IRF1*. There

was also no evidence of association for *IL1RN* rs315921 or *HDLBP* rs6752050, though the frequencies of the respective minor alleles were considerably lower among AA than EA.

By comparing the SNP phenotype association results between EA and AA, in some instances the regional LD patterns suggested further localization of the putative causal variant(s). These findings are described in further detail under **Supplemental Material.**

Between-study heterogeneity and gene-environment interaction

In the combined meta-analysis, the *FGG* SNP cluster tagged by rs2066861 showed strong inter-cohort differences in association with fibrinogen (p-value for heterogeneity = 1×10^{-9}). **Table 5** shows the individual study association results for the 3 fibrinogen structural gene region non-redundant SNPs associated with fibrinogen phenotype in EA. While the SNP regression coefficients for rs1800787 and rs6054 are consistent across studies, rs2066861 is strongly associated with lower fibrinogen only in ARIC and CHS, the two largest studies that used the Clauss functional fibrinogen assay. By testing several study characteristics and summary measures, the type of fibrinogen assay explained almost all (96.3%) of the between-study variance associated with rs2066861 in *FGG*. Similar between-cohort differences for rs2066861 were observed among the AA cohorts (p-value for heterogeneity = 7×10^{-5}). Within FHS, there was no evidence for heterogeneity of results between the parent, offspring, and generation 3 cohorts. There was no significant interaction between the significant SNPs and sex, current smoking, or obesity in either European Americans or African-Americans (data not shown).

DISCUSSION

In this large population-based study, we were able to simultaneously confirm and more precisely characterize the genetic variants associated with fibrinogen in EA[5, 6] and also compare and contrast results of the association findings within an under-represented minority population of AA. In EA, we identified multiple SNPs independently associated with fibrinogen at *FGB-FGA-FGG*, *NLRP3*, *IL1RN*, and *IRF1-SCL22A5*. We note concordance of EA and AA fibrinogen association results at two common fibrinogen loci (*FGB* rs1800787 and *FGG* rs2066861), as well as at several additional loci within inflammatory genes (*IL6R*, *IL1RN*, *NLRP3*) for which there is strong prior evidence of association with fibrinogen based on EA GWAS or CARe IBC results. We also identified a new low frequency non-synonymous *FGB* variant (rs6054 encoding β-fibrinogen Pro265Leu) associated with lower fibrinogen levels.

The current study includes by far the largest AA sample (n=6,657) evaluated for genetic factors associated with plasma fibrinogen. Our results confirm the association of several SNPs (rs2066874, rs2070017, and rs6058) previously identified through candidate gene analyses in single cohort studies of CARDIA and CHS AAs.[3, 16] We also report a novel association with rs10050257, located ~5kb upstream of the *FGA* transcription start site. We used a genotyping array that has denser linkage disequilibrium and broader population coverage of previously identified candidate gene regions for AA than current GWAS platforms. Currently, our ability to perform replicate the novel rs10050257 association is limited by (a) the unavailability of additional African-American samples genotyped using the IBC genotyping array; (b) the relatively low allele frequency of these African-American SNPs, which are not present on current GWAS genotyping platforms.

While some loci were consistently associated with fibrinogen between EA and AA, others were not. Several fibrinogen-associated SNPs in *FGA, FGB,* and *FGG* were exclusive to AAs. Other loci were associated with fibrinogen phenotype only among EA. The latter could possibly be due to the smaller AA size as compared to the EA sample or to different LD patterns between these populations. Based on population-specific effect sizes and/or allele frequency differences, it is possible that some of these loci might account in part for the higher fibrinogen levels in AA compared to EA.

Despite the consistent heritability of fibrinogen[7-9], none of the previously or newly identified significant SNPs in our study explain a large portion of the variation in fibrinogen. Together, the common and low frequency SNPs explained 2.2%-15% of the variation in circulating fibrinogen among EA cohorts and 1.8%-3.8% among AA cohorts. For the newly identified lower frequency variants, the percent explained by rs6054 and rs1800961 together ranged from 0.3%-1.0%. We also found no evidence that gene by environment interactions are important in determining fibrinogen levels in either racial/ethnic group, though the number of environmental factors examined was limited.

Characterization of the *cis*-acting and coding variants within the fibrinogen structural genes have contributed to our understanding of fibrinogen synthesis and the potential role of fibrinogen and inflammation in athero-thrombotic disease. Fibrinogen is composed of three chains of amino acids, α, β, and γ, and each chain is encoded by a distinct gene (*FGA*, *FGB*, and *FGG*), all of which reside in a cluster on chromosome 4. Higher fibrinogen levels have been observed with either the *FGB* -455G>A (rs1800790) or -148C>T polymorphism (rs1800787)[22]. Another SNP in perfect LD with rs1800787 (rs4508864), has been previously associated with *FGB* expression in

liver tissue[23]. Interleukin-6 (IL-6) is the primary mediator of fibrinogen synthesis in response to inflammation, and sequences responsive to IL-6 are present in the promoter regions of the 3 genes encoding fibrinogen. The *FGB* rs1800787 polymorphism is located in between a hepatocyte nuclear factor-3 (HNF-3) site and a CCAAT box/enhancer-binding protein (C/EBP) site within the *FGB* promoter that comprise a an IL-6-response element. The rs1800787 -148 C/T polymorphism influences IL-6 induced *FGB* promoter activity by interfering with the cooperation between adjacent HNF-3 and C/EBP promoter binding sites[24]. Interestingly, we additionally note that rs10050257, the newly identified *FGA* promoter region SNP associated with fibrinogen in African Americans is also located within C/EBP and HNF-3 consensus transcription factor binding sequences. Additional in vitro transcriptional analysis of the *FGA* promoter is required to confirm the mechanism of association for rs10050257.

While evidence for association of common *FGA*, *FGB*, and *FGG* variants with thrombotic risk has been inconsistent,[25-29] familial dysfibrinogenemias due to rare fibrinogen gene mutations have been associated with either thrombosis or hemorrhage.[30] Mutations in the fibrinogen genes leading to amyloidosis have also been described[31]; and recent evidence suggests that fibrinogen may be involved in the pathogenesis of Alzheimer's disease[32]. The β-fibrinogen Pro265Leu polymorphism (rs6054) is located within an evolutionarily conserved intra-chain disulfide loop that appears to affect fibrinogen secretion and assembly[33]; this SNP is predicted to alter 'normal' function.[34] The role of the rare Pro265Leu variant in clinical athero-thrombotic disease remains to be determined.

Altered splicing regulation may be another mechanism by which fibrinogen gene variants contribute to phenotypic differences in fibrinogen. Both rs6054 and rs6058 (a synonymous SNP located near the splice site of exon 6 of *FGB*) are predicted to alter splicing enhancer elements.[34] SNPs rs2066861 and rs2066865 (*FGG* 10034C>T) tag a common *FGG* haplotype and are located in a region of alternative pre-mRNA processing that results in the formation of the fibrinogen gamma chain isoform, fibrinogen γ' that appears to be protective against deep vein thrombosis (DVT)[35]. *FGG* 10034C>T results in a decrease in the ratio of fibrinogen γ' to γA and therefore increases risk of DVT[36] and possibly stroke[29]. These *FGG* SNPs are also in linkage disequilibrium with the Thr312Ala polymorphism (rs6050) of *FGA*, which is located within the αC domain of fibrinogen, and is important for lateral aggregation and factor XIII-induced cross-linking of fibrin. The Ala312 fibrinogen-α chain variant has been associated with venous thrombosis[37] or post-stroke mortality in subjects with atrial fibrillation.[38] The Ala312 variant has also been associated with altered clot structure, and therefore may predispose to clot embolization.[39]

Fibrinogen can be measured using either a functional Clauss method or an antigen method. In most individuals, the results of the two assays are highly correlated, but discrepancies can occur since the immunoassay and functional assay measure different properties of fibrinogen[40, 41]. In the current study, we tested for between-study differences in genotype-fibrinogen association results according to type of fibrinogen assay at the stage of meta-analysis. The use of the fibrinogen immunoassay in two of the six CARe studies allowed us to confirm that the common *FGG* haplotype associated with the fibrinogen γ' chain isoform is differentially associated with decreased fibrinogen levels as measured by functional assay, but not by immunoassay. The same

FGG haplotype was recently associated with higher D-dimer levels. Together, these findings have potentially important implications for thrombotic risk assessment[42].

Several of the genes harboring polymorphisms associated with fibrinogen are implicated in pathways that link the sensing and regulation of cellular injury, inflammation and metabolic stress, and autoimmunity. Hepatic fibrinogen synthesis is regulated by cytokines IL-1 and IL-6[43], and *IL6R* and *IL1RN* encode important regulatory proteins for each of these cytokines. One of our significant SNPs, rs4537545, influences *IL6R* expression in lymphocytes[44], and is in strong LD with rs8192244, which encodes a functional Ala358Asp substitution at the site where the IL-6 receptor is cleaved to form soluble IL-6 receptor. Interleukin (IL)-1 receptor antagonist, the protein encoded by *IL1RN* mediates a variety of IL-1 related inflammatory responses. The NLRP3 inflammasome complex functions as an upstream activator of IL-1, IL-18, and NF-kappaB signaling. Mutations in either *NLRP3* or *IL1RN* result in dysregulated IL-1β production and autoinflammatory disease[45]. Chromosome 5q31 contains a cluster of coordinately regulated genes involved in the immune response, including a 250 kb risk haplotype associated with Crohn's disease susceptibility[46]. The protein encoded by *CPS1* is an enzyme that catalyzes the initial step of the hepatic urea cycle. The non-synonymous *CPS1* variant rs7422339 (Thr1405Asn) has been associated with vascular and inflammatory disease [47], and was recently associated with homocysteine[48].

Several other fibrinogen phenotype-associated genes have functional associations with lipid metabolism, another important determinant of vascular risk. A variant of *PCCB*, which encodes the beta subunit of the amino acid catabolic enzyme propionyl-CoA carboxylase, was recently associated with small HDL particle concentration[49]. Previous studies have consistently found that

HDL cholesterol is inversely associated with fibrinogen[50]. High density lipoprotein-binding protein (HDLBP) may function in the removal of excess cellular cholesterol and has been co-localized with apolipoprotein E in cholesterol-loaded plaque macrophages[51]. The Thr130Ile variant of *HNF4A* (rs1800961) is located in the DNA binding domain of the transcription factor HNF-4, and has been associated with decreased transcriptional activity *in vitro*, increased risk of T2D[52] and lower HDL cholesterol levels[53].

In summary, both common and less frequent sequence variants of inflammatory and hemostatic genes are associated with fibrinogen. It appears that at least some loci contributing to fibrinogen levels differ between AA and EA. Additional studies are needed to better understand the relative contribution of genetic and environmental factors to the chronic systemic inflammatory burden that may underlie inter-population differences in fibrinogen and potentially contribute to CVD risk.

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Authorship Contributions and Disclosure of Conflicts of Interest:

Contributions: CLW, LAL, KCT, EML, ADJ, LAH, CP, YL, QY, APR, and COJ contributed to analysis and interpretation of the data; BJK took part in designing the IBC candidate gene array; CLW, APR drafted the manuscript; DG, NLS, COJ, DRJ, ARF, ADJ, BJK, JAD, JGW, GT, RPT, and WT contributed to substantial revision of the manuscript; senior investigators participating in data collection for cohorts represented in CARe are CJO, SR, HAT, JGW, ARF, DRJ, APR

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Table 1. Characteristics of the Study Participants*

	ARIC		CARDIA		CFS		CHS		FHS†	MESA	
	AA	EA	AA	EA	AA	EA	AA	EA	EA	AA	EA
N	2853	9406	1178	1370	341	244	719	3859	6502	1566	2253
Female, %	63	54	60	52	58	50	63	56	53	54	52
Current smoker, %	29	24	32	21	18	8	16	11	14	18	12
Diabetes, %	13	5	2	1	12	5	16	7	11	15	4
Age, years	53±6	54±6	25±4	26±3	41±19	45±20	73±6	73±6	49±14	62±10	63±10
Body mass index, kg/m^2	30.6±6.3	28.3±5.3	25.8±5.9	23.7±4.0	33.1±9.8	31.9±9.3	28.5±5.6	26.4±4.5	27.4±5.5	30.2±5.9	27.8±5.1
Systolic blood pressure, mm Hg	128±21	118±17	112±11	109±11	123±18	122±15	142±29	135±21	121±17	132±22	123±1
Diastolic blood pressure, mm Hg	80±12	72±10	70±11	69±9	74±11	73±8	75±11	70±11	75±10	75±10	70±10
Total cholesterol, mg/dL	215±45	215±41	179±33	176±33	184±42	192±42	209±39	212±39	194±36	189±36	196±36
HDL cholesterol, mg/dL	55±17	51±17	54±13	52±13	45±13	43±12	58±16	54±16	54±16	52±15	52±16
Fibrinogen, mg/dL	320±71	297±61	363±89	320±69	323±85	328±70	346±75	320±64	357± 75	362±80	336±70

*mean±SD for continuous variables, % for categorical variables

†combines parent, offspring, and generation 3 cohort

Table 2. Non-redundant candidate gene SNPs associated with fibrinogen in European-Americans

Chromosome	Gene	dbSNP reference number	Minor allele frequency	Meta-analysis regression coefficient (SE)	Meta-analysis p-value	Comment
1q21	IL6R	rs7529229 (T>C)	0.41	-0.050 (0.009)	8.3xE-08	Confirmation of previous GWAS result
1q44	NLRP3	rs4925659 (G>A)	0.38	0.052 (0.010)	5.3xE-08	Confirmation of previous GWAS result
1q44	NLRP3	rs12239046 (C>T)	0.39	-0.049 (0.010)	3.3xE-07	Fine-mapping of previous GWAS result
2q13	IL1RN	rs315921 (G>A)	0.18	-0.057 (0.012)	2.6xE-06	Confirmation of previous GWAS result
2q13	IL1RN	rs4251961 (T>C)	0.38	0.040 (0.0102)	2.1xE-05	Fine-mapping of previous GWAS result
2q34	CPS1	rs7422339 (C>A)	0.32	-0.060 (0.010)	1.9xE-09	Confirmation of previous GWAS result
2q37	HDLBP	rs6752050 (T>C)	0.15	-0.063 (0.013)	1.2xE-06	New association in current study
3q22	PCCB	rs3821445 (A>G)	0.20	0.056 (0.012)	1.2xE-06	Confirmation of previous GWAS result
4q32	FGB	rs1800787 (C>T)	0.21	0.151 (0.012)	1.4xE-39	Confirmation of previous GWAS result
4q32	FGB	rs6054 (C>T)	0.004	-0.580 (0.068)	1.2xE-17	New association in current study
4q32	FGG	rs2066861 (C>T)	0.23	-0.068 (0.011)	4.2xE-10‡	Fine-mapping of previous GWAS result
5q31	SLC22A5-IRF1	rs6874639† (A>G)	0.19	-0.128 (0.014)	4.3 E-20	Confirmation of previous GWAS result
5q31	IRF1	rs6873426† (G>T)	0.31	0.089 (0.014)	2.1 E-10	Fine-mapping of previous GWAS result
20q13	HNF4A	rs1800961 (C>T)	0.03	-0.124 (0.027)	4.5 E-6	New association in current study

dbSNP = NCBI Single Nucleotide Polymorphism Database
SE= standard error of regression coefficient

†SNP genotype imputed from HapMap CEU (European-American) genotype data

‡Indicates meta-analysis p-value for heterogeneity <0.01

Table 3. Non-redundant candidate gene tagSNPs associated with fibrinogen in African-Americans

Chromosome	Gene	dbSNP reference number	Minor allele frequency	Meta-analysis regression coefficient (SE)	Meta-analysis p-value	Comment
4q32	FGG	rs2066874 (T>C)	0.03	-0.333 (0.050)	2.9xE-11	Replication of previous candidate gene study
4q32	FGA	rs2070017 (C>T)	0.11	-0.163 (0.028)	4.8xE-09	Replication of previous candidate gene study
4q32	FGA	rs10050257 (T>G)	0.06	-0.213 (0.037)	7.7xE-09	New association in current study
4q32	FGB	rs1800787 (C>T)	0.10	0.146 (0.029)	4.7xE-07	Replication of previous candidate gene study
4q32	FGB	rs6058 (G>T)	0.07	-0.170 (0.036)	9.5xE-07	Replication of previous candidate gene study
4q32	FGA-FGG	rs10034922† (C>T)	0.19	-0.121 (0.024)	3.9xE-07	New association in current study
4q32	FGB	rs4463047† (C>T)	0.13	-0.179 (0.036)	7.3xE-07	New association in current study

dbSNP = NCBI Single Nucleotide Polymorphism Database
SE= standard error of regression coefficient

†SNP genotype imputed from HapMap European-American (CEU) +Yoruban (YRI) genotype data

Table 4. Comparison of SNPs associated with circulating levels of fibrinogen in European-Americans and African-Americans

Chromosome	Coordinate	Gene	SNP	Allele 1	Allele 2	Variant Type	European Americans			African Americans		
							CAF*	Beta	p-value	CAF*	Beta	p-value
1	152687402	IL6R	rs7529229	t	c	intron	0.41	-0.050	8.3xE-08	0.64	-0.012	0.51
1	152685503	IL6R	rs8192284	a	c	coding-nonsynon	0.40	-0.046	9.3xE-07	0.14	-0.060	0.02
1	245670086	NLRP3	rs4925659	g	a	intron	0.38	0.052	5.3xE-08	0.17	0.018	0.44
1	245668218	NLRP3	rs12239046	c	t	intron	0.39	-0.049	3.3xE-07	0.49	-0.039	0.02
2	113588522	IL1RN	rs315921	g	a	5' upstream	0.18	-0.057	2.6xE-06	0.03	0.030	0.54
2	113590938	IL1RN	rs4251961	t	c	5' upstream	0.38	0.040	2.1xE-05	0.18	0.097	1.8xE-05
2	211248752	CPS1	rs7422339	c	a	coding-nonsynon	0.32	-0.060	1.9xE-09	0.37	-0.021	0.25
2	241877453	HDLBP	rs6752050	t	c	untranslated	0.15	-0.063	1.2xE-06	0.03	-0.033	0.51
3	137485499	PCCB	rs3821445	a	g	intron	0.20	0.056	1.2xE-06	0.11	-0.005	0.85
4	155703465	FGB	rs1800787	c	t	5' upstream	0.21	0.151	1.4xE-39	0.10	0.146	4.7xE-07
4	155709058	FGB	rs6054	c	t	coding-nonsynon	0.004	-0.580	1.2xE-17	0.001	-0.343	0.14
4	155746886	FGG	rs2066861	c	t	5' upstream	0.23	-0.068	4.2xE-10	0.29	-0.073	6.8xE-05

4	155749031	FGG	rs2066874	t	c	intron	8 E-05	-0.289	0.62	0.03	-0.333	2.9xE-11
4	155734845	FGA	rs10050257	t	g	5' upstream	0.0009	-0.288	0.10	0.06	-0.213	7.7xE-09
4	155729726	FGA	rs2070017	c	t	intron	0.002	-0.303	0.03	0.11	-0.163	4.8xE-09
4	155709794	FGB	rs6058	g	t	coding-synon	0.0001	-0.676	0.02	0.07	-0.170	9.5xE-07
4	155740078	FGA-FGG	rs10034922†	c	t	intergenic	0.01	0.115	0.09	0.19	-0.121	3.9xE-07
5	131677085	SLC22A4	rs270607†	a	g	intron	0.30	-0.063	2.0xE-08	0.24	-0.065	0.005
5	131806615	SLC22A5-IRF1	rs6874639†	a	g	intergenic	0.19	-0.128	4.3xE-20	0.32	-0.0273	0.29
20	42475778	HNF4A	rs1800961	c	t	coding-nonsynon	0.03	-0.124	4.5xE-6	0.006	-0.098	0.38

*coded allele frequency of Allele 2
†SNP genotype imputed from HapMap CEU or CEU + YRI

Table 5. Cohort-specific association results for fibrinogen gene SNPs in European-Americans

SNP	Gene	ARIC N=9554	CARDIA N=1311	CFS N=252	CHS N=3918	FHS N=6733	MESA N=2288	p-value for between-study heterogeneity
rs1800787	FGB	0.132 ± 0.018 P=1 x 10^{-13}	0.164 ± 0.04 P=0.0004	NA	0.150 ± 0.027 P=3 x 10^{-8}	0.178 ± 0.023 P=2 x 10^{-14}	0.159 ± 0.037 P=2 x 10^{-5}	0.62
rs6054	FGB	-0.588 ± 0.112 P=1 x 10^{-7}	-0.904 ± 0.350 P=0.01	NA	-0.648 ± 0.192 P=0.0007	-0.526 ± 0.111 P=2 x 10^{-6}	-0.545 ±.0218 P=0.013	0.87
rs2066861	FGG	-0.116 ± 0.017 P=8 x 10^{-12}	-0.0588 ± 0.045 P=0.20	0.0224 ±0.115 P=0.85	-0.159 ± 0.02 P=1 x 10^{-9}	0.0216 ± 0.02 P=0.33	0.057± 0.03 P=0.11	1.48 x 10^{-9}

For each study, the number in the top row indicate regression beta coefficient for standardized fibrinogen ± standard error, based on an additive genetic model. The last column indicates the p-value for heterogeneity based on the combined meta-analysis.

Figure Legends

Figure 1. Shows the imputed regional association results for chromosome 4 SNPs associated with fibrinogen levels. The left y-axis displays the -log(p-value), the right y-axis the recombination rate, and the x-axis the SNP position on the chromosome. The degree of linkage disequilibrium (LD) (r^2) is shown by various colors (legend in top right had corner). The symbol shapes represent the type of SNP: inverted triangles represent non-synonymous coding variants, squares represent synonymous coding variants, and circles represent intron and other variant types.

Figure 2. Shows the imputed regional association results for chromosome 5 SNPs associated with fibrinogen levels. The left y-axis displays the -log(p-value), the right y-axis the recombination rate, and the x-axis the SNP position on the chromosome. The degree of linkage disequilibrium (LD) (r^2) is shown by various colors (legend in top right had corner). The symbol shapes represent the type of SNP: inverted triangles represent non-synonymous coding variants, squares represent synonymous coding variants, and circles represent intron and other variant types.

Figure 1: Chromosome 4 regional association plot for imputed fibrinogen-associated SNPs in European-Americans

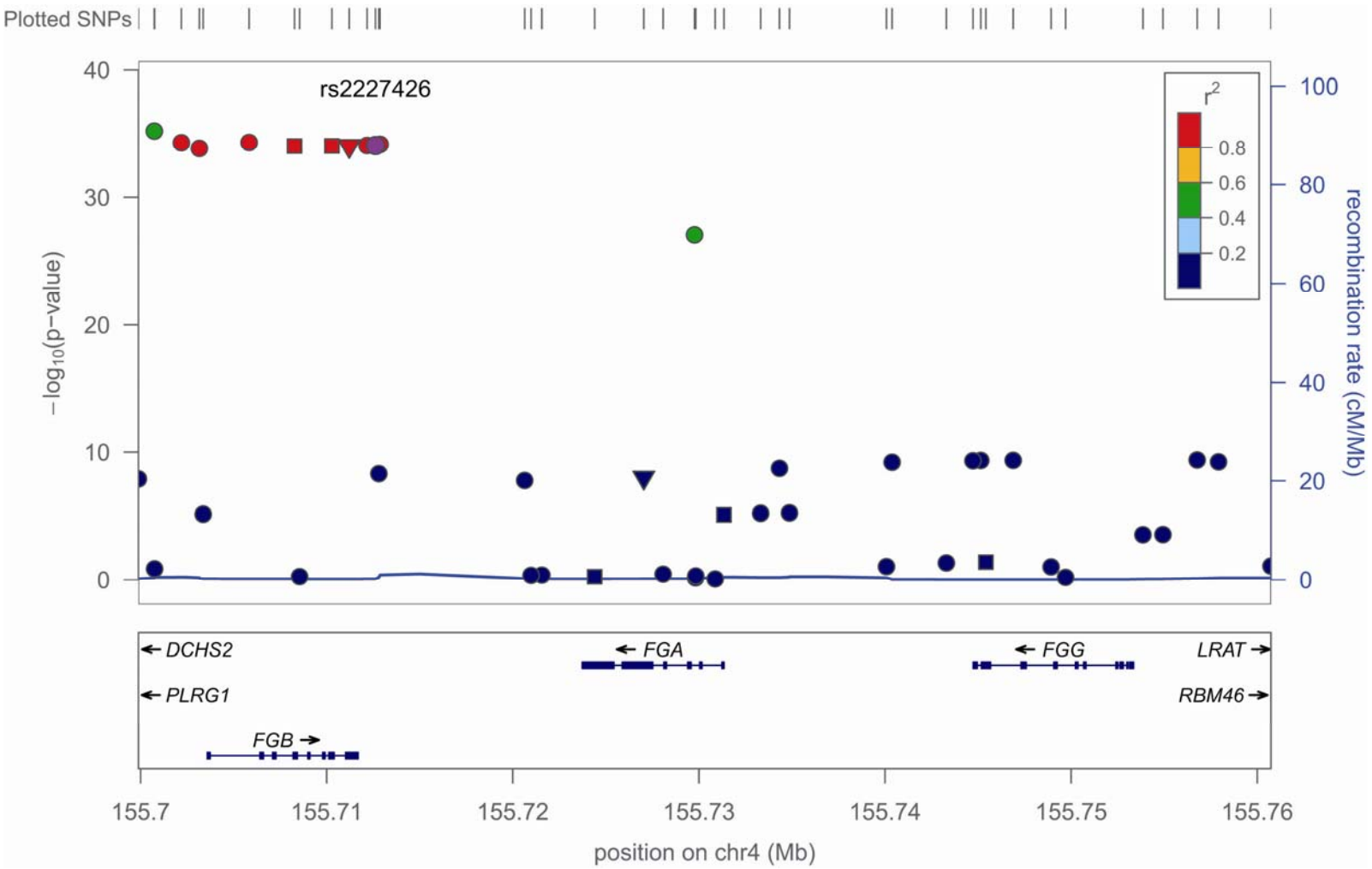

Figure 2. Chromosome 5 regional association plot for imputed fibrinogen-associated SNPs in European-Americans