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Multiple Novel Loci, Including Those Related to Crohn's Disease, Psoriasis and Inflammation, Identified in a Genome-Wide Association Study of Fibrinogen in 17,686 Women: the Women's Genome Health Study

Jacqueline S. Danik, MD, MPH[1,2,*,†], **Guillaume Pare, MD, MSc**[1,*,†], **Daniel I. Chasman, PhD**[1,2], **Robert Y.L. Zee, PhD, MPH**[1,2], **David J. Kwiatkowski, MD, PhD**[2,3], **Alex Parker, PhD**[4], **Joseph P. Miletich, MD**[4], and **Paul M Ridker, MD, MPH**[1,2]

[1]Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Boston, MA 02115

[2]Donald W. Reynolds Center for Cardiovascular Research, Brigham and Women's Hospital, Boston, MA 02115

[3]Translational Medicine Division, Brigham and Women's Hospital, Boston, MA 02115

[4]Amgen, Inc., Cambridge, MA 02139

Abstract

Background—Fibrinogen is a multifunctional circulating glycoprotein involved in wound-healing, thrombosis, platelet aggregation and inflammation, and elevated levels predict vascular disease. Despite evidence of such crucial biological functions and moderate heritability, comprehensive analysis of the influence of genetic variation on fibrinogen is not available.

Methods and Results—To address this issue, we undertook a genome-wide association study evaluating the potential relationships between 337,343 single nucleotide polymorphisms (SNPs) and plasma fibrinogen levels among 17,686 apparently healthy women participating in the Women's Genome Health Study (WGHS). As C-reactive protein is also an inflammatory marker known to predict cardiovascular diseases, we compared the determinants of fibrinogen levels with those of C-reactive protein.

Four novel loci were identified, in addition to the fibrinogen gene cluster, which were associated with fibrinogen levels at genome-wide levels of significance (range of P-values from 8.82×10^{-09} to 8.04×10^{-39}). Two of the loci related to common chronic inflammatory diseases: the first, at locus 5q31.1 (SLC22A5, SLC22A4, IRF1) lies immediately adjacent to a locus linked to Crohn's disease (P-value for lead SNP 1.24×10^{-12}) and the second, at locus 17q25.1 (CD300LF, SLC9A3R1, NAT9) has been associated with psoriasis (P-value for lead SNP 7.72×10^{-11}). A third locus at 1q21.3 (IL6R) lies within the interleukin 6 receptor gene, a critical component of the inflammatory cascade (P-value for lead SNP 1.80×10^{-11}). A novel locus at 2q34 (CPS1) participates in the urea cycle (P-value 8.82×10^{-09}). The majority of implicated SNPs showed little evidence of dual association with C-reactive protein levels.

*Correspondence: Jacqueline Suk Danik, Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, 900 Commonwealth Avenue East, Boston, MA 02215. Tel 617-278-0808 FAX 617-232-3514 email: jdanik@partners.org.
†These authors contributed equally to this work.

Conflict of Interest Disclosures P.M.R. has also received grant support from Aztra-Zeneca, Novartis, Sanofi-Aventis, and is listed as a coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease. J.P.M reports holding equity in Amgen, Inc. J.S.D, G.P, D.C., R.Y.L.Z, D.J.K and A.P have no other conflicts to disclose.

Conclusions—An agnostic survey of the human genome identifies novel loci related to common chronic inflammatory diseases as genetic determinants of fibrinogen levels, in addition to loci that relate to the inflammatory cascade, the urea cycle and the fibrinogen gene cluster.

Keywords

fibrinogen; genetics; inflammation; coagulation; women

Introduction

Fibrinogen is a circulating glycoprotein involved in wound-healing, thrombosis, platelet aggregation and inflammation which also has roles in cell adhesion, vasoconstriction, and chemotactic activity 1. Like the acute phase reactant C-reactive protein (CRP), plasma fibrinogen levels also associate with increased risk of myocardial infarction, stroke, and vascular mortality2,3, and these two inflammatory biomarkers may provide complementary information4. In addition to environmental factors that affect fibrinogen level, there is evidence of substantial heritability of fibrinogen (25-51%) in twin and family studies 5,6. To date, evaluations of genetic determinants of fibrinogen have typically used a candidate gene approach and focused on the FGA, FGB and FGG genes, which encode fibrinogen's alpha, beta and gamma polypeptide chains. In these studies, variants within the promoter and genic regions of FGB have been associated with stable and acute phase fibrinogen levels7–9 as well as vascular events10,11. In aggregate, however, these polymorphisms explain only a small fraction of the estimated heritable effects on fibrinogen and the multi-functional nature of the protein itself suggests that other loci should have substantive effects. We therefore undertook a genome-wide association study evaluating potential relationships between 337,343 SNPs and plasma fibrinogen among 17,686 apparently healthy women participating in the Women's Genome Health Study (WGHS)[12]. As C-reactive protein is also an inflammatory marker known to predict cardiovascular diseases, we also directly compared the determinants of fibrinogen levels with those of C-reactive protein13.

Methods

The study cohort was derived from participants of the Women's Genome Health Study12, the genetic arm of the Women's Health Study. In brief, participants in WGHS include American women with no prior history of cardiovascular disease, cancer, or other major chronic illness who provided a baseline blood sample during the enrollment phase of the Women's Health Study between 1992 and 1995 as well as consent for blood based analyses related to the risk of incident chronic diseases. All baseline blood samples underwent ascertainment in a single core laboratory for fibrinogen levels using an immunoturbidimetric assay (Kamiya Biomedical, Seattle, Wash), which was standardized to a calibrator from the World Health Organization[14]. The coefficients of variation obtained from blinded simultaneously analyzed quality controls were <5% for fibrinogen. DNA extracted from the baseline blood samples underwent SNP genotyping using the Illumina Infinium II assay to query a genome-wide set of 315,176 haplotype-tagging SNP markers (Human HAP300 panel) as well as a focused panel of 45,882 missense and haplotype tagging SNPs selected to enhance coverage of genomic regions in which we had a strong *a priori* interest owing to presence of genes believed to be of relevance to metabolic, cardiovascular, and inflammatory diseases.

Before performing any genetic analyses, all SNPs were evaluated for high call rates (>90%) and the percentage of missing SNPs for each individual was calculated (<2%). For SNPs with adequate data, Hardy-Weinberg disequilibria ($P>10^{-6}$) were evaluated to identify potential genotyping errors. In total, 337,343 SNPs passed criteria for use. We also compared the Illumina-based SNP data for each individual participant for a panel of 44 common SNPs that

have previously been ascertained in this population using alternative genotyping technologies; this step is used as a secondary check to ensure accurate specimen labeling prior to any analyses.

For the purpose of this analysis, we limited our evaluation to 17,686 non-diabetic WGHS participants who were of Caucasian ancestry and were not taking lipid-lowering agents.

Because population stratification can result in inflated type I error in genome-wide association analysis, a principal component analysis using 1443 ancestry informative SNPs was performed using PLINK15 in order to confirm self-reported ancestry. Briefly, these SNPs were chosen based on a fixation index16 (Fst) > 0.4 in HapMap populations (YRB, CEU, CHB+JPT) and inter-SNP distance at least 500 kb in order to minimize linkage disequilibrium. Different ethnic groups were clearly distinguished with the two first components. Based on this analysis, 69 individuals were excluded from further evaluation as they did not cluster with other Caucasians, leaving 17,686 participants for the current study population. Two additional steps were taken to rule out the possibility that residual stratification within Caucasians was responsible for the associations observed. First, association analysis was done with correction by genomic control. Second, a principal component analysis[17] was performed in previously identified Caucasians (only) using 64,208 SNPs chosen to have pair-wise linkage disequilibrium lower than r^2=0.2. The first ten components were then used as covariates in the association analysis. As adjustment by these covariates did not change the conclusions, we present analysis among Caucasian participants without further correction for sub-Caucasian ancestry.

Analysis was performed to define any novel gene locus associated with fibrinogen levels. In all statistical analyses, we adjusted plasma levels of fibrinogen on an *a priori* basis for age, smoking, body mass index, hormone therapy, and menopausal status, the major environmental determinants of fibrinogen levels. For all genotype-phenotype association analyses, we assumed an additive model of inheritance and initially conducted univariable linear regression analyses to test the null hypothesis that fibrinogen levels did not differ by individual SNP genotypes (PLINK v1.03)[15]. To identify any clusters of SNPs that might be associated with fibrinogen levels, we used a genome-wide criterion of statistical significance of $<5\times10^{-08}$.

Once any locus with genome-wide significance was identified, a forward selection linear multiple regression model was used to further define the extent of the genetic association. Briefly, all genotyped SNPs within 100 kb of the most significantly associated SNP at each locus and passing quality control requirements were tested for possible incorporation into a multiple regression model. In stepwise fashion, a SNP was added to the model if it had the smallest P-value among all the SNPs not yet included in the model and if it was statistically significant after adjusting for multiple comparisons.

Subsequently, in models that included all SNPs that non-redundantly provided information on fibrinogen levels within a given loci, we used a similar multiple regression model to calculate the total proportion of variation in fibrinogen accounted for by the common SNPs evaluated.

Last, to address the hypothesis that plasma levels of fibrinogen and C-reactive protein have different genetic determinants, fibrinogen levels were regressed on SNPs of genome-wide significance for C-reactive protein, and CRP levels regressed on SNPs of genome-wide significance for fibrinogen in fully-adjusted models.

The study was approved by the institutional review committee at Brigham and Women's Hospital and the subjects gave informed consent.

Results

Among the 17,686 women in this cohort, median fibrinogen levels were 350.6 mg/dL (range 29.1-1104.5 mg/dL). The distribution of P-values for the association of each individual SNP with plasma fibrinogen levels according to chromosomal position and number is shown in Figure 1, while Table 1 presents a listing of the genome-wide significant SNPs ($P<5\times10^{-08}$) and model-selected SNPs along with their beta coefficients, p-values and median levels of fibrinogen for homozygous carriers of the minor allele, heterozygotes and homozygous carriers of the major allele. Nineteen SNPs were individually associated with fibrinogen at a genome-wide level of significance and cluster in one of five chromosomal regions: 5 in chromosome locus 1q21.3 (IL6R), one in chromosome locus 2q34 (CPS1), 10 in chromosome locus 4q32.1 (the fibrinogen gene cluster), 1 in chromosome 5q31.1 (within or near genes of solute carrier family 22 (members 5 and 4)), and 2 in chromosome 17q25.1 (near genes SLC9A3R1 and NAT9, as well as a member of an immunoglobulin gene family, CD300LF; other members of this family in this region include CD300A, CD300LN, CD300C and CD300E). For four of the loci, effects of genotype on plasma fibrinogen level conform to an additive model. However, rs7422339 (CPS1) showed evidence (p=0.0002) for non-additive effects of the minor allele as judged by a likelihood ratio test comparing the additive regression model to an alternative genotype model with an additional degree of freedom. Specifically, the association for CPS1 tended toward a dominant genetic model, with median fibrinogen values of 354 mg/dL (N=8281) for major allele homozygotes, 348 mg/dL (N=7481) for heterozygotes and 349 mg/dL (N=1706) for minor allele homozygotes.

Also presented in Table 1 are the results of forward selection models that summarize evidence of nonredundant contributions to fibrinogen level for each of the five loci. At the IL6R, CPS1 and CD300LF loci, only one lead SNP was included by model selection (rs8192284, rs7422339 and rs10512597 respectively). Two SNPs were included at 4q32.1 (FGB, FGA, FGG; rs6056, rs1800788) and 5q31.1 (SLC22A5, SLC22A4, IRF1; rs1016988, rs10479002). The genetic contexts of the five loci are shown in Figure 2.

As shown in the quantile-quantile plots (Figure 3), p-values larger than 0.001 conform to the expected null distribution. The excess of p-values smaller than 0.001 was due largely to the associations of the candidate loci; after further adjustment of fibrinogen residuals by model-selected SNPs, there is a significant decrease in excess of small p-values in genome-wide association testing (Figure 3, right panel).

Table 2 shows a comparison of phenotypic variance explained by independent genetic factors and clinical covariates. Of the genetic variance, which explained a total of 1.93% of the fibrinogen phenotype, 12.4% was attributable to polymorphism within the IL6R locus, 8.8% to the CPS1 locus, 50.3% to fibrinogen gene cluster, 17.6% to the 5q31.1 locus and 10.9% to the 17q25.1 locus. Clinical covariates (age, body mass index, smoking and hormone use) accounted for a significant proportion of the phenotypic variance (14.0%).

Replication of the SLC22A5/IRF1, the IL6R, and the known FGB findings are also provided in the accompanying manuscript from Dehghan et al (p-values1.01×10^{-13}, 7.42×10^{-06} and 1.84×10^{-27} respectively). With respect to CD300LF and CPS1, the Dehghan data also support replication, albeit at p-values of 0.001 and 0.01. Even after correction for the five loci tested, these remain significant with p-values ≤ 0.01, and consistent direction of effect.

Because fibrinogen and CRP levels correlate (r=0.4), we assessed the degree of overlap between genome-wide significant determinants of these inflammatory biomarkers. As shown in Table 3, of the genes related to fibrinogen, the IL6R SNP (rs8192284; $p=1.04\times10^{-22}$) and the CD300LF SNP (rs10512597; $p=9.85\times10^{-04}$) were also associated with CRP levels. By

contrast, of the genes related to CRP[13], only the IL6R, GCKR (rs780094; p=0.002) and LEPR SNP (rs1892534, p=0.009) also showed evidence of association with fibrinogen.

Discussion

In this genome-wide study of 337,343 polymorphisms among 17,686 women, four novel loci were associated with fibrinogen in addition to the known association with the fibrinogen gene cluster. Two of the novel loci relate to known human chronic inflammatory diseases but are genetically associated with fibrinogen levels for the first time, one to a critical component of the inflammatory cascade and one to the urea cycle.

The locus at 5q31.1 (SLC22A5, SLC22A4, IRF1) implicated in our study, and in the accompanying study by Dehghan et al, is immediately adjacent to a 250 kb locus that has previously been linked to Crohn's disease and has been referred to as the IBD5 region18. Recent confirmation of the association of this locus with Crohn's disease has emerged from the Wellcome Trust Consortium19. Because this region is rich in candidate genes and is characterized by extensive linkage disequilibrium, one cannot be certain of the causal variant or the underlying biological mechanism. Of note, candidate genes encompassed by this region include a cytokine gene cluster (IL5, IL4, IL13) as well as a regulator of interferon alpha production (IRF1) among others. Other genes in this region are SLC22A5 and SLC22A4, which are high affinity sodium-dependent uptake transporters that function in the transport of l-carnitine and in the elimination of cationic drugs in the intestine. Specific SNPs of these genes have been shown to affect transcriptional efficiency of SLC22A420, due to an allelic difference in affinity to runt-related transcription factor 1 (RUNX1)21. Preliminary data have also implicated this region to other diseases with autoimmune etiologies such as type I diabetes22.

The association at locus 17q25.1 is intronic to the CD300LF and RAB37 genes. CD300LF is a member of an immunoglobulin superfamily gene cluster that may serve as an inhibitory receptor to regulate the maturation and differentiation of immune cells, helping to contain inflammation[23]; RAB37 is a GTPase expressed in mast cells[24]. This locus has been associated with psoriasis, and is referred to as PSORS2 [25,26]. Particular focus has been on the nearby LD block which encompasses SLC9A3R1 and NAT9; a SNP that lies between the two genes may lead to loss of RUNX1 binding, a common theme for other inflammatory diseases such as rheumatoid arthritis and lupus[27]. This is however, to our knowledge, the first report of a relationship of this region to circulating fibrinogen levels.

The IL6R SNP, rs8192284, associated with fibrinogen is a non-synonymous SNP that is also associated with CRP[13]. The biological relevance of this SNP was supported by recent data from the Health ABC study, where the same missense SNP accounted for a significant percentage of variance of both soluble IL6R levels and plasma IL-6 levels[28]. IL-6 is an important upstream messenger cytokine in inflammation that changes the program of protein synthesis in the liver from "housekeeping" proteins, such as albumin, to a family of acute phase proteins made in the liver, such as CRP and fibrinogen. Our finding that polymorphism in the IL6R gene is a determinant of fibrinogen expression is consistent with data linking IL-6 to hepatic production of fibrinogen. These data support the close biological relationship between fibrinogen levels and IL-6, and is concordant with the reported relationship of IL-6 with incident diabetes and atherothrombosis[29].

With regard the CPS1 SNP, rs7422339, carbamoylphosphate synthetase I is a mitochondrial matrix enzyme that catalyses the first and rate-limiting step of the hepatic urea cycle. The hepatic urea cycle is responsible for the elimination of ammonia in the form of urea as well as the synthesis of arginine, a precursor of the potent vasodilatator nitric oxide. Specifically, the

CPS1 SNP rs7422339 associated with fibrinogen in our study encodes for the substitution of asparagine for threonine (T1405N) in the region critical for N-acetyl-glutamate binding and results in 20-30% higher enzymatic activity[30]. This variation has been shown to influence nitric oxide metabolite concentrations and vasodilation following agonist stimulation31, as well as the risk of veno-occlusive disease after bone marrow transplantation; such data may be concordant with data linking fibrinogen with vascular disease.

Last, the IL6R SNP (rs8192284), the GCKR SNP (rs780094) and SLC9A3R1 SNP (rs10512597) were related to both fibrinogen and CRP, suggesting they may be central regulators of inflammation. However, the majority of implicated SNPs showed little evidence of dual association, showing that fibrinogen and CRP may have different genetic architecture, even though both are predictive of vascular disease.

Conclusions

In aggregate, our genome-wide study identifies four novel loci related to fibrinogen, the 5q31.1 locus related to IBD, the 17q25.1 locus related to psoriasis, IL6R, a critical component of inflammation, and CPS1, all of which are replicated in the accompanying manuscript from Dehghan et al. We also confirm the fibrinogen gene cluster locus. These data identify new components of fibrinogen regulation, a glycoprotein with multiple critical functions in humans including clotting, thrombosis and inflammation. The link to regions of the genome associated with common human autoimmune diseases may provide further insights into their pathophysiology.

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References

1. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448–54. [PubMed: 9971870]
2. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. The Framingham Study. Jama 1987;258:1183–6. [PubMed: 3626001]
3. Danesh J, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. Jama 2005;294:1799–809. [PubMed: 16219884]
4. Mora S, Rifai N, Buring JE, Ridker PM. Additive value of immunoassay-measured fibrinogen and high-sensitivity C-reactive protein levels for predicting incident cardiovascular events. Circulation 2006;114:381–7. [PubMed: 16864722]
5. Hamsten A, Iselius L, de Faire U, Blomback M. Genetic and cultural inheritance of plasma fibrinogen concentration. Lancet 1987;2:988–91. [PubMed: 2889959]
6. Livshits G, Schettler G, Graff E, Blettner M, Wahrendorf J, Brunner D. Tel Aviv-Heidelberg three-generation offspring study: genetic determinants of plasma fibrinogen level. Am J Med Genet 1996;63:509–17. [PubMed: 8826427]
7. Humphries SE, Cook M, Dubowitz M, Stirling Y, Meade TW. Role of genetic variation at the fibrinogen locus in determination of plasma fibrinogen concentrations. Lancet 1987;1:1452–5. [PubMed: 2885451]
8. van 't Hooft FM, von Bahr SJ, Silveira A, Iliadou A, Eriksson P, Hamsten A. Two common, functional polymorphisms in the promoter region of the beta-fibrinogen gene contribute to regulation of plasma fibrinogen concentration. Arterioscler Thromb Vasc Biol 1999;19:3063–70. [PubMed: 10591688]

9. Kathiresan S, Yang Q, Larson MG, Camargo AL, Tofler GH, Hirschhorn JN, Gabriel SB, O'Donnell CJ. Common genetic variation in five thrombosis genes and relations to plasma hemostatic protein level and cardiovascular disease risk. Arterioscler Thromb Vasc Biol 2006;26:1405–12. [PubMed: 16614319]
10. Scarabin PY, Bara L, Ricard S, Poirier O, Cambou JP, Arveiler D, Luc G, Evans AE, Samama MM, Cambien F. Genetic variation at the beta-fibrinogen locus in relation to plasma fibrinogen concentrations and risk of myocardial infarction. The ECTIM Study. Arterioscler Thromb 1993;13:886–91. [PubMed: 8499409]
11. Zito F, Di Castelnuovo A, Amore C, D'Orazio A, Donati MB, Iacoviello L. Bcl I polymorphism in the fibrinogen beta-chain gene is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels. A case-control study in a sample of GISSI-2 patients. Arterioscler Thromb Vasc Biol 1997;17:3489–94. [PubMed: 9437197]
12. Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, Buring JE. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. Clin Chem 2008;54:249–55. [PubMed: 18070814]
13. Ridker PM, Pare G, Parker A, Zee RY, Danik JS, Buring JE, Kwiatkowski D, Cook NR, Miletich JP, Chasman DI. Loci related to metabolic-syndrome pathways including LEPR, HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women's Genome Health Study. Am J Hum Genet 2008;82:1185–92. [PubMed: 18439548]
14. Whitton CM, Sands D, Hubbard AR, Gaffney PJ. A collaborative study to establish the 2nd International Standard for Fibrinogen, Plasma. Thromb Haemost 2000;84:258–62. [PubMed: 10959698]
15. 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;81:559–75. [PubMed: 17701901]
16. Hudson RR, Slatkin M, Maddison WP. Estimation of levels of gene flow from DNA sequence data. Genetics 1992;132:583–9. [PubMed: 1427045]
17. 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;38:904–9. [PubMed: 16862161]
18. Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 2001;29:223–8. [PubMed: 11586304]
19. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–78. [PubMed: 17554300]
20. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 2004;36:471–5. [PubMed: 15107849]
21. Tokuhiro S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, Suzuki M, Nagasaki M, Ohtsuki M, Ono M, Furukawa H, Nagashima M, Yoshino S, Mabuchi A, Sekine A, Saito S, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. Nat Genet 2003;35:341–8. [PubMed: 14608356]
22. Santiago JL, Martinez A, de la Calle H, Fernandez-Arquero M, Figueredo MA, de la Concha EG, Urcelay E. Evidence for the association of the SLC22A4 and SLC22A5 genes with type 1 diabetes: a case control study. BMC Med Genet 2006;7:54. [PubMed: 16796743]
23. Speckman RA, Wright Daw JA, Helms C, Duan S, Cao L, Taillon-Miller P, Kwok PY, Menter A, Bowcock AM. Novel immunoglobulin superfamily gene cluster, mapping to a region of human chromosome 17q25, linked to psoriasis susceptibility. Hum Genet 2003;112:34–41. [PubMed: 12483297]

24. Masuda ES, Luo Y, Young C, Shen M, Rossi AB, Huang BC, Yu S, Bennett MK, Payan DG, Scheller RH. Rab37 is a novel mast cell specific GTPase localized to secretory granules. FEBS Lett 2000;470:61–4. [PubMed: 10722846]
25. Nair RP, Henseler T, Jenisch S, Stuart P, Bichakjian CK, Lenk W, Westphal E, Guo SW, Christophers E, Voorhees JJ, Elder JT. Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. Hum Mol Genet 1997;6:1349–56. [PubMed: 9259283]
26. Tomfohrde J, Silverman A, Barnes R, Fernandez-Vina MA, Young M, Lory D, Morris L, Wuepper KD, Stastny P, Menter A, et al. Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. Science 1994;264:1141–5. [PubMed: 8178173]
27. Helms C, Cao L, Krueger JG, Wijsman EM, Chamian F, Gordon D, Heffernan M, Daw JA, Robarge J, Ott J, Kwok PY, Menter A, Bowcock AM. A putative RUNX1 binding site variant between SLC9A3R1 and NAT9 is associated with susceptibility to psoriasis. Nat Genet 2003;35:349–56. [PubMed: 14608357]
28. Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, Choy E, Hu D, Tamraz B, Pawlikowska L, Wassel-Fyr C, Huntsman S, Waliszewska A, Rossin E, Li R, Garcia M, Reiner A, Ferrell R, Cummings S, Kwok PY, Harris T, Zmuda JM, Ziv E. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. Am J Hum Genet 2007;80:716–26. [PubMed: 17357077]
29. Kado S, Nagase T, Nagata N. Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. Acta Diabetol 1999;36:67–72. [PubMed: 10436255]
30. Summar ML, Hall L, Christman B, Barr F, Smith H, Kallianpur A, Brown N, Yadav M, Willis A, Eeds A, Cermak E, Summar S, Wilson A, Arvin M, Putnam A, Wills M, Cunningham G. Environmentally determined genetic expression: clinical correlates with molecular variants of carbamyl phosphate synthetase I. Mol Genet Metab 2004;81(Suppl 1):S12–9. [PubMed: 15050969]
31. Summar ML, Gainer JV, Pretorius M, Malave H, Harris S, Hall LD, Weisberg A, Vaughan DE, Christman BW, Brown NJ. Relationship between carbamoyl-phosphate synthetase genotype and systemic vascular function. Hypertension 2004;43:186–91. [PubMed: 14718356]
32. McVean GA, Myers SR, Hunt S, Deloukas P, Bentley DR, Donnelly P. The fine-scale structure of recombination rate variation in the human genome. Science 2004;304:581–4. [PubMed: 15105499]
33. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5. [PubMed: 15297300]

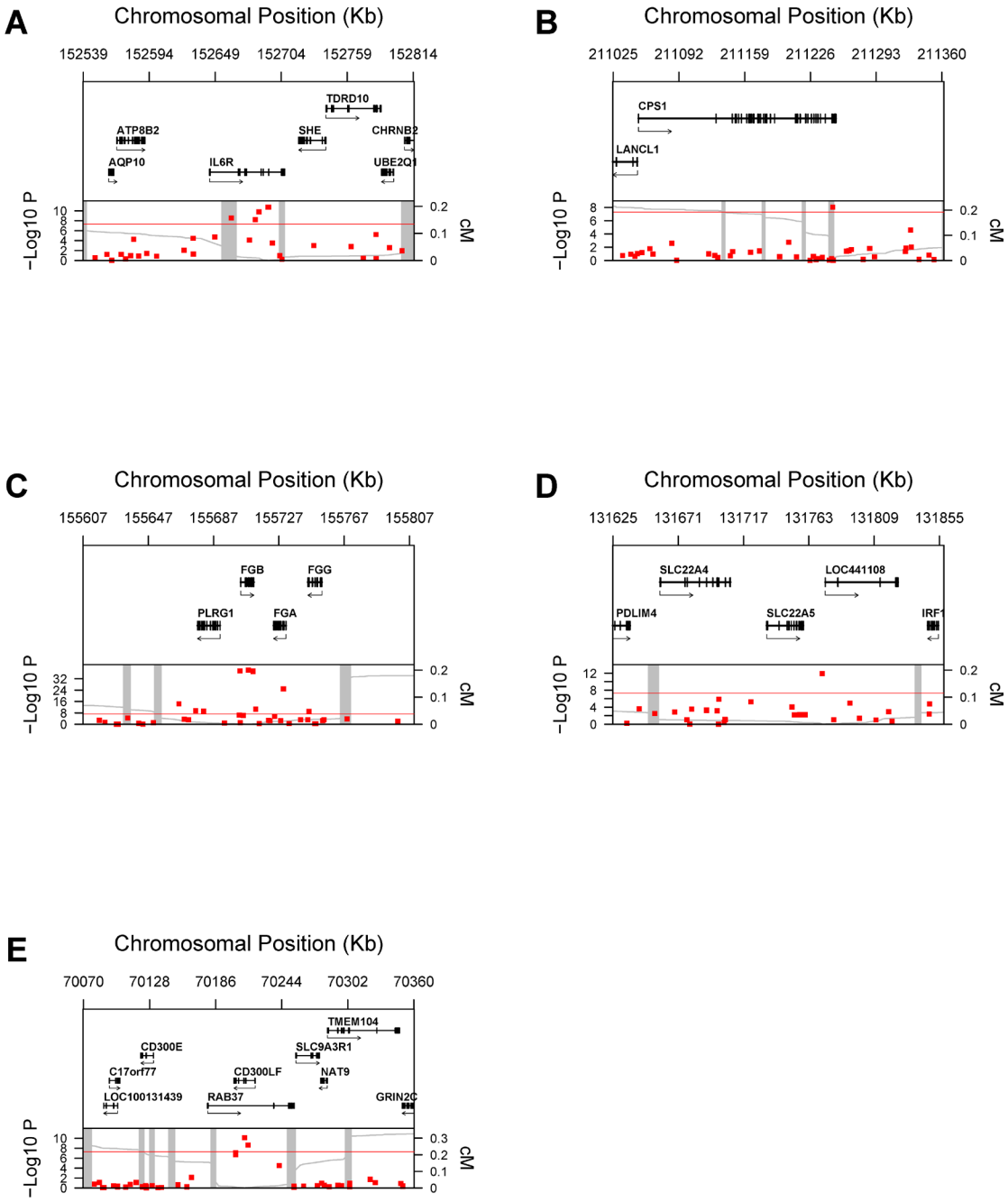

Figure 1.
The distribution of P-values for the association of individual SNPs with plasma fibrinogen levels according to chromosome number and position.

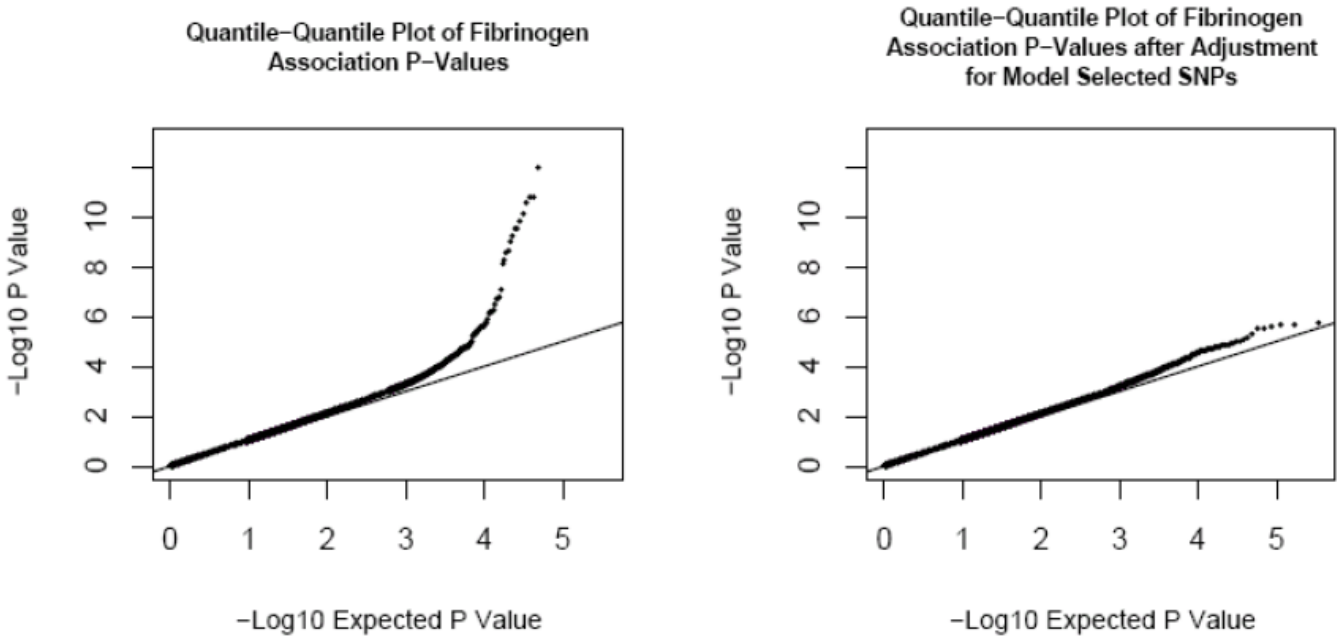

Figure 2.

Genetic Context of Loci with Genome-Wide Significant Associations with Fibrinogen Data are presented that map each SNP according to its physical location at each of the 5 loci, as well a plot of the P-values in relation to the distance from known recombination hotspots according to HapMap. Top panel: genes from RefSeq release 25 are shown. Only one isoform is shown when multiple splicing variants are known. Lower panel: SNPs are shown according to their physical location and P-values (red dots). Also shown is the genetic distance in cM from the lowest P-value SNP (light grey line) along with the position of recombination hotspots (light grey vertical bars). Recombination rates and hotspots are based on HapMap data, as described by McVean et al.[32].

The IL6R region is shown in Figure 2-A, the CPS1 region in Figure 2-B, the fibrinogen gene cluster (4q32.1) in Figure 3-C, the SLC22A5, SLC22A4, IRF1 locus (5q31.1) in Figure 2-D and the CD300LF, SLC9A3R1, NAT9 locus in Figure 2-E.

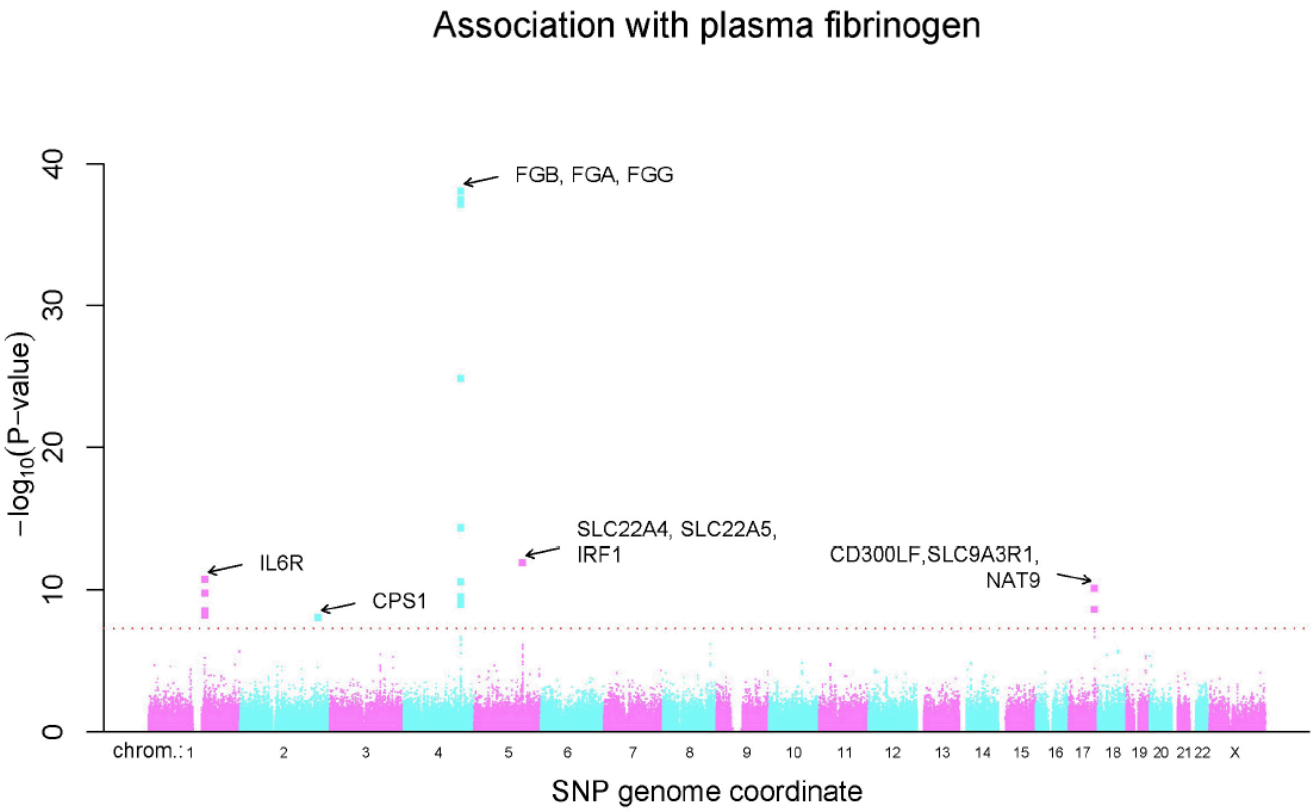

Figure 3.
Quantile-Quantile Plot of Actual and Expected P-Values for Association with Fibrinogen

Table 1

SNPs (beta-coefficients and P-value) associated with plasma fibrinogen levels in the Women's Genome Health Study (WGHS) after adjustment for age, smoking status, body mass index, menopausal status and current hormone replacement therapy

								Median Fibrinogen level (mg/dL)[e]			Genome-wide Association Values		Non-redundant SNPs at each Locus (from model selection)	
Nearest Candidate Gene(s)	**Loci**	**SNP**	**Position[a] (bp)**	**Function**	**MAF[b]**	**HW[c]**	**A1-A2[d]**	**A1A1**	**A1A2**	**A2A2**	**Beta**	**P-value[f]**	**Beta**	**P-value[g]**
IL6R	1q21.3	rs6684439	152662463	intron	0.40	0.36	G-A	353	350	345	-4.69	2.77×10^{-09}		
		rs4845623	152682401	intron	0.42	0.36	A-G	353	351	346	-4.55	6.08×10^{-09}		
		rs4537545	152685503	intron	0.41	0.20	G-A	353	351	345	-5.01	1.66×10^{-10}		
		rs4129267	152692888	intron	0.40	0.36	G-A	353	351	345	-5.30	1.84×10^{-11}		
		rs8192284[h]	152693594	coding-nonsynonymous	0.40	0.33	A-C	353	351	345	-5.30	1.80×10^{-11}	-5.44	6.57×10^{-12}
CPS1	2q34	**rs7422339**	211248752	coding-nonsynonymous	0.31	0.96	C-A	354	348	349	-4.84	8.82×10^{-09}	-4.85	7.92×10^{-09}
FGB, FGA, FGG	4q32.1	rs1388070	155520388	unknown	0.35	0.80	A-G	347	352	356	5.12	3.23×10^{-10}		
		rs4482740	155665755	unknown	0.36	0.43	G-A	346	352	360	6.32	4.40×10^{-15}		
		rs7654425	155676058	unknown	0.34	0.34	A-G	355	348	344	-5.19	2.98×10^{-10}		
		rs7698829	155680917	intron	0.14	1.00	A-G	352	348	339	-7.00	6.39×10^{-10}		
		rs1800790	155703158	locus[i]	0.21	0.04	G-A	345	358	370	12.31	2.91×10^{-38}		
		rs6056	155708271	coding-synonymous	0.18	0.16	G-A	346	359	370	12.94	8.04×10^{-39}	14.08	2.45×10^{-42}
		rs4220	155711209	coding-nonsynonymous	0.18	0.15	G-A	346	359	370	12.79	6.65×10^{-38}		
		rs1044291	155712802	unknown	0.34	0.56	G-A	355	348	344	-5.47	2.76×10^{-11}		
		rs2070016	155729764	intron	0.15	0.19	A-G	347	358	365	11.23	1.29×10^{-25}		
		rs1049636	155745420	intron	0.30	0.11	A-G	352	350	345	-5.19	9.95×10^{-10}		
		rs1800788	155703364	locus	0.20	0.46	G-A	350*	351*	350*	1.70	0.082*	5.13	3.68×10^{-07}
SLC22A5, SLC22A4, IRF1	5q31.1	**rs1016988**	131772473	unknown	0.20	0.65	A-G	354	346	338	-6.84	1.24×10^{-12}	-6.49	2.29×10^{-11}
		rs10479002	131699561	coding-synonymous	0.04	1	G-C	350*	358*	383*	9.51	1.27×10^{-06}*	8.36	2.33×10^{-05}
CD300LF, SLC9A3R1, NAT9	17q25.1	**rs10512597**	70211428	intron	0.18	0.07	G-A	352	347	341	-6.45	7.72×10^{-11}	-6.61	3.21×10^{-11}
		rs1037170	70214509	intron	0.27	0.59	A-G	352	349	345	-5.22	2.29×10^{-09}		

Table 1 Legend

[*] These 2 SNPs were identified in forward selection models that include the other lead SNPs in bold. See text for details.

[a] Based on NCBI Build 36.1

[b] MAF: Minor allele frequency based on the combined samples.

[c] HW: Deviation from Hardy-Weinberg equilibrium P-value based on the combined samples.

[d] A1=major allele, A2=minor allele. A1A1=homozygotes for major allele. A1A2=heterozygotes. A2A2=homozygotes for minor allele

[e] Crude median fibrinogen levels (mg/dL) observed among homozygous carriers of the major allele, heterozygotes and homozygous carriers of the minor allele for each selected SNP

[f] SNPs of genome-wide significance with P <5×10-08 are shown for residuals of fibrinogen regressed on each SNP.

[g] P-values from forward selection models are shown, after adjustment for age, smoking habit, body mass index, menopausal status and current hormone therapy.
We evaluated for inclusion in this selection model 139 SNPs distributed across the 5 loci. P-values <0.00036 were considered to correct for the total number of SNPs considered.

[h] In bold are non-redundant SNPs in each of 5 loci that contribute to fibrinogen levels in forward selection model

[i] Within 2kb of a gene

Abbreviations
IL6R = interleukin 6 receptor
CPS1 = carbamoyl phosphate synthetase I
FGB = fibrinogen, beta polypeptide chain
FGA = fibrinogen, alpha polypeptide chain
FGG = fibrinogen, gamma polypeptide chain
SLC22A5 = solute carrier family 22 (organic cation transporter), member 5
SLC22A4 = solute carrier family 22 (organic cation transporter), member 4
IRF1= interferon regulatory factor 1
CD300LF = immunoglobulin superfamily, member 13;
SLC9A3R1=solute carrier family 9, isoform A3, regulatory factor 1;
NAT9= a member of the N-acetyltransferase family

Table 2

Proportion of Phenotypic Variance According to Individual Clinical and Genetic Covariates

Category	Variable	Proportion of Variance Explained % (r^2)	Proportion of Variance Explained by Category % (r^2)
Clinical covariates	Age	3.07	13.96
	BMI (in WHO categories)	8.31	
	Smoking status (current vs. other)	1.37	
	Menopausal Status	<0.001	
	Hormone user	1.21	
1q21.3 (IL6R) Locus	rs8192284	0.24	0.24
2q34 (CPS1) Locus	rs7422339	0.17	0.17
4q32.1 (FGB, FGA, FGG) Locus	rs6056	0.84	0.97
	rs1800788	0.13	
5q31.1 (SLC22A5, SLC22A4, IRF1) Locus	rs1016988	0.25	0.34
	rs10479002	0.09	
17q25.1 (CD300LF, SLC9A3R1, NAT9) Locus	rs10512597	0.21	0.21
TOTAL			15.89

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Table 3

Relationship of SNPs of Genome-Wide Significance to Fibrinogen with CRP Levels and Relationship of SNPs of Genome-Wide Significance to CRP with Fibrinogen Levels

Nearest Candidate Gene(s)	SNPs	Association with Fibrinogen		Association with CRP*	
		Beta-Coefficient	P-value	Beta-Coefficient	P-value
Genes/SNPs Associated with Fibrinogen at Genome-wide Significance					
IL6R	rs8192284	-5.299	1.80×10^{-11}	-0.109	**1.04×10^{-22}**
CPS1	rs7422339	-4.843	8.82×10^{-09}	0.004	0.750
FGB, FGA, FGG	rs6056	12.940	8.04×10^{-39}	-0.002	0.867
SLC22A5, SLC22A4, IRF1	rs1016988	-6.839	1.24×10^{-12}	-0.022	0.112
CD300LF, SLC9A3R1, NAT9	rs10512597	-6.450	7.72×10^{-11}	-0.046	**9.85×10^{-04}**
Genes/SNPs Associated with CRP at Genome-wide Significance*					
IL6R	rs8192284	-5.299	**1.80×10^{-11}**	-0.109	1.04×10^{-22}
CRP	rs3091244	-0.111	0.890	0.224	7.10×10^{-88}
LEPR	rs1892534	-2.067	**0.009**	-0.148	2.24×10^{-40}
GCKR	rs780094	2.410	**0.002**	0.131	2.74×10^{-32}
Gene Desert	rs10778213	1.998	0.010	-0.089	3.75×10^{-16}
HNF1A	rs7310409	-0.587	0.462	-0.176	9.61×10^{-56}
APOE	rs769449	-1.337	0.251	-0.270	5.98×10^{-57}

*CRP was log-transformed

Abbreviations

CRP = C-reactive protein

LEPR = leptin receptor protein

GCKR = glucokinase regulatory protein

HNF1 = hepatic nuclear factor-1 (transcription factor 1, hepatic [TCF1])

APOE = apolipoprotein E