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

The Women’s Genome Health Study

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**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 crucial biological function 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. 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 probability values from $8.82 \times 10^{-9}$ to $8.04 \times 10^{-10}$). Two of the loci are related to common chronic inflammatory diseases: the first, at locus 5q31.1 (SLC22A5, SLC22A4, IRF1), lies immediately adjacent to a locus linked to Crohn disease ($P$ value for lead SNP, $1.24 \times 10^{-12}$) and the second, at locus 17q25.1 (CD300LF, SLC9A3R1, NAT9), has been associated with psoriasis ($P$ value for lead SNP, $7.72 \times 10^{-11}$). 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, $1.80 \times 10^{-11}$). A novel locus at 2q34 (CPSI) participates in the urea cycle ($P=8.82 \times 10^{-9}$). The majority of implicated SNPs showed little evidence of dual association with C-reactive protein levels.

**Conclusions**—A genome-wide 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. (Circ Cardiovasc Genet. 2009;2:134-141.)

**Key Words:** fibrinogen ■ genetics ■ inflammation ■ coagulation ■ women

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. Like the acute-phase reactant C-reactive protein (CRP), plasma fibrinogen levels also associate with increased risk of myocardial infarction, stroke, and vascular mortality, and these 2 inflammatory biomarkers may provide complementary information. In addition to environmental factors that affect fibrinogen level, there is evidence of substantial heritability of fibrinogen (25% to 51%) in twin and family studies. 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 α, β, and γ polypeptide chains. In these studies, variants within the promoter and genic regions of FGB have been associated with stable and acute phase fibrinogen levels as well as vascular events. In aggregate, however, these polymorphisms explain only a small fraction of the estimated heritable effects on fibrinogen, and the multifunctional nature of the protein.

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itself suggests that other loci should have substantive effects. We therefore undertook a genome-wide association study evaluating potential relationships between 337,343 single-nucleotide polymorphisms (SNPs) and plasma fibrinogen among 17,686 apparently healthy women participating in the Women’s Genome Health Study. As CRP is also an inflammatory marker known to predict cardiovascular diseases, we also directly compared the determinants of fibrinogen levels with those of CRP.

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Methods

The study cohort was derived from participants of the Women’s Genome Health Study. The genetic arm of the Women’s Health Study. In brief, participants in Women’s Genome Health Study 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 baseline blood sample during the enrollment phase of the Women’s Health Study. As CRP is also an inflammatory marker known to predict cardiovascular diseases, we also directly compared the determinants of fibrinogen levels with those of CRP.

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 univarible linear regression analyses to test the null hypothesis that fibrinogen levels did not differ by individual SNP genotypes (PLINK version 1.03). To identify any clusters of SNPs that might be associated with fibrinogen levels, we used a genome-wide criterion of statistical significance of ×10^-8.

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 a stepwise fashion, a SNP was added to the model if it had the smallest probability 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 nonredundantly 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.

Finally, to address the hypothesis that plasma levels of fibrinogen and CRP have different genetic determinants, fibrinogen levels were regressed on SNPs of genome-wide significance for CRP, 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 probability values for the association of each individual SNP with plasma fibrinogen levels according to chromosomal position and number is shown in Figure 1, whereas Table 1 presents a listing of the genome-wide significant SNPs (P<5×10^-8) and model-selected SNPs along with their β coefficients, probability 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 1 of 5 chromosomal regions: 5 in chromosome locus 1q21.3 (interleukin 6 receptor [IL6R]), 1 in chromosome locus 2q34 (carbamoyl phosphate synthetase I [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 NAP7, 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 4 of the loci, effects of genotype on plasma fibrinogen level conform to an additive model. However, rs7422339 (CPS1) showed evidence (P=0.0002) for nonadditive 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 5 loci. At the IL6R, CPS1, and CD300LF loci, only 1 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 5 loci are shown in Figure 2.

As shown in the quantile-quantile plots (Figure 3), P values <0.001 conform to the expected null distribution. The excess of P values <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 probability values in genome-wide association testing (Figure 3, right).

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 article by Dehghan et al.18 (P values 1.01×10⁻¹³, 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 5 loci tested, these remain significant with P values ≤0.01 and have 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=1.04×10⁻²²) and the CD300LF SNP (rs10512597; P=9.85×10⁻⁰⁴) were also associated with CRP levels. By contrast, of the genes related to CRP, only the IL6R, GCKR (rs780094; P=0.002), and LEPR SNP (rs1892534, P=0.009) showed evidence of association with fibrinogen.

Discussion

In this genome-wide study of 337 343 polymorphisms among 17 686 women, 4 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, 1 to a critical component of the inflammatory cascade and 1 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 disease and has been referred to as the IBD5 region.21 Recent confirmation of the association of this locus with Crohn disease has emerged from the Wellcome Trust Consortium.22 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 cyto-
Table 1. SNPs Associated With Plasma Fibrinogen Levels in the Women’s Genome Health Study After Adjustment for Age, Smoking Status, Body Mass Index, Menopausal Status, and Current Hormone Replacement Therapy

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<th>Nearest Candidate Gene(s)</th>
<th>Loci</th>
<th>SNP</th>
<th>Position* (bp)</th>
<th>Function</th>
<th>MAF†</th>
<th>HW‡</th>
<th>A1−A2§</th>
<th>A1A1</th>
<th>A1A2</th>
<th>A2A2</th>
<th>Median Fibrinogen Level, mg/dL</th>
<th>Genome-Wide Association Values</th>
<th>Nonredundant SNPs at Each Locus (From Model Selection)</th>
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FGB indicates fibrinogen, β-polypeptide chain; FGA, fibrinogen, α-polypeptide chain; FGG, fibrinogen, γ-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.

*Based on NCBI Build 36.1.
†Minor allele frequency based on the combined samples.
‡Deviation from Hardy-Weinberg equilibrium P value was based on the combined samples.
§A1, major allele; A2, minor allele; A1A1, homozygotes for major allele; A1A2, heterozygotes; A2A2, homozygotes for minor allele.
¶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.
†SNPs of genome-wide significance with P<5×10⁻⁸ are shown for residuals of fibrinogen regressed on each SNP.
‡‡Values in bold are nonredundant SNPs in each of 5 loci that contribute to fibrinogen levels in forward selection models. See text for details.
§§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.
**Values in bold are nonredundant SNPs in each of 5 loci that contribute to fibrinogen levels in forward selection model.
††Within 2 kb of a gene.
†††These 2 SNPs were identified in forward selection models which include the other lead SNPs in bold. See text for details.

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kine gene cluster (IL5, IL4, IL13) as well as a regulator of interferon-α 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 SLC22A4,23 because of an allelic difference in affinity to runt-related transcription factor 1 (RUNX1).24 Preliminary data have also implicated this region to other diseases with autoimmune etiologies such as type I diabetes.25

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 inflammation26; RAB37 is a GTPase expressed in mast cells.27 This locus has been associated with psoriasis and is referred to as PSORS2.28,29 Particular focus has been on the nearby LD block that encompasses SLC9A3R1 and NAT9; an SNP that lies between the 2 genes may lead to loss of RUNX1 binding, a common theme for other inflammatory diseases, such as rheumatoid arthritis and lupus.30 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 nonsynonymous 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.31 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 are concordant with the reported relationship of IL-6 with incident diabetes and atherothrombosis.32

With regard to the CPS1 SNP, rs7422339, carbamoylphosphate synthetase I is a mitochondrial matrix enzyme that catalyzes 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 vasodilator 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% to 30% higher enzymatic activity.33 This variation has been shown to influence nitric oxide metabolite concentrations and vasodilation following agonist stimulation,34 as well as the risk of veno-occlusive disease after bone marrow transplantation; such data may be concordant with those linking fibrinogen with vascular disease.

Finally, the IL6R SNP (rs8192284), the GCKR SNP (rs780094), and the SLC9A3R1 SNP (rs10512597) were related to both fibrinogen and CRP, suggesting that 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 4 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 article by Dehghan et al.18 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

Table 2. Proportion of Phenotypic Variance According to Individual Clinical and Genetic Covariates

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

FGB indicates fibrinogen, β-polypeptide chain; FGA, fibrinogen, α-polypeptide chain; FGG, fibrinogen, γ-polypeptide chain; BMI, body mass index; WHO, World Health Organization.
genome associated with common human autoimmune diseases may provide further insight into their pathophysiology.

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Disclosures
Dr Ridker has also received grant support from Astra-Zeneca, Novartis, and Sanofi-Aventis, and is listed as a co-inventor on patents held by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease. Dr Miletich reports holding equity in Amgen, Inc. The other authors have no conflicts to disclose.

References

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

<table>
<thead>
<tr>
<th>Nearest Candidate Gene(s)</th>
<th>SNPs</th>
<th>β Coefficient</th>
<th>P</th>
<th>β Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes/SNPs associated with fibrinogen at genome-wide significance</td>
<td>rs8192284</td>
<td>−5.299</td>
<td>1.80 × 10^{-11}</td>
<td>−0.109</td>
<td>1.04 × 10^{-22}</td>
</tr>
<tr>
<td>Genes/SNPs associated with CRP* at genome-wide significance</td>
<td>rs3091244</td>
<td>−0.111</td>
<td>0.890</td>
<td>0.224</td>
<td>7.10 × 10^{-18}</td>
</tr>
</tbody>
</table>

CRP indicates leptin receptor protein; GCKR, glucokinase regulatory protein; HNF1, hepatic nuclear factor-1 transcription factor 1, hepatic [TCF1]; APOE, apolipoprotein E. **CRP** was log-transformed.


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;81:559–575.


20. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–575.


22. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447:661–678.


**CLINICAL PERSPECTIVE**

Fibrinogen is a multifunctional circulating glycoprotein involved in wound healing, thrombosis, platelet aggregation, and inflammation. Elevated fibrinogen levels predict vascular disease, but little is known of the influence of genetic variation on fibrinogen. This study evaluated the potential relationship of 337 343 SNPs with plasma fibrinogen levels among 17 686 apparently healthy women participating in the Women’s Genome Health Study. Four novel loci were identified, in addition to the fibrinogen gene cluster, which were associated with fibrinogen levels at genome-wide levels of significance (P values range from 8.82×10^{-9} to 8.04×10^{-10}). Two of the associations are related to common chronic inflammatory diseases: the first, at 5q31.1 (SLC22A5, SLC22A4, IRF1), lies immediately adjacent to a locus linked to Crohn disease (P value for lead SNP 1.24×10^{-15}) and the second, at a locus on 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 is 1.80×10^{-11}). A novel locus at 2q34 (CPS1) lies within a gene participating in the urea cycle (P value is 8.82×10^{-9}). Replication of these findings was seen in separate cohorts. Although the fibrinogen gene cluster locus was expected, the unexpected link to regions of the genome associated with common human autoimmune diseases such as Crohn disease and psoriasis may provide further insights into their pathophysiology.
Novel Loci, Including Those Related to Crohn 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, Guillaume Paré, Daniel I. Chasman, Robert Y.L. Zee, David J. Kwiatkowski, Alex Parker, Joseph P. Miletich and Paul M Ridker

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