Fibrinogen (coagulation factor I) is a major player in thrombus formation; it is cleaved by thrombin to form fibrin, which is the most abundant component of a blood clot. Beyond the role played in the coagulation and cardiovascular diseases (CVDs), fibrinogen is a proinflammatory factor in autoimmune and inflammatory diseases (such as rheumatoid arthritis, vasculitides, inflammatory bowel disease, multiple sclerosis, chronic obstructive pulmonary diseases, kidney disorders, and posttransplantation fibrosis) and in several types of cancer. Fibrinogen has been demonstrated to interfere with the immunoinflammatory responses through binding to inflammatory cells via ligand-receptor interactions that are different from those involved in the coagulation cascade. Fibrinogen also stimulates angiogenesis in vitro and in vivo and acts as a mitogen for fibroblasts. Therefore, fibrinogen is a multifaceted molecule that plays various roles in several human diseases.

Fibrinogen: Genetic and Environmental Influences

Fibrinogen is a large, soluble plasma hexameric glycoprotein (360 kDa) composed of pairs of 3 polypeptides (Aα, Bβ, and γγ) that are encoded by the FGA (MIM+134820), FGB (MIM+134850), and FGG (MIM+134830) genes, all mapping in a cluster on chromosome 4 (4q28). The FGA, FGB, and FGG genes are expressed almost exclusively in hepatocytes; the expression is regulated by both proximal promoters and enhancers and posttranscriptionally by miRNAs and is influenced by functional regulatory variants and acute-phase stimulation. The highly variable range of plasma fibrinogen levels in the general population (1.5–4 g/L) is the dynamic result of genetic and environmental factors. The genetic makeup, along with race, sex, and age, contributes to the nonmodifiable levels of fibrinogen. Environmental factors influence the modifiable levels of fibrinogen and include diet, hormone levels, and lifestyle choices such as physical activity, alcohol consumption, and smoking/smoking history. In addition, cardiovascular risk factors such as body mass index, obesity, total and low-density lipoprotein cholesterol levels, systolic blood pressure, drugs/medications, and inflammatory molecules such as interleukin-6 also contribute to the variation. Partitioning the genetic and nongenetic contributors to individual plasma levels could help define the proportion of modifiable fibrinogen levels, with potential clinical translational implications. Common variants of both genes coding for fibrinogen chains and nonfibrinogen genes contributing to the heritable component of fibrinogen account for only ≈2% of the variance.

Fibrinogen: A Marker of Risk for CVD

An increased plasma level of fibrinogen is an established marker for coronary artery disease (CAD), stroke, and peripheral vascular disease (both arterial and venous). More recently, an increased plasma level of fibrinogen has also been described in atrial fibrillation with high ventricular frequency. In proteomic analysis, serum levels of the fibrinogen Aα chain fragment were found to be higher in chronic thromboembolic pulmonary hypertension. Therefore, the spectrum of CVD in which fibrinogen is emerging as either a marker of increased risk or a contributor to the pathogenesis is expanding. It has been proposed that the fibrinogen level may be included in the risk stratification of CVD. A recent analysis of 52 prospective studies that included 246,669 participants ≥40 years of age without a prior CVD history estimated that the addition of C-reactive protein (CRP) or fibrinogen levels to conventional risk factors for the prediction of cardiovascular risk in people at intermediate risk for a cardiovascular event could help prevent 1 additional event over a period of 10 years for every 400 to 500 people screened under current treatment guidelines.14

Fibrinogen: The Circulating Level Assays

An obvious question is whether the circulating fibrinogen level is the right or most proximal trait to its possible genetic determinants. At present, all existing studies exploring the association between fibrinogen and CVD are focused on plasma levels; none are focused on its derivatives or fibrinogen dysfunction. The assays used for testing fibrinogen levels are heterogeneous, including activity assays or antigen-based assays. The most common test for
Fibrinogen measurement is a functional assay (or activity assay) that is based on the time for fibrin clot formation (von Clauss method); the other common test is an immunonephelometric assay (antigen assay) that is based on the measure of fibrinogen antigen. There is substantial variation in the values obtained from testing the same samples in different laboratories. Further standardization could add reliability to the measured values and make results more comparable. A better standardization of assays testing fibrinogen is especially important when we consider that an increase of 1 g/L plasma fibrinogen is associated with a >2-fold increase in CAD, stroke, and vascular mortality.

**Fibrinogen: The Genetic Architecture**

Genetic determinants influencing the phenotype are partially known (Figure). The individual characterization of the genetic architecture and the proportion of heritability of fibrinogen levels could contribute to the identification of subjects with an increased risk of CVD.

Mutations in the FGA, FGB, and FGG genes cause mendelian diseases such as the rare autosomal-recessive afibrinogenemia (<0.2 g/L), the autosomal-dominant hypofibrinogenemia (0.2–0.8 mg/L), and the autosomal-dominant dysfibrinogenemia (normal levels but abnormal function). Heritable disorders of the fibrinogen demonstrate both hemorrhagic (prevalent) and prothrombotic (less common) phenotypes. Common variants comprising mostly single-nucleotide polymorphisms (SNPs) in the FGA, FGB, and FGG genes contribute to variations in plasma levels and an increased risk of thrombosis.

Mendelian randomization studies that expanded the analysis of genetic determinants of plasma fibrinogen levels from a single common SNP (the most common tested SNP is rs1800790 [−455G>A]) to multiple SNPs and haplotypes in the entire fibrinogen gene cluster demonstrated...
several contributory functional sites associated with the plasma fibrinogen levels. The rs1800790 and rs2070011 SNPs were related to the common functional variation of the gene cluster and a possible causal relationship of plasma fibrinogen levels with CAD.\textsuperscript{18} Although there is evidence that common SNPs in the \textit{Fibrinogen} gene are consistently and strongly associated with difference in plasma fibrinogen levels, their association with CVD remains unconfirmed. The core of the problem is that increased fibrinogen levels are markers of risk for CVD, but genetic variants contributing to the increased levels do not segregate with the CVD phenotypes. Large databases of exome sequencing (http://evs.gs.washington.edu/EVS/, http://www.ncbi.nlm.nih.gov/SNP/, and http://www.1000genomes.org/) now include hundreds of variants of \textit{FGA, FGB, and FGG} genes, the majority with a very low minor allele frequency, but the variants with very low minor allele frequency have not been investigated in large association studies and their functional role remains unknown. Therefore, the possibility that less common variants with larger functional effects in homogeneously selected subgroups of patients may potentially contribute to plasma levels of fibrinogen exists.

### Genome-Wide Association Studies

**Interrogating Inflammatory Markers Do Not Identify Loci in Fibrinogen Gene Cluster**

Fibrinogen levels, but not their genetic determinants, seem to correlate with the CVD. However, because fibrinogen is also a proinflammatory factor, loci associated with inflammatory or immune pathways could also be plausible candidates for association with fibrinogen levels. In the Fibrinogen Studies Collaboration, 10% of fibrinogen levels were explained by inflammatory markers (notably, a positive association with CRP).\textsuperscript{19} However, genome-wide association (GWA) studies specifically interrogating inflammatory markers such as CRP did not identify fibrinogen loci.\textsuperscript{20} A large meta-analysis of GWA studies of genetic variants associated with CRP levels in CVD identified 18 loci (7 known and confirmed and 11 novel loci).\textsuperscript{20} The mismatch suggests that the genetic architecture of CRP may only partly explain CRP levels in patients with CVD, but these genetic contributors do not explain the association of CRP with CVD. The possibility exists, however, that either the nongenetic determinants of CRP levels largely prevail over the genetic determinants or that ≥1 still-unknown intermediate phenotypes or trait (ie, atherosclerosis, or risk factors, or novel biomarkers) between gene products and their effects could be better candidates for the association between CVD phenotypes and genetic variants.

### Genetic Architecture of Fibrinogen Levels: GWA Studies and Meta-Analysis

GWA studies are based on array platforms that contain millions of SNPs and explore in thousands of individuals the associations between common genetic variants and diseases or simple phenotypic traits such as fibrinogen levels. An online catalog of published GWA studies is available at http://www.genome.gov/gwastudies. GWA studies are able to identify disease or risk loci located in or near genes that were not previously known as potentially involved in the tagged disease or trait and common loci associated with different diseases or traits that were not previously suspected as sharing the same etiologic pathways. Characterization of the tagged phenotypic trait is essential for unraveling the underlying genetic contributors in GWA studies. The proximity of the trait to the genetic determinants may decrease the number of candidate or contributing loci, thus increasing the power of the studies aimed at their identification.

In this issue of \textit{Circulation}, Sabater-Lleal et al\textsuperscript{21} present the results of a large multiethnic meta-analysis of GWA study in >100000 European, black, and Hispanic American subjects. The authors identified 24 fibrinogen-associated lead SNPs in 23 loci but no strong causal association between circulating fibrinogen and CVD. The meta-analysis included data from 28 GWA studies with a sample size of 40695 cases and 85582 controls for CAD, 4752 cases and 24030 controls for stroke, and 3208 cases and 46167 controls for venous thromboembolism. The authors confirmed 8 known loci (\textit{IL6R}, \textit{NLRP3}, \textit{IL1RN}, \textit{CPSS}, \textit{PCCB}, \textit{FGB}, \textit{IRF1}, and \textit{CD300LF}) partly coinciding with those associated with levels of CRP (\textit{IL6R}, \textit{NLRP3}, and \textit{IRF1}).\textsuperscript{19} In addition, the meta-analysis identified 15 novel independent loci (\textit{JMJD1C, LEPR, PSMG1, CHD9, SPPL2A, PLEC1, FARP2, MS4A6A, TOMM7/IL6, ACTN1, HGFAC, IL1R1, DIP2B, and SHANK3/CPT1B}), known and novel, together accounting for 3.7% (range, 1.4%–7.6%) of plasma fibrinogen variation. Most SNPs were in or close to genes involved in immunoinflammatory and adipocytokine pathways, thus confirming the closeness of inflammatory and thrombotic factors. The most significant contribution of this study is the confirmation that only a small proportion of plasma fibrinogen variation is explained by genetic architecture. The novel discovery is the 15 loci playing an important role in inflammation and immune-response pathways. The biological plausibility of a potential role for these genes in contributing to fibrinogen levels is provided by the molecular interactions between inflammation and coagulation and by the role of fibrinogen as a proinflammatory mediator. All novel genes are potential candidates for further exploration of their role in CVD. The concern is that the combined effect of all 24 fibrinogen-associated lead SNPs was not significant for CAD, stroke, or thromboembolism. The conclusion that “clinical outcome analysis of these loci does not support a causal relationship between circulating levels of fibrinogen and CAD, stroke, or thromboembolism” seems to exclude both further possibilities that GWA studies unravel the contribution of the genetic architecture of fibrinogen plasma levels and the association between fibrinogen genetic makeup and CVD.

### How to Unravel the Missing Heritability of Fibrinogen Levels?

The enormous effort by the current meta-analysis\textsuperscript{21} confirms that the dilemma of increased fibrinogen levels and increased risk of CVD is not salvageable with GWA studies, and any attempts to increase sample size are not likely to provide a more optimistic answer. The missing heritability calls for further investigation because the clinical need remains and will increase in the near future when other diseases such as cancer
and inflammatory/autoimmune diseases in which fibrinogen is emerging as an active player and marker will invoke characterization of its genetic architecture to partition the role of environmental and genetic factors. The possibility exists that GWA studies are potential tools to unravel the dilemma; however, they are probably not the ideal tools, especially since the standards for measuring levels of plasma fibrinogen are far from being optimized, with >40 available assays and a heterogeneity of clinical cardiovascular phenotypes enrolled in each study. Alternatively, the fibrinogen levels, as currently measured, are not the ideal traits to achieve the objective of clarifying their genetic architecture. Either intermediate traits such as the fragment of fibrinogen α chain of 2989-Da peptide (KMADEGSEADHEGTHSTKRGHAKSRPV) identified in the sera of patients with chronic thromboembolic pulmonary hypertension by tandem mass spectrometry or “dysfibrinogenemia,” which is unrelated to the levels of the fibrinogen but is associated with fibrinogen gene variants, may be a more appropriate trait for association studies. A recent advancement in research on chronic thromboembolic pulmonary hypertension revealed a high proportion of patients with dysfibrinogenemia,22 A research approach that combines new parallel sequencing of a few or multiple candidate genes, starting from those identified by GWA studies, with proteomic analysis searching for intermediate traits/biomarkers in clinically homogeneous population in which confounding comorbidities associated with increased fibrinogen levels are excluded could open novel avenues for elucidating the full genetic architecture of plasma fibrinogen levels.

Disclosures

None.

References

Y, Sun W, Sarin AP, Fontes JD, Badola S, Astor BC, Hofman A, Pouta
J, Jamshidi Y, Nolte IM, Soranzo N, Spector TD, Völzke H, Parker AN,
Aspelund T, Bates D, Young L, Tsai K, Siscovick DS, Guo X, Rotter JI,
Uda M, Schlessinger D, Rudan I, Hicks AA, Penninx BW, Thorand B,
Gieger C, Coresh J, Willemsen G, Harris TB, Uiterlinden AG, Järvelin
MR, Rice K, Radke D, Salomaa V, Willems van Dijk K, Boerwinkle E,
Vasan RS, Ferrucci L, Gibson QD, Bandinelli S, Sniesther H, Boomsma DI,
Xiao X, Campbell H, Hayward C, Pramstaller PP, van Duijn CM, Peltonen
L, Psaty BM, Gudnason V, Ridker PM, Homuth G, Koenig W, Ballantyne
CM, Wittemen JC, Benjamin EJ, Perola M, Chasman DI. Meta-analysis of
genome-wide association studies in >80 000 subjects identifies multiple

AD, Teumer A, Reiner AP, Folkersen L, Basu S, Rudnicka AR, Trompet
S, Malarstig A, Baumert J, Bis JC, Guo X, Hottenga JJ, Shin SY, Lopez
P, Huffman JE, Zemunik T, Redline S, Mehra R, Pulanic D, Rudan I,
Wright AF, Kolcic I, Polasek O, Wild SH, Campbell H, Curb JD, Wallace
R, Liu S, Eaton CB, Becker LC, Bandinelli S, Rääkkönen K, Widen E,
Palotie A, Forngae M, Green D, Gross M, Davies G, Harris SE,
Liewald DC, Starr JM, Williams FMK, Grant PJ, Spector TD, Strawbridge
RJ, Silveira A, Sembild B, Rivadeneira F, Uitterlinden AG, Franco OH,
Hofman A, van Dongen J, Willemsen G, Boomsma DI, Yao J, Swords
Jenny N, Haritunians T, McKnight B, Lunley T, Taylor KD, Rotter JI,
H, Kocher T, Goel A, Franzosi MG, Seedorf U, Clarke R, Steri M, Tarasov
KV, Sanna S, Schlessinger D, Stott DJ, Sattar N, Buckley BM, Rumley
A, Lowe GD, McArdis WL, Chen MH, Tohter GH, Song J, Boerwinkle E,
Folsom AR, Rose LM, Franco-Cereceda A, Teichert M, Ikram MA, Mosley
TH, Bevan S, Dichgans M, Rothwell PM, Sudlow CLM, Hopewell JC,
Chambers JC, Saleheen D, Kooneer JS, Danesh J, Nelson CP, Erdmann I,
Reilly MP, Kathiresan S, Schunkert H, Morange PE, Ferrucci L, Eriksson
JC, Jacobs D, Deary JJ, Soranzo N, Witteman JCM, de Geus EJC, Tracy
S, Markus HS, Watkins H, Samani NJ; VTE Consortium; STROKE
Consortium; Wellcome Trust Case Control Consortium 2 (WTCCC2); C4D
Consortium; CARDioGRAM Consortium; Wallaschofski H, Smith
NL, Tregouet D, Ridker PM, Tang W, Strachan DF, Hamsten A, O’Donnell
CJ. Multiethnic meta-analysis of genome-wide association studies in
>100 000 subjects identifies 23 fibrinogen-associated loci but no strong
evidence of a causal association between circulating fibrinogen and car-

22. Morris TA, Marsh JJ, Chiles PG, Magaña MM, Liang NC, Soler X,
Desantis DJ, Ngo D, Woods VL Jr. High prevalence of dysfibrinogenemia
among patients with chronic thromboembolic pulmonary hypertension.

KEY WORDS: Editorials ■ blood coagulation ■ cardiovascular diseases ■
fibrinogen ■ genetics ■ inflammation ■ risk factors
Fibrinogen: A Circulating Factor in Search of Its Genetic Architecture
Eloisa Arbustini, Nupoor Narula and Andrea M. D'Armini

Circulation. 2013;128:1276-1280; originally published online August 22, 2013;
doi: 10.1161/CIRCULATIONAHA.113.005125

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/128/12/1276

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/