Fibrinogen: A Circulating Factor in Search for Its Genetic Architecture

Running title: Arbustini et al.; Genetics and plasma fibrinogen levels

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Introduction

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 (CVD), fibrinogen is a proinflammatory factor in autoimmune and inflammatory diseases (such as rheumatoid arthritis, vasculitides, inflammatory bowel disease, multiple sclerosis, chronic obstructive pulmonary diseases and kidney disorders and post-transplant-fibrosis) as well as in several types of cancer. Fibrinogen has been demonstrated to interfere with the immuno-inflammatory responses through binding to inflammatory cells via ligand–receptor interactions that are different from those involved in the coagulation cascade. Fibrinogen also stimulates angionogenesis 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.

Since family studies have demonstrated a major genetic component to variance of fibrinogen (44%), and increased levels of fibrinogen have been proved to be a marker of risk for cardiovascular diseases, the elucidation of the genetic architecture of plasma fibrinogen levels is becoming a major undertaking in translational research and an emerging clinical need.

Fibrinogen: Genetic and Environmental Influences

Fibrinogen is a large, soluble plasma hexameric glycoprotein (360KDa) comprised of pairs of three polypeptides (Aα, Bβ and γ) that are encoded by 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, post-transcriptionally, by miRNAs and is influenced by functional regulatory variants and acute-phase stimulation.
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 make-up contributes to the non-modifiable levels of fibrinogen along with race, gender and age. Environmental factors influence the modifiable levels of fibrinogen and include diet, hormone levels, and life styles such as physical activity, alcohol consumption and smoking/smoking history. In addition, cardiovascular risk factors such as body mass index (BMI), obesity, total and low-density lipoprotein (LDL) cholesterol levels, systolic blood pressure, drugs/medications and inflammatory molecules such as interleukin 6 also contribute to the variation. Partitioning the genetic and non-genetic 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 non-fibrinogen genes contributing to heritable component of fibrinogen only account for about 2% of the variance.

**Fibrinogen: A Marker of Risk for Cardiovascular Diseases (CVD)**

Increased plasma level of fibrinogen is an established marker for coronary artery disease, stroke, and peripheral vascular disease (both arterial and venous). More recently, increased plasma level of fibrinogen has also been described in atrial fibrillation with high ventricular frequency. Using proteomic analysis, serum levels of fibrinogen Aα chain fragment were found to be higher in chronic thromboembolic pulmonary hypertension (CTEPH). Therefore, the spectrum of CVD where fibrinogen is emerging as either marker of increased risk or 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 of 40 years of age or older and without a prior CVD history, estimated that the addition of 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 one additional event over a period of 10 years for every 400 to 500 people screened under current treatment guidelines\textsuperscript{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 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 upon 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\textsuperscript{15}. Guidelines are available at www.bcshguidelines.com/pdf/fibrinogenassays0503.pdf. There is a substantial variation in the values obtained from testing 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 considering that an increase of 1 g/L of plasma fibrinogen is associated with more than a two-fold increase in CAD, stroke, and vascular mortality\textsuperscript{10}.

**Fibrinogen: The Genetic Architecture (Figure 1)**

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

Mutations in *FGA, FGB and FGG* genes cause Mendelian diseases such as the rare
autosomal recessive afibrinogenemia (<0.2g/L), the autosomal dominant hypofibrinogenemis (0.2-0.8mg/L) the autosomal dominant dysfibrinogenemia (normal levels but abnormal function).

Heritable disorders of the fibrinogen demonstrate both hemorrhagic (prevalent) and pro-thrombotic (less common) phenotypes. Common variants comprising mostly SNP in FGA, FGB and FGG genes contribute to variations in plasma levels and 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 SNP and haplotypes in the entire fibrinogen gene cluster demonstrated several contributory functional sites associated with the plasma fibrinogen levels. The rs1800790 and rs2070011 SNP were related to the common functional variation of the gene cluster and a possible causal relationship of plasma fibrinogen levels with CAD. Although there is evidence that common SNP in 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/, http://www.1000genomes.org/) now include hundreds of variants in FGA, FGB, FGG genes, the majority with a very low Minor Allele Frequency (MAF), but the latter 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 (GWA) 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, since 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 C-reactive protein)\textsuperscript{19}. However, 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 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, however, does exist that either the non-genetic determinants of CRP levels largely prevail over the genetic determinants or that one, or more than one, still unknown intermediate phenotypes/traits (i.e. atherosclerosis or risk factors or novel biomarkers) between gene products and their effects on the tagged trait 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 SNP and explore in thousands of individuals the associations between common genetic variants and diseases or simple phenotypic traits, such as fibrinogen levels. An on-line catalog of published GWASs is available at http://www.genome.gov/gwastudies. GWA studies are able to identify disease or risk loci located in or near to 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 *Circulation*, O’Donnell and coworkers present the results of a large multi-ethnic meta-analysis of GWA study in over 100,000 European, African American and Hispanic-American subjects. The authors identified 24 fibrinogen-associated lead SNP 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 40,695 cases and 85,582 controls for coronary artery disease, 4,752 cases and 24,030 controls for stroke, and 3,208 cases and 46,167 controls for venous thromboembolism. The authors confirmed 8 known loci (*IL6R, NLRP3, IL1RN, CPS1, PCCB, FGB, IRF1 and CD300LF*) partly coinciding with those associated with levels of CRP (*IL6R, NLRP3, IL1RN*). In addition, the meta-analysis identified 15 novel independent loci (*JMJD1C, LEPR, PSMG1, CHD9, SPPL2A, PLEC1, FARP2, MS4A6A, TOMM7/IL6, ACTN1, HGFAC, IL1R1, DIP2B and SHANK3/CPT1B*) together, known and novel, accounting for 3.7% (range 1.4-7.6%) of plasma fibrinogen variation. Most SNP 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 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 as well as by the role of fibrinogen as 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 SNP 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 make-up and CVD.

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

The enormous effort by the current meta-analysis21 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 further increase in the near future when other diseases, such as cancer, 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 one of the possible tools to unravel the dilemma but probably not the ideal ones, especially because the standards for measuring levels of plasma fibrinogen are far from being optimized, with more that 40 assays available and the clinical cardiovascular phenotypes enrolled in each different study are different and not homogenous. 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 Aα chain of 2989-Da peptide (KMADEAGSEADHEGTHSTKRGHAKSRPV) identified in the sera of patients with CTEPH by tandem mass spectrometry14, or “dysfibrinogenemia” which is unrelated to the levels of the
fibrinogen but is associated with fibrinogen gene variants, may more appropriate traits for association studies. Recent advancement of research in CTEPH revealed a high proportion of patients with dysfibrinogenemia. Research approach that combines new high-throughput sequencing technologies for massive parallel sequencing of a few or multiple candidate genes, starting from those identified by GWAS, with proteomic analysis searching for intermediate traits/biomarkers in clinically homogeneous population in which confounding co-morbidities that are associated with increased fibrinogen levels are excluded, could open novel avenues for elucidating the full genetic architecture of plasma fibrinogen levels.

Conflict of Interest Disclosures: None.

References:


Figure Legend:

**Figure 1.** The figure summarizes the known fibrinogen abnormalities and related phenotypes. A part from the Mendelian autosomal dominant (AD) and recessive (AR) disorders including an autosomal form of renal amyloidosis is caused by *FGA* mutations that confer fibrillogenic properties to the mutated protein, common genetic variants in the fibrinogen gene cluster are associated with increased levels of plasma fibrinogen in cardiovascular diseases (CVD) in which discrepant and uncertain results available to date do not provide conclusive results. The missed paradigm that continues to challenge research is that increased fibrinogen levels seem to confer increased risk of CVD with the contribution of common variants in both fibrinogen and non-
fibrinogen genes but there is no association between the currently known genetic architecture of fibrinogen levels and cardiovascular diseases. The identification of 15 novel loci\textsuperscript{21} is a promising base for further progression in unraveling the genetic architecture of fibrinogen plasma levels. These loci indicate as novel candidates both genes whose product could have biologically plausibility such as molecules involved in inflammatory, immunologic (in red), adipocytokines (dark red), or tyreotrophin (blue) pathways and structural and regulatory genes that code for factors whose potential role in the overall fibrinogen architecture is unknown (green). Some of the SNPs related to the novel loci could address to more than candidate 1 gene (rs2286503 and rs6010044), with the more distant gene as more plausible than the closest one.
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