Addressing some challenges of congenital fibrinogen disorders in 2023 and beyond

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ABSTRACT

Congenital fibrinogen disorders (CFD) include several types and subtypes of fibrinogen deficiency, resulting from monoallelic or biallelic mutations in one of the three fibrinogen genes. While it is relatively easy to make an accurate diagnosis based on activity and antigen levels of fibrinogen and genotype, prediction of the clinical phenotype is challenging. Even among patients with the same genotype, the clinical features are heterogeneous and unpredictable. The development of next-generation sequencing rises the possibility to integrate genetic modifiers to explain the subtle relationship between genotype and clinical phenotype. A recent development in integrative hemostasis assays can also help in the determination of patients at risk of bleeding or thrombosis. In this short review, we go through these topics and explain why CFD could be considered an oligogenic rather than a monogenic disease.

Introduction

Congenital fibrinogen disorders (CFD) encompass a heterogeneous group of fibrinogen deficiencies.1 Diagnosis of CFD is based on the assessment of functional and antigenic fibrinogen levels, allowing to differentiate afibrinogenemia (complete absence of fibrinogen), hypofibrinogenemia (decreased levels of functional fibrinogen), dysfibrinogenemia (normal levels of dysfunctional fibrinogen) and hypodysfibrinogenemia (decreased levels of dysfunctional fibrinogen). Assessment of fibrinogen antigen is mandatory to distinguish dysfibrinogenemia from hypofibrinogenemia. However, fibrinogen immunological assays are not widely available in hemostasis laboratories. The prothrombin-derived fibrinogen assay is an accurate alternative in view of its good correlation with the fibrinogen antigen level in dysfibrinogenemia.2 In addition, a recent communication from the Subcommittee FXIII and Fibrinogen of the International Society of Thrombosis and Hemostasis proposes a classification in several subtypes according to the genotype and the clinical manifestations.3

Since the first description of afibrinogenemia in 1920 by Rabe and Salomon and the first report on dysfibrinogenemia in 1958 by Imperato and Dettori,4,5 hundreds of families with CFD have been identified in the last decades. Gathering and studying CFD cases has improved our knowledge of fibrinogen molecules and enhanced our understanding of the multiple functions of fibrinogen.6 Nevertheless, several aspects regarding the diagnosis, the prediction of outcomes, and the clinical management still require to be solved. The emergence of next-generation sequencing (NGS) rises the possibility to integrate genetic modifiers to explain the subtle relationship between genotype and clinical phenotype. Recent development in integrative hemostasis assays can help in the determination of patients at risk of bleeding or thrombosis. In this short review, we provide some insights into these two selected aspects and give some perspectives for further clinical and fundamental research.
Correlation between genotype and phenotype

Genotype is necessary to confirm the diagnosis of CFD, it distinguishes between hypofibrinogenemia and hypodysfibrinogenemia or severe hypofibrinogenemia and afibrinogenemia and simplifies family screening and prenatal diagnosis. Of note, proper experimental validation at the protein or RNA level is important to confirm the pathogenic effect of new fibrinogen variants, for instance by protein modeling, mass spectrometry, or protein expression.13,15

A few rare fibrinogen variants are strongly associated with a clinical phenotype. This is the case of the fibrinogen storage disease (hypofibrinogenemia type 2D according to the ISTH classification) and the thrombotic-related fibrinogen variants (dysfibrinogenemia type 3B) (Table 1).8,22 Fibrinogen storage diseases are usually suspected in familial history of cryptogenic liver disease associated with hypofibrinogenemia.21 Diagnosis relies on histological observation of fibrinogen inclusion in hepatocytes and genotype.13 The molecular reasons for fibrinogen inclusions are still not understood. Among others, it has been reported that conformational changes in the region of the globular domain of fibrinogen involved in the “end-to-end” interaction can cause an abnormal exposure of hydrophobic patches in the fibrinogen γ chain, which becomes available for interactions with lipids enhancing the accumulation of fibrinogen and apolipoprotein B.12

Thrombotic-related dysfibrinogenemia variants were first described in communication by SSC ISTH in 1995. An association between thrombophilia and the fibrinogen variant was clearly observed in 26 probands with a history of personal and familial thrombosis at a young age without other biological risk factors. Different mechanisms, often overlapping, may account for the increased risk of thrombosis in these fibrinogen variants: i) elevated levels of circulating thrombin resulting from the failure in fibrinogen binding; ii) altered strength, architecture, and stability of the fibrin network; iii) decreased fibrinolysis resulting from impaired binding of plasminogen or tissue-type plasminogen activator to abnormal fibrin.24 In addition, many defects in dysfibrinogenemia affect fibrinogen clottability to different degrees and can lead to a mild bleeding phenotype. In a recent review, Li et al. identified several fibrinogen variants that could be related to bleeding. These included: i) mutations in the NH2-terminal portion of the Aα chain that hamper fibrinogen fitting into the active site cleft of thrombin and drastically slow the conversion of fibrinogen into monomeric fibrin; ii) mutations adding new N-linked glycosylation sites introduce bulky and negatively charged carbohydrate side chains and undermine the alignment of fibrin monomers during polymerization; iii) mutations generating unpaired cysteine form extra disulfide bonds between the abnormal fibrinogen chains and produce highly branched and fragile fibrin networks; iv) truncation mutations in the fibrinogen cα regions impair the lateral fibril aggregation, as well as factor XIII crosslinking, endothelial cell, and platelet binding.23 However, in a multicentric study of 101 patients with dysfibrinogenemia with a mean follow-up of 8.8 years the most frequent mutations (i.e., FGA Arg35His/Cys and FGG Arg301His/Cys) were statistically associated neither with major bleeding (HR 0.8, 95% CI 0.1–4.1, P=0.79) nor with thrombosis (HR 0.8, 95% CI 0.3–2.4, P=0.68).26

One of the most striking clinical features of CFD is the heterogeneity of symptoms even among patients with the same genotype. Based on historical records on dysfibrinogenemia and more recent cohort studies, it is accepted that at diagnosis about 55% of patients are asymptomatic, 25% have a tendency to bleed and 20% have a thrombotic phenotype (in venous and arterial territories).26–30 Similarly, while most patients with afibrinogenemia suffer from severe, sometimes life-threatening, bleeding, about 20% also experience thrombotic events and a few are even asymptomatic.31 The thrombotic occurrence in afibrinogenemia is a clinical conundrum. Among others, concomitant thrombophilia and fibrinogen supplementation especially with fresh frozen plasma or cryoprecipitates, may increase the risk of

<table>
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<th>Native protein</th>
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*also named Paris V, Chapel Hill III; **also named Christchurch II, London VIII, St-Germain III, Vicenza III; ***at homozygous state.
thrombosis in afibrinogenemia. Of note, when evaluating the patient’s clinical phenotype, it is crucial to take into account the family history and environmental risk factors that can contribute to the heterogeneity of symptoms, beyond the genotype. In hypofibrinogenemia, the bleeding phenotype is strongly correlated to the fibrinogen concentration. However, to date, no prospective data have been published allowing us to determine the real incidence of bleeding in hypofibrinogenemia or leading to identify factors modulating their clinical phenotype.

So far, most studies on CFD employed traditional Sanger sequencing to identify molecular defects, thus missing other mutations that could influence the patient’s clinical phenotype. NGS now provides information on the most known genetic modifiers of fibrinogen and the hemostatic balance. In addition, NGS allows the development of a large data set and the identification of mutations not detected by standard PCR amplification. However, strong bioinformatic tools are required to analyze the amount of data and especially filtering variants against the allele frequencies. When considering NGS in the investigation of a rare bleeding disorder, it is important to highlight that: i) additional analyses may be required to determine whether a variant is pathogenic or not; ii) that physicians may have to deal with incidental findings and ethical issues; iii) that NGS has a great accuracy for single nucleotide changes, but complex rearrangements are not detected.

Increased fibrinogen concentrations are associated with decreased fibrin clot permeability and tighter clots when associated with a polymorphism (rs5985) of the F13A gene encoding factor XIII subunit A Val34Leu. Coding polymorphisms in the fibrinogen genes also influence the fibrin clot structure. The common FGB BArg478Lys polymorphism (rs4220) is associated with thin fibrin fibers, small pores, increased stiffness, and hypofibrinolysis, while the FGA Thr331Ala polymorphism (rs6050) alters FXIII-dependent fibrin cross-linking. In the future, the largest studies focusing on the significance of polymorphisms and the impact of variants of other genes involved in coagulation will provide a global view of the hemostatic balance of patients with CFD.

Global hemostasis assays and fibrin clot study

Integrative hemostatic assays, such as thrombin generation assay and studies of fibrin clots may provide additional information on the global hemostatic balance of the patient. In afibrinogenemia, thrombin generation has been tested to monitor the effect of fibrinogen replacement on the overall thrombotic potential. In a series of 20 patients, infusion of a standard dose of fibrinogen concentrate led to a statistically significant increase in endogenous thrombin potential even though without reaching values observed in healthy controls. This study was not powered to determine whether basal decreased or increased thrombin generation was correlated with a risk of bleeding or thrombosis. In dysfibrinogenemia, thrombin generation measured by ST Genesiaa with both Bleed- and ThromboScreen was not correlated with the clinical phenotype in a series of 22 patients. Given the strong interplay between thrombin and fibrin, it could be worth deeply investigating how the absence or the presence of dysfunctional fibrinogen may have an impact on the complex mechanism of thrombin generation or, on the contrary, on the fibrinolytic potential.

Several lines of growing evidence have pointed out the important role of fibrin structure in thrombosis and/or bleeding. The structure of the fibrin clot is a major determinant of the mechanical properties and of the clot resistance to fibrinolysis by tissue plasminogen activator and the plasmin system. Therefore, many efforts have been done in determining the effect of dysfunctional fibrinogen on the fibrin clot network and properties. On one side, patients with a bleeding phenotype have abnormal polymerization profiles, increased lysis, and thick fibrin fiber with large pores. On the other side, patients with a thrombotic phenotype have fibrin networks composed of thin fiber strands that have small pores and are more rigid and less permeable. If these methods are often investigated to explain the clinical presentation of single cases, data on large series are limited. Recently, defective fibrin polymerization and prolonged fibrinolysis have been reported in 24 patients with dysfibrinogenemia compared to healthy individuals, even though without statistical significance. Overall, a comprehensive functional assessment of properties and ultrastructure of the fibrin clot in a large cohort of patients is lacking. Moreover, fibrin clot studies in dysfibrinogenemia rarely take into account other genetic variations that might influence or modulate the fibrin clot (i.e., intermediate phenotype), and no prospective data are available so far. Overall, such studies could allow us to better predict the clinical outcomes and offer a more personalized therapeutic strategy toward precision medicine.

Conclusions

We should start considering CFD as an oligogenic rather than just a monogenic disease and focus research on the assessment of common variants that might contribute to clinical variations between patients with CFD. Today we are far from having clinically relevant information on most genetic modifiers and their impact on the hemostasis balance. Understanding the genetic-related thrombin generation and fibrin clot mechanisms involved in a patient’s phenotype will help in understanding the pathophysiological mechanisms underlying the bleeding and/or thrombosis in CFD.

References

6. Casini A, Moerloose P, Neerman-Arbez M. One Hundred

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