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] hundred 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 fibrin(ogen).[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.[7,8]

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).[9-22] Fibrinogen storage diseases are usually suspected in familial history of cryptogenic liver disease associated with hypofibrinogenemia.[23] 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 fibrin(ogen) 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 αC regions impair the lateral fibril aggregation, as well as factor XIII crosslinking, endothelial cell, and platelet binding.[25] 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 1. Variants of hypofibrinogenemia associated with fibrinogen storage disease and variants of dysfibrinogenemia associated with thrombosis.

Mutant fibrinogen	Gene	Exon	Native protein	Reference
Fibrinogen storage disease				
Aguadilla	FGG	9	p.Arg401Trp	9
AI duPont	FGG	8	p.Thr340Pro	10
Angers	FGG	8	p.delGVYYQ372-376	11
Ankara	FGG	8	p.His366Asp	12
Beograd	FGG	9	p.Gly392Ser	13
Brescia	FGG	8	p.Gly310Arg	14
Pisa	FGG	8	p.Asp342Asn	13
Trabzon	FGG	9	p.Thr397Ile	15
Thrombotic-related dysfibrinogenemia				
Caracas V	FGA	5	p.Ser551Cys	16
Dusart*	FGA	5	p.Arg573Cys	17
Ijmuiden**	FGB	2	p.Arg44Cys	18
Melun	FGG	8	p.Asp390Val	19
Naples***	FGB	2	p.Ala98Thr	20
New York	FGB	2	p.del39-102	21
Nijmegen	FGB	2	p.Arg74Cys	22

*also named Paris V, Chapel Hill III; **also named Christchurch II, London VIII, St-Germain III, Vicenza III; ***at homozygous state.

thrombosis in afibrinogenemia.[32] 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.[33] In hypofibrinogenemia, the bleeding phenotype is strongly correlated to the fibrinogen concentration.[34] 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.[8] NGS now provides information on the most known genetic modifiers of fibrinogen and the hemostatic balance.[35,36] 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.[37]

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.[38] Coding polymorphisms in the fibrinogen genes also influence the fibrin clot structure. The common FGB BβArg478Lys polymorphism (rs4220) is associated with thin fibrin fibers, small pores, increased stiffness, and hypofibrinolysis,[39] while the FGA AαThr331Ala polymorphism (rs6050) alters FXIII-dependent fibrin cross-linking.[40] 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 hemostasis 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.[41] 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 Genesiaâ with both Bleed- and ThromboScreen was not correlated with the clinical phenotype in a series of 22 patients.[42] Given the strong interplay between thrombin and fibrin,[43] 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.[44]

Several lines of growing evidence have pointed out the important role of fibrin structure in thrombosis and/or bleeding.[45] 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.[46] Therefore, many efforts have been done in determining the effect of dysfunctional fibrinogen on the fibrin clot network and properties.[47] On one side, patients with a bleeding phenotype have abnormal polymerization profiles, increased lysis, and thick fibrin fiber with large pores.[48] 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.[49] 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.[50] 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),[24] 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 CDF. 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

1. Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. Blood 2015;125:2052-61.
2. Luo M, Xiang L, Yan J, et al. Fibrinogen Clauss and prothrombin time derived method ratio can differentiate dysfibrinogenemia from hypofibrinogenemia and hyperfibrinogenemia. Thromb Res 2020;194:197-9.
3. Casini A, Undas A, Palla R, et al. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. J Thromb Haemost 2018;16:1887-90.
4. Rabe F, Saloman E. Ueber-faserstoffmangel im blute bei einem falle von hemophilie. Arch Intern Med 1920:2-14.
5. Imperato C, Dettori AG. Congenital hypofibrinogenemia with fibrinoasthenia. Helv Paediatr Acta 1958;13:380-99.
6. Casini A, Moerloose P, Neerman-Arbez M. One Hundred

Years of Congenital Fibrinogen Disorders. Semin Thromb Hemost 2022;48:880-8.

7. Margaglione M, Vecchione G, Santacroce R, et al. A frameshift mutation in the human fibrinogen Aalpha-chain gene (Aalpha(499)Ala frameshift stop) leading to dysfibrinogen San Giovanni Rotondo. Thromb Haemost 2001;86:1483-8.

8. Asselta R, Plate M, Robusto M, et al. Clinical and molecular characterisation of 21 patients affected by quantitative fibrinogen deficiency. Thromb Haemost 2015;113:567-76.

9. Brennan SO, Maghzal G, Shneider BL, et al. Novel fibrinogen gamma375 Arg-->Trp mutation (fibrinogen aguadilla) causes hepatic endoplasmic reticulum storage and hypofibrinogenemia. Hepatology 2002;36:652-8.

10. Brennan SO, Davis RL, Conard K, et al. Novel fibrinogen mutation gamma314Thr-->Pro (fibrinogen AI duPont) associated with hepatic fibrinogen storage disease and hypofibrinogenaemia. Liver Int 2010;30:1541-7.

11. Dib N, Quelin F, Ternisien C, et al. Fibrinogen angers with a new deletion (gamma GVYYQ 346-350) causes hypofibrinogenemia with hepatic storage. J Thromb Haemost 2007;5:1999-2005.

12. Callea F, Giovannoni I, Sari S, et al. Fibrinogen Gamma Chain Mutations Provoke Fibrinogen and Apolipoprotein B Plasma Deficiency and Liver Storage. Int J Mol Sci 2017;18.

13. Asselta R, Robusto M, Braidotti P, et al. Hepatic fibrinogen storage disease: identification of two novel mutations (p.Asp316Asn, fibrinogen Pisa and p.Gly366Ser, fibrinogen Beograd) impacting on the fibrinogen gamma-module. J Thromb Haemost 2015;13:1459-67.

14. Brennan SO, Wyatt J, Medicina D, et al. Fibrinogen brescia: hepatic endoplasmic reticulum storage and hypofibrinogenemia because of a gamma284 Gly-->Arg mutation. Am J Pathol 2000;157:189-96.

15. Burcu G, Bellacchio E, Sag E, et al. Structural Characteristics in the gamma Chain Variants Associated with Fibrinogen Storage Disease Suggest the Underlying Pathogenic Mechanism. Int J Mol Sci 2020;21.

16. Marchi R, Lundberg U, Grimbergen J, et al. Fibrinogen Caracas V, an abnormal fibrinogen with an Aalpha 532 Ser-->Cys substitution associated with thrombosis. Thromb Haemost 2000;84:263-70.

17. Koopman J, Haverkate F, Grimbergen J, et al. Molecular basis for fibrinogen Dusart (A alpha 554 Arg-->Cys) and its association with abnormal fibrin polymerization and thrombophilia. J Clin Invest 1993;91:1637-43.

18. Koopman J, Haverkate F, Grimbergen J, et al. Abnormal fibrinogens IJmuiden (B beta Arg14----Cys) and Nijmegen (B beta Arg44----Cys) form disulfide-linked fibrinogen-albumin complexes. Proc Natl Acad Sci U S A 1992;89:3478-82.

19. Bentolila S, Samama MM, Conard J, et al. [Association of dysfibrinogenemia and thrombosis. Apropos of a family (Fibrinogen Melun) and review of the literature]. Ann Med Interne (Paris) 1995;146:575-80.

20. Koopman J, Haverkate F, Lord ST, et al. Molecular basis of fibrinogen Naples associated with defective thrombin binding and thrombophilia. Homozygous substitution of B beta 68 Ala----Thr. J Clin Invest 1992;90:238-44.

21. Al-Mondhiry H, Bilezikian SB, Nossel HL. Fibrinogen "New York"--an abnormal fibrinogen associated with thromboembolism: functional evaluation. Blood 1975;45:607-19.

22. Engesser L, Koopman J, de Munk G, et al. Fibrinogen Nijmegen: congenital dysfibrinogenemia associated with impaired t-PA mediated plasminogen activation and decreased binding of t-PA. Thromb Haemost 1988;60:113-20.

23. Asselta R, Paraboschi EM, Duga S. Hereditary Hypofibrinogenemia with Hepatic Storage. Int J Mol Sci 2020;21.

24. Casini A, Neerman-Arbez M, Ariens RA, de Moerloose P. Dysfibrinogenemia: from molecular anomalies to clinical manifestations and management. J Thromb Haemost 2015;13:909-19.

25. Li Y, Ding B, Wang X, Ding Q. Congenital (hypo-)dysfibrinogenemia and bleeding: A systematic literature review. Thromb Res 2022;217:36-47.

26. Casini A, Blondon M, Lebreton A, et al. Natural history of patients with congenital dysfibrinogenemia. Blood 2015;125:553-61.

27. Castaman G, Giacomelli SH, Biasoli C, et al. Risk of bleeding and thrombosis in inherited qualitative fibrinogen disorders. Eur J Haematol 2019;103:379-84.

28. Zhou J, Ding Q, Chen Y, et al. Clinical features and molecular basis of 102 Chinese patients with congenital dysfibrinogenemia. Blood Cells Mol Dis 2015;55:308-15.

29. Simurda T, Zolkova J, Kolkova Z, et al. Comparison of clinical phenotype with genetic and laboratory results in 31 patients with congenital dysfibrinogenemia in northern Slovakia. Int J Hematol 2020;111:795-802.

30. Wypasek E, Klukowska A, Zdziarska J, et al. Genetic and clinical characterization of congenital fibrinogen disorders in Polish patients: Identification of three novel fibrinogen gamma chain mutations. Thromb Res 2019;182:133-40.

31. Casini A, von Mackensen S, Santoro C, et al. Clinical phenotype, fibrinogen supplementation, and health-related quality of life in patients with afibrinogenemia. Blood 2021;137:3127-36.

32. Casini A, Neerman-Arbez M, de Moerloose P. Heterogeneity of congenital afibrinogenemia, from epidemiology to clinical consequences and management. Blood Rev 2021;48:100793.

33. Santacroce R, Cappucci F, Pisanelli D, et al. Inherited abnormalities of fibrinogen: 10-year clinical experience of an Italian group. Blood Coagul Fibrinolysis 2006;17:235-40.

34. Peyvandi F, Palla R, Menegatti M, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. J Thromb Haemost 2012;10:615-21.

35. Couzens A, Lebreton A, Masclaux F, et al. Hemizygous FGG p.Ala108Gly in a hypofibrinogenemic patient with a heterozygous 14.8 Mb deletion encompassing the entire fibrinogen gene cluster. Haemophilia 2022;28:e132-e5.

36. Bor MV, Feddersen S, Pedersen IS, et al. Dysfibrinogenemia-Potential Impact of Genotype on Thrombosis or Bleeding. Semin Thromb Hemost 2022;48:161-73.

37. Guipponi M, Masclaux F, Sloan-Bena F, et al. A homozygous duplication of the <I>FGG</i> exon 8-intron 8 junction causes congenital afibrinogenemia. Lessons learned from the study of a large consanguineous Turkish family. Haematologica 2022;107:1064-71.

38. Ariens RA, Philippou H, Nagaswami C, et al. The factor XIII V34L polymorphism accelerates thrombin activation

of factor XIII and affects cross-linked fibrin structure. Blood 2000;96:988-95.

39. Ajjan R, Lim BC, Standeven KF, et al. Common variation in the C-terminal region of the fibrinogen beta-chain: effects on fibrin structure, fibrinolysis and clot rigidity. Blood 2008;111:643-50.

40. Carter AM, Catto AJ, Kohler HP, et al. alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism. Blood 2000;96:1177-9.

41. Khayat C, Marchi R, Durual S, et al. Impact of Fibrinogen Infusion on Thrombin Generation and Fibrin Clot Structure in Patients with Inherited Afibrinogenemia. Thromb Haemost 2022;122:1461-8.

42. Szanto T, Lassila R, Lemponen M, et al. Whole Blood Thromboelastometry by ROTEM and Thrombin Generation by Genesia According to the Genotype and Clinical Phenotype in Congenital Fibrinogen Disorders. Int J Mol Sci 2021;22.

43. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev 2007;21:131-42.

44. Simpson ML, Goldenberg NA, Jacobson LJ, et al. Simulta-neous thrombin and plasmin generation capacities in normal and abnormal states of coagulation and fibrinolysis in children and adults. Thromb Res 2011;127:317-23.

45. Zabczyk M, Ariens RAS, Undas A. Fibrin clot properties in cardiovascular disease: from basic mechanisms to clinical practice. Cardiovasc Res 2023;119:94-111.

46. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. Blood 2013;121:1712-9.

47. Undas A. How to Assess Fibrinogen Levels and Fibrin Clot Properties in Clinical Practice? Semin Thromb Hemost 2016;42:381-8.

48. Marchi R, Vilar R, Durual S, et al. Fibrin clot properties to assess the bleeding phenotype in unrelated patients with hypodysfibrinogenemia due to novel fibrinogen mutations. Thromb Res 2021;197:56-64.

49. Collet JP, Woodhead JL, Soria J, et al. Fibrinogen Dusart: electron microscopy of molecules, fibers and clots, and viscoelastic properties of clots. Biophys J 1996;70:500-10.

50. Casini A, Duval C, Pan X, et al. Fibrin clot structure in patients with congenital dysfibrinogenaemia. Thromb Res 2016;137:189-95.